

Age-Related Changes in Human Corneal Epithelial Thickness Measured With Anterior Segment Optical Coherence Tomography

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PURPOSE. To measure corneal and limbal epithelial thickness (ET) in normal subjects and to evaluate its variation with age by using anterior segment optical coherence tomography (AS-OCT).

METHODS. A total of 180 normal subjects (180 healthy eyes) were enrolled and divided into four groups according to age: A (0–20 years), B (21–40 years), C (41–60 years), and D (>60 years). Cornea and limbus were imaged with OCT. Corneal ET (CET) was obtained automatically by the built-in analysis software of the OCT system. Limbal ET (LET) in four quadrants was manually measured from OCT images.

RESULTS. Corneal ET of a central 2-mm diameter zone in groups A, B, C, and D were $53.4 \pm 2.8 \mu\text{m}$, $53.4 \pm 2.7 \mu\text{m}$, $53.2 \pm 3.0 \mu\text{m}$, and $52.9 \pm 3.3 \mu\text{m}$, respectively, N showing no significant change with aging. In the paracentral zone extending to 6-mm diameter, correlation analysis suggested that CET was inversely associated with age ($P < 0.05$). Limbal ET in the nasal and the temporal quadrants were similar and decreased with aging, the averages were $58.3 \pm 8.1 \mu\text{m}$, $54.1 \pm 6.1 \mu\text{m}$, $51.2 \pm 6.1 \mu\text{m}$, $51.6 \pm 5.2 \mu\text{m}$ for groups A, B, C and D, respectively; while age seemed to have no effect on LET of the superior and the inferior quadrant.

CONCLUSIONS. The paracentral corneal epithelium, as well as the nasal and temporal limbal epithelium, became thinner with aging, while the central CET seemed to remain constant. Measurement with AS-OCT of the corneal and limbal ET could aid in clinical assessment and planning treatments of the cornea.

Keywords: corneal epithelium, limbus, aging, optical coherence tomography

As the outermost layer that covers the front of an eye, corneal epithelium not only functions as a barrier that block the passage of foreign material, but also plays an important role in maintaining high optical quality. Stem cells in the basal layer of limbal epithelium hold a physiological significance in the renewal and metabolism of corneal epithelium, particularly under stress situations.^{1,2} A full understanding of the biological characteristics of human corneal and limbal epithelium affords us a great insight into the ocular surface in health and disease. Recently, epithelial thickness (ET) profiles have drawn increasing attention, because clinical and laboratory evidence suggests that ET change can not only affect total corneal power and bring undesired refractive shift, but also indicate structural or functional alteration in various conditions, such as contact lens wear, keratoconus, and disorders implicating limbal stem cell deficiency. Therefore, accurate measurement of corneal and limbal ET is of potential value in detecting early changes of ectatic corneal diseases, monitoring corneal remodeling, designing refractive surgery and assessing eligibility of other treatments.^{3–6} Until now, however, the normative database of ET has not been well established, and its association with age

was not explicitly illustrated. In an aging eye, changes related to corneal epithelium involved the surface of its superficial cells becoming smoother due to the loss of microvilli, microplicae, and glycocalyx.⁷ Basal epithelial cell density at the limbus-cornea and the limbus-palisade regions decreased significantly with advancing age; while in central cornea, morphology and density of both the superficial and the basal cells remained constant throughout life.^{8,9}

The advent of several imaging modalities, such as high-frequency scanning ultrasound biomicroscopy,^{10,11} confocal microscopy through focusing,^{7,12,13} and optical coherence tomography (OCT),^{5,14,15} have facilitated measurement of corneal and limbal ET. Among them, OCT has been reported as a repeatable and reproducible method with its advantages such as higher scanning speed and resolution. This noninvasive technique has been applied in clinical evaluation of various disorders, including keratoconus, corneal edema, and dry eye.^{16–19} Ge et al.²⁰ noted that the axial resolution of different OCT devices may not affect the measurement results of corneal ET, but higher optical resolution could yield better image quality that allowed for higher precision.

