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# **Modelling the normal retinal nerve fibre layer thickness as measured by Stratus optical coherence tomography**

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## Abstract

**Background:** The variation in retinal nerve fibre layer thickness (RNFLT) as measured by Stratus optical coherence tomography (OCT) in healthy subjects may be reduced when the effect on RNFLT measurements of factors other than disease is corrected for, and this may improve the diagnostic accuracy in glaucoma. With this perspective we evaluated the isolated and combined effects of factors potentially affecting the Stratus OCT RNFLT measurements in healthy subjects.

**Methods:** We included 178 healthy eyes of 178 subjects between 20 and 80 years of age. Participants underwent an extensive eye examination. Stratus OCT RNFLT was measured by three standard protocols, two with high and one with standard image resolution. Effects on RNFLT of age, gender, refractive error, axial length, lens nuclear colour and opalescence, intra-ocular pressure (IOP), and optic disc size were examined by univariate and multivariate analyses.

**Results:** Age, refractive error, axial length, and lens nuclear colour and opalescence affected RNFLT in univariate analyses, whereas gender, IOP, and optic disc size had no significant effect. In multivariate analyses only age in combination with refractive error, or with axial length, was significant and explained 14.7–17.6% ( $R^2$ ) of the total variation of RNFLT, approximately 50% more than age alone. RNFLT decreased by 2.6–2.9  $\mu\text{m}$  per increasing decade of age and increased by 1.5–1.8  $\mu\text{m}$  per more positive diopter of spherical equivalent using full-circle measurements of the three standard protocols. These effects varied between measurement sectors.

**Conclusions:** RNFLT as measured by Stratus OCT standard protocols was significantly affected by age and refractive status. The effect on global RNFLT of a difference in refractive error of 10 diopters corresponded to the effect of a difference in age of 60 years. Theoretically, the effect of refractive status may be explained by artefacts of RNFLT measurement circle placement. The results suggest that the diagnostic accuracy of Stratus OCT may be improved by considering refractive status in addition to age when RNFLT is measured. For this purpose spherical equivalent seems as effective as axial length.

**Keywords:** Nerve fibre layer thickness - Optical coherence tomography - Healthy subjects - Age - Refractive error - Lens - Optic disc

## Introduction

Objective measurements of retinal nerve fibre layer thickness (RNFLT) can be of value in glaucoma diagnosis and follow-up. RNFLT can be measured objectively in vivo by the laser-based imaging technique, optical coherence tomography (OCT) [5, 10, 17]. The Stratus OCT (model 3000, Carl Zeiss Meditec, Dublin, CA), a commercially available third-generation instrument, has improved image resolution and scan rate compared with earlier OCT versions. Promising results using RNFLT quantification of this instrument in glaucoma diagnosis have been reported [6, 14, 15]. Knowledge of factors other than disease affecting OCT RNFLT measurements may decrease the random variability and improve the diagnostic sensitivity. Such factors may be related to anatomical RNFLT variations and specifically related to the RNFLT measurement technique of the Stratus OCT. The potential effects on peripapillary OCT RNFLT measurements of several factors such as gender [5, 9, 19], age [5, 9, 12, 18], refractive status [3, 5, 9, 12], optic disc size [5, 16], lens fluorescence, and OCT image quality, probably related to presence of media opacities [9], have been studied, but often with an earlier generation OCT instrument of poorer image resolution than the Stratus OCT. Different software algorithms for defining the RNFLT were used, and some studies did not examine the combined effects of different factors on RNFLT measurements. Age is a directly accessible parameter and has been reported to affect the quantity of nerve fibres in histological studies [2, 11, 13] and the RNFLT measurements in studies with OCT instruments of earlier generations [5, 9, 12, 18].

The software provided by the manufacturer has normal age-corrected reference values for two different Stratus OCT RNFLT scan protocols, one of standard and one of high image resolution. A relatively large RNFLT variability remains among healthy subjects even after elimination of the effect of age. A model of the normal RNFLT including factors other than age which can explain a greater part of the variability between subjects would beneficially narrow the normal limits and consequently result in better diagnostic accuracy.

