In Vivo Analysis of Ciliary Muscle Morphologic Changes with Accommodation and Axial Ametropia

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Purpose. To use anterior segment optical coherence tomography (AS-OCT) to analyze ciliary muscle morphology and changes with accommodation and axial ametropia.

Methods. Fifty prepresbyopic volunteers, aged 19 to 34 years were recruited. High-resolution images were acquired of nasal and temporal ciliary muscles in the relaxed state and at stimulus vergence levels of −4 and −8 D. Objective accommodative responses and axial lengths were also recorded. Two-way, mixed-factor analyses of variance (ANOVAs) were used to assess the changes in ciliary muscle parameters with accommodation and determine whether these changes are dependent on the nasal–temporal aspect or axial length, whereas linear regression analysis was used to analyze the relationship between axial length and ciliary muscle length.

Results. The ciliary muscle was longer ($r = 0.34$, $P = 0.02$), but not significantly thicker ($F = 2.84$, $P = 0.06$), in eyes with greater axial length. With accommodation, the ciliary muscle showed a contractile shortening ($F = 42.9$. $P < 0.001$), particularly anteriorly ($F = 177.2$, $P < 0.001$), and a thickening of the anterior portion ($F = 46.2$, $P < 0.001$). The ciliary muscle was thicker ($F = 17.8$, $P < 0.001$) and showed a greater contractile response on the temporal side.

Conclusions. The accommodative changes observed support an anterior, as well as centripetal, contractile shift of ciliary muscle mass. (*Invest Ophthalmol Vis Sci.* 2010;51:6882–6889) DOI:10.1167/iovs.10-5787

Details concerning how ciliary muscle contraction influences zonular tension and the roles of the posterior zonules, iris, and vitreous body in accommodation remain unclear.[1–4] The process is indisputably governed by ciliary muscle contraction,[5–7] with the forward and inward shift of its mass toward the lens equator, causing a reduction in zonular tension[4,8] that allows the capsule to mold the young lens into a thicker, more convex and dioptrically powerful form.[5,9]

The position of the ciliary body, screened by the iris, has hindered in vivo observation of its morphology and accommodative movements.[10–12] In vivo primate studies, using iridectomized animals allow direct visualization of the ciliary muscle and its relationship with the lens equator.[13] The rhesus monkey (*Macaca mulatta*) is considered the optimum animal model for the study of human accommodation, because of close similarities in accommodative structures[14,15] and mechanism[6,8,16] and the development of presbyopia on comparable relative timescales.[17–19] Implantation of an electrode into the Edinger-Westphal nucleus permits accurate control of the amplitude and duration of the accommodative response.[13,20,21] Studies in which Edinger-Westphal–stimulated accommodation was used in the rhesus monkey have demonstrated linear associations between accommodative response and various biometric correlates, including anterior chamber depth and lens thickness[20] and movements of the ciliary processes and lens edge.[7] In further studies, investigators have concluded that, although the ciliary body maintains its ability to move centripetally with accommodative effort,[22] the forward shift of the ciliary body is lost with age in the rhesus monkey.[23,24] In addition, ultrasound biomicroscopy (UBM) of the ciliary region in cyclopleged monkeys in vivo reveals significant nasal versus temporal biometric asymmetry.[25] To date, nasal–temporal asymmetry of the ciliary body has not been detected in vivo in humans.

In vitro monkey studies have been conducted to analyze postmortem ciliary muscle sections and identify age-dependent changes that could contribute to presbyopia. Isolated ciliary muscle strips from both young and presbyopic monkeys continue to contract in response to pharmacologic agents,[26,27] and there is no decrease in the quantity or affinity of muscarinic receptors with age.[19] However, presbyopic monkey samples in which the posterior attachment of the ciliary muscle is intact, do not contract in response to muscarinic agonists.[27] In addition, the elastic tendons forming the posterior attachments thicken with age and show increased levels of microfibrils.[28] These findings indicate that decreased compliance of the posterior insertion of rhesus monkey ciliary muscle, which is essential to allow its forward and inward accommodative movement and the restoration of its position during disaccommodation,[29] could be an important factor in the development of presbyopia.

In humans, analysis of postmortem ciliary body tissue from eyes exposed to high doses of pharmacologic agents before dissection has demonstrated that the muscle maintains its ability to contract throughout life.[10] Further in vitro studies have identified nasal–temporal asymmetry in human ciliary body morphology, with the temporal aspect being significantly longer at all ages,[30,31] although this disparity has not been observed in vivo.

More recently, UBM has been used in a small number of in vivo investigations to analyze configurational changes of human ciliary muscle with accommodation. Ma and Chen[32] observed that the thickness of the anterior, but not the posterior, portion of the ciliary body increases with accommodation in young eyes. In presbyopic subjects, the contractility of the ciliary muscle (inferred by centripetal movement) in response to pilocarpine significantly increases after cataract extraction.[11] Lenticular sclerosis may therefore hinder ciliary body contractility by exerting tension via the zonules. Cataract sur-

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gery, wherein the thickened, presbyopic lens mass is removed and a significantly thinner intraocular lens (IOL) is implanted into the capsular bag, which undergoes postoperative fibrosis and contraction, may alter anterior segment geometry in such a way as to allow centripetal movement to be restored.