TABLE 1. Demographic Characteristics of Healthy Volunteer Subjects

Group	Subjects, n	Sex, n		Age Range, y	Mean Age, y
		Men	Women		
A	45	20	25	<20	14.29 ± 3.66
B	45	20	25	21-40	30.16 ± 5.50
C	54	24	30	41-60	51.54 ± 6.26
D	36	16	20	>60	67.47 ± 5.40
<40 y	90	40	50	7-40	22.22 ± 9.23
>40 y	90	40	50	40-83	57.91 ± 9.82
Total	180	80	100	7-83	40.07 ± 20.26

This study aimed to evaluate human corneal and limbal epithelial thickness profile in a healthy population with a broad age range by Fourier-domain OCT.

MATERIALS AND METHODS

Subjects

This study received the approval of the Ethics Committee of Eye & Ear, Nose Throat Hospital of Fudan University, and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects (or the legal guardian of those aged younger than 18 years).

Volunteers from the community were recruited from April to July 2013. To select normal subjects, each volunteer received a complete ophthalmologic evaluation including the ocular surface disease index (OSDI) questionnaire,¹⁷ slit-lamp biomicroscopy, intraocular pressure, refraction, best spectacle-corrected visual acuity, Schirmer I test, and tear break-up time. Eligible subjects had OSDI score <13 and normal ophthalmic examination results. Exclusion criteria included a history of contact lens wear, prior ocular trauma or surgery, current or long-term topical medication, and ocular or systemic diseases that may affect the cornea.

In total, the study included 180 normal subjects (80 men and 100 women; age range, 7-83 years). The subjects were divided into four groups according to age: A (0-20 years), B (21-40 years), C (41-60 years), and D (>60 years). Each group had the same sex ratio (male:female = 4:5). Characteristics of the enrolled subjects are summarized in Table 1.

Imaging Instrumentation

We used the Fourier-domain OCT system (RTVue-100; Optovue Inc., Fremont, CA, USA) with a cornea anterior module long adapter lens (1.96-mm scan depth and 6-mm scan width) in

this study. The device worked at 830-nm wavelength and had a scan speed of 26,000 axial scans per second. A *Pachymetry+Cpwr* scan pattern was used to map the cornea over a 6-mm diameter area, the settings were 8 meridional B-scans, consisting of 1024 A-scans each 5- μ m axial resolution. Following correct fixation and centering on the pupil, each scan was acquired within 5 seconds. Images of limbal area in each quadrant (superior, inferior, nasal, and temporal) were obtained using the cross-line scan mode. The subject was asked to fixate at a peripheral target to maintain the perpendicularity of the OCT beam at the surface of the targeted tissue, which was essential for obtaining accurate thickness values.

All tests were performed by one trained operator. To avoid potential artifacts or interferences from other ocular examinations, OCT scans were performed first, on one eye (randomly selected) of each subject for three times.

Measurement of Corneal Epithelial Thickness

Running on built-in software, version A6 .9.0.27 (Optovue, Inc.), the OCT system automatically generated a total corneal thickness (CT) map and a CET map for each pachymetry scan. Data output included the mean values in 17 sectors: (1) one central zone within 0- to 2-mm diameter, (2) eight paracentral zones from 2- to 5-mm diameter, and (3) eight peripheral zones from 5- to 6-mm diameter. The superior, inferior, minimum (Min), maximum (Max) CET, Min-Max (CET difference of Min and Max), and standard deviation (topographic variability of CET) corresponding to the central 5-mm diameter area were obtained in the statistics report. Representative examples were shown in Figure 1.

Measurement of Limbal Epithelial Thickness

The LET was determined based on a manual segmentation method reported by Francoz et al.,²¹ but with the following adjustments. First, a mean LET was calculated from measurement of thickness over the entire limbus, which referred a corneoscleral transitional zone, usually 1.0- to 1.5-mm wide extending centripetally from the scleral spur, according to the anatomical definition. The maximum LET was also included in the measurement (Fig. 2). Second, an external software program (*Image-Pro Plus*; Media Cybernetics, Inc., Rockville, MD, USA) was employed in image analysis instead of the internal software provided by the OCT system, because the former had a function of calculating thickness between two lines or traces, whereas the latter could only generate thickness value at a target point by manually placing the cursor there. For each image of the limbal area, LET was