With this perspective, the purpose of this study was to examine the isolated and combined effects on Stratus OCT RNFLT as measured using different standard scan protocols of a number of potentially important factors in a large representative group of healthy subjects covering a wide age range. We studied the effects of age, gender, refractive status, lens nuclear opalescence and colour, intra-ocular pressure (IOP), and optic disc size.

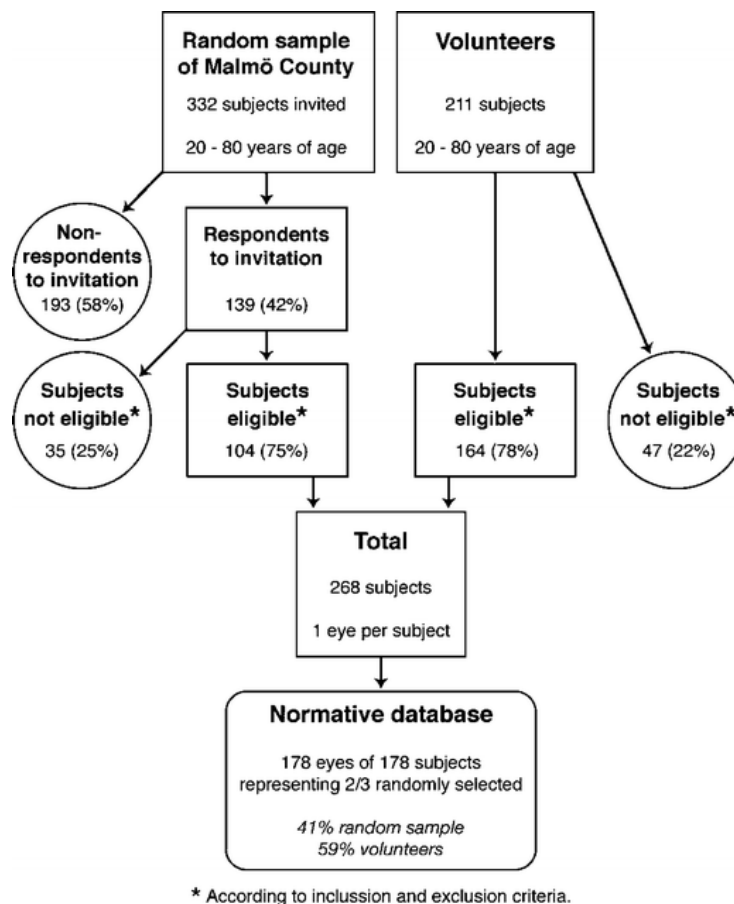
## Materials and methods

### Participant recruitment

Healthy subjects of 20–80 years of age were eligible. A group of 332 subjects with equal distribution between age-decades and between genders within each age-decade were randomly selected among the population of Malmö County. Subjects who had been examined at the Department of Ophthalmology,

Malmö University Hospital within the 4 years prior to randomization were not eligible. A second group was comprised of volunteers, medical students or staff at Malmö University Hospital, members of social societies for elderly persons, and relatives/friends of these subjects.

Figure 1 presents a flow chart of subject recruitment/inclusion.



**Fig. 1** Subject recruitment/inclusion flow chart. One hundred and four subjects from a random sample of Malmö County and 164 volunteers were included. Of these 268 subjects approximately two-thirds (178 eyes) were randomized to create a normative database

## Examination protocol

All subjects were asked to fill in a questionnaire that was mailed or handed out prior to examination. The questionnaire addressed contacts with ophthalmologists, presence of eye symptoms (prior or current), family history of glaucoma, and current use of medication. The answers of the questionnaire were expanded during an interview, and the subject's geographical/ethnic origin was registered.

All participants were subjected to a full ophthalmological examination of both eyes. Visual acuity (VA) was assessed on a Snellen decimal scale with an autorefractor (model 595, Humphrey Instruments, San Leandro, CA). If VA was  $<1.0$  a manual refraction was performed using a Snellen chart. Perimetry was performed with frequency doubling technology (FDT) (Welch Allyn, Skaneateles Falls, NY) screening tests (C20-1). If FDT results were obviously flawed by the subject misunderstanding the test instructions, a re-test was allowed. Pupils were dilated and fundus, RNFL and stereo optic nerve head (ONH) digital photographs were obtained followed by circumpapillary OCT measurements. A slit-lamp examination was performed that included lens opacity classification according to the Lens Opacity Classification System (LOCS) II [7, 8], and IOP was measured with Goldmann applanation tonometry. Axial length was measured using ultrasonography (Microscan, model 100A, Sonomed, Lake Success, NY). The average of five measurements with a standard deviation  $\leq 0.1$  mm was taken as the axial length of the eye.