In addition to accommodative changes, the morphologic characteristics of the ciliary muscle have been studied with regard to refractive error in vivo, using UBM[33,34] and anterior segment optical coherence tomography (AS-OCT).[35] Oliveira et al.[34] first reported increased ciliary body thickness in adults with high axial myopia, contravening the intuitive expectation that this structure would be attenuated in longer, myopic eyes and the findings of van Alphen,[36] who demonstrated thinning of human ciliary muscle with in vitro globe expansion. The association between axial myopia and greater ciliary body thickness has been confirmed in subjects with unilateral high myopia[33] and in children aged 8 to 15 years,[35,37] although the reason for this thickening remains unclear. Bailey et al.[35] suggested that ciliary muscle hypertrophy leads to poorer contractile responses and the accommodative dysfunction that is central to the hyperopic defocus model of myopigenesis, in which the resultant retinal defocus is accompanied by axial elongation.[38–40] However, it is unknown whether accommodative dysfunction constitutes a cause or effect of myopia.[41,42]

The morphology and accommodative configuration of human ciliary muscle is therefore of significant interest, in relation to the mechanism of accommodation and, as a consequence of recent findings, its link with refractive error. Although in vitro studies have furthered understanding of human and primate accommodation, the precise impact of postmortem tissue changes cannot be known,[43,44] and samples from dissected eyes may not represent normally responding ciliary muscle. In addition, the effects of ischemia may alter the response of the muscle to topically applied pharmacologic agents.[10] Thus, in vivo approaches to analyzing the ciliary body may be more valid, particularly because the entire accommodative apparatus remains intact. Hitherto, UBM has been most frequently used to acquire high-resolution in vivo images of the relaxed and accommodating ciliary body in humans[11,32,45,46] and primates.[17,23,25] However, the contact nature of the technique and requirement for subjects to be supine could alter anterior segment geometry.[47,48]

AS-OCT represents a relatively new methodology for imaging the anterior segment, including the ciliary body.[35,37] The noncontact approach is advantageous, and subjects can adopt a natural, upright position. In high-resolution corneal imaging mode, axial resolution of 8 μm is possible.[49] Accommodative changes in some biometric factors, including anterior chamber depth and lens thickness have been determined by AS-OCT (Davies LN, et al. *IOVS* 2008;49:ARVO E-Abstract 3777).[50] Although the relaxed ciliary body has been imaged with AS-OCT to investigate the link between thickness and refractive error,[35,37] no previously published study has analyzed in vivo accommodative changes in this structure with AS-OCT.

The purpose of this in vivo study was to further the authors' previous work (Sheppard AL, et al. *IOVS* 2009;50:ARVO E-Abstract 2796) and use AS-OCT to provide new data regarding human ciliary muscle morphology and accommodative characteristics. Furthermore, nasal versus temporal asymmetry in ciliary muscle morphology and response was investigated, along with a possible link to axial ametropia.

Methods

Sample Size Estimation

Before recruitment of subjects for the main study, a pilot investigation was conducted to highlight potential problems in the protocol and to acquire initial data to assist in the determination of the required sample size for the project. Pilot data were necessary for sample size estimation in this incidence to determine standard deviations of the accommodative biometric measures, which have not been studied in this manner in vivo. Fifteen young subjects (mean age, 23.2 ± 3.1 years) participated in the pilot study and were imaged in both the relaxed state and at 4.0-D stimulus vergence. The differences in means between the two demand levels and standard deviations of ciliary muscle thickness and length parameters were used in sample size calculations (Sigmaplot Statistical and Graphing Software, ver. 11; Systat Software Inc., Chicago, IL). The maximum number of subjects required for any of the individual parameters was 23, which was therefore used as a minimum level for subject recruitment, to ensure adequate statistical power of the results.

Subjects

Fifty prepresbyopic volunteers, 19 to 34 years of age (mean, 25.8 ± 4.5) with no previous history of ocular abnormality or intraocular surgery, were recruited using e-mail announcements at Aston University. Participants in the pilot study were remeasured during the main investigation, which took place several months later. Subjects with all types of refractive error were included, provided their prescription (including astigmatic component, if applicable) was amenable to correction with daily disposable soft contact lenses (Focus Dailies and Focus Dailies Toric: nelfilcon A, 69% water content; Ciba Vision, Duluth, GA). The parameter ranges for these contact lenses meant that the subjects who had spherical refractive errors greater than +6.00 or −10.00 DS were excluded from the investigation, as were those with oblique cylinders >0.50 DC or orthogonal cylinders >1.50 DC. The study was approved by the Ethics Committee of Aston University and was performed in accordance with the tenets of the Declaration of Helsinki. Written, informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

Measurements

Refractive error was determined in both eyes from the mean of five open-view distance autorefractor readings (WAM-5500 Auto Ref–Keratometer; Grand Seiko Co. Ltd., Hiroshima, Japan). The WAM-5500 is a binocular open-field autorefractor and keratometer that has been validated and found to provide repeatable and accurate results, compared with subjective refraction.[51] The refraction of subjects with spherical or astigmatic error >0.50 D in either eye was corrected with the disposable soft contact lenses (Focus Dailies or Focus Dailies Toric; Ciba Vision). Functional emmetropia was necessary to ensure near-identical accommodative demand for each subject. All further measurements were taken from the right eye only.

