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# Pseudomyopia and cycloplegic changes in young cynomolgus macaques

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**Purpose:** Primate eyes feature a physiologic accommodative system identical to that of humans. Nonetheless, their biometric changes before and after cycloplegia have not been investigated. In this study, we aimed to evaluate the characteristics of pseudomyopia and explore factors associated with cycloplegic changes in young cynomolgus macaques.
**Methods:** Refractive and biometric measurements were performed on both eyes before and after cycloplegia in 88 cynomolgus macaques aged 3−6 years without ocular diseases. Pseudomyopia was defined as autorefraction-estimated noncycloplegic spherical equivalent (NSE) < −0.50 D and cycloplegic spherical equivalent (CSE) ≥ −0.50 D. Linear mixed models were established to identify factors associated with cycloplegic differences.
**Results:** After excluding low-quality ophthalmic data, 149 eyes were included in the final analysis. The median NSE was −0.38 (interquartile range: −1.50, 0.25) D and the median CSE was 0.13 (−0.50, 0.90) D, exhibiting a robust Pearson's correlation coefficient of 0.89 (95% confidence interval: 0.78−0.92). Pseudomyopia was observed in 35 of the 149 eyes, with a greater hyperopic shift (median, 1.42 D) compared to that in non-myopic and true-myopic eyes (median, 0.50 D for both, p<0.001). Pseudomyopic eyes demonstrated active accommodation before cycloplegia and exhibited biometric changes after cycloplegia akin to those in true-myopic eyes. Under cycloplegia, non-myopic and pseudomyopic eyes exhibited similar biometric measurements. A greater hyperopic shift correlated with a higher hyperopic NSE and greater reductions in lens thickness.
**Conclusions:** The use of 1% cyclopentolate yielded discernible alterations in refractive and biometric parameters in 3- to 6-year-old cynomolgus macaques. Notably, compared with non-myopia and true myopia, pseudomyopia exhibited associations and discrepancies in accommodative status. These findings underscore the importance of investigating the role of the lens in animal models employed in myopia research.

Countries in eastern and southeastern Asia experience an epidemic of myopia, including high myopia. Compelling evidence from multiple studies supports the causative involvement of both nature (genetics and heredity) and nurture (environment and lifestyle), including time spent outdoors, genetical preposition, on- versus off-pathway stimulation, etc [1]. According to the National Health Commission report, the prevalence of myopia in Chinese children and adolescents had reached 53.60% by 2018, raising concerns about its long-term impact on visual health [2]. Contributing factors to this rise include increased screen time, academic pressure, and lifestyle changes, all of which have intensified the burden of near work on the visual system, resulting in over-accommodation, which may ultimately lead to the development of pseudomyopia [3].

Pseudomyopia, also known as spasm of accommodation or false myopia, is a transient refractive error caused by excessive contraction of the ciliary muscle. As the ciliary muscle contracts, the ciliary body moves anteriorly and centripetally, releasing the tension on the zonular fibers. The release of zonular tension allows the lens capsule to reshape the lens into a more spherical form [4,5]. This process is characterized by a reduction in lens equatorial diameter [6,7], accompanied by an increase in lens thickness and alterations in the anterior and posterior lens surface curvatures [8,9]. These changes ultimately result in increased refractive power of the lens.

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Pseudomyopia can develop as a result of prolonged near work, emotional stress, or ocular fatigue [10], and can be reversed by cycloplegic agents which temporarily relax the ciliary muscle [11].

Myopia, also known as nearsightedness, is a refractive error where light rays entering the eye parallel to the optic axis focus in front of the retina when ocular accommodation is relaxed. This is usually due to a larger anteroposterior length of the eyeball, but can also be caused by an overly curved cornea or increased optical power of the lens [12]. Despite advancements in research on the etiology and progression of myopia, knowledge gaps continue to exist, particularly regarding the link between pseudomyopia and true myopia. Pseudomyopia has been established as an independent risk factor for the progression of myopia in school-aged children [13]. Prolonged excessive accommodation may contribute to myopia development through a mechanism involving prolonged retinal defocus both at a distance and in close proximit, coupled with the typical lag in accommodation during sustained near work, leading to axial elongation [14-16].