Informed consent was obtained from all subjects, and the study was performed according to the Helsinki Declaration and approved by the Committee for Research Ethics at Lund University.

## Inclusion criteria

All of the following criteria had to be satisfied for inclusion:

- Absence of glaucomatous atrophy of the RNFL (widespread or localized) in both eyes assessed by subjective evaluation of RNFL photographs by two observers.
- Absence of glaucomatous ONH excavation, i.e. neuro-retinal rim thinning vertically and/or notching in both eyes by subjective evaluation of stereo ONH photographs by two observers.
- All visual field reliability indices, false-positive and false-negative answers, and fixation losses,  $<30\%$  in both eyes.
- A reliable FDT test within 99% of normal limits in all test areas in both eyes, or, if outside normal limits: a reliable Swedish interactive thresholding algorithm (SITA) standard 24–2 visual field obtained with the Humphrey Field Analyzer II (Carl Zeiss Meditec) in both eyes within 4 weeks with the glaucoma hemifield test (GHT) “within normal limits” or “borderline” and the pattern standard deviation (PSD) within the 95% normal limit.
- IOP  $<22$  mmHg in both eyes and inter-eye difference less than 4 mmHg.
- VA  $\geq 0.5$  in both eyes, or if VA was reduced to below 0.5 due to amblyopia or media opacities in one eye the contra-lateral eye was eligible.

## Exclusion criteria

Presence of any of the following led to exclusion:

- History, records or findings of diabetic retinopathy, optic neuritis or cerebrovascular insult affecting the visual function.
- A history of blunt or penetrating ocular trauma.
- Findings of retinal pathology apart from mild age-related drusen maculopathy.
- A refractive error greater than  $\pm 5$  diopters (D) sphere or  $\geq 3$  D cylinder.



- Presence of corneal opacities or cortical (LOCS C>II) or subcapsular (LOCS P>0) cataracts within the optical axis of the eye. We did not exclude subjects based on LOCS nuclear opalescence or colour grading results.
- Overt OCT image artefacts apart from those expected due to presence of blood vessels or software algorithm errors in the RNFL definition (the inner border) in scan series essential for analyses as appraised by subjective evaluation.

Subjects with a family history of glaucoma or eyes with intra-ocular lens (IOL) implants were not excluded.

## Test methods

### Optical coherence tomography

To examine the RNFL we applied the Stratus OCT software version A4.0.3 and scan protocols using circumpapillary scans with a preset diameter of 3.40 mm centred around the ONH. The right eye was always examined first. Scans were obtained at the maximum power of 750 mW. One photographer experienced in the technique obtained all OCT measurements.

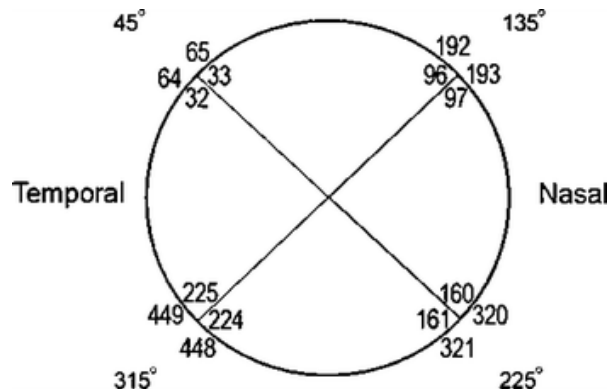
We recorded six OCT scan series per eye, two each of:

- A fast RNFLT scan protocol (three scans, each of 256 measurement points, continuously captured in 1.8 s)
- A RNFLT scan protocol with high resolution (three scans, each of 512 measurement points, each scan separately captured in 1.2 s)
- A RNFLT scan protocol with regular resolution (three scans, each of 256 measurement points, each scan separately captured in 0.6 s)

To address the purpose of the present study we report only results of RNFLT of the standard scan protocols “A” and “B” (above). If both series of a scan protocol were eligible, the series recorded first was used to calculate RNFLT values. The RNFLT was evaluated based on:

- (1) Average values of the three scans of “A”, referred to as protocol “Fast”
- (2) Average values of the three scans of “B”, referred to as protocol “High average”
- (3) The first of the three scans of “B”, referred to as protocol “High single”

The full-circle RNFLT was calculated (MATLAB version 7.0.4, Service Pack 2) for each scan and, corresponding to the different scan protocols, as the mean of the 512 or 256 RNFLT values. The RNFLT of the temporal, superior, nasal, and inferior quadrants were defined as the mean of measurement points included in the sectors from 315° to 45°, from 45° to 135°, from 135° to 225°, and from 225° to 315°, respectively (Fig. 2). The full-circle and quadrant RNFLT calculations were equivalent to the RNFLT analysis of the commercial OCT software.



**Fig. 2** Quadrant sections and number of measurement points of the peripapillary OCT scan circle. RNFLT of a single section was calculated as the average RNFLT of all corresponding measurement points. Measurement point numbers indicated inside and outside the circle apply to the 256 and 512 measurement point density protocols, respectively

## Fundus photography

We obtained digital black-and-white 35° and 50° RNFL photographs in red-free and in blue illumination (495 nm), digital 20° ONH colour stereo photographs, and digital 50° colour fundus photographs centred on the fovea for both eyes with a non-telecentric Topcon TRC-50IX (Topcon, Tokyo, Japan) fundus camera equipped with a Kodak Megaplug camera 1.4i/10 (Eastman Kodak, San Diego, CA, USA) black-and-white digital array sensor and a Sony Power HAD, DXC 950P, three-CCD colour video camera (Sony Corporation, Tokyo, Japan).

## Optic disc area assessments

The outline of the optic disc margin corresponding to the inner border of the scleral ring was accomplished manually guided by 3D viewing of the digital ONH colour stereo photographs (see above), using the IMAGEset 2000 software version 2.55 (Topcon, Tokyo, Japan). The optic disc area corrected for the camera magnification factor was calculated automatically. We mathematically corrected these measurements to additionally account for the effect of differences in axial length. This correction was based on the observed relation in 20 eyes between axial length and differences in disc area between the Topcon ONH photograph IMAGEset 2000 software measurements and measurements corrected for the camera magnification factor and axial length of the eye according to Bengtsson and Krakau [4] based on ONH photographs of the Zeiss telecentric fundus camera (Zeiss, Oberkochen, Germany).

Reproducibility of optic disc area assessments, measured as the coefficient of variation between two repeated IMAGEset 2000 measurements in 20 subjects randomly selected from the study population of 178, was 1.7%.

## Statistics

When both eyes were eligible, one eye was randomly chosen per subject for data analysis. Standard statistical analyses were made using SAS software version 8.2 (SAS Institute, Cary, NC, USA). A  $p$  value  $\leq 0.05$  indicated statistical significance. Simple linear regression analyses (least mean square), two-sample  $t$ -test or ANOVA were used in univariate analyses of factors potentially affecting RNFLT. All the factors significantly affecting RNFLT of each OCT protocol in univariate analyses were further analysed in multivariate analyses by multiple linear regression (least mean square, backward selection). Regression coefficients for significant factors were compared between OCT protocols.

## Results

### Demographic and clinical data

All participants were examined between 23 February 2004 and 25 February 2005. Figure 1 presents a subject recruitment/inclusion flow chart. Of the original 332 randomly invited subjects, 58% did not respond or did not wish to participate. Of the 350 subjects examined, 82 (23%) were excluded. The major causes of exclusion of subjects or eyes were refractive errors, media opacities, visual field test results, and/or IOP exceeding or outside criteria. The single cause of exclusion was bad OCT image quality or software RNFL definition problems in three subjects.

We included 178 healthy eyes of 178 subjects representing a randomized sample of two-thirds of all eligible healthy subjects (Fig. 1). The randomized selection of these subjects was done with the purpose of creating a normative database.

Gender, age, VA, refractive error (spherical equivalent), axial length, LOCS nuclear colour and opalescence, IOP, and optic disc area are listed in Table 1.