Objective accommodative responses were determined with the WAM-5500 autorefractor while subjects fixated Maltese cross targets in free space at −4 and −8-D stimulus vergences, presented in random order. The constant angular subtense of the targets was 4.6°. Average target luminance and Michelson contrast values were 34.5 cd/m^2 and 82% and 30.5 cd/m^2 and 80%, for the 4- and 8-D stimuli, respectively. The left eye was occluded with a patch during measurement of the response, and subjects were instructed to carefully focus[52] on the center of the maltese cross to induce both voluntary and reflex accommodation.[53] We ensured at this stage that participants had sufficient subjective accommodative amplitude to maintain clarity of the 8-D stimulus, a requirement for ciliary muscle imaging. Five readings were obtained at each stimulus level, and the mean of these values was used in conjunction with the distance autorefractor results to determine the objective accommodative response.

Axial lengths were obtained from the mean of five partial coherence laser interferometry (PCI) readings, with an ocular biometer (IOLMaster; Carl Zeiss Meditec, Inc., Dublin, CA). The biometer is a high-resolution, noncontact device developed principally for determination of ocular biometry before cataract extraction with intraocular lens implantation and has a resolution of 0.01 mm for axial length

measures.[54,55] For subjects corrected with soft contact lenses, axial length measurement was conducted at the end of data collection after lens removal.

Ciliary Muscle Image Acquisition and Analysis

Images were obtained of the nasal and temporal ciliary muscle of the right eye at stimulus vergence levels of −0.19, −4, and −8 D. The high vergence level of −8 D was selected to induce near-maximum non-pharmacologically induced accommodative changes in ciliary muscle. The AS-OCT system (Visante; Carl Zeiss Meditec. Inc., Dublin, CA) was set to high-resolution corneal mode for all imaging. According to the manufacturer, this setting provides axial resolution of approximately 8 μm. The device employs low-coherence interferometry, with a 1310-nm superluminescent light-emitting diode. The scanning spot moves rapidly across the eye, acquiring 512 A-scans in 0.25 seconds in high-resolution mode, to generate a two-dimensional image covering an area measuring 10 mm in width and 3 mm in depth. The scanning plane was set horizontally, at 0°, throughout the investigation.

Maltese cross targets were used for fixation, positioned at an angle of 40°, such that the eccentric gaze of the subject, while the head was in the primary position on the chin and forehead rest, allowed images centered on the ciliary muscle to be captured. Forty degrees represented the minimum level of horizontal eye movement needed to view the distant targets, beyond the AS-OCT device, and meant that the optical axis of the instrument was through the sclera, rather than the cornea, reducing optical distortion. The distant target was viewed through a mirror, resulting in a stimulus vergence of −0.19 D. Near targets, subtending 4.6°, were suspended in free space from an adjustable apparatus mounted on the AS-OCT headrest (Fig. 1). Average luminance and Michelson contrast values were 38 cd/m^2 and 81% and 32 cd/m^2 and 78% for the 4- and 8-D stimuli, respectively. For each of the two sides of the eye imaged, all targets were positioned along the same axis, with subjects asked to ensure that the near stimuli appeared directly over the distant maltese cross to reduce the possibility of varied acquisition planes. Targets of the varied stimulus vergence levels were presented in random order, and multiple images were acquired

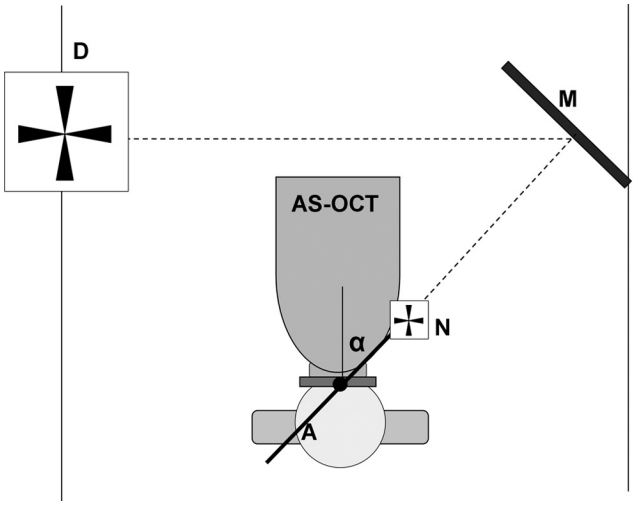

FIGURE 1. Schematic diagram of the laboratory setup for imaging nasal ciliary muscle using the AS-OCT. The subject views a near Maltese cross (N) at either −4.0- or −8.0-D stimulus vergence, attached to an adjustable apparatus (A) mounted on the AS-OCT headrest. Angle α = 40°. The relaxed ciliary muscle is imaged by removing the near target from the apparatus, allowing the subject to view, via a mirror (M), a distant maltese cross (D) positioned on the laboratory wall, resulting in a stimulus vergence of −0.19 D. For clarity, the mirror and distant target used for imaging temporal ciliary muscle have been omitted from the diagram, although these were present throughout the investigation.

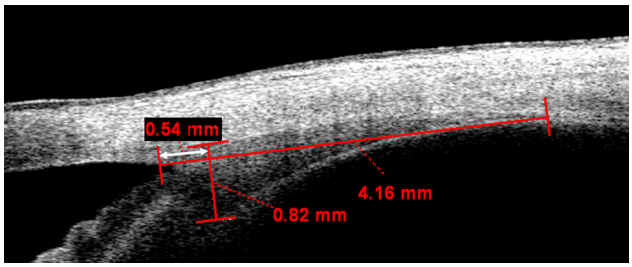

FIGURE 2. Measurement of ciliary muscle length, from the scleral spur to posterior visible limit (here, 4.16 mm) and ciliary muscle anterior length, from the point of maximum width (indicated by the 0.82-mm caliper) to the scleral spur (here, 0.54 mm; *double-headed arrow*).

of nasal and temporal ciliary muscle in each accommodative state, ensuring good visibility of the muscle in at least three images wherever possible. During the image capture process, which lasted approximately 5 to 10 seconds per scan, subjects were again asked to carefully focus on the center of the cross. A code was used to store the multiple image sets for each subject on the AS-OCT hard drive, such that the examiner performing post hoc analysis of ciliary muscle biometry was masked to the stimulus demand level and the refractive error of the individual.