In experimental animal models investigating the mechanisms of myopia, researchers primarily apply form-deprivation or hyperopic defocus to create myopia in a variety of species, including nonhuman primates [17]. However, upon removal of the abnormal visual cues (deprivation or hyperopic defocus lenses), the young animals exhibit rapid and systematic reduction in the experimentally induced myopia [18-20]. Hence, induced myopia may not offer a comprehensive understanding of the natural process of emmetropization, as it differs from human juvenile myopia, which cannot be reversed [21]. Accordingly, it is important to develop a naturally occurring myopia model resembling human myopia.

Primate eyes feature a physiologic accommodative system identical to that of humans. The movement of the accommodative structures is consistent with the classic mechanism of accommodation described by Helmholtz [22,23]. In recent years, research has focused on investigating the prevalence and characteristics of naturally occurring myopia in primates [24-27]. Nonetheless, the effects of cycloplegics, pharmacological agents used to temporarily paralyze accommodation, on refractive changes in young primates remain poorly understood. Addressing this knowledge gap is crucial for advancing our understanding of myopia development and refining clinical interventions aimed at preventing and managing this widespread visual health concern. This study aimed to evaluate the differences in refractive and biometric parameters before and after cycloplegia and to investigate factors associated with pseudomyopia in young cynomolgus macaques.

## METHODS

*Animals:* This study was conducted in accordance with the principles of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the Association for Research of Vision and Ophthalmology guidelines. The animal procedures strictly followed the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Huazhen Biosciences (approval number HZ-EXF001) and Zhongshan Ophthalmic Center (approval number W202203).

We used naturally raised adult cynomolgus macaques (*Macaca fascicularis*) aged 3−6 years [28]. As most of them had completed the emmetropization process and begun to develop myopia [24], they were considered ideal candidates for investigating the emmetropization process of naturally occurring juvenile myopia. A total of 88 cynomolgus macaques (46 males and 42 females) without surgical history were sourced from Huazhen Biotechnology Co., Ltd, Guangzhou, China. The cynomolgus macaques were born and raised at the Huazhen Laboratory Animal Breeding Center at approximately 26°C and 40−70% room humidity at a 12-h light/dark cycle.

*Autorefractive and ocular biometry measurements:* The macaques were fasted and water deprived for eight hours before examination. All examinations were performed in both eyes and completed within 120 minutes under anesthesia. Sedation was achieved using intramuscular injections of 5 mg/kg Zoletil 50 (VIRBAC Carros, France) and 1−2 mg/kg xylazine hydrochloride (Dunhua Shengda Animal Medicine Co., Ltd Dunhua, China). The monkeys were stabilized on a countertop with each eyelid kept open using a speculum during the examination. Corneal humidity was maintained using normal saline solution to ensure good optical quality of the ocular surface.

After non-cycloplegic measurements were completed, cycloplegia was achieved with the application of 1% cyclopentolate (S.A. ALCON-COUVREUR N.V. Puurs, Belgium). Three drops of 1% cyclopentolate were instilled every 5 min, and 20 min after the last administration of cyclopentolate, the absence of light reflex was confirmed. Cycloplegic autorefractive and biometric measurements were obtained strictly following the standards mentioned above.

Pre- and post-cycloplegic autorefractive measurements (KR-800, Topcon Corporation, topcon.co.jp) were performed in the prone position with the subject's chin on a chin rest, ensuring the monkey was at its primary gaze. Spherical refraction (SR), cylinder refraction (CR), and corneal power

(CP) measurements were repeated at least five times for each eye. Data were retained only when the error was less than 0.25 D.