**Table 1** Demographic and clinical data in 178 eyes of 178 healthy subjects

Gender (female/male ratio)	97/81
Age (years) <sup>a</sup>	57.1 (20 – 79)
Visual acuity (Snellen decimal) <sup>a</sup>	1.0 (0.5 – 1.0)
Refractive error (diopters) <sup>a</sup>	+0.1 (–5.4 – +4.4)
Axial length (mm) <sup>a</sup>	23.5 (21.3 – 27.9)
LOCS nuclear colour (0,1,2) <sup>a</sup>	1 (0 – 2)
LOCS nuclear opalescence (0,1–4) <sup>a</sup>	1 (0 – 2)
Goldmann IOP (mmHg) <sup>a</sup>	14.5 (8 – 21)
Optic disc area (mm <sup>2</sup> ) <sup>a</sup>	2.05 (1.11 – 3.49)

LOCS, Lens Opacity Classification System II [7, 8]

<sup>a</sup>Median (min.–max.)

An ethnic/geographical origin other than Scandinavian was registered in 14 subjects (7.9%). A positive family history of glaucoma was registered in 32 subjects (18.0%). The included eyes of two subjects (1.1%) were pseudophakic.

## Univariate analyses

Age, refractive error, axial length, LOCS nuclear opalescence and colour all came out as significant factors that affected the full circle RNFLT, whereas gender, IOP, or optic disc area were statistically non-significant (Tables 2a and 2b).

**Table 2a** Univariate analyses of different factors potentially affecting the Stratus OCT RNFLT as measured using three standard protocols: simple regression analyses

Factors	OCT protocol	Regression coefficient [μm/....]	<i>p</i> value	<i>R</i> <sup>2</sup> [%]
Age (decade)	Fast	−2.1	<0.0001	9.1
	High average	−1.8	<0.0001	8.4
	High single	−1.9	<0.001	8.0
Refractive error <sup>a</sup> (diopters)	Fast	0.9	0.035	2.5
	High average	0.8	0.034	2.5
	High single	0.7	0.075	1.8
Axial length (mm)	Fast	−2.6	<0.0001	5.4
	High average	−2.5	<0.001	6.1
	High single	−2.4	0.002	5.3
Intra-ocular pressure Goldmann (mmHg)	Fast	−0.48	0.089	1.6
	High average	−0.49	0.054	2.1
	High single	−0.46	0.088	1.7
Optic disc area (mm <sup>2</sup> )	Fast	0.92	0.640	0.1
	High average	1.43	0.420	0.4
	High single	1.45	0.435	0.3

<sup>a</sup>Spherical equivalent

**Table 2b** Univariate analyses of different factors potentially affecting the Stratus OCT RNFLT as measured using three standard protocols: two-sample *t*-test, and ANOVA tests

Factors	OCT protocol	Full circle [mean]		RNFLT	<i>p</i> value <sup>a</sup>
		<i>Female</i>		<i>Male</i>	
Gender (female, male)	Fast	100.6		101.7	0.449
	High average	96.3		97.8	0.282
	High single	96.3		98.0	0.245
		0	1	2	
Nuclear opalescence LOCS (0, 1–4)	Fast	103.6	100.0	96.9	0.018
	High average	99.1	96.1	93.5	0.036
	High single	99.3	96.0	93.7	0.037
		0	1	2	
Nuclear colour LOCS (0, 1, 2)	Fast	104.4	99.4	97.2	<0.001
	High average	99.8	95.5	93.7	<0.002
	High single	100.0	95.7	93.4	<0.002

LOCS, Lens Opacity Classification System II [7, 8]

<sup>a</sup>Two sample *t*-test for gender; ANOVA for nuclear opalescence and colour

## Multivariate analyses and RNFLT modelling

Age was a significant factor for full-circle RNFLT in the multiple regression analysis, which included, in addition to age, the factors, refractive error, axial length and LOCS II lens classification for nuclear colour and opalescence. This was true for all three OCT protocols. During backward selection age, refractive error and axial length were the factors left before the final selection step in which age and additionally either refractive error or axial length reached statistical significance depending on OCT protocol examined. The significant inverse correlation ( $r=-0.64$ ,  $p<0.0001$ ) between refractive error and axial length made both these factors significant when analysed in separate models in combination with age (Table 3).

The coefficient of determination ( $R^2$ ) of the RNFLT model improved by approximately 50%, e.g. from 9.1 to 17.6, using the “fast” protocol, when adding the refractive error or axial length into the model (Tables 2a and 3).

**Table 3** Multivariate analyses including only factors which reached significance in univariate analyses of RNFLT as measured by three Stratus OCT standard protocols

Multiple linear regression analyses	Model including all factors <sup>a</sup>	Model including significant factors <sup>b</sup>	Alternative model <sup>c</sup>
<b>Full circle RNFLT</b>			
<b>OCT protocol: Fast</b>	Model R <sup>2</sup> (%)		
	20.0	17.6	17.1
	<i>p</i> value		
Age (years)	0.003	<0.0001	<0.0001
Refractive error <sup>d</sup> (diopters)	0.029	<0.0001	–
Axial length (mm)	0.099	–	<0.0001
LOCS nuclear colour	0.118	–	–
LOCS nuclear opalescence	0.908	–	–
<b>OCT protocol: High average</b>	Model R <sup>2</sup> (%)		
	19.4	17.2	16.7
	<i>p</i> value		
Age (years)	0.003	<0.0001	<0.0001
Refractive error <sup>d</sup> (diopters)	0.051	–	<0.0001
Axial length (mm)	0.049	<0.0001	–
LOCS nuclear colour	0.152	–	–
LOCS nuclear opalescence	0.789	–	–
<b>OCT protocol: High single</b>	Model R <sup>2</sup> (%)		
	17.6	15.8	14.7
	<i>p</i> value		
Age (years)	0.007	<0.0001	<0.0001
Refractive error <sup>d</sup> (diopters)	0.107	–	0.0003
Axial length (mm)	0.053	<0.0001	–
LOCS nuclear colour	0.133	–	–
LOCS nuclear opalescence	0.800	–	–

LOCS, Lens Opacity Classification System II [7, 8]

<sup>a</sup>The multiple regression model including all factors significant in univariate analyses of RNFLT

<sup>b</sup>The multiple regression model including all factors significant in the multivariate analysis

<sup>c</sup>Like <sup>b</sup>but with refractive error replaced by axial length and visa versa

<sup>d</sup>Spherical equivalent

The regression coefficients of the multiple regression models for age and spherical equivalent and for age and axial length are presented in Table 4 for full circle, superior, nasal, and inferior quadrants. None of the regression coefficients of the two models were significant in the temporal quadrant. No major differences in regression coefficients were present among OCT protocols, but differences were seen between refractive status regression coefficients among peripapillary measurement sectors, with the steepest slopes in the inferior quadrant, followed by the superior and nasal quadrants.

**Table 4** Magnitude of influence of significant factors in two regression models of global and sector-wise Stratus OCT RNFLT of three standard protocols

OCT RNFLT parameter	OCT protocol	Model including age and refractive error		Model including age and axial length	
		Regression coefficients		Regression coefficients	
		Age [ $\mu\text{m}/\text{decade}$ ]	Spherical equivalent [ $\mu\text{m}/\text{diopter}$ ]	Age [ $\mu\text{m}/\text{decade}$ ]	Axial length [ $\mu\text{m}/\text{mm}$ ]
Full circle	Fast	-2.9*	1.8*	-2.4*	-3.2*
	High average	-2.6*	1.6*	-2.1*	-3.0*
	High single	-2.6*	1.5*	-2.2*	-3.0*
Superior quadrant	Fast	-4.1*	2.2‡	-3.5*	-4.0‡
	High average	-3.6*	2.1‡	-3.0*	-4.0†
	High single	-3.4*	1.8†	-2.9†	-3.6‡
Nasal quadrant	Fast	-3.6*	1.9‡	-3.1*	-3.8†
	High average	-3.2*	1.4\$	-2.9*	-3.8†
	High single	-3.5*	1.4\$	-3.3*	-4.0†
Inferior quadrant	Fast	-3.9*	3.2*	-3.0†	-5.9*
	High average	-3.6*	3.1*	-2.7†	-5.8*
	High single	-3.8*	3.2*	-2.9†	-5.9*

\* $p$  value<0.0001

†0.0001 $\leq p$  value<0.001

‡0.001 $\leq p$  value<0.01

\$0.01 $\leq p$  value<0.05

## Discussion

We had the opportunity to examine the isolated but also the combined effects of several factors potentially affecting the RNFLT as measured by Stratus OCT. In our study only age and refractive status (refractive error or axial length) in combination significantly affected the Stratus OCT RNFLT among the factors age, gender, refractive error, axial length, nuclear lens opalescence and/or colour, IOP and optic disc area. Refractive status (refractive error or axial length) and age in combination explained approximately 50% more of the total normal variation in global RNFLT than age alone.

Age has been shown to influence the RNFLT significantly in different OCT studies, although the studies report some differences regarding the extent to which global and peripapillary sectors were affected [5, 9, 12]. To our knowledge the large effect of refractive status on OCT RNFLT measurements has not been shown previously. For global RNFLT the effect of a difference in refractive error of 10 diopters equalled the effect of a difference in age of 60 years.

We suggest that accounting for refractive status in addition to age should be considered when the Stratus OCT RNFLT values are interpreted. Refractive status and age both affected all OCT RNFLT protocols, and to approximately the same extent. The strong correlation between refractive error and axial length probably explain why the two factors each became significant in different OCT RNFLT protocols in the multiple regression analysis in which both factors were present prior to selection, but also why these two factors were significant in all OCT RNFLT protocols in combination with age.

The effect of refractive error on OCT RNFLT was reported weak and non- or borderline significant in studies with an earlier generation OCT instrument [5, 9, 12]. Like the present study, Bayraktar et al. [3] observed that the peripapillary OCT RNFLT was negatively correlated to axial length, but also using an earlier generation instrument (OCT model 2000), and they did not account for the effect of age. The theoretical reasons for refractive status to influence OCT measurements as discussed by Wakitani et al. [20] include that the diameter of the OCT measurement circle as actually projected onto the retina as an artefact becomes larger the longer the axial length of the eye. In the present study the magnitude of influence on RNFLT measurements of that correlation depends on RNFL anatomy, and the observed variation in regression coefficients of refractive error/axial length between peripapillary measurement locations probably reflects this. Although the effect of refractive status may be due to artefacts of OCT RNFLT measurement circle placement rather than based on anatomical differences in eyes of different refractive status, our study was not designed to answer this question.

The model including both age and refractive error seems to be preferable in clinical settings, since this obviates the need for axial length measurement, but whether this is true in all clinical situations, e.g. when a myopic shift in refraction is induced by increasing lens nuclear sclerosis/opalescence, warrants further examination. Theoretically, the model including axial length should be advantageous in pseudophakic eyes.

OCT RNFLT has been reported to be significantly correlated to lens fluorescence measurements [9]. In the present study lens nuclear opalescence or colour also came out as a significant factor in univariate



analyses but not in multivariate analyses. This might be explained by age and refractive status, being correlated to age-related lens changes (data not shown).

Savini et al. [16] recently reported a significant effect of optic disc size as measured by Stratus OCT on peripapillary OCT RNFLT measurements using a fixed scan circle diameter and suggested an increased number of nerve fibres [11] or a closer location of the scan circle to the ONH margin in eyes with a large ONH as explanations. Our study did not confirm this result, which may be explained by the fact that the optic disc and RNFLT OCT measurements of the Savini study were not corrected for axial length.

An effect of IOP in patients on RNFLT as measured by OCT has been proposed [1]. We did not observe any statistically significant association between IOP and RNFLT in subjects with normal IOP and without apparent signs of glaucomatous damage, although  $p$  values did almost reach statistical significance in univariate analyses. Furthermore, IOP did not reach statistical significance when analysed in combination with age or with age and refractive status. Consequently, we believe that the effect of different IOP levels within the normal range probably is without clinical significance for Stratus OCT RNFLT measurements in healthy subjects.

Given fulfilment of all inclusion/exclusion criteria there was no difference in RNFLT between subjects with and those without a family history of glaucoma ( $p=0.98$ ).

In conclusion, our results suggest that refractive status (refractive error or axial length) in addition to age should be considered when the normal RNFL thickness is measured by Stratus OCT, while gender, IOP, and/or optic disc area are not important. Lens nuclear opalescence and/or colour were not significant in multivariate analyses. The effect of refractive error on RNFLT measurements seems to be equivalent to that of axial length.

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