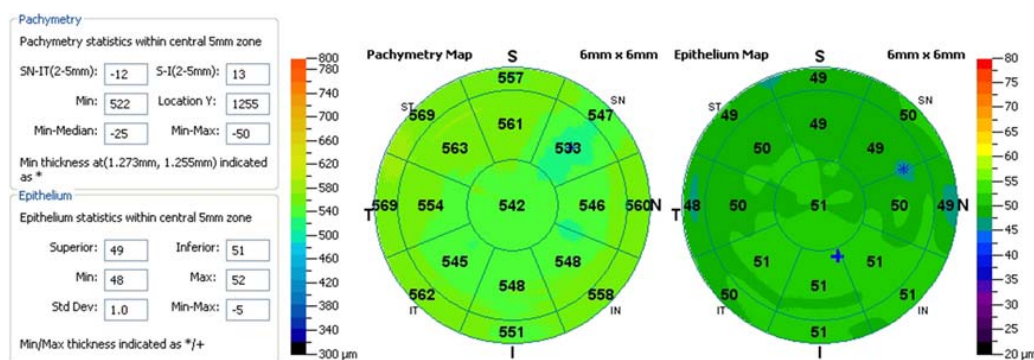


FIGURE 1. Main report of image analysis exported by OCT system, showing total corneal and corneal epithelial thickness distribution over the central 6-mm diameter area.

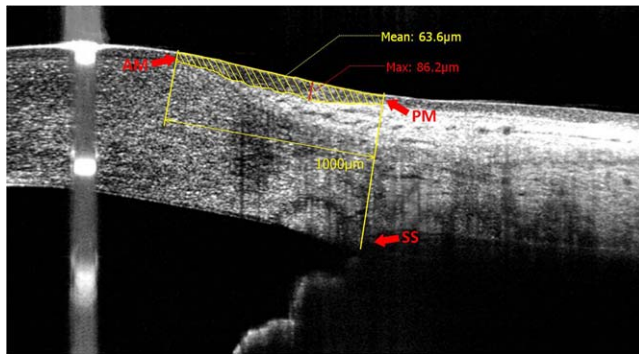


FIGURE 2. Limbus anatomical landmarks and evaluation of limbal epithelium in an OCT cross-line scan image. *Shaded region* indicates the target limbal epithelium. The mean/maximum LET was measured as the average/maximum distance between anterior and posterior epithelial surface (*two yellow curves*). AM, anterior margin of limbus; PM, posterior margin of limbus; SS, scleral spur.

determined as the average of 3 independent measurements, and the examiner was unaware of the subject's age. In order to evaluate interobserver variability, a second examiner was asked to perform the measurements on the same images, using the same image-analysis protocol while being masked to the first results. A Wilcoxon paired test demonstrated no significant differences between the two examiners.

Statistical Analysis

Statistical analyses were performed with statistical software (SPSS for Windows, version 19.0; SPSS, Inc., Chicago, IL, USA). Basic descriptive statistics were calculated on all data gathered, and values were reported as means ± SD. Difference in each parameter across age groups was tested with one-way ANOVA (or nonparametric Kruskal-Wallis test where appropriate). Comparisons between groups were conducted with Student's *t*-test (or Mann-Whitney *U* test where appropriate). Spearman's

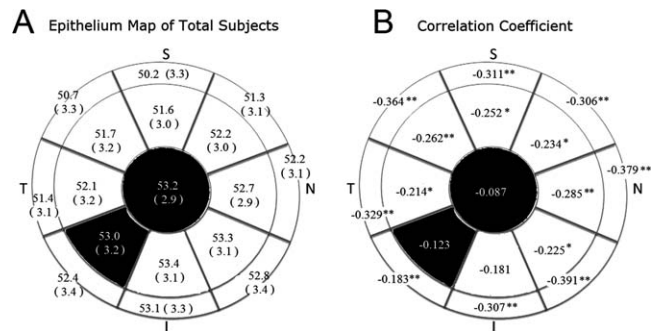


FIGURE 4. Distribution of CET (μm) of total subjects and correlation with age. (A) Mean (SD) CET in 17 sectors. (B) Correlation coefficient in 17 sectors. *Black region* indicates NO significant correlation; while *white region* indicates a negative linear correlation (Spearman's test, significance level at $P < 0.05$; * $P < 0.01$, ** $P < 0.001$).

correlation analysis was used to determine the significance level of the association of age with CET and LET. Two-tailed *P* values of <0.05 was considered significant.

