As first described by Alfred Vogt in 1921, the corneal epithelium has the ability to alter its thickness profile to try and re-establish a smooth, symmetrical optical surface and either partially or totally mask the presence of an irregular stromal surface. Such compensatory epithelial thickness changes have since been described after myopic 3-10 and hyperopic11 excimer laser ablation and radial keratotomy.12 Epithelial thickness changes after myopic excimer laser ablation have been associated with refractive regression.3-10 The majority of studies were based only on central epithelial thickness measurements and found a correlation between epithelial thickening and refractive regression. This suggests that lenticular epithelial thickness changes partially replace the ablated stromal tissue. Using very high-frequency (VHF) digital ultrasound, we have previously mapped the epithelial thickness change across a 10-mm corneal diameter and demonstrated that the epithelial thickness change after myopic excimer laser ablation is lenticular.13,14 The impact of lenticular epithelial thickness changes on refractive accuracy and stability of excimer laser ablation can be expected because the original excimer laser ablation profile formulae described by Barraquer’s 1964 law of thicknesses15 as well as Munnerlyn et al16 assumed that the epithelium would regrow with a uniform thickness after photorefractive keratectomy.

ABSTRACT

PURPOSE: To longitudinally follow epithelial thickness profile changes after myopic LASIK for 1 year to determine when and how epithelial changes occur and when epithelial stability is reached.

METHODS: Epithelial thickness was measured with Artemis very high-frequency digital ultrasound (ArcScan Inc) across the central 10-mm corneal diameter in 11 eyes before and 1 day, and 1, 3, 6, and 12 months after myopic femtosecond LASIK. For each consecutive pair of time points, maps of the mean change in epithelial thickness and paired t test P value were plotted.

RESULTS: Mean preoperative spherical equivalent refraction was $-3.34 \pm 1.51$ dioptries (D) (range: $-1.50$ to $-5.13$ D). Overnight, there was a central 5-mm zone of epithelial thickening ($0.5$ to $1.5 \mu m$, $P<.05$) surrounded by epithelial thinning ($4$ to $6 \mu m$, $P<.05$) extending to the 8-mm zone. Between 1 day and 1 month, the epithelium thickened within the 7-mm zone by up to $5 \mu m$ ($P<.05$) in a lenticular pattern with greater thickening centrally and progressively less thickening centrifugally. Between 1 and 3 months, the epithelium continued to thicken in the central 7-mm zone by approximately $1 \mu m$ ($P<.05$). No change in epithelial thickness occurred after 3 months ($P>.05$).

CONCLUSIONS: A lenticular change occurred in the epithelial thickness profile, with more thickening centrally than paracentrally; 22% of the total increase in central thickness occurred overnight, 58% between 1 day and 1 month, and 20% between 1 and 3 months, with stability between 3 and 12 months. The lenticular epithelial changes contribute to the observed myopic shift after myopic LASIK during the first 3 months. [J Refract Surg. 2012;28(3):195-201.] doi:10.3928/1081597X-20120127-02

Change in Epithelial Thickness Profile 24 Hours and Longitudinally for 1 Year After Myopic LASIK: Three-dimensional Display With Artemis Very High-frequency Digital Ultrasound

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Some studies have also investigated the longitudinal changes in epithelial thickness using a 50-MHz ultrasonic pachymeter,⁴ confocal microscopy,⁵,⁶,⁸ confocal microscopy through focusing,⁹ and 1310-nm optical coherence tomography.⁷ However, these studies only report changes in central epithelial thickness. Artemis VHF digital ultrasound (ArcScan Inc, Morrison, Colorado) is to date the only published method measuring individual corneal layers, including the epithelium and stroma, continuously over a large area (8 to 10 mm).¹⁷ The repeatability of epithelial thickness measurements has been shown to be better than 1.32 μm within the central 6-mm diameter, with a central repeatability of 0.58 μm.¹⁷

The purpose of this study was to qualitatively and quantitatively characterize longitudinal changes in the thickness profile of the epithelium in a population of eyes after myopic LASIK using the Artemis VHF digital ultrasound arc-scanning system.

**PATIENTS AND METHODS**

This study was a prospective, noncomparative case series of 11 consecutive volunteers with low to moderate myopia recruited from a population of patients seeking refractive surgery at the London Vision Clinic, London, United Kingdom. Inclusion criteria were medical suitability for LASIK with a myopic spherical equivalent refraction between −1.50 and −5.50 diopters (D). Written informed consent was obtained from all patients. The study adhered to the tenets of the Declaration of Helsinki and was performed in accordance with an institutional review board approved protocol.