Image analysis was performed by one examiner (ALS) using the inbuilt Visante system software (ver. 2.0), which utilizes edge-detection algorithms to locate corneal surfaces and assign appropriate refractive indices to each portion of the image and adjust its dimensions. An index of 1.000 (air) is applied to the region anterior to the cornea, 1.338 (cornea) for the area within the corneal boundaries, and 1.343 (aqueous humor) for structures posterior to the cornea.[50] A refractive index of 1.000 was applied to the whole image, using the Edit Surfaces option, before measurements were taken. After this adjustment, the Visante calipers were used to measure a range of ciliary muscle biometric characteristics. The software allows up to seven calipers to be positioned simultaneously on each image, with the option of hiding from view those not required—for example, if they obscure the region required for another measure. Overall visible ciliary muscle length was defined as the anteroposterior distance from the scleral spur, representing the anterior insertion, to the posterior tip of the ciliary muscle (Fig. 2). Because intraindividual variability in the relative visibility of these landmarks on different images, each subject's images for one stimulus vergence level were examined before measurements were taken, with adjustment of brightness and contrast settings where necessary, to facilitate localization of these points. In addition, the software allows the operator to magnify the image and more accurately place the calipers. Anterior length was measured from the point of maximum width of the ciliary muscle to the scleral spur. To obtain this measurement, a caliper was first placed along the widest portion of the ciliary muscle, and a second caliper, perpendicular to the first, was used to determine the distance from the widest region to the scleral spur (Fig. 2). In addition to lengths, a range of width measurements were obtained, judged from the ciliary muscle–sclera boundary to the pigmented ciliary epithelium. Width measurements (including determination of maximum width) were always obtained with a caliper positioned perpendicular to the ciliary muscle–sclera boundary. Three key width measures were acquired, selected because of the known anterior shift of the ciliary muscle with contraction.[29,32] Using knowledge of the predetermined overall length, the width of the muscle was determined at the point that fell 25% of the total length posterior to the scleral spur (CM25; Fig. 3). Similar measures were obtained at locations 50% and 75% of the overall ciliary muscle length posterior to the scleral spur (CM50 and CM75, respectively; Fig. 3). In addition, the ciliary muscle thickness at a set location 2 mm posterior to the scleral spur was determined (CM2). Bailey et al.[35] included this width measurement in image analysis and found that it correlated negatively with refractive error in children.

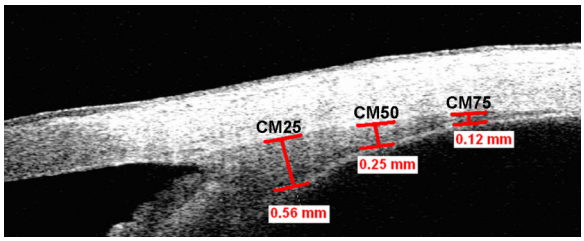

FIGURE 3. Measurement of ciliary muscle width parameters (same eye as in Fig. 2). CM25, CM50, and CM75 are ciliary muscle widths at 25%, 50%, and 75% of total ciliary muscle length, respectively.

The refractive index of 1.000 applied during image analysis would not be appropriate for analysis of thickness measures and would cause an overestimation of these parameters, as the index of the ciliary muscle is significantly higher than this value. Reports in the literature suggest the refractive index of the ciliary muscle to be in the region of 1.382,[56,57] so all thickness measures were divided by this to provide more realistic data. After analysis of the three images of nasal and temporal ciliary muscle for each accommodative state, in which the muscle appeared best defined, mean values for each of the length parameters and width parameters (adjusted for refractive index) were entered into a spreadsheet (Excel; Microsoft, Redmond, WA) and used for statistical analyses. Knowledge of the objective responses to the −4- and −8-D stimuli allowed determination of the changes in ciliary muscle parameters per diopter of accommodation for each subject. Using mean values of ciliary muscle response per diopter of objective accommodation in all 50 participants, allowed description of the cohort as a whole.

Statistical Analysis

The relationship between axial length and both ciliary muscle overall length and anterior length was determined by linear regression analysis (SigmaPlot; Systat Software, Inc.). To assess the differences in ciliary muscle parameters with accommodation and to determine whether any changes observed were dependent on either axial length or nasal–temporal aspect, we performed two-way, mixed-factor ANOVAs (SPSS, ver. 15; SPSS Inc., Chicago, IL). Eyes were classified into tertiles according to axial length, for use in the ANOVAs. Demand, the within-subject factor, was assigned three levels, of −0.19, 4, and 8 D, whereas the side (nasal or temporal) and axial length (short, medium or long) were designated as between-subjects factors. A significance level of $\alpha = 0.05$ was in all analyses.