RESULTS

Corneal Epithelial Thickness

Figure 3 showed CET distribution of the four age groups. A decline of CET with aging was observed from data on the pachymetry maps, ANOVA test indicated that epithelial thickness in the majority of the central 6-mm cornea was significantly different across age categories. Correlation analysis further demonstrated that except for the central 2-mm sector and the inferotemporal sector in the paracentral area, CET was negatively correlated with age ($P < 0.05$) (Fig. 4). As the age increased, the minimum value generally decreased, while the maximum remained constant ($55.9 \pm 3.4 \mu\text{m}$); higher topographic variability of CET was also observed (Table 2). Moreover, pairwise comparisons indicated no significant difference between the two youngest categories (A and B) or the two oldest categories (C and D; $P > 0.05$). Age categories were then collapsed into two categories: one consisted of subjects aged younger than 40 years (i.e., group A + group B), the other with subjects aged older than 40 years (i.e., group C + group D). No statistically significant difference of the central CET between the two categories was noted; while the older subjects had a thinner epithelial layer in 2- to 6-mm cornea (Fig. 5).

Limbal Epithelial Thickness

The LET profile of the four age groups was presented in Tables 3 and 4. Quadrant comparisons indicated that the limbal epithelium in the nasal and temporal quadrants was thinner than in the superior and inferior quadrants ($P < 0.001$). When compiled according to the four age categories, a clear decline in the mean ET of either nasal or temporal limbus was evident, ANOVA test indicated a significant age-dependent tendency ($P < 0.001$). Pairwise comparisons suggested the substantial difference across four age groups was mainly due to markedly higher LET value of the subjects aged younger than 20 years. However, as for mean ET of either superior or inferior limbus, no significant correlation with age was observed ($P > 0.05$), and the greatest value was measured in group B. We also demonstrated age had the same effects on the maximum LET.

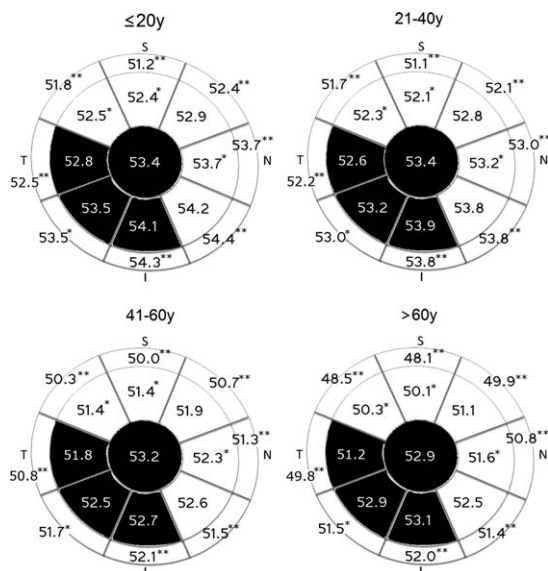


FIGURE 3. Distribution of CET (μm) in each age group. Black sectors indicate NO significant age dependence of regional CET, while white sectors indicate a significant difference across age groups (one-way ANOVA test, significance level at $P < 0.05$; * $P < 0.01$, ** $P < 0.001$). I, inferior; S, superior; N, nasal; T, temporal.

TABLE 2. Corneal Epithelium Statistics (µm) Within Central 5-mm Diameter Area in Each Age Group and Correlation With Age

	Total Subjects	Age Group				Correlation With Age	
		A	B	C	D	r	P
Superior	51.9 ± 2.9	52.7 ± 2.6	52.4 ± 2.9	51.7 ± 2.8	50.7 ± 3.2	-0.259	0.000*
Inferior	53.1 ± 3.0	53.7 ± 2.8	53.5 ± 2.9	52.5 ± 2.9	52.5 ± 3.5	-0.189	0.011*
Minimum	48.8 ± 3.3	49.9 ± 2.7	49.9 ± 3.2	48.2 ± 3.0	46.7 ± 3.2	-0.367	0.000*
Maximum	55.9 ± 3.4	56.2 ± 3.0	55.8 ± 3.0	55.6 ± 3.4	56.3 ± 4.2	-0.065	0.389
Min-Max	-7.2 ± 3.6	-6.4 ± 2.1	-5.7 ± 2.4	-7.4 ± 3.7	-9.6 ± 4.9	-0.326	0.000*
SD	1.6 ± 0.8	1.5 ± 0.5	1.3 ± 0.6	1.7 ± 0.8	2.2 ± 1.1	0.348	0.000*

Statistic data are presented as mean ± SD.