Myopic LASIK was performed by one author (D.Z.R.) with the MEL 80 excimer laser and VisuMax femtosecond laser (Carl Zeiss Meditec, Jena, Germany). The intended flap thickness was 110 μm in 10 eyes and 100 μm in 1 eye. The intended flap diameter was 8 mm in 7 eyes and 8.5 mm in 4 eyes. The M (medium) contact glass was used in all eyes. The side-cut angle was 110° in all eyes. A non-linear aspheric ablation profile with a 6-mm optical zone was used in all eyes, which is now commercially available as part of the Laser Blended Vision module for the CRS-Master (Carl Zeiss Meditec).¹⁸

Both eyes of each patient were scanned with the Artemis 1 VHF digital ultrasound arc-scanner, as described previously,¹⁴,¹⁷ before and 1 day and 1, 3, 6, and 12 months after LASIK. All patients were scanned by an expert user (D.Z.R.) and the scans were analyzed by a trained and experienced observer (T.J.A.). For three-dimensional scan sets, the scan sequence consisted of four meridional B-scans at 45° intervals. Epithelial thickness data were interpolated between scan sets to generate a 10×10-mm Cartesian matrix in 0.1-mm steps.

**STATISTICAL ANALYSIS**

Paired $t$ tests were performed to compare the epithelial thickness between right and left eyes along each scan line. One eye from each patient was then randomly selected, using Microsoft Excel’s (Microsoft Corp, Redmond, Washington) random number generator, for inclusion in the aggregate analysis. Maps of the mean epithelial thickness were plotted for each time point. For each eye and each consecutive pair of time points, the epithelial thickness change was calculated as the epithelial thickness at the later time point minus the epithelial thickness at the earlier time point (so that positive values represent epithelial thickening and negative values represent epithelial thinning). Maps of the mean change in epithelial thickness were plotted and paired $t$ tests were performed for each consecutive pair of time points and between before and 12 months after LASIK. All statistics were carried out for all eyes using vertical mirrored symmetry superimposition; thickness values for left eyes were reflected in the vertical axis and superimposed onto right eye values so that nasal/temporal characteristics could be combined. All maps were plotted using DeltaGraph v5.0 (SPSS Inc, Richmond, California). On the $t$ test maps, regions where the data were interpolated are indicated by a semi-transparent overlay; the $t$ test results are only strictly valid along meridional scan lines where the data were obtained directly, but the map is presented to ease the visual assessment of where the epithelial thickness changes were statistically significant.

The cross-sectional semi-meridional average epithelial thickness profile was determined for each eye and each time point by averaging the epithelial thickness within the 0.03-mm zone centered on the corneal vertex and within 35 annular bands each 0.06-mm wide centered on the corneal vertex with central radii increasing in 0.1-mm steps. These cross-sectional semi-meridional average epithelial thickness profiles were then averaged across eyes for each time point and plotted against the radial distance from the corneal vertex. A similar analysis was also performed and plotted to show the cross-sectional semi-meridional average change in epithelial thickness for each consecutive pair of time points.

To assess the consistency of longitudinal changes across the population, the mean central (within 1.5 mm) and para-central epithelial (annulus between 3 and 3.4 mm) were plotted for each eye for all time points.

Mean spherical equivalent refraction was calculated at each time point and plotted against time to represent the refractive stability. The change in spherical equivalent refraction was calculated for each eye and each pair of consecutive time points.
Multivariate linear regression analysis was performed to determine whether central epithelial thickening was correlated with spherical equivalent refraction treated and average simulated keratometry (Atlas topographer, Carl Zeiss Meditec).

The mean change in corneal vertex stromal thickness was calculated for each pair of consecutive time points. Descriptive statistics, comparative statistics, and linear regression analysis were performed in Microsoft Excel 2007. A $P$ value of .05 was considered statistically significant.

RESULTS

Artemis VHF digital ultrasound scanning was not performed for one patient at 1-day follow-up due to flap trauma caused by eye rubbing while sleeping. Artemis data were available for all patients for all other visits.

Mean patient age was 31.6±7.5 years (median 30 years, range: 23.9 to 52.1 years). Mean manifest refraction was $-3.03±1.37$ D sphere (range: $-1.50$ to $-4.50$ D), and $0.61±0.41$ D cylinder (range: 0.00 to 1.25 D). Mean spherical equivalent refraction was $-3.34±1.51$ D (range: $-1.50$ to $-5.13$ D). Mean thinnest corneal thickness was 525.0±27.3 μm (range: 483 to 568 μm) by Artemis and 536.3±25.9 μm (range: 495 to 583 μm) by hand-held ultrasound (Corneo-Gage Plus 50 MHz; Sonogage, Cleveland, Ohio). No statistically significant difference was noted in epithelial thickness between right and left eyes at any time point ($P$>.05).

**EPITHELIAL THICKNESS ANALYSIS**

**Preoperative.** Figure 1 shows that the epithelium on average was 5.3 μm thinner superiorly than inferiorly and 1.0 μm thinner temporally than nasally. Figure 2 shows that after annular averaging the epithelium was 2.3 μm thinner centrally than paracentrally.

** Overnight.** The epithelium had changed overnight characterized by a central 4.8-mm zone within which the epithelium either remained unchanged or became thicker (0.5 to 1.5 μm); the epithelial thickening was statistically significant ($P$<.05) between the 1- and 2-mm radii ($P$>.90). This area of thickening was surrounded by an annular zone of epithelial thinning (4 to 7 μm, $P$<.05, power >.90) extending to the 8-mm zone.

**1 Day to 1 Month.** The epithelium thickened at all points within the 7-mm zone by up to 5 μm between 1 day and 1 month, which was statistically significant ($P$<.05, power >.90) at the majority of locations except for inferiorly. Figure 3 shows that the epithelial thickening between 1 day and 1 month followed a lenticular profile with more thickening centrally and...
Epithelial Thickness Profile Changes Measured With the Artemis/Reinstein et al

Progressively less thickening centrifugally; the mean epithelial thickening was 4.4 μm at the corneal vertex, 3.5 μm at the 2-mm radius, and 2.3 μm at the 3-mm radius.

1 to 3 Months. The epithelium continued to thicken in the central 4-mm zone by approximately 1 μm, which was statistically significant (P<0.05, power >0.90) in a number of locations centrally. No statistically significant epithelial thickness changes occurred outside of this zone. Figure 3 shows that the epithelial thickening between 1 month and 3 months followed a lenticular profile with more thickening centrally and progressively less thickening centrifugally; the mean epithelial thickening was 1.7 μm at the corneal vertex, 1.0 μm at the 2-mm radius, and 0.3 μm at the 3-mm radius.

3, 6, and 12 Months. No statistically significant changes were noted in epithelial thickness between 3 and 6 months or between 6 and 12 months (P>0.05).

Preoperative to 12 Months. Figure 4 shows that the overall change in epithelium at 12 months was characterized by a central 4.8-mm zone of epithelial thickening (P<0.05, power >0.90) surrounded by an annulus of epithelial thinning (P<0.05, power >0.90) between the 5.6- and 8-mm diameters except superiorly. The maximum amount of epithelial thickening was 7.5±4.2 μm located 1.0 mm temporally. Only a small annular ring (0.3-mm wide) was seen in between the zones of thickening and thinning where the epithelium was unchanged after LASIK.

Longitudinal Changes. Figure 5A shows central epithelial thickening overnight in 80% (8/10) of eyes and the central epithelium was thicker at 12 months than preoperatively in 91% (9/11) of eyes. Figure 5B shows paracentral epithelial thinning in 100% (10/10) of eyes and the paracentral epithelium was thinner at 12 months than preoperatively in 100% (11/11) of eyes. These plots demonstrate the consistency of the epithelial changes across all eyes in the population.

Tables A and B (available as supplemental material in the PDF version of this article) show the mean epithelial thickness and mean change in epithelial thickness, respectively, at increasing radial distances from the corneal vertex at each time point.

Refractive Stability

Figure 6 shows the stability of spherical equivalent refraction over time. Table C (available as supplemental material in the PDF version of this article) presents the change in spherical equivalent refraction between consecutive postoperative time points and the percentage of eyes in which the spherical equivalent refraction changed by >0.50 D.

Multivariate linear regression found a statistically significant positive correlation between central epithelial thickening and spherical equivalent refraction treated (P=.03), but no correlation with average simulated keratometry was found (P=.62).