Repeatability

The repeatability of ciliary muscle measures and examiner interpretation was assessed initially by imaging and analyzing the ciliary muscle of a single subject 10 times at −0.19 D stimulus vergence. The subject removed and repositioned his or her head on the AS-OCT forehead and chin rest before each image was acquired. Furthermore, to assess intersession repeatability, the temporal ciliary muscle of a subset of 10 subjects was imaged at −0.19 D (minimal) and 8.0 D (maximum) stimulus vergence at a second session, within 2 weeks of the initial visit. The temporal ciliary muscle aspect was chosen for intersession repeatability measures, as it has been shown that the scleral spur is most easily discernible on the nasal side[58]; thus, it would be expected that temporal ciliary muscle measures would be associated with greater variability. The bias for each ciliary muscle parameter was calculated from the mean difference in measures between visits, and paired *t*-tests were used to determine whether the levels of bias were significantly different from 0. The limits of agreement (LoA; the interval over which 95% of the differences between the two visits lie[59,60]) were established by using the standard deviation of differences with the following formula:

$$\text{LoA} = \text{bias} \pm (1.96 * \text{SD of differences}) \quad (1)$$

TABLE 2. Repeatability of Ciliary Muscle Biometric Measures

Parameter	Measure
Overall length, μm	4570 ± 32.2
Anterior length, μm	870 ± 26.4
CM25, μm	485 ± 9.6
CM50, μm	326 ± 11.4
CM75, μm	166 ± 5.1
CM2, μm	369 ± 27.5

Data (mean ± SD) were assessed by imaging and analyzing a single subject 10 times at the minimum accommodative state.

RESULTS

A wide range of MSE refractive error was found in the cohort, from −9.50 to +0.88 D (mean: −2.00 ± 2.62 D) and consequently, axial lengths were broadly spread, from 22.17 to 28.12 mm (mean, 24.49 ± 1.13 mm). Mean objective accommodative responses to the 4.0 and 8.0 D stimuli were 2.82 ± 0.58 and 5.44 ± 0.97 D, respectively. The general characteristics of ciliary muscle biometric parameters at stimulus levels of 0.19, 4.0, and 8.0 D are summarized in Table 1.

Repeatability

The repeatability results of ciliary muscle measures assessed by imaging and analyzing a single subject 10 times at −0.19 D stimulus vergence are shown in Table 2, while intersession repeatability is summarized in Table 3. None of the amounts of bias reported in Table 3 are significantly different from 0 (using paired *t*-tests), at either the minimum or maximum accommodative stimulus level.

Ciliary Muscle Biometry and Changes with Accommodation

Mean relaxed ciliary muscle overall length was 4630 ± 470 and 4810 ± 690 μm on the nasal and temporal aspects, respec-

TABLE 1. Nasal and Temporal Ciliary Muscle Parameters with Accommodative Stimulus Level

	Nasal			Temporal		
CM Parameter	**0.19 D**	**4.0 D**	**8.0 D**	**0.19 D**	**4.0 D**	**8.0 D**
Overall length, μm	4630 ± 470	4470 ± 460	4440 ± 480	4810 ± 690	4620 ± 590	4520 ± 610
Anterior length, μm	860 ± 120	780 ± 110	750 ± 130	900 ± 140	740 ± 150	680 ± 150
CM25, μm	535 ± 51	550 ± 51	564 ± 58	550 ± 51	571 ± 58	586 ± 72
CM50, μm	297 ± 43	297 ± 36	297 ± 43	347 ± 43	333 ± 43	333 ± 51
CM75, μm	152 ± 22	152 ± 15	152 ± 22	174 ± 22	166 ± 22	166 ± 22
CM2, μm	347 ± 58	340 ± 58	340 ± 58	405 ± 58	384 ± 65	384 ± 65

Data are expressed as the mean ± SD. $n = 50$ eyes.

TABLE 3. Intersession Repeatability Data of Ciliary Muscle Biometric Parameters at Two Demand Levels

Parameter	0.19-D Stimulus Level			8-D Stimulus Level		
	Bias	SD of Differences	95 % LoA	Bias	SD of Differences	95 % LoA
Overall length, μm	−15.0	105.9	−222.8, +192.8	−39.7	95.8	−227.5, 148.1
Anterior length, μm	−4.0	46.0	−94.2, +86.2	18.3	16.3	−13.7, +50.3
CM25, μm	−5.8	11.4	−28.1, +16.4	5.6	10.3	−14.7, +25.8
CM50, μm	2.9	12.4	−21.3, +27.2	2.5	10.3	−17.6, +22.6
CM75, μm	0.3	3.7	−6.9, +7.5	0.6	10.3	−19.5, +20.7
CM2, μm	−3.5	12.1	−27.4, +20.2	6.8	15.5	−23.5, +37.1

None of the measures of bias was significantly different from 0 (paired *t*-test).

tively, although the difference between sides was not significant ($F = 1.67$, $P = 0.2$). Anterior length comprised, on average, 18.5% of overall ciliary muscle length in the relaxed state and was also independent of the nasal–temporal aspect ($F = 2.18$, $P = 0.12$). A positive correlation was identified between axial length and both overall ciliary muscle length and anterior length (Fig. 4; $r = 0.34$, $P = 0.02$, and $r = 0.49$, $P < 0.001$, respectively). Figure 5 shows a sample image of ciliary muscle morphology on the temporal side in a lengthened eye (axial length, 28.12 mm) and an emmetropic eye in the shortest tertile (axial length, 23.70 mm). The overall ciliary muscle length and anterior length are both noticeably greater in the longer eye.