* Correlation is significant at $P < 0.05$ (Spearman's test, r : correlation coefficient).

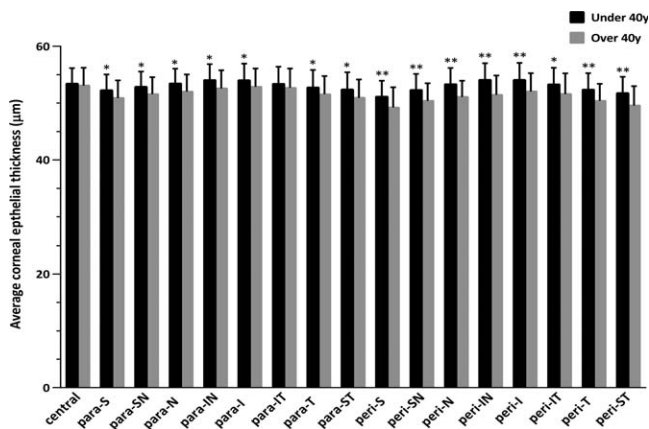


FIGURE 5. Comparison of average CET in each sector between the subjects under and over 40 years. There was no significant difference in the central 2-mm cornea or the inferotemporal sector of paracentral cornea (* $P < 0.01$, ** $P < 0.001$). IN, inferonasal; IT, inferotemporal; para-/peri-, paracentral/peripheral zone divided into eight sectors; SN, supranasal; ST, supratemporal.

DISCUSSION

The average central CET ($53.2 \pm 2.9 \mu\text{m}$) measured by this OCT system (Optovue, Inc.) was within the range ($48.0\text{--}59.9 \mu\text{m}$) reported in peer-reviewed literature.^{3-6,12-14,21-29} Our study demonstrated the epithelial layer of the cornea had a nonuniform thickness profile, as suggested in previous studies.^{5,10,29} In an aging eye, the topographic variability of CET would increase, which was considered as a natural tendency to counterbalance the underlying stromal irregularities; however, age effect on thickness of the epithelial layer was not fully elucidated.^{11-15,21} Present findings showed that the differences of CET in 2- to 6-mm diameter area between under and over 40 years were ranged between 1.19 to 2.65 μm , which were slightly larger than the repeatability measurement fluctuations reported by Ma et al.³⁰ With such data validity, our study demonstrated that epithelial thickness in paracentral cornea decreased with aging. However, these differences, though significant, were below the resolution of the AS-OCT device used, perhaps future technologies like swept-source imaging will help us to confirm our initial findings and elucidate the anatomy better.

Corneal epithelium is renewed and maintained by limbal epithelial stem cells (LESCs) during normal homeostasis or

TABLE 3. Mean LET (µm) in Four Quadrants in Each Age Group and Correlation With Age

	Total Subjects	Age Group				Correlation With Age	
		A	B	C	D	r	P
Nasal	53.4 ± 6.8	58.2 ± 7.4	52.8 ± 5.2	50.8 ± 6.0	51.5 ± 6.0	-0.345	0.000*
Temporal	54.2 ± 7.4	58.3 ± 8.9	55.4 ± 7.3	51.6 ± 6.2	51.6 ± 4.5	-0.380	0.000*
Superior	67.4 ± 10.6	66.7 ± 11.1	70.2 ± 9.5	67.3 ± 9.3	65.4 ± 12.1	-0.073	0.418
Inferior	67.7 ± 10.7	66.3 ± 9.0	70.4 ± 11.7	66.2 ± 11.3	67.7 ± 10.7	-0.041	0.653

LET data are presented as mean ± SD.