Corneal Vertex Stromal Thickness

Table C (available as supplemental material in the PDF version of this article) presents the change in corneal vertex stromal thickness for each consecutive pair of time points. The corneal vertex stromal thickness became thinner in all eyes between 1 day and 1 month by an average of 10 μm. The corneal vertex stromal thickness was unchanged between 1 and 3 months. The corneal vertex stromal thickness became slightly thinner (mean 2.6 μm) between 3 and 6 months, but then thickened by approximately the same amount between 6 and 12 months (mean 3.1 μm).
DISCUSSION

As described previously by our group in a study that reported lenticular epithelial thickness changes 3 to 6 months after myopic LASIK, the epithelial thickness profile was found to change in a lenticular fashion after myopic LASIK, with greater epithelial thickening centrally than paracentrally and epithelial thinning outside the 2.8-mm radius. The epithelium started to change immediately, with a large change overnight that represented 22% of the total increase in epithelial thickness at the location of maximum epithelial thickening. The majority (58%) of central epithelial thickening occurred between 1 day and 1 month and the final 20% occurred between 1 and 3 months. The epithelial thickness profile was stable between 3 and 12 months.

The mean preoperative epithelial thickness profile was nonuniform in thickness characterized by thinner epithelium superiorly than inferiorly and thinner epithelium temporally than nasally, which matched the mean epithelial thickness profile previously reported.
by our group for a population of normal eyes measured using Artemis VHF digital ultrasound.\textsuperscript{19} Similarly, the epithelium was on average slightly thinner inferiorly than superiorly within the central 6-mm zone at all postoperative time points. This phenomenon has also been reported after hyperopic LASIK where the thinnest epithelium was 1-mm superiorly rather than centrally as might have been expected.\textsuperscript{11}

The result in the present study that the epithelial thickness profile was stable between 3 and 12 months is in accordance with one previous study, which reported no change in central epithelial thickness after 3 months.\textsuperscript{4} Other studies have reported that the central epithelial thickness was stable as early as 1 week\textsuperscript{6} and 1 month\textsuperscript{5-8} after LASIK. The largest change in spherical equivalent refraction of \( \pm 0.38 \) D was observed between 1 day and 1 month, which corresponded with the largest change in epithelial thickness. Given the lenticular profile of the epithelial thickness changes, it is likely that these would partly explain the myopic shift, although redistribution of edema and other biomechanical shifts within the stromal substance are likely to also have had an influence on the observed refractive changes. We intend to use ray tracing to analyze the refractive power of the epithelial thickness changes reported herein to evaluate the refractive influence of the different biomechanical effects in a future publication. The spherical equivalent refraction continued to regress (0.09 D) between 3 and 12 months, during which time the epithelial thickness profile was unchanged. This suggests that the refractive changes after 3 months were due to biomechanical factors mentioned earlier or natural myopization.

The present study included epithelial thickness measurements up to 1 year after LASIK, meaning that longer-term changes in epithelial thickness could not be assessed. However, central epithelial thickening has been reported 2 years,\textsuperscript{20} 3 years,\textsuperscript{6,9,21} and 7 years\textsuperscript{8} after excimer laser ablation, all of which found no change in central epithelial thickness after 3 months. We have also previously reported lenticular epithelial thickness changes similar to those after myopic excimer laser ablation up to 26 years after radial keratotomy.\textsuperscript{12} Therefore, it seems likely and reasonable to assume that the lenticular epithelial thickness changes observed after LASIK will be stable and permanent.

The present study found that the epithelium changed significantly overnight characterized by epithelial thickening at 1.5-mm super temporally and epithelial thinning outside the 2.5-mm radius. Overnight epithelial thickness changes after LASIK have only been investigated in one previous study using 1310-nm optical coherence tomography, which found no change in central epithelial thickness.\textsuperscript{7} The results from the present study indicate that the majority of overnight epithelial thickness changes occurred paracentrally, possibly in response to edema although this was not measured. This finding also demonstrates the speed with which the epithelium remodels in response to changes in curvature. We have previously demonstrated such overnight changes in epithelial thickness in a case of free cap rotation where the epithelium was seen to regularize following the rotation of the free cap back to its original position.\textsuperscript{22}

Another interesting finding was that the epithelium was up to 6 \( \mu \)m thinner 12 months after LASIK compared with before treatment para centrally outside the 5.6-mm diameter zone. Given that epithelial changes occur to compensate for changes in stromal curvature, para central epithelial thinning implies relative stromal thickening in this region, as has been previously described by our group.\textsuperscript{14}

A potential for inaccuracy exists due to hydration changes within the corneal epithelium while the eye is immersed in \( 33^\circ \)C saline; however, we have previously reported that the corneal thickness remained unchanged for the first four of five repeated measurements performed over the course of 5 minutes using the Artemis immersion technique.\textsuperscript{17} Given that three-dimensional Artemis scan sets take approximately 2 to 3 minutes per eye, we are confident that measurements were obtained before hydration had an impact on epithelial thickness.

The epithelium appears to play a significant role in the early postoperative period after myopic LASIK, which results in the need for an initial overcorrection for the refraction to regress back to the target. The present study found the epithelial thickness to be stable after 3 months, which suggests that other factors are likely to be responsible for any refractive changes observed after this time.

\textbf{AUTHOR CONTRIBUTIONS}

Study concept and design (D.Z.R., T.J.A., M.G.); data collection (D.Z.R., T.J.A.); analysis and interpretation of data (D.Z.R., T.J.A., M.G.); drafting of the manuscript (T.J.A.); critical revision of the manuscript (D.Z.R., M.G.); statistical expertise (T.J.A.)

\textbf{REFERENCES}

### TABLE A

**Mean Epithelial Thickness at Increasing Radial Distances From the Corneal Vertex**

<table>
<thead>
<tr>
<th>Distance (mm)</th>
<th>Preop</th>
<th>1 Day</th>
<th>1 Month</th>
<th>3 Months</th>
<th>6 Months</th>
<th>12 Months</th>
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<tbody>
<tr>
<td>0.00</td>
<td>53.8</td>
<td>53.6</td>
<td>57.9</td>
<td>59.6</td>
<td>59.1</td>
<td>59.5</td>
</tr>
<tr>
<td>0.50</td>
<td>53.7</td>
<td>54.2</td>
<td>58.3</td>
<td>59.9</td>
<td>59.7</td>
<td>59.7</td>
</tr>
<tr>
<td>1.00</td>
<td>54.1</td>
<td>55.2</td>
<td>59.1</td>
<td>60.5</td>
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<td>60.1</td>
</tr>
<tr>
<td>1.50</td>
<td>54.6</td>
<td>56.0</td>
<td>59.5</td>
<td>60.9</td>
<td>60.4</td>
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<td>2.00</td>
<td>55.2</td>
<td>55.8</td>
<td>59.3</td>
<td>60.4</td>
<td>60.0</td>
<td>59.5</td>
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<tr>
<td>2.50</td>
<td>55.7</td>
<td>54.5</td>
<td>57.5</td>
<td>58.0</td>
<td>57.9</td>
<td>57.2</td>
</tr>
<tr>
<td>3.00</td>
<td>56.1</td>
<td>52.4</td>
<td>55.1</td>
<td>55.4</td>
<td>55.9</td>
<td>55.2</td>
</tr>
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</table>

### TABLE B

**Mean Change in Epithelial Thickness at Increasing Radial Distances From the Corneal Vertex**

<table>
<thead>
<tr>
<th>Distance (mm)</th>
<th>Preop to 1 Day</th>
<th>1 Day to 1 Month</th>
<th>1 to 3 Months</th>
<th>3 to 6 Months</th>
<th>6 to 12 Months</th>
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</thead>
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<tr>
<td>0.00</td>
<td>−0.2</td>
<td>+4.4</td>
<td>+1.7</td>
<td>−0.6</td>
<td>+0.4</td>
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<td>0.50</td>
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<td>+0.1</td>
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<tr>
<td>1.00</td>
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<td>−0.4</td>
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<td>+1.3</td>
<td>−0.5</td>
<td>−0.2</td>
</tr>
<tr>
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<td>−0.6</td>
</tr>
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<tr>
<td>3.00</td>
<td>−3.8</td>
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</table>

### TABLE C

**Change in Spherical Equivalent Refraction and Corneal Vertex Stromal Thickness**

<table>
<thead>
<tr>
<th></th>
<th>1 Day to 1 Month</th>
<th>1 Month to 3 Months</th>
<th>3 Months to 6 Months</th>
<th>6 Months to 12 Months</th>
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</thead>
<tbody>
<tr>
<td>SEQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD change (D)</td>
<td>−0.38±0.41</td>
<td>−0.06±0.17</td>
<td>+0.01±0.34</td>
<td>−0.10±0.25</td>
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<tr>
<td>% eyes in which SEQ changed &gt;0.50 D</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corneal Vertex Stromal Thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD (range) (µm)</td>
<td>−10.7±6.2</td>
<td>1.3±4.3</td>
<td>−2.6±4.1</td>
<td>3.1±5.3</td>
</tr>
<tr>
<td></td>
<td>(−0.3 to −21.5)</td>
<td>(−10.0 to 10.1)</td>
<td>(−11.5 to 6.8)</td>
<td>(−10.1 to 11.1)</td>
</tr>
</tbody>
</table>

SEQ = spherical equivalent refraction, SD = standard deviation

Note. Negative values represent stromal thinning, positive values represent stromal thickening.