A statistically significant reduction was found in both overall ciliary muscle length and anterior length during accommodative effort (Fig. 4; $F = 42.9$, $P < 0.001$ and $F = 177.2$, $P < 0.001$, respectively). The shortening with accommodation was not dependent on axial length for either overall or anterior length ciliary muscle measures ($F = 0.43$, $P = 0.79$ and $F = 0.60$, $P = 0.67$), although the anterior length showed a significantly greater accommodative reduction on the temporal, compared to the nasal, side ($F = 20.6$, $P < 0.001$). The greatest magnitude of changes in ciliary muscle overall length and anterior length occurred between the 0.19- to 4.0-D, rather than 4.0- to 8.0-D, stimulus levels. For the temporal aspect, overall length decreased on average by 80 ± 100 and 50 ± 120 μm per diopter of accommodative response between the 0.19- to 4.0-D and 4.0- to 8.0-D demand levels, respectively. Anterior length reduced by 60 ± 40 and 30 ± 30 μm per diopter of response between the lower and higher demand levels, respectively. It is therefore apparent that most of the shortening in overall ciliary muscle length is as a result of a reduction in the anterior portion, which constitutes only approximately 19% of overall length in the relaxed state. The mean reductions in anterior length were significantly smaller on the nasal side: −30 ± 14 μm from 0.19 to 4.0 D and −20 ± 20 μm from 4.0 to 8.0 D.

Considering ciliary muscle thickness, the most anterior portion measured, CM25, represented the thickest region at all accommodative stimulus levels, with the more posterior CM50 and CM75 becoming progressively thinner (Table 1). The proportional measures of ciliary muscle thickness (CM25, CM50, and CM75) were not dependent on axial length (CM25: $F = 0.16$, $P = 0.86$; CM50: $F = 0.83$, $P = 0.44$; CM75: $F = 2.17$, $P = 0.12$), although CM50 and CM75 were significantly thicker on the temporal than on the nasal aspect ($F = 21.2$, $P < 0.001$ and $F = 15.3$, $P < 0.001$ for CM50 and CM75, respectively). There was a trend, although not statistically significant, for CM25 to be thicker on the temporal side, as well ($F = 3.16$, $P = 0.08$). With accommodative effort, there was a statistically significant thickening of CM25 ($F = 46.2$, $P < 0.001$), but neither CM50 nor CM75 changed significantly with accommodation ($F = 1.90$, $P = 0.15$; $F = 1.84$, $P = 0.16$, respectively). A mean increase in CM25 of 7.1 ± 6.4 μm per diopter of accommodative response was identified.

CM2 thickness measures, taken at a constant 2 mm posterior to the scleral spur are unrelated to the overall length of the ciliary muscle. A trend for CM2 to increase with axial length

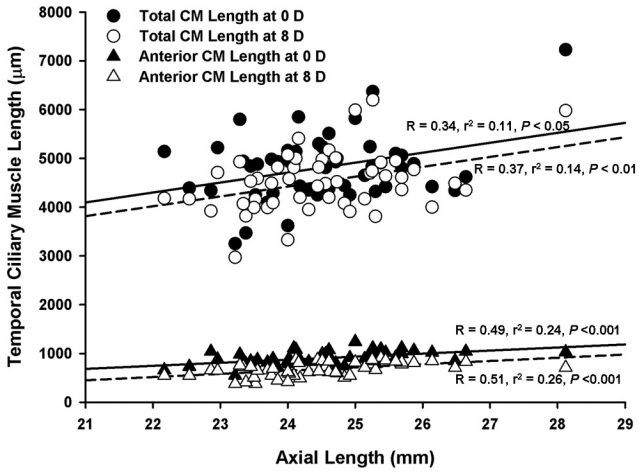

FIGURE 4. Ciliary muscle total length and anterior length in relaxed state and in response to 8-D stimulus. Both ciliary muscle length measures, particularly anterior length, show a positive correlation with axial length. With accommodation, a contractile shortening of ciliary muscle occurs, most of which is accounted for by a reduction in the anterior length, which comprises only approximately 19% of overall length in the relaxed state.

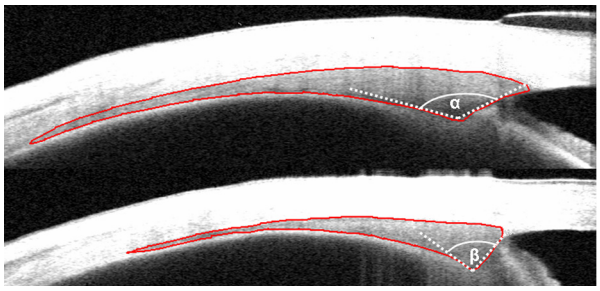

FIGURE 5. Sample images of temporal ciliary muscle morphology in a long, myopic eye (axial length, 28.12 mm; *top*) and an emmetropic eye (axial length, 23.7 mm; *bottom*). Ciliary muscle overall length (6580 and 4800 μm in the long and short eyes, respectively) and anterior length (from the thickest point to the scleral spur; 1030 and 810 μm in the long and short eyes, respectively) were both noticeably greater in the longer eye. In addition, the ciliary muscle inner apical angle was larger in the myopic eye ($\alpha = 138°$, $\beta = 92°$).

