

Vertical and Horizontal Thickness Profiles of the Corneal Epithelium and Bowman's Layer after Orthokeratology

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PURPOSE. To investigate thickness profile changes of the corneal epithelium and Bowman's layer at the vertical and horizontal meridians with overnight myopia orthokeratology (OK) lenses.

METHODS. Twenty subjects (age range: 19–33 years) wore reverse-geometry rigid gas-permeable OK lenses in both eyes for 30 days. Before lens wear and after 1, 7, and 30 days of overnight lens wear, evaluation of lens fitting, visual acuity examination, corneal topography, and ultra-high resolution optical coherence tomography (UHR-OCT) were performed. The central, midperipheral, and peripheral cornea were imaged in both the horizontal and vertical meridians. Custom software was produced to acquire the thickness profiles of the epithelium and Bowman's layer.

RESULTS. Unaided visual acuity and refraction were improved significantly after OK lens wear. The central corneal epithelium thinned in the horizontal and vertical meridians after one night of lens wear ($P < 0.05$). In the horizontal meridian, the epithelium thickened at the temporal and nasal midperipheries ($P < 0.05$), while the superior midperipheral epithelium thinned in the vertical meridian. There were no changes in the thickness profile of Bowman's layer during the study period.

CONCLUSIONS. Overnight wear of OK lenses caused the central corneal epithelium to thin in both the vertical and horizontal meridians, while the midperipheral nasal and temporal epithelium became thicker and the superior midperipheral epithelium became thinner. The thickness of the central or midperipheral Bowman's layer in either meridian did not change. Improved vision acuity after overnight OK lens wear can be attributed to changes in the corneal epithelium and not Bowman's layer. (*Invest Ophthalmol Vis Sci.* 2013;54:691–696) DOI:10.1167/iops.12-10263

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Orthokeratology (OK) is used to temporarily reduce the refractive error in myopic patients by the programmed application of specially designed rigid contact lenses.^{1,2} The improvement of uncorrected visual acuity and reduction in myopia are attained by flattening and thinning of the central cornea. OK lenses for myopia provide reliable refractive correction for up to -4.5 diopters (D) on lens removal.^{3–5} With the development of higher gas-permeable lens materials, overnight OK lens has come into greater use as a treatment modality to correct refractive error.^{6–8}

Previous studies have reported the time course of refractive and topographic thickness changes of the cornea and its sublayers in myopia OK lens wearers.^{9–11} In an early study, Swarbrick et al., using a modified optical pachymeter, found central epithelial thinning and midperipheral corneal thickening after reverse-geometry lens wear.⁹ Albarbi et al. obtained similar results using an overnight OK lens-wearing modality.¹⁰ Wang et al. used optical coherence tomography (OCT) to measure the topographic thickness of the cornea and epithelium after one night of OK lens wear.¹¹ However, all of these studies were focused on thickness changes of the total cornea and the epithelium at the horizontal meridian only.

Due to limitations in imaging technology, at the vertical meridian not much is known regarding the effect of OK lenses on the topographic thickness and corneal sublayers, including Bowman's layer. In previous studies, we used spectral-domain OCT with ultra-high resolution ($\sim 3 \mu\text{m}$) to measure the topographical thickness of the total cornea, epithelium, and Bowman's layer.^{12,13} The purpose of the present study was to investigate topographic thickness changes of the epithelium and Bowman's layer at both the vertical and horizontal meridians over a 30-day period of overnight myopia OK lens wear.

SUBJECTS AND METHODS

Subjects

Twenty myopic subjects (3 men and 17 women; mean \pm standard deviation age: 25.9 ± 4.4 years; range: 19–33 years) with no history of rigid gas-permeable lens wear or any current ocular or systemic disease were recruited. All subjects had myopia of no more than -4.00 D and astigmatism of no more than -1.50 D. The spherical equivalent refractive error at baseline was -2.46 ± 0.92 D (range: -0.75 to approximately -3.75 D). All procedures adhered to the Declaration of Helsinki, and the protocol was reviewed and approved by the Ethics Committee of Wenzhou Medical College. Informed consent was obtained from each subject before commencement of the study.

Orthokeratology Lenses

Orthokeratology lenses (E&E Optics Ltd., Hong Kong, China) of reverse-geometry design were used. The lenses were 10.6 mm in overall diameter with a 6.0 mm optic zone diameter. The central thickness was

TABLE. Spherical Equivalent Refractive Error, Flat Keratometry, and Steep Keratometry ($n = 20$ Right Eyes from 20 Subjects)

Time	Spherical Equivalent, D	Flat Keratometry, D	Steep Keratometry, D
Baseline	-2.46 ± 0.92	43.07 ± 1.43	44.08 ± 1.36
Day 1	$-0.62 \pm 0.77^*$	$42.15 \pm 1.33^*$	43.54 ± 1.21
Day 7	$-0.03 \pm 0.08^*$	$41.66 \pm 1.36^*$	$43.03 \pm 1.49^*$
Day 30	$-0.10 \pm 0.30^*$	$41.54 \pm 1.52^*$	$43.20 \pm 1.55^*$

All values are represented as mean \pm standard deviation.

* $P < 0.05$, compared with the baseline.

0.22 mm. The lenses were manufactured with the Boston XO material in which the gas permeability, DK, was 100×10^{-11} (cm²/mL O₂)/(s/mL/mm Hg).

Lens Fitting

The lenses were selected based on simulated keratometry (simK) readings and the corneal asphericity along the flattest meridian, measured with a corneal topographer (Medmont E300 Corneal Topographer; Medmont Pty Ltd., Victoria, Australia). The procedures and ideal lens fitting were according to the manufacturer's instructions. Briefly, at the trial fitting visit, each subject wore the trial lenses for 30 minutes in the open-eye condition before assessment of the fitting. An ideal lens fit included good centration, adequate lens movement during blinking, and no sign of lens binding. When viewed with fluorescein staining, a centrally flattened treatment zone diameter of almost 4 mm surrounded by a steepened annulus indicated an acceptable lens fitting. The experimental lenses were ordered from the manufacturer based on the parameters of the trial lenses that gave the best fit. At each visit after OK lens wear, the lens fitting was evaluated before lens removal by an experienced optometrist (JJ). If the lens provided optimal fitting, such as good on-eye centration based on the fluorescein pattern, subjects were enrolled in the study; otherwise they were excluded.

Corneal Topography

Changes in corneal keratometry were monitored with the Medmont E300 Corneal Topographer (Medmont Pty Ltd.) during the follow-up visits. Keratometry of each eye was taken at least three times before lens wear and at each subsequent visit. Steep and flat keratometry at the corneal apex was used to determine refractive corneal changes after lens wear.

Thickness Profile of the Corneal Epithelium and Bowman's Layer

A custom-built ultra-high resolution OCT (UHR-OCT) instrument was used to assess changes in topographic thickness of the corneal epithelium and Bowman's layer across both the horizontal and vertical meridians as reported in our previous study.^{12,13} Briefly, the system included a specially designed spectrometer with ~ 3 μ m axial resolution and approximately 2.0 mm imaging depth. A telecentric scanning probe designed for imaging the anterior segment of the eye was mounted on a standard slit lamp. The scan speed was set to 24K A-scans per second, and the scan width was set to 8.021 mm in the horizontal meridian and 7.989 mm in the vertical meridian.

Each subject was asked to wear the OK lens overnight for at least 1 month. After the baseline initial visit, follow-up visits were scheduled at 1, 7, and 30 days after lens wear. All measurements, including lens fitting evaluation, visual acuity, corneal topography, fluorescein staining, and UHR-OCT imaging were conducted in that order at baseline before lens wear and at each follow-up time after OK lens wear. At baseline, two repeated UHR-OCT images were taken of one eye in each of 10 subjects to test the repeatability of the topographic thicknesses of the epithelium and Bowman's layer.

OCT images were taken 2 to 4 hours after lens removal to reduce the influence of corneal edema. To eliminate interoperator variance, all UHR-OCT measurements were performed by the same operator (YL). The procedure for UHR-OCT imaging has been described previously.¹³ Briefly, each subject was asked to sit in front of a slit lamp on which the UHR-OCT probe was mounted. An external fixation target was provided for the subject. The central UHR-OCT beam, indicated on a monitor, was set on the corneal apex where a specular reflection was normally detected. For imaging of the peripheral cornea, at both the horizontal and vertical meridians, the subject was first asked to look straight ahead and the UHR-OCT beam was aligned and set across the corneal apex where a specular reflection appeared. Then the subject was asked to turn the test eye to look at the nasal, temporal, superior, and inferior fixation targets that were set to approximately 15 cm from the subject's eye and 30° subtended on the eye.

As described in our previous paper,¹³ custom software was used to yield the thickness profiles of the epithelium and Bowman's layer. First, boundaries of the epithelium and Bowman's layer were semimanually segmented through the definition of 4 to 5 points. A polynomial function was fitted to each interface boundary using the least-square curve-fitting method based on the outlined points, which helped to obtain the boundary points along all A-scan lines. Then a refraction correction algorithm was used to correct the surfaces.¹⁴ For the central cornea, only 1000 pixels around the corneal apex, equivalent to a 3.90 mm chord distance, were selected for data analysis (Fig. 1A). For the peripheral cornea at both the horizontal and vertical meridians, the end of Bowman's layer was chosen as the landmark to define the outer limits (Fig. 1B). The analysis was based on 1000 pixels extending from the end of Bowman's layer toward the central cornea. Both central and peripheral regions were divided into 10 equal zones (Fig. 1). The corneal apex was located at zones 5 to 6 for the central cornea (Fig. 1A). The end of Bowman's layer extended from the beginning of zone 1 to the end of zone 10, toward the central cornea (Fig. 1A).

Data Analysis

Statistica software (v7.0; Statsoft Inc., Tulsa, OK) was used for descriptive statistics and data analysis. Quantitative variables were expressed as means \pm standard deviations. Analysis of variance (ANOVA) was used for overall changes of topographic thicknesses over time or over zones for each region along each meridian during the OK lens wear. Tukey post hoc pairwise comparison analysis was used to determine if there were pairwise differences between zones or times. Statistical significance was accepted when $P < 0.05$.

RESULTS

Spherical equivalent refractive error decreased during the 30 days of this study (ANOVA, $P < 0.05$, Table). The myopic refractive error was -2.46 ± 0.92 D at baseline and decreased to -0.62 ± 0.77 D after one night of lens wear. It continued to decrease to -0.03 ± 0.08 D at 7 days. After 7 days, there was no significant change (post hoc, day 7 versus day 30, $P > 0.05$), although there was a significant decrease, -0.10 ± 0.30 D, compared to the baseline (post hoc, baseline versus day 30, $P < 0.05$).

Flat and steep keratometry values decreased during the 30 days of this study (ANOVA, $P < 0.05$ each, Table). At day 1 of lens wear, the simK power in the flat meridian was significantly reduced compared with the baseline (post hoc, $P < 0.05$, Table). Compared to day 1, the simK continued to become more flat after 7 days of OK lens wear ($P < 0.05$). After this time, there were no more significant changes (post hoc, $P > 0.05$). In the steep meridian, the simK decreased significantly between baseline and day 7 (post hoc, $P < 0.05$); however, the changes between baseline and day 1 and between day 7 and day 30 were not significant (post hoc, $P > 0.05$).

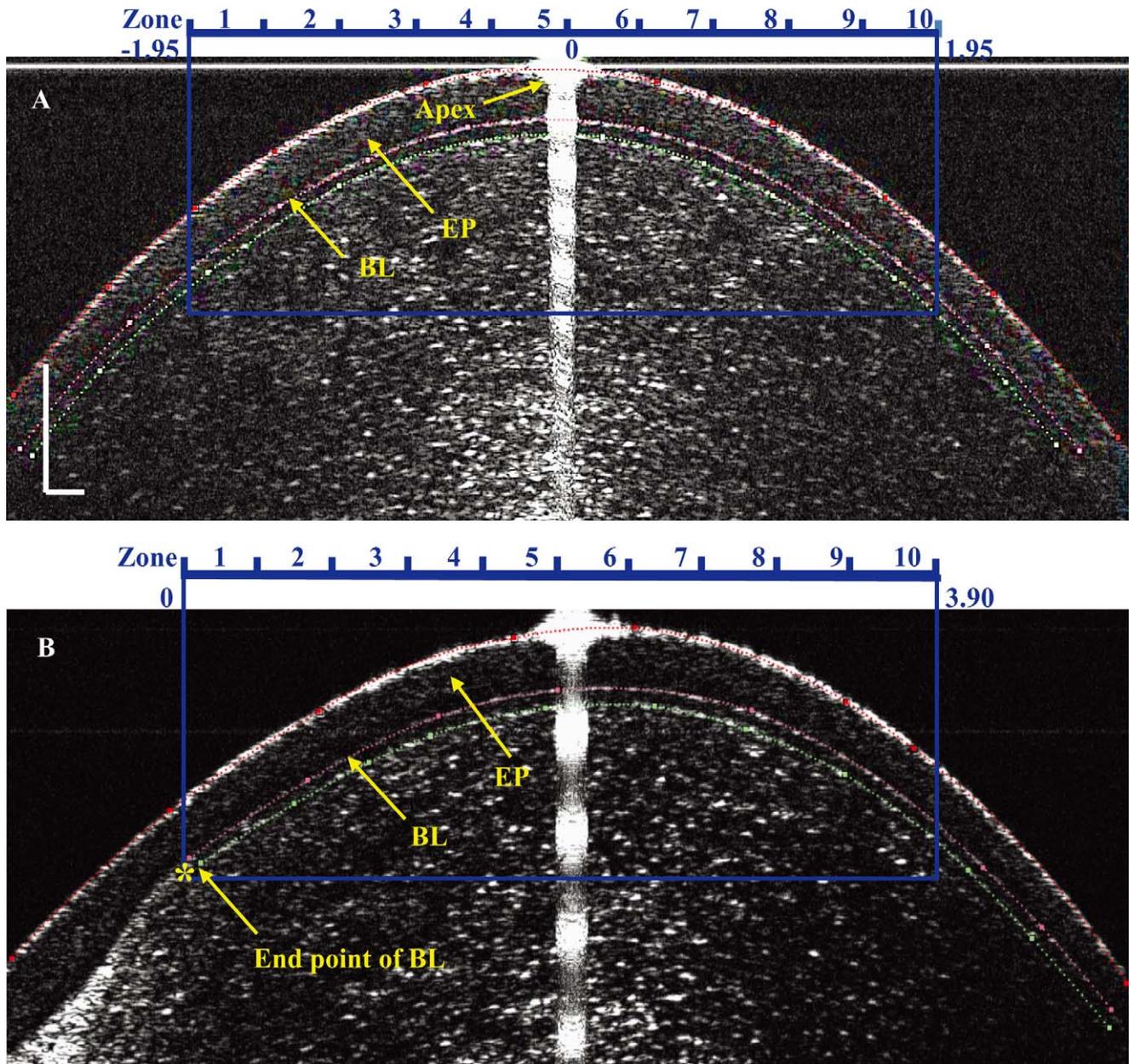


FIGURE 1. UHR-OCT corneal images with boundary outlines of the corneal epithelium and Bowman's layer. The images were acquired at the central (A) and peripheral (B) regions of the cornea. The boundaries were semi-automatically segmented. (A) For the central cornea, 1000 pixels around the cornea apex, equivalent to a 3.90 mm chord distance, were selected for data analysis. (B) For the peripheral cornea, the end of Bowman's layer was chosen as the landmark to define the outer limits, and 1000 pixels (3.90 mm chord distance) extending from the end of Bowman's layer toward the central cornea were selected for analysis. Both central and peripheral regions were divided into 10 equal zones. The corneal apex was located between zone 5 and 6 for the central cornea (A). For the peripheral cornea (B), Bowman's layer extended from the beginning of zone 1 to the end of zone 10, toward the central cornea. *Yellow asterisk:* the end point of Bowman's layer. EP, epithelium; BL, Bowman's layer. *Scales bars:* 200 μm .

UHR-OCT corneal images at the vertical and horizontal meridians in the central, midperipheral, and peripheral regions were used to evaluate topographical thickness of the epithelium and Bowman's layer from limbus to limbus. The epithelium and Bowman's layer were visualized clearly at baseline (Figs. 1A, 1B).

The repeatability of the central epithelium thickness measurements, determined as the standard deviation of the difference between two measurements, was $\pm 1.55 \mu\text{m}$ (range: ± 0.92 to approximately $\pm 1.92 \mu\text{m}$) and $\pm 1.83 \mu\text{m}$ (range: ± 1.26 to approximately $\pm 2.09 \mu\text{m}$) averaged over 10 zones along the horizontal and vertical meridians, respectively. For

the peripheral cornea, the thickness repeatability was $\pm 1.88 \mu\text{m}$ (range: ± 1.15 to approximately $\pm 2.35 \mu\text{m}$) and $\pm 1.89 \mu\text{m}$ (range: ± 0.82 to approximately $\pm 2.83 \mu\text{m}$) along the horizontal and vertical meridians, respectively. For Bowman's layer, the thickness repeatability was $\pm 2.1 \mu\text{m}$ and $\pm 2.2 \mu\text{m}$ at the central region and midperiphery, respectively. All of the *P* values for the repeated measurements were greater than 0.05.

At baseline, the distribution trend of the topographic epithelium thickness was different between the horizontal and vertical meridians. In the vertical meridian, the central epithelial thickness, $52.6 \mu\text{m}$, gradually decreased to $50.0 \mu\text{m}$ in the inferior region and to $44.4 \mu\text{m}$ in the superior region (*P*

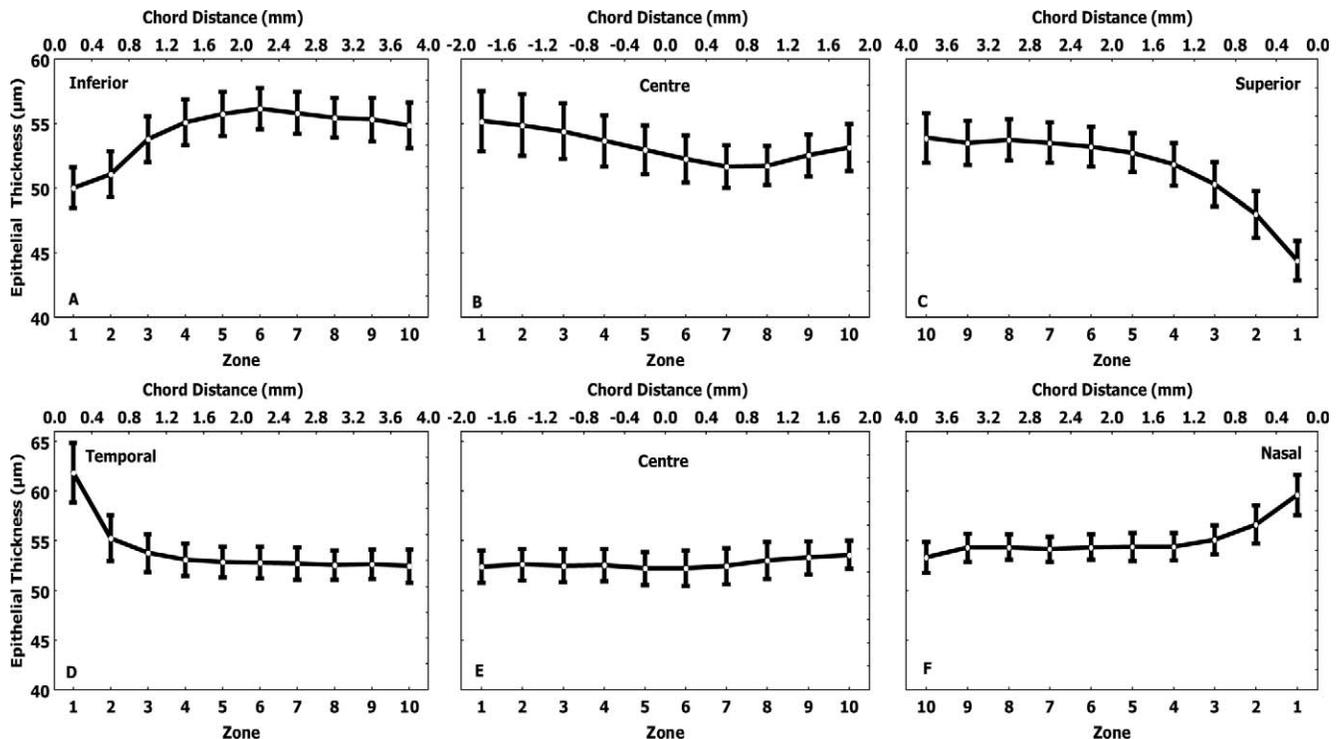


FIGURE 2. Thickness profile of the corneal epithelium at baseline. The epithelial thickness profiles at the vertical (A–C) and horizontal (D–F) meridians were obtained by scanning different locations. Each scan consisted of 1000 lateral A-scans over a chord distance of 3.90 mm (upper scale) and was divided into 10 equal zones (lower scale). For the peripheral profiles, zone 1 began at the edge of Bowman’s layer. The thickness of the epithelium gradually increased from the central cornea toward the periphery in the horizontal meridian ($P < 0.001$), while it decreased from the central cornea toward the periphery in the vertical meridian ($P < 0.001$). Error bars: 95% confidence intervals.

< 0.001 each, Figs. 2A–C). In the horizontal meridian, the thickness of the epithelium gradually increased from the central region toward the periphery. At the central region, the epithelial thickness was $52.2 \mu\text{m}$, which was significantly smaller than at the nasal, $59.6 \mu\text{m}$, and temporal sides, $61.9 \mu\text{m}$ ($P < 0.001$ each, Figs. 2D–F).

During the 30-day period of the study, significant epithelial thinning occurred in central region zones 3 to 9 across the vertical meridian (post hoc tests, $P < 0.05$, Fig. 3B). By day 1, the epithelium had thinned 5.4% at zone 6, which had the maximum epithelial thinning (post hoc tests, $P < 0.05$, Fig. 3B), and it continued to thin by 14.3% compared to the baseline for the same zone ($P < 0.05$, Fig. 3B). After day 7, there was no further decrease of central epithelial thickness along the vertical meridian (day 7 versus day 30, $P > 0.05$, Fig. 3B). There was no epithelial thickening at either the inferior or superior region (Figs. 3A, 3C). In fact, the epithelium on the superior side became thinner in zones 9 to 10 during the study period (post hoc, $P < 0.05$, Fig. 3C).

In the horizontal meridian (Figs. 3D–F), the central epithelium in zones 1 to 7 became significantly thinner (post hoc tests, $P < 0.05$, Fig. 3E). On day 1 the epithelium thinned approximately 7.2% of maximum at zone 4 ($P < 0.05$, Fig. 3E), and by day 7 it had thinned by 16.4% compared to the baseline for the same zone ($P < 0.05$, Fig. 3E). There were no significant changes after day 7 ($P > 0.05$). In the midperipheral regions, the epithelial thickness at the temporal side (zones 6–9, Fig. 3D) and the nasal side (zones 7–10, Fig. 3F) increased significantly over the 30-day lens wear period (post hoc tests, $P < 0.05$). Midperipheral epithelial thickening was present by day 7 (nasal side, 5.7% at zone 8; temporal side, 6.1% at zone 8; post hoc tests, $P < 0.05$). After day 7, neither the nasal nor the

temporal region continued to increase in thickness (day 7 versus day 30, post hoc tests, $P > 0.05$, Figs. 3D, 3F).

At baseline, the thickness of Bowman’s layer was uniform, $16.6 \mu\text{m}$ at the center and $16.8 \mu\text{m}$ at the midperiphery (ANOVA, $P > 0.05$), respectively. After OK lens wear, there were no significant changes in the thickness of Bowman’s layer in either the horizontal or vertical meridian (ANOVA, $P > 0.05$).

DISCUSSION

We have previously used ocular anterior segment imaging by UHR-OCT to determine the topographical thickness of the epithelium and Bowman’s layer in normal subjects.^{12,13} In the current study, we used this method for the first time to simultaneously measure the thickness profile of these layers across the central and peripheral cornea in both vertical and horizontal meridians of OK lens wearers before and after lens wear. The repeatability of baseline measurements showed the high precision of UHR-OCT in both central and peripheral measurements. This high level of precision is necessary to quantify thickness changes in the epithelium and Bowman’s layer after OK lens wear.

This study demonstrated that overnight wear of OK lenses for myopia caused changes in the thickness profile of the corneal epithelium. These changes may contribute to the reduction of refraction in myopic patients. Interestingly, the profile of changes in the vertical meridian was different from that in the horizontal meridian. In addition, there were no significant changes in the thickness profile of Bowman’s layer over the study period. This indicates that Bowman’s layer probably does not play a role in the reduction of myopia.

Epithelial thinning occurred in the central area approximately 2 mm from the corneal apex in both the horizontal and

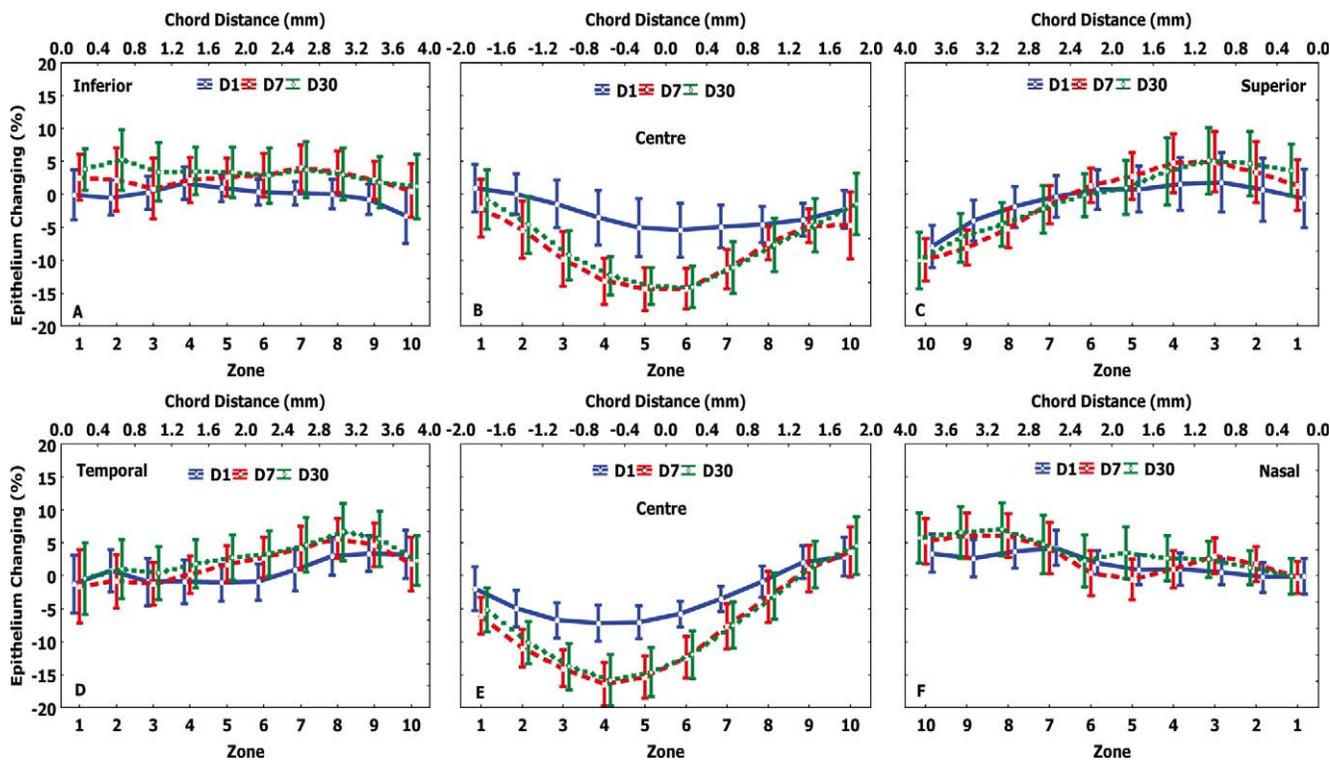


FIGURE 3. Changes in the corneal epithelial profile during the 30 days of overnight OK lens wear. At the vertical meridian (A–C), the central zones (B) became thinner ($P < 0.05$) during the 30 days of OK lens wear. In the inferior region (A), there was no significant change in thickness ($P > 0.05$). In the superior region (C), the epithelium became thinner ($P < 0.05$). At the horizontal meridian (D–F), the central zones (E) became thinner ($P < 0.05$) while the temporal (D) and nasal (F) epithelia were thickened by day 7 ($P < 0.05$). Error bars: 95% confidence intervals.

vertical meridians. This is consistent with the concept that myopia OK lenses induce apical corneal epithelial thinning.^{1,5,8,15–17} After one night of OK lens wear, the maximum thinning magnitudes of central epithelium were 7% and 6% at the horizontal and vertical meridians, respectively. The magnitude reached 14% at the vertical meridian and 16% at the horizontal meridian after seven nights of wear. Similar results have been reported in previous studies.^{1,11,15} Haque et al. reported that 15% of central epithelial thinning occurred 14 hours after lens removal following four consecutive nights of wearing myopia OK lenses.¹⁸ Wang et al. showed approximately 5% thinning of the central epithelium immediately after the first night of wear.¹¹ Alharbi and Swarbrick measured epithelial thickness by optical pachymetry 8 to 10 hours after OK lens overnight wear.¹⁰ They found approximately 8% and 17% central epithelial thinning after 1 and 10 nights, respectively, of myopia OK lens wear.

In the horizontal meridian, zones 6 to 8 in the temporal and nasal regions correspond to the midperipheral region of cornea. Our method did not provide a location projection of the reverse curve of the OK lens on the corneal zones because of different diameters among individuals and use of landmarks. Nevertheless, we found that the epithelium thickened by approximately 6% in these midperipheral regions in both the nasal and temporal regions during overnight OK lens wear. Wang et al. demonstrated that the corneal epithelium was significantly thickened in the midperiphery,¹¹ which was in agreement with the results of our study. However, Alharbi and Swarbrick found no significant thickening of the epithelium in the midperiphery of the horizontal meridian.¹⁰ The different methods or different time points used in those studies might explain these differences.

Two main factors could cause the epithelial thickening. One is edema due to the lack of sufficient oxygen supply

underneath the lens of the overnight closed eye.¹¹ Another factor that might contribute to horizontal midperipheral thickening is the deformation of epithelial cells due to the wearing of OK lenses.^{19–22} Our measurements were taken 2 to 4 hours after OK lens removal; therefore corneal swelling at that time was probably minimal.^{1,5,9,11} Thus, the thickness profile changes of the epithelium in the horizontal meridian may indicate that central cells were compressed and that the cells in the midperipheral region were vertically elongated, which has been shown in previous studies.^{19–22} Further work is necessary to study the nature of cellular changes underlying the central epithelial thinning and midperipheral epithelial thickening induced by the OK lens.

Interestingly, we found no midperipheral epithelial thickening in the vertical meridian. On the contrary, the epithelium tended to become thinner at the superior midperipheral region. There may be several explanations for the opposite changes in the midperipheral vertical and horizontal meridians. First, the upper eyelid might generate greater pressure on the superior cornea than the inferior cornea.^{23–25} This could produce less edema or even a thinning of the epithelium at the superior midperiphery. Second, although the fitting of the OK lens was acceptable in the clinic and the visual acuity was satisfactory to all subjects, superior decentration of the lens might have occurred in most of our subjects. This could have caused greater pressure on the superior side of the cornea. We have shown that the profile of epithelial thickness at the vertical meridian is different from that at the horizontal meridian.¹³ This difference might induce a different regional response to the presence of overnight OK lens wear. This hypothesis needs to be tested in a future study.

To the best of our knowledge, this is the first report of the Bowman's layer thickness profile in both the vertical and

horizontal meridians. The thickness in both meridians was approximately 16.7 μm at the central and midperipheral regions, similar to that reported in previous studies,^{12,20} and it was relatively uniform along both meridians. This was also the first attempt to report the response of the whole Bowman's layer at the horizontal and vertical meridians for overnight OK lens wear. Over the 30 days of the study, we found no significant changes in Bowman's layer at any of the locations along the horizontal or vertical meridian. However, Nieto-Bona et al. reported that Bowman's layer thinned after 15 days of overnight OK lens wear.²⁰ They measured the thickness by confocal microscopy, and the use of this invasive modality could explain the difference in results.

There are some limitations in the present study. Although the subjects were asked to fixate on targets with the same distance and angle relative to the cornea, measurement errors may be induced by a fixation target that is too close (15 cm) and by possible shifting of the measurement locations after OK lens treatment. These measurement errors were minimized by alignment of the OCT scan beam on the measured meridians and use of the end of Bowman's membrane as the landmark. Our reliability test showed that the measurement precision of epithelial thickness profile was less than 2.0 μm for both the central and peripheral measurements. Significant epithelial thinning in the central cornea after overnight OK lens wear and corneal thickening at the horizontal midperipheral region indicated that our method appeared to have the ability to detect the thickness alternation in the epithelium induced by OK lens treatment rather than the measurement errors. There may have been some overlap in the regions of the central and peripheral cornea used to yield the epithelial profile. For instance, zone 10 at the periphery may overlap with zone 1 or 10 at the central cornea. However, our conclusion based on zones 2 to 9 in the center and zones 2 to 9 in the periphery may not be impacted by the overlap. Finally, the small sample size ($n = 20$) also may be a limitation. Nevertheless, there was sufficient power to detect epithelial thickness topographic changes after overnight OK lens wear. We noticed a difference in the central epithelial thinning measured in the horizontal meridian compared in the vertical meridian. This may be due to the interpolation of the epithelial thickness on the apex where the specular reflex was located. The difference was evident only in the measurement on day 1, which may also indicate that epithelial changes occur rapidly between measurements. This idea will be examined in future studies.

In summary, overnight wear of myopia OK lenses for 30 days caused specific changes of region in the horizontal and vertical thickness profiles of the corneal epithelium. In contrast, there was no response of Bowman's layer to OK lens wear during the period of this study.

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