* Correlation is significant at $P < 0.05$ (Spearman's test, r : correlation coefficient).

TABLE 4. Maximum LET (µm) in Four Quadrants in Each Age Group and Correlation With Age

	Total Subjects	Age Group				Correlation With Age	
		A	B	C	D	r	P
Nasal	73.7 ± 10.8	80.0 ± 12.1	75.2 ± 9.3	71.0 ± 9.5	68.3 ± 8.3	-0.381	0.000*
Temporal	74.7 ± 12.6	79.4 ± 14.2	76.9 ± 13.9	73.0 ± 11.2	69.7 ± 8.6	-0.302	0.001*
Superior	91.2 ± 17.9	87.9 ± 16.2	97.0 ± 15.3	94.8 ± 20.8	84.6 ± 16.2	-0.072	0.423
Inferior	96.8 ± 20.6	91.4 ± 14.7	106.2 ± 20.7	96.8 ± 24.1	92.4 ± 19.0	-0.061	0.505

LET data are presented as mean ± SD.

* Correlation is significant at $P < 0.05$ (Spearman's test, r : correlation coefficient).

after wounding.^{31,32} In the course of proliferation and differentiation, these cells migrate centripetally while detaching from the basement membrane outward to the surface to form 5 to 7 layers of corneal epithelium, and finally shed by desquamation.³³ Investigations into the normal corneoscleral transitional zone suggested that the diameter and density of cells in both the limbus-cornea and limbus-palisade changed with advancing age, whereby the mean diameter increased with a corresponding decrease in cell density.^{9,34} Zheng and Xu³⁵ utilized in vivo confocal microscopy to observe the microstructure of limbus and found that the presence of Vogt palisades, which was believed to harbor LSCs, declined with aging; whereas the limbal basal epithelial cells showed age-related size enlargement and quantity reduction. Such results implied that as the age increased, LSCs kept a dynamic evolution with attenuating proliferative potential, leading to degenerated capacity in maintaining the integrity and stability of the corneal epithelium. This may be an explanation of our findings that the old had thinner corneal epithelium compared with the young.

Interestingly, age seemed to have no effect on central CET, which concurred with previous findings.^{11-15,21} Majo et al.³⁶ proposed that limbus only contributed cells to the corneal epithelium in response to wound healing, whereas during normal homeostasis the renewal was carried out by stem cells scattered throughout the corneal epithelium itself. In other studies, Chang et al.^{37,38} found that the capacity for epithelial cell proliferation and migration appeared to be as active in the central cornea as in the limbus; epithelial recovery in response to wound healing remained equal even after ablation of the limbus, cell density of the central corneal epithelium could increase faster than at the periphery. Laboratory evidence suggested that epithelial cells separated from human central cornea were capable of forming clonogenic spheres which were positive for p63 immunolabeling, indicating that central corneal epithelium contained cells with stem cell- or progenitor cell-like properties. These novel findings could be an explanation why the central corneal epithelium was less susceptible to age influences, also supplemented the conventional view that maintenance and repair of the corneal epithelium were only carried out by cells proliferating and moving centripetally from the limbus.

In terms of limbal epithelium evaluation, we observed that mean LET in the nasal and temporal quadrants was thinner than in the superior and inferior quadrants; the former exhibited significant age-related decrease while the latter seemed to remain constant with aging. The regenerative capacity of LSCs might account for such variations.

It was well recognized that the biological functions and activities of LSCs were regulated by exquisite programs. Various extrinsic (e.g., ultraviolet radiation) or intrinsic (e.g., cytokines) signals could cause failure to maintain a normal microenvironment in the limbal region, thus giving rise to the development of ocular disorders.⁴¹⁻⁴³ Histological and slit-lamp examinations have shown the presence of melanocytes, among the basal cells of the rete pegs in Vogt palisades, correlated with the pigment distribution in the limbal area.^{9,44,45} This melanin pigment is supposed to protect LSCs from the deleterious effect of ambient ultraviolet radiation.⁴⁶ According to Zheng and Xu's³⁵ study, aging also gave rise to a reduction in melanocytes quantity, which could impair LSCs repository due to the decreasing amount of pigment. In an aging eye, LSCs in nasal and temporal limbus were more likely to be the victims of solar damage than those in the superior and inferior limbus, because the former had higher risk of exposure to ultraviolet radiation without eyelids covering up. In addition, an intrinsic weakness in LESC reserves was implied by less prominent Vogt palisades in the nasal and temporal limbus,^{47,48}

suggesting that these regions might be less likely to undergo effective self-repair and self-renewal in normal homeostasis or after injury.

However, age-related changes of LET presented in this study were not observed previously. In fact, very little was known about epithelial thickness profiles at limbal region. With different OCT systems, epithelial thickness in the nasal and temporal limbus was measured to be 60.1 to 79.6 μm , usually thinner than that in the superior and inferior limbus which was reported to be 68.0 to 106.8 μm .^{21,22,27,39} Our findings showed lower LET in each quadrant compared with these values. Such discrepancy could be related to intrinsic differences of instruments. For instance, configurations, properties, and software algorithms of different OCT devices were not calibrated identically.⁴⁰ On the other hand, both in vivo and histological studies have suggested that the epithelial layer gradually became thinner from central cornea to limbus,^{21,27,39,49} while our results failed to demonstrate such variations. This could be attributed to different methods in image processing of the raw OCT scans. Specifically, the pachymetry map-based CET was accurately acquired by the internal software with an automated algorithm, while LET was measured by manual segmentation using an external software. Results yielded from different measurement protocols were incomparable, therefore the changing patterns of epithelial thickness from central cornea to limbus could not be inferred from the present findings.

The current study suffered from several limitations. First, given the fact that the OCT system was unable to discriminate the precorneal tear film, of which the thickness was reported $4.79 \pm 0.88 \mu\text{m}$ in a recent study,⁵⁰ a possible uncertainty was raised: whether the age-related changes we have observed were indeed attributed to the corneal epithelium thinning or faked by the tear film incorporated in the measurement. It was proposed that steady tear film thinning, mainly due to evaporation, eventually caused tear film breakup at different locations. Since the tear breakup time decreased with aging,^{51,52} we postulate that the tear film thinning rate increases with aging. This could lead to a reduction of tear film thickness (TFT) which might skew the results in the present study. However, OCT images were acquired within 5 seconds since the eyes opened, not long enough to observe the breakup of tear film (dry eyes were excluded in subject selection); the decline of TFT with aging was still lack of evidence. In addition, observations showed that the inferior tear film was thinner than the superior in normal eyes.^{53,54} Such a spatial distribution pattern of TFT was quite different from that of CET. We hope future imaging technologies with higher resolution and a function of automatically excluding tear film in the measurement, will help us evaluate CET more accurately and confirm its association with aging. Second, although data from the central 6-mm cornea is essential for vision and adequate for keratoconus screening, it is insufficient for design and fitting of contact lenses, or for monitoring disorders involving the peripheral cornea and planning clinical procedures for them. OCT scans covering the entire cornea are preferable for obtaining more information. The last limitation lies in the subjective interpretation of images when determining the limbus. In some cases, blurred visualization of the scleral spur made it difficult to precisely define the posterior margin of limbus and to guarantee the accuracy of LET measurements. Since LET, for the first time, was defined using the proposed averaging approach, these measurement data should be considered preliminary and subject to further refinement to verify reliability and validity. After all, so few was reported about LET and its association with the properties of the limbal epithelial cells. Future studies combined with other clinical or laboratory investigations such as confocal

microscopy and immunostaining of the targeted tissues or cells, will be needed to help understand the physiology or pathogenesis of human limbus in health and diseases.

To date, reports of CET measurement in normal population were not adequate. Results of our study have supplemented some normative information about human corneal epithelium. In conclusion, age seemed to have no effect on epithelial thickness of the central 2-mm cornea, or of the superior and inferior limbus; while in the majority of paracentral cornea up to 6 mm in diameter, as well as in the nasal and temporal limbus, the epithelial layer became thinner with increasing age. Studies of diverse populations and larger sample sizes may further validate these initial findings. Human corneal and limbal epithelial thickness profiles in normal corneas, measured by AS-OCT, could serve as a suitable basis for investigations into ocular surface pathology.

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