was identified, although it was not statistically significant at the 0.05 level ($F = 2.84$, $P = 0.06$). CM2 was found to be significantly thicker on the temporal side, compared with the nasal aspect ($F = 17.8$, $P < 0.001$). Mean values in the relaxed state were 347 ± 58 and 405 ± 58 μm, on the nasal and temporal sides, respectively. Accommodative effort caused a statistically significant thinning of CM2 ($F = 13.1$, $P < 0.001$), particularly on the temporal side ($F = 3.4$, $P = 0.04$), with nearly all of the change occurring between the 0.19- and 4.0-D stimuli, rather than 4.0 to 8.0 D. Mean changes in CM2 thickness per diopter of accommodative response were −2.2 ± 11 μm from 0.19 to 4.0 D and 0 ± 19 μm from 4.0 to 8.0 D on the nasal side. Temporally, these changes were greater at −7.0 ± 13 μm/D of accommodative response from 0.19- to 4.0-D stimuli and −1.1 ± 12 μm between the 4.0- and 8.0-D levels.

Discussion

There is a paucity of literature documenting in vivo changes in human ciliary muscle biometry with accommodation. Recent advances in ophthalmic imaging technology allow the ciliary muscle to be more easily visualized and imaged with sufficient resolution to detect accommodative changes. To date, this study is the largest to report the relaxed and accommodated morphologic characteristics of ciliary muscle biometry, by using AS-OCT in an adult population.

In the unaccommodated state, the ciliary muscle was found to be significantly longer, in overall length and anterior length, in eyes with a greater axial length. No statistically significant nasal versus temporal difference in ciliary muscle length measures was identified. The proportional measures of ciliary muscle thickness (CM25, CM50, and CM75) were not dependent on axial length, although CM2, measured at a fixed distance of 2 mm posterior to the scleral spur, showed a trend toward being thicker in longer eyes ($F = 2.84$, $P = 0.06$). Regarding nasal versus temporal thickness characteristics, CM50, CM75, and CM2 were all significantly greater temporally. For the most anterior location measured, CM25, the nasal–temporal asymmetry was less pronounced ($F = 3.16$, $P = 0.08$). Nasal versus temporal differences in human ciliary muscle thickness have not been reported in an in vivo study, and the relevance of this asymmetry is unclear. It is feasible that a stronger contractile response would occur on the side where the ciliary muscle is thickest. The greater accommodative shortening of the anterior portion of the ciliary muscle on the temporal side ($F = 20.6$, $P = <0.001$) observed in this study supports this premise. Nasal–temporal variation in ciliary muscle morphology and contractile response could have implications in strategies being developed to surgically restore accommodation to presbyopic eyes, such as accommodating intraocular lenses and capsular bag refilling. Ocular asymmetry in the anatomy of the ciliary region has been documented in living rhesus monkeys,[25] and it has been suggested that nasal–temporal anatomic variations are a functional necessity in primates, enabling alignment of the lenticular axes on the object of regard and maintaining binocular single vision during the convergent eye movements that accompany accommodation.

Published studies have identified a strong negative correlation between refractive error and axial length and ciliary body thickness, particularly at the point 2 mm posterior to the scleral spur (CB2). Bailey et al.[35] used AS-OCT with an applied refractive index of 1.000 to measure ciliary body thickness in children. After adjustment of their results to the 1.382 index used in the present investigation, they found the mean nasal CB2 to be 415 μm in emmetropes and 455 μm in myopes, whereas Schultz et al.[37] reported a mean nasal CB2 of 437 μm in children with refractive errors from −6.00 to +3.44 D, but of the same age range. We used methodology equivalent to that used in these two investigations and identified a considerably thinner mean nasal CM2 (equivalent to CB2) in adults aged 19 to 34 years, of 340 μm in emmetropes, and 356 in myopes. AS-OCT findings therefore imply that the ciliary muscle is at its thickest during childhood and becomes thinner in adulthood, when eye growth has ceased, and refractive error is stabilized. UBM analysis of a cohort of adults of average age 51.8 years identified the mean CB2 to be 490 μm in myopes, and 362 μm in emmetropes, but did not assess youthful subjects.[34] Further investigation using identical methodology across a broad range of age groups is needed to clarify the effect of age on ciliary muscle thickness and the possible implications for development of refractive error. Furthermore, thickness measurements taken at a fixed distance from the scleral spur do not take into account the fact that ciliary muscle overall length varies significantly with refractive error, so a point 2 mm from the scleral spur may represent an anatomically different region of the ciliary body in varying refractive error groups. Proportional thickness measures such as CM25, CM50, and CM75 may be more valid in analyzing subjects of different refractive error and ensuring that similar regions of the ciliary muscle are compared.

Although the present investigation has identified a weaker association between ciliary muscle thickness measures and refractive error than in published studies, a positive correlation between ciliary muscle length and axial length was found (Fig. 4). The correlation with axial length was greater for anterior length measures ($r = 0.49$, $r^2 = 0.24$; $P < 0.001$) than for overall ciliary muscle length ($r = 0.34$, $r^2 = 0.11$; $P = 0.017$). A possible explanation for this observation is that the scleral spur was more readily identifiable in many images than the posterior limit of the ciliary muscle, meaning that the anterior length measures were associated with slightly reduced variability compared with the overall values of muscle length, as indicated by the standard deviations of the repeatability measurements (Tables 2, 3).

Hitherto, no in vivo study has documented human ciliary muscle lengths. However, the mean overall relaxed ciliary muscle length measures determined in this investigation, 4.63 mm nasally and 4.81 mm temporally, are in accordance with previous in vitro reports. Pardue and Sivak[10] found a mean ciliary muscle length of 3.87 mm in atropine-treated eyes, whereas Aiello et al.[30] determined mean lengths of 4.79 mm on the nasal side and 5.76 mm temporally, although their sample consisted of just five adult eyes. The finding that the ciliary muscle is longer in myopic eyes is unsurprising: One would expect the morphology of the ciliary body to alter with elongation of the globe. van Alphen[36] observed marked thinning of the ciliary body with in vitro globe expansion, although the findings of the present study indicate that the ciliary muscle is not simply stretched as the eye elongates. The ciliary muscle was not found to be attenuated in myopic subjects as would be predicted from stretching alone, in fact, no significant relationship between ciliary muscle thickness and refractive error was identified. It seems likely, therefore, that axial elongation is accompanied by some radial growth—thickening of the ciliary muscle during myopigenesis. The greater anterior ciliary muscle length in longer eyes indicates that the structure grows in the anteroposterior direction as the globe elongates, with the scleral spur as the fixed anchor point. Figure 5 supports this assertion: The inner apical angle of the ciliary muscle is clearly larger in the myopic eye than in the emmetropic one. However, in vivo measurements of this angle are necessary to confirm the finding.

The key accommodative changes in ciliary muscle morphology identified in the present investigation are a contractile shortening of both overall length and anterior length, and a

thickening of CM25, the most anterior of the thickness measures. These observations support the generally accepted model of ciliary muscle action during accommodation, whereby most of the muscle mass shifts anteriorly and inward to reduce zonular tension.[1,2,4,61] Although the accommodative forward shift of ciliary muscle has been observed in vivo in rhesus monkeys using UBM,[17,23] the movement has not been visualized previously in human subjects, as the iris normally prevents exploration of the ciliary region with most available imaging techniques. Furthermore, a recently published human in vivo MRI study found no change in the anteroposterior position of the ciliary muscle with accommodative effort in either young or presbyopic subjects,[62] suggesting that only a centripetal, rather than a forward and centripetal, shift of muscle mass occurs during accommodation. A case report of accommodation in a 19-year-old albino using AS-OCT also failed to observe a concurrent forward shift of the ciliary body with its centripetal accommodative movement.[63] However, the images were not centered on the ciliary region, nor captured in high-resolution mode, and accommodative changes in a single albino subject cannot be considered representative of the population as a whole. The present study found that anterior ciliary muscle length decreased significantly with accommodation, whereas CM25 thickened. Between the 0.19- and 4-D stimulus levels, anterior length reduced on average by 30 ± 30 μm per diopter of response nasally and 60 ± 40 μm/D of accommodation, temporally. This shortening of anterior length represents a movement of the thickest region of the ciliary muscle toward the cornea (i.e., evidence of forward shift of the main muscle mass), whereas the thickening of CM25 by approximately 8 ± 11 μm/D of response on both sides is indicative of centripetal ciliary muscle movement.

Therefore, in vivo support of anterior, as well as centripetal, ciliary muscle movement with accommodation in a relatively large cohort of human subjects is provided for the first time. However, in addition to the implications of the study, there are limitations that should be considered. First, the objective measures of accommodation were acquired before AS-OCT scanning and may not represent the precise level of accommodation exerted by each subject during image capture. Simultaneous measurement of refraction and ciliary muscle imaging would be preferable, although the dimensions of the Visante device, which screens much of the subject's face, may hinder such attempts. An appliance mounted on the AS-OCT headrest, with a beam splitter linked to an autorefractor, is being considered and could be custom manufactured, to facilitate simultaneous measurement of accommodation during scanning.

To visualize the complete ciliary muscle, it was necessary for subjects to view an eccentrically positioned target at an angle of 40°. Therefore, the images of temporal ciliary muscle were captured during convergence, but the nasal muscle was imaged during abduction, which does not represent the natural convergent state of the eye during accommodation. It is not possible to use AS-OCT to image the entire right nasal ciliary muscle during convergence. However, peripheral refraction measures have been found not to vary depending on whether a subject moves his or her eye or head,[64,65] implying that the state of convergence does not directly alter refraction. Neither the accommodative response therefore, nor the ciliary muscle parameters should have been significantly confounded by state of convergence.

As with any two-dimensional imaging technique, there is the potential for image acquisition planes to vary between measurements. To reduce the associated impact, multiple images were acquired and analyzed at each stimulus level and the average values used for statistical analyses. After completion of data collection, an update to the Visante software was released (ver. 2.0). All analysis was performed with the new software, which gives the option of zooming while viewing images, allowing for easier identification of ciliary muscle boundaries. The software update also includes a novel enhanced high-resolution imaging mode that takes several scans and automatically generates an averaged image of improved quality than has been possible. In addition, we are currently exploring the possibility of using custom-developed software with edge-detection algorithms, to derive objective measures of ciliary muscle parameters and allow more rapid and reliable image analysis.

In summary, our study is the first to report in vivo biometric characteristics of relaxed and contracting human ciliary muscle among a relatively large adult cohort, using AS-OCT. The ciliary muscle is longer in eyes with axial myopia, and there are nasal–temporal asymmetries in both morphology and accommodative changes in all refractive error groups. The main accommodative changes observed were a shortening of muscle length, particularly in the anterior portion, and a thickening at CM25. These findings support the broadly accepted theory of the anterior as well as inward shift of human ciliary muscle mass during accommodation.

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