Pre- and post-cycloplegic biometric measurements, including central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), and axial length (AL), were obtained using an LENSTAR LS 900 optical biometer (Haag-Streit AG, haag-streit.com). The instrument calibration was checked at the beginning of each day using a model eye provided by the manufacturer. Data were recorded five times along with standard ultrasonic waveforms. Errors between measurements less than 0.02 mm were recorded and averaged.

*Definitions:* The spherical equivalent (SE) was calculated as SE=SR + 0.5 CR. The refractive status was defined based on the autorefraction-estimated cycloplegic SEM (CSE), with a CSE > +0.50 D defined as hyperopia, −0.50 D ≤ CSE ≤ +0.50 D as emmetropia, and CSE < −0.50 D as myopia. The differences in SE (DSE) before and after cycloplegia were calculated by subtracting the CSE from the noncycloplegic SEM (NSE), with a negative DSE indicating a higher myopic measure in the NSE. Cycloplegic measurements such as CP, ACD, LT, and AL were noted as cCP, cACD, cLT, and cAL. The differences between pre- and post-cycloplegic measurements are noted as dCP, dACD, and dLT.

Cynomolgus macaques were grouped by age into three categories: the three-year-old group (aged 3–3.99 years), the four-year-old group (aged 4–4.99 years), and the five-year-old group (aged 5–6.7 years). Myopia was classified into three subgroups according to the guidelines of the Chinese Medical Association of Ophthalmology, Division of Refraction [13,29] as follows: non-myopia, if both NSE and CSE ≥ −0.50 D; pseudomyopia, if NSE < −0.50 D while CSE ≥ +0.50 D; and true myopia, if both NSE and CSE < −0.50 D.

*Statistical analysis:* The analysis included data from both eyes and was conducted using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY). Normally distributed data were presented as mean values ± standard deviation, while skewed data were presented as median values with interquartile ranges. Pearson' correlation coefficient was calculated to evaluate the correlation between NSE and CSE measurements. Paired *t* tests were used to compare pre- and post-cycloplegic measurements. A one-way ANOVA (ANOVA) or independent-samples Kruskal–Wallis test was applied to identify intergroup differences. Bonferroni's correction was employed for post-hoc analysis. Univariate linear and multivariate linear mixed model analyses were used to identify factors associated with DSE and CSE. Measurements of the bilateral eyes were nested within the subject to account for within-subject variability. Multivariate models were adjusted for age and other ocular characteristics, with p<0.10 in the univariate analysis. P values less than 0.05 were considered to indicate statistical significance.

## RESULTS

*Animal characteristics:* After excluding low-quality ophthalmic data, 149 eyes were included in the final analysis (Figure 1). The mean age was 4.46±0.88 (range, 2.96−6.65) years. Overall, compared with males, females had a steeper cornea (60.25±1.80 D versus 57.85±2.03 D; t=-7.59, p<0.001), smaller ACD (2.67±0.16 mm versus 2.72±0.14 mm; t=2.17; p=0.03), and shorter AL (17.05±0.56 mm versus 17.67±0.51 mm; t=7.08, p<0.001).

*Pre-post cycloplegia differences by age:* Before cycloplegia, among the 149 eyes analyzed, 20 exhibited hyperopia, 59 exhibited emmetropia, and 70 displayed myopia. Following cycloplegia, a significant shift was observed: 35 out of the 70 initially myopic eyes transitioned to either hyperopia or emmetropia, while 30 of the 59 initially emmetropic eyes shifted to hyperopia (Figure 2A).

Refractive and biometric data in different age groups are presented in Table 1. No statistical differences were observed in CSE and AL among the three age groups. The CP of the 3-year-old group was significantly smaller than those of the 4- and 5-year-old groups (p=0.03 and p=0.02). After cycloplegia, all age groups exhibited increases in hyperopia and ACD, and decrease in LT. The 3-year-old age group demonstrated the greatest increase in hyperopia, although statistical significance was not achieved (p=0.66). The increase in ACD in the 5-year-old group was much greater than that observed in the 4-year-old group (p=0.01).

*Characteristics of pseudomyopia in cynomolgus macaques:* Overall, the median NSE was −0.38 (−1.50, 0.25) D, and the median CSE was 0.13 (−0.50, 0.90) D. The median DSE was −0.75 D (−1.38, 0.02), with a strong positive correlation (Pearson's correlation coefficient=0.89, 95% confidence interval: 0.78−0.92). According to the definitions, 79 eyes met the criteria for non-myopia, 35 eyes for pseudomyopia, and 35 eyes for true myopia (Figure 2B).

As shown in Table 2, the median NSE for the non-myopia, pseudomyopia, and true myopia groups was 0.25 (-0.13, 0.63) D, -1.13 (-1.75, -0.75) D, and -2.25 (-4.04, -1.42) D, respectively. Pseudomyopic eyes demonstrated a smaller ACD and greater pre-cycloplegic LT (ACD: 2.60±0.16 mm; LT: 3.08±0.14 mm) compared with non-myopic (ACD: 2.62±0.13 mm; LT: 3.05±0.11 mm) and true-myopic eyes (ACD: 2.62±0.13 mm; LT: 3.05±0.11 mm; ANOVA, p=0.27 and p=0.30, respectively). AL was significantly greater in

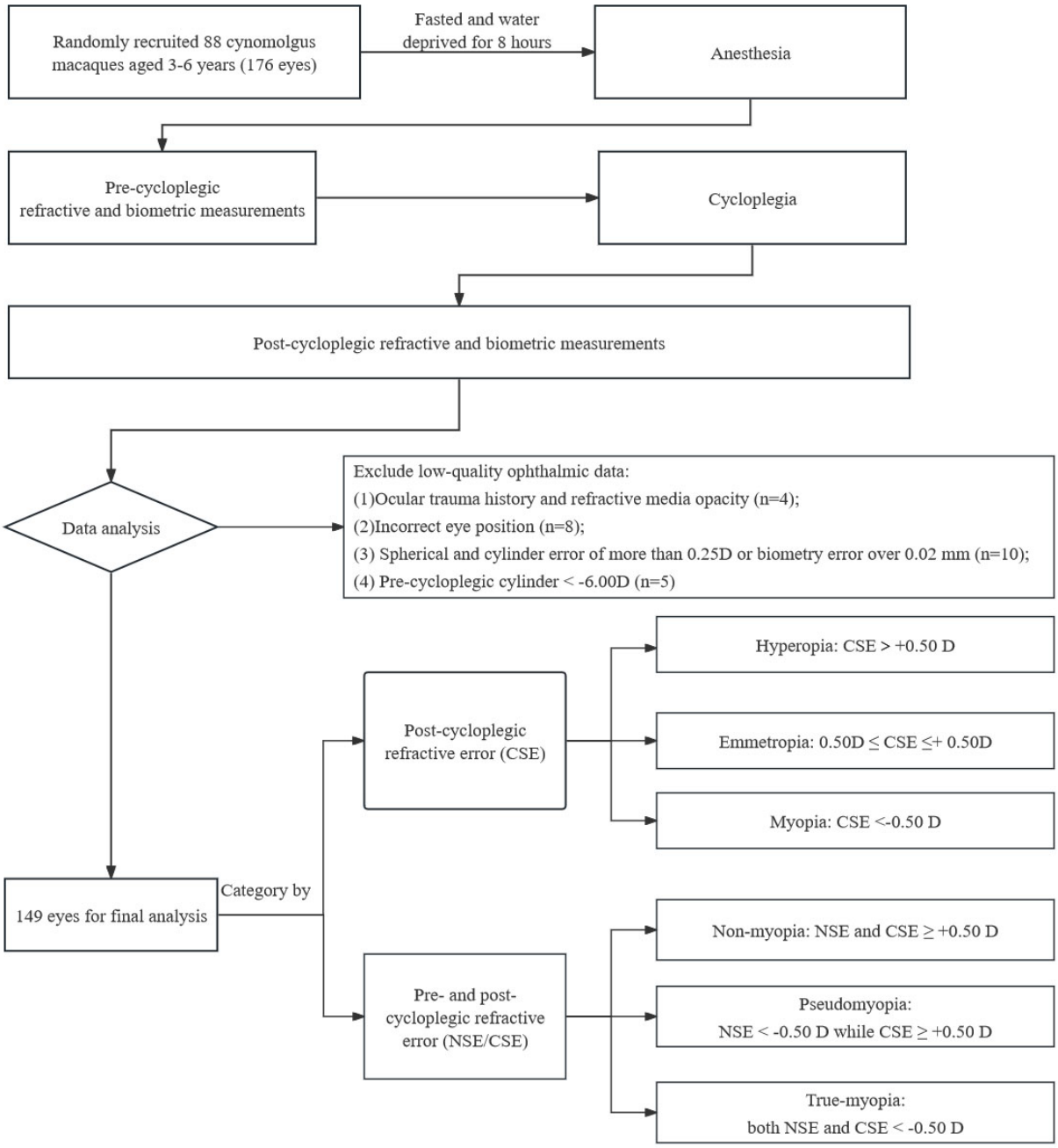


Figure 1. Flowchart of the experiment process and refractive error categorization.

true-myopic (17.62±0.81 mm) than in non-myopic (17.28±0.54 mm, ANOVA, p=0.022).

After inducing cycloplegia, significant changes were observed in SR, SE, ACD, and LT in all three groups (paired t-test, all p<0.001). Pseudomyopic eyes demonstrated a greater hyperopic shift in DSE compared to non-myopic and true-myopic eyes (-1.42 (-2.25, -0.92) D versus -0.50 (-1, 0.13) D versus -0.50 (-1.38, 0.21) D; p<0.001 for both). Regarding biometric parameters, ACD increased the most in true-myopic eyes (-0.09±0.03 mm), followed by pseudomyopic eyes (-0.07±0.04 mm) and non-myopic eyes (-0.06±0.03 mm; p=0.005 for true versus non-myopia). LT showed a greater decrease in pseudomyopic and true-myopic eyes (0.05±0.04 mm in both) than in non-myopic eyes (0.03±0.03 mm; p<0.01 for both). There were no significant changes in AL.

Interestingly, when ocular accommodation was fully relaxed, non-myopic and pseudomyopic eyes demonstrated similar biometric measurements between each other and distinct differences with the measurements of true-myopic eyes. Though insignificantly, ACD in non-myopic and pseudomyopic eyes (2.68±0.14 mm and 2.68±0.17 mm, respectively) was smaller than that in true-myopic eyes (2.74±0.16 mm, ANOVA, p=0.226 and p=0.241, respectively), while LT in non-myopic and pseudomyopic eyes (3.02±0.12 mm and 3.03±0.13 mm, respectively) was greater than that in true-myopic eyes (2.99±0.14 mm, ANOVA, p=0.676 and p=0.549, respectively).

*Factors associated with DSE and CSE:* In the univariate analysis (Table 3), NSE (coefficient (95% confidence interval): 0.148 (0.074 to 0.223); p<0.001), CSE (−0.088 (−0.167 to −0.008); p=0.031), cCP (0.078 (0.009 to 0.146); p=0.026), dCP (−0.286 (−0.403 to −0.17); p<0.001), dACD (4.186 (−0.331 to 8.704); p=0.069), and dLT (−7.574 (−11.551 to −3.596); p<0.001) were associated with DSE and included in the multivariate analysis. As both NSE and CSE were significantly correlated with each other, two separate multivariate models were established to avoid multicollinearity. In Model 1, in which NSE was an independent variable, DSE was significantly associated with NSE (0.137 (0.062 to 0.212); p<0.001), dCP (−0.308 (−0.43 to −0.186); p<0.001), and dLT (−7.692 (−12.638 to −2.746); p=0.003). In Model 2, in which CSE was an independent variable, DSE was significantly associated with CSE (−0.093 (−0.173 to −0.013); p<0.001), dCP (−0.308 (−0.434 to −0.182); p<0.001), and dLT (−10.345 (−15.329 to −5.36); p<0.001).

As shown in Table 4, CSE was not associated with sex (−0.18 (−0.81 to 0.45); p=0.57) but was significantly associated with age (−0.369 (−0.722 to −0.017); p=0.04), cCP (−0.216 (−0.351 to −0.081); p=0.002), cACD (−4.007 (−5.934 to −2.079); p<0.001), cLT (2.694 (0.281 to 5.108); p=0.029), cAL (−1.54 (−1.988 to −1.093); p<0.001), DSE (−0.356 (−0.679 to −0.033); p=0.031), dCP (0.361 (0.115 to 0.607); p=0.004), dACD (9.921 (0.863 to 18.979); p=0.032) and dLT (−7.76 (−16.051 to 0.531); p=0.066). In the multivariate analysis, CSE was determined by cCP (−0.769 (−0.902 to −0.635); p<0.001), cLT (−1.994 (−3.857 to −0.131); p=0.036), cAL (−3.474 (−4.013 to −2.936); p<0.001), and slightly affected by DSE (−0.418 (−0.596 to −0.239); p<0.001) and dCP (−0.372 (−0.541 to −0.202); p<0.001).

## DISCUSSION

Cynomolgus and rhesus macaques are commonly used as experimental subjects in ophthalmology research. For rhesus macaques, prior studies have comprehensively documented refractive and biometric data spanning from postnatal to older animals, whereas studies on young cynomolgus macaques are limited. Understanding the refractive status of non-human primates with naturally occurring refractive errors will help establish an animal model for studying the pathogenesis of myopia.

This study, conducted on 3−6-year-old cynomolgus macaques, revealed that the prevalence of pseudomyopia was 23.49%. Pseudomyopic eyes had similar biometric measurements to those of non-myopic eyes under relaxed conditions,. However, they exhibited active accommodation before

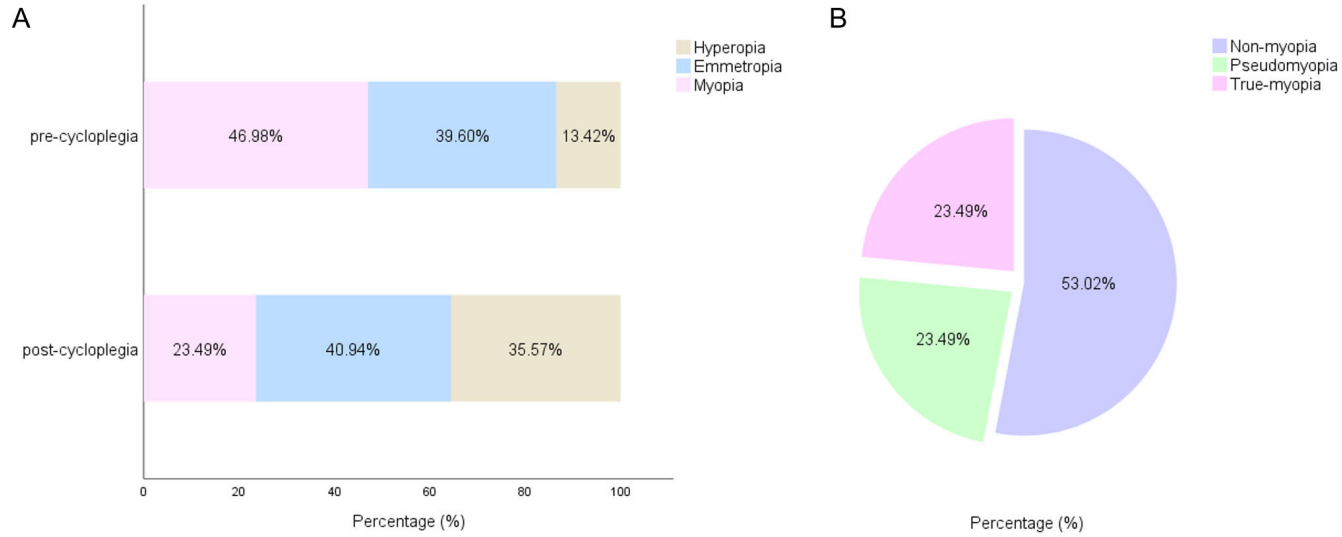


Figure 2. Distribution of pre- and post-cycloplegic refraction (**A**) and myopia type (**B**) in cynomolgus macaque.

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**Table 1. Cycloplegic changes in refractive and biometric parameters in cynomolgus macaques by age.**

| Parameter* | 3-year-old | 4-year-old | 5-year-old | P value | Pairwise comparison§ |
|---|---|---|---|---|---|
| N (eyes) | 42 | 61 | 46 | | |
| cSR (D) | 1.00 (0.25, 1.56) | 1.00 (0.25, 1.50) | 0.75 (0.25, 1.50) | 0.57† | |
| cCR (D) | −1.75 (−2.77, −1.00) | −1.25 (−2.00, −0.75) | −1.04 (−2.13, −0.75) | 0.19† | **$P_a$=0.03** |
| cSE (D) | 0.13 (−0.31, 0.87) | 0.25 (−0.48, 0.88) | 0 (−1.13, 1.00) | 0.75† | $P_b$=1.00 |
| cCP (D) | 58.06±2.36 | 59.22±2.21 | 59.39±2.05 | **0.01**‡ | **$P_c$=0.02** |
| cCCT (μm) | 454.15±33.14 | 455.43±31.44 | 454.95±38.3 | 0.98‡ | |
| cACD (mm) | 2.68±0.15 | 2.71±0.17 | 2.70±0.15 | 0.63‡ | |
| cLT (mm) | 3.03±0.13 | 3.00±0.14 | 3.03±0.12 | 0.24‡ | |
| cAL (mm) | 17.35±0.54 | 17.40±0.63 | 17.38±0.67 | 0.91‡ | $P_a$=0.41 |
| Pre-post difference | | | | | |
| dSR (D) | −0.87±0.92 | −0.92±1.18 | −0.69±0.95 | 0.50‡ | **$P_b$=0.04** |
| dCR (D) | 0.15±1.55 | 0.58±1.07 | −0.12±1.80 | **0.04**‡ | $P_c$=1.00 |
| dSER (D) | −0.80±0.94 | −0.63±1.01 | −0.75±0.93 | 0.66‡ | $P_a$=0.94 |
| dCP (D) | 0.28±1.17 | −0.26±1.27 | −0.05±1.25 | 0.10‡ | **$P_b$=0.01** |
| dCCT (μm) | −7.33±15.22 | −11.02±14 | −12.5±21.65 | 0.35‡ | $P_c$=0.18 |
| dACD (mm) | −0.07±0.03 | −0.08±0.03 | −0.06±0.03 | **0.01**‡ | |
| dLT (mm) | 0.03±0.03 | 0.04±0.04 | 0.04±0.04 | 0.45‡ | |
| dAL (mm) | 0.01±0.05 | 0±0.06 | 0.01±0.04 | 0.43‡ | |

Abbreviations: SR, spherical refraction; CR, cylinder refraction; SE, spherical equivalent; CP, corneal power; CCT, central corneal thickness; ACD, anterior chamber depth; LT, lens thickness; AL, axial length. Data are presented as mean ± standard deviation or median (interquartile range). * The prefix 'c' denotes cycloplegic measurements, and the prefix 'd' indicates differences in measurements before and after cycloplegia. † Calculated using the independent-samples Kruskal–Wallis test. ‡ Calculated using ANOVA. § Pairwise comparisons adjusted using Bonferroni's correction. $P_a$, 3- versus 4-year-old group; $P_b$, 4- versus 5-year-old group; $P_c$, 3- versus 5-year-old group; p values <0.05 were considered to indicate statistical significance (bold font).

Table 2. Cycloplegic changes in refractive and biometric parameters in cynomolgus macaques by myopia type.

| Variables | Non-myopia | | Pseudomyopia | | True myopia | | Pre-post differences | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Non-myopia | Pseudomyopia | True myopia | P |
| SR (D) | 0.50 (0.25, 1.08) | **1.25 (0.83, 1.75)*** | −0.25 (−1.25, 0.25) | **0.75 (0.25, 1.25)*** | −0.75 (−2.33, −0.25) | **-0.25 (−1.5, 0.5)*** | −0.75 (−1.25, 0) | −1.5 (−1.75, −0.5) | −0.83 (−1.5, 0) | **0.006**† |
| CR (D) | −0.75 (−1.25, −0.5) | **-1.25 (−2, −0.75) *** | −1.5 (−2.33, −0.50) | −1 (−1.75, −0.75) | −1.42 (−2.83, −0.5) | −2.5 (−3.75, −1) | 0.42 (−0.25, 0.75) | 0 (−1.5, 0.75) | 0.50 (−0.75, 1.67) | **0.025**† |
| SEM (D) | 0.25 (−0.13, 0.63) | **0.75 (0.13, 1.25)*** | −1.13 (−1.75, −0.75) | **0 (−0.13, 0.75)*** | −2.25 (−4.04, −1.42) | **-1.42 (−2.75, −0.88)*** | −0.50 (−1, 0.13) | −1.42 (−2.25, −0.92) | −0.50 (−1.38, 0.21) | **<0.001**†§ |
| CP (D) | 58.54±1.85 | 58.49±2.12 | 58.89±2.13 | 58.56±1.95 | 59.75±2.08 | **60.37±2.34*** | 0.05±1.16 | 0.33±1.16 | −0.62±1.36 | **0.004**‡§ |
| CCT (μm) | 442.22±28.46 | **453.78±30.12*** | 449.09±26.31 | 453.77±36.83 | 444.97±36.46 | **458.65±39.42*** | −11.55±14.63 | −4.69±23.39 | −13.68±13.35 | 0.06 |
| ACD (mm) | 2.62±0.13 | **2.68±0.14*** | 2.60±0.16 | **2.68±0.17*** | 2.66±0.16 | **2.74±0.16*** | −0.06±0.03 | −0.07±0.04 | −0.09±0.03 | **0.005**‡ |
| LT (mm) | 3.05±0.11 | **3.02±0.12*** | 3.08±0.14 | **3.03±0.13*** | 3.04±0.14 | **2.99±0.14*** | 0.03±0.03 | 0.05±0.04 | 0.05±0.04 | **<0.001**†‡ |
| AL (mm) | 17.28±0.54 | 17.27±0.54 | 17.40±0.48 | 17.40±0.49 | 17.62±0.81 | 17.62±0.81 | 0.01±0.05 | 0.01±0.07 | 0±0.03 | 0.466 |

Abbreviations: SR, spherical refraction; CR, cylinder refraction; SE, spherical equivalent; CP, corneal power; CCT, central corneal thickness; ACD, anterior chamber depth; LT, lens thickness; AL, axial length. Data are presented as mean ± standard deviation or median (interquartile range). * Calculated using the pair test. † Pairwise comparisons between the non-myopia and pseudomyopia groups. ‡ Pairwise comparisons between the non-myopia and true myopia groups. § Pairwise comparisons between the pseudomyopia and true myopia groups.

Table 3. Univariate and multivariate linear mixed analysis of factors correlated with differences in spherical equivalent.

| Variables* | Univariate model | | Multivariate model 1 | | Multivariate model 2 | |
|---|---|---|---|---|---|---|
| | Coefficient, 95% CI | P value | Coefficient, 95% CI | P value | Coefficient, 95% CI | P value |
| Sex† | 0.148 (−0.164 to 0.461) | 0.349 | