Objective: To study how customizing the peripapillary scan diameter on the basis of optic nerve head (ONH) diameter affects retinal nerve fiber layer (RNFL) thickness measurements using Stratus optical coherence tomography (OCT).

Methods: Retinal nerve fiber layer was examined using 1 fixed-diameter circular scan (3.4 mm) and 2 customized-diameter scans (at 0.5 mm and 1 mm from the ONH edge) in 81 healthy subjects.

Results: Using fixed-diameter scans, the mean RNFL thickness increased with larger ONH vertical diameters ($r=0.3425$, $P=0.002$), whereas using customized-diameter scans, negative correlations were detected ($r=-0.3004$ [$P=0.006$] at 0.5 mm and $r=-0.2369$ [$P=0.03$] at 1 mm from the ONH edge). The mean values obtained by customized-diameter scans showed lower standard deviations in most measurements, meaning a tendency toward lower interindividual variability.

Conclusions: When RNFL thickness is measured at a constant distance from the ONH edge, larger discs exhibit a thinner RNFL. Hence, the correlation between large discs and thicker RNFLs observed using the standard fixed-diameter scan probably represents a technical artifact reflecting the shorter distance between the scan and the ONH edge. A new normative database, stratified not only on the basis of age but also on the basis of ONH size, is suggested.

Arch Ophthalmol. 2007;125(7):901-905

RETIINAL NERVE FIBER LAYER (RNFL) thickness measurement using optical coherence tomography (OCT) has gained popularity as a diagnostic tool in several diseases of the optic nerve.1-3 Traditionally, OCT assesses RNFL thickness by means of a peripapillary circular scan with a fixed diameter of 3.4 mm because this diameter has been found to offer the best reproducibility.4

The possible effect of optic nerve head (ONH) size on RNFL thickness measurements using OCT was questioned by Carpineto et al 5 in 2003. Results of subsequent studies6,7 have shown that larger ONHs correlate with thicker RNFLs, as measured by OCT using the fixed-diameter circular scan. The following 2 possible explanations have been suggested for this result6: larger optic discs have more fibers (as shown by histological studies8,9), or the use of a fixed-diameter scan produces an artifact. The latter hypothesis derives from the notion that RNFL thickness decreases at increasing distances from the ONH10: if a fixed-diameter circular scan is used, the distance between the scan and the ONH margin will be reduced in the presence of a large ONH. Such an artifact may lead to an overestimation of RNFL thickness in patients with large ONHs because the measurements would be made closer to the optic disc edge. This study was conceived to determine whether the correlation of larger ONHs with thicker RNFLs can still be observed after measuring the peripapillary RNFLs by means of a circular scan in which the diameter is changed according to the ONH size so as to maintain a constant distance between the ONH edge and the scan.

METHODS

PATIENTS

This was a prospective observational cross-sectional study carried out in a private practice (Centro Salus, Bologna, Italy). Eighty-one eyes of 81 healthy subjects of white race aged between 20 and 50 years were enrolled (45 men and 36 women, mean±SD age, 34.4±8.3 years). The study population consisted of volunteers from the staff of Centro Salus and patients with minor refractive disor-
Inclusion criteria were the following: normal appearance of the optic disc, best-corrected visual acuity above 20/25, intraocular pressure lower than 21 mm Hg, no significant ocular involvement such as diabetes mellitus. In addition, patients older than 50 years were excluded to minimize the effect of age and lens opacities on RNFL thickness measurements. To avoid possible artifacts, we also excluded patients with peripapillary atrophy.

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PROCEDURE

The OCT3 (Stratus OCT software version 4.0.1; Carl Zeiss Meditec, Inc, Dublin, California) was used to measure the thickness of the peripapillary RNFL and the ONH size. The examination was performed under mydriasis by one of us (G.S.). Stratus OCT uses low-coherence interferometry to generate cross-sectional images of the retina, optic disc, and RNFL with 10-µm axial resolution and 20-µm transverse resolution. The instrument contains an interferometer that resolves posterior pole structures by measuring the echo delay time of light that is reflected and backscattered from different layers in the retina and optic disc. The RNFL thickness algorithm searches for the RNFL in a 2-pass process: it looks first for the highest rates of change in reflectivity at the vitreoretinal interface; it looks second for reflectivity above a threshold value in its adjacent highly reflective layer. The threshold is individually determined for each scan as a multiple of the local maximum reflectance to adjust for variations in optical alignment, drying of the corneal surface, or changes in pupil size. The nerve fiber layer thickness is defined as a multiple of the number of pixels between the anterior and posterior edges of the RNFL.

To determine the RNFL thickness, each eye was imaged using the following 3 scans, all sampling 512 data points along a circle concentric with the ONH (Figure 1): (1) with a fixed diameter of 3.4 mm (RNFL thickness 3.4 acquisition protocol); (2) with a variable radius given by the formula \( R = r + 0.5 \) mm, where \( r \) is the vertical radius of the ONH as measured using OCT (nerve head circle acquisition protocol); and (3) with a variable radius given by the formula \( R = r + 1 \) mm, where \( r \) is the vertical radius of the ONH as measured using OCT (nerve head circle acquisition protocol).

In other words, the second and third measurements were intended to measure the RNFL at fixed distances of 0.5 mm and 1 mm, respectively, from the ONH edge. In all cases, the recorded fundus flash image was used by the operator to look for the best centering around the ONH.

The ONH evaluation consisted of 6 radial scans centered on the ONH, spaced 30° apart (fast optic disc acquisition protocol). Each radial scan included 128 points. The machine automatically defined the edge of the optic disc as the end of the retinal pigment epithelium or choriocapillaris and used smoothing with fit to circle to fill the gaps between scans. The resultant image was manually corrected when the machine did not accurately identify the retinal pigment epithelium or choriocapillaris edge. A straight line connected the edges of the retinal pigment epithelium or choriocapillaris, and a parallel line was constructed 150 µm anteriorly. Structures below this line were defined as the disc cup and above this line as the neuroretinal rim.

These OCT data were exported to a personal computer, and the left eye data were converted to the right eye format. Only good-quality OCT data, as judged by the appearance of the RNFL and the optic disc pictures, were used for further analysis. Fewer than 6 images with artifacts or with missing parts or images showing seemingly distorted anatomy were excluded, including those with inadequate signal strength.

The peripapillary RNFL thickness variables evaluated in this study included the following: mean (360° measure), temporal...
quadrant (316°-338°), superior quadrant (46°-135°), nasal quadrant (136°-225°), and inferior quadrant (226°-315°).

### STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad InStat version 3a for Macintosh (GraphPad Software, San Diego, California). For statistical evaluations, only 1 randomly chosen eye was considered for each patient. Possible relationships between the ONH vertical diameter and RNFL thickness of each quadrant and the 360° mean measurement were analyzed using linear regression. The mean values were compared using repeated-measures analysis of variance; differences among standard deviations were evaluated using Bartlett test. P < .05 was considered statistically significant.

#### RESULTS

Stratus OCT measurements resulted in a mean ± SD ONH vertical diameter of 1.68 ± 0.17 mm (range, 1.34-2.09 mm [median, 1.67 mm]). In 10 cases (12%), the vertical diameter was less than 1.5 mm, in 8 cases (10%) it was greater than 1.9 mm, and in the remaining 62 cases (78%) it was between 1.5 and 1.9 mm. The mean ± SD radii for customized scans were 1.34 ± 0.09 mm and 1.84 ± 0.09 mm for examinations performed at 0.5 mm and 1 mm, respectively, from the optic disc border.

The mean values of RNFL thickness are given in the table. Compared with examinations performed using a fixed diameter, using a circular scan with a customized diameter provided higher values for all measurements when the distance from the optic disc was 0.5 mm and provided slightly lower values for all measurements when the distance from the optic disc was 1 mm (P < .001, analysis of variance). Standard deviations were always lower for measurements taken at a fixed distance of 1 mm from the ONH edge (although using Bartlett test, the difference was not statistically significant in the inferior quadrant), while the scan at a fixed distance of 0.5 mm from the ONH edge produced higher standard deviations than the fixed-diameter scan (Table).

As far as the fixed-diameter circular scan is concerned, linear regression analysis detected a positive relationship between the 360° mean RNFL thickness measurement and the ONH vertical diameter (r = 0.3425, r² = 0.1173, P = .002) (Figure 2). A similar result was observed for the superior quadrant (r = 0.3197, r² = 0.1022, P = .004) and, to a lesser extent, for the inferior quadrant (r = 0.2209, r² = 0.0488, P = .048). Conversely, linear regression analysis showed a negative relationship between the optic disc vertical diameter and the 360° mean RNFL thickness measurement obtained with both customized-diameter scans (at 0.5 mm from the ONH edge; r = −0.3004, r² = 0.0902, P = .006; at 1 mm from the ONH edge; r = −0.2369, r² = 0.0561, P = .03) (Figure 3).

Linear regression analysis also detected a significant correlation between the ONH vertical diameter and the superior quadrant measured using the scan at 1 mm from the ONH edge (r = −0.2169, r² = 0.047, P = .05). Customized scans did not allow us to detect further statistically significant relationships between the ONH vertical diameter and the RNFL thickness in the other quadrants.

Although OCT has been extensively investigated as a tool for assessing RNFL thickness in different optic nerve diseases, only a few attempts have been made to understand how the relationship between ONH size and scan radius affects such measurements. Recently, increasing attention has been focused on this issue. It has already been reported that ONH size positively correlates to RNFL thickness. Two explanations were proposed for this correlation. First, the results may simply confirm previous histological studies showing that the optic nerve fiber count increases with enlarging ONH size. Although these studies analyzed the retrobulbar portion of the optic nerve, it may be extrapolated that a similar correlation exists in the peripapillary RNFL. Second, the results may be the consequence of an artifact in OCT measurements of RNFL thickness (in large ONHs, in fact, the fixed-diameter scan is performed closer to the ONH edge, where the RNFL is inevitably thicker). The present study aimed to analyze how adjusting the OCT scan diameter on the basis of the ONH vertical diameter (so that the distance to the disc edge does not change) affects the correlation between ONH size and RNFL thickness.

Three findings deserve specific attention. First, we confirmed the results of the previous investigation (which was carried out on a separate sample) by showing that the fixed-diameter circular scan gives higher RNFL values in patients with larger ONHs (Figure 1). Similar data have been reported by Carpineto et al, who studied 30

### Table. Retinal Nerve Fiber Layer Thickness for Each Scan Diameter

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>3.4-mm Fixed Diameter</th>
<th>0.5 mm From the ONH Edge</th>
<th>1 mm From the ONH Edge</th>
<th>Analysis of Variance</th>
<th>Bartlett Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>360° Mean</td>
<td>103.1 ± 9.5</td>
<td>132.3 ± 11.6</td>
<td>97.3 ± 8.4</td>
<td>&lt; .001</td>
<td>.01</td>
</tr>
<tr>
<td>Temporal</td>
<td>72.8 ± 10.5</td>
<td>90.2 ± 13.5</td>
<td>68.9 ± 9.6</td>
<td>&lt; .001</td>
<td>.006</td>
</tr>
<tr>
<td>Superior</td>
<td>127.8 ± 13.8</td>
<td>160.2 ± 17.4</td>
<td>121.1 ± 11.3</td>
<td>&lt; .001</td>
<td>.001</td>
</tr>
<tr>
<td>Nasal</td>
<td>80.6 ± 15.3</td>
<td>112.2 ± 19.5</td>
<td>76.0 ± 13.8</td>
<td>&lt; .001</td>
<td>.006</td>
</tr>
<tr>
<td>Inferior</td>
<td>131.0 ± 16.6</td>
<td>166.6 ± 18.9</td>
<td>122.8 ± 14.9</td>
<td>&lt; .001</td>
<td>.10</td>
</tr>
</tbody>
</table>

Abbreviation: ONH, optic nerve head.

a Data are given as mean ± SD unless otherwise indicated.
healthy subjects using the traditional 3.4-mm-diameter scan and a custom scan with a fixed distance of 0.85 mm from the ONH border. In their study, the correlation between scan RNFL thickness (as measured using the 3.4-mm fixed-diameter scan) and ONH diameter was even stronger ($r = 0.988$) than it was in our study.

Second, because both customized-diameter scans detected lower RNFL thickness values in larger ONHs than in smaller ONHs (Figure 2 and Figure 3), we conclude that the higher RNFL values obtained for these optic discs using the fixed-diameter scan are caused by a technical artifact and actually reflect the shorter distance between the scan and the ONH edge, rather than a larger number of fibers. Surprisingly, Carpineto et al.\textsuperscript{7} did not reveal such a correlation between ONH size and RNFL thickness when RNFL thickness was measured using the customized-diameter scan. The difference between our study results may be accounted for by several factors such as the variations in the sample size and ages, our selection of subjects aimed at avoiding large errors of refraction, or the method used to calculate ONH size.

However, our results do not rule out the possibility that larger discs contain more fibers. In fact, it is reasonable to deduce that RNFL fibers emerging from a large ONH must be distributed over a wider circumference: as a consequence, the larger spatial distribu-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Linear regression analysis shows the increasing values of 360° mean retinal nerve fiber layer (RNFL) thickness for larger discs ($r = 0.3425$, $r^2 = 0.1173$, $P = .002$) when a fixed-diameter scan is used.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Linear regression analysis shows the decreasing values of 360° mean retinal nerve fiber layer (RNFL) thickness for larger discs ($r = -0.2369$, $r^2 = 0.0561$, $P = .03$) when a customized-diameter scan at 1 mm from the optic nerve head edge is used.}
\end{figure}
tion will result in thinner RNFLs when larger ONHs are analyzed.

Third, the use of a customized-diameter scan at 1 mm from the ONH edge will probably allow clinicians to achieve lower interindividual variability when assessing RNFL thickness using OCT. In fact, measurements showed lower standard deviations when the custom scan at 1 mm from the ONH edge was used. Conversely, the results obtained using the custom scan at 0.5 mm from the disc border showed higher standard deviations. This is probably because, when the distance from the optic disc was small, we found it more difficult to obtain a good centering of the circular scan around the disc, and centering errors are known to increase the variability of OCT measurements of RNFL thickness. Further studies involving more patients (especially in the lower and upper ranges of ONH size) will be needed to assess whether custom scans can actually reduce interindividual variability in the measurement of RNFL thickness using OCT.

Together, these observations suggest the need for new normative databases stratified not only on the basis of age, as in the case of the commercially available database, but also on the basis of ONH size. From a clinical point of view, the use of such a database should increase the diagnostic usefulness of OCT in detecting RNFL defects in patients with glaucoma with large ONHs, among whom Stratus OCT demonstrates a lower sensitivity.

The present study has some limitations. The sample size was not large, and the age range was only moderately restricted. In addition, we measured the optic disc diameter by means of the fast optic disc acquisition protocol with manual correction, and this method still requires full validation.

**CONCLUSIONS**

We confirmed that OCT measurements of RNFL thickness are affected by ONH size. When a fixed-diameter circular scan is used, larger discs show higher values; conversely, when the diameter is adjusted on the basis of ONH size, larger discs show lower values. These correlations need to be confirmed in histopathological studies, ideally involving nerve fiber morphometry in patients whose RNFL has been previously measured using OCT. In the meanwhile, a normative database of peripapillary RNFL thickness should be created to correct for ONH size. This would probably reduce the interindividual variability of RNFL thickness measurements using OCT and increase the sensitivity of this technology for diseases such as glaucoma. Otherwise, ophthalmologists should be aware that, when using standard-diameter peripapillary OCT scans, RNFL thinning may be underread in patients with larger optic discs and may be overread in patients with smaller optic discs.

**Submitted for Publication:** July 20, 2006; final revision received November 24, 2006; accepted December 7, 2006.

**Correspondence:** Giacomo Savini, MD, Centro Salus, Via Saffi 4/H, 40131 Bologna, Italy (giacomo.savini@alice.it).

**Financial Disclosure:** None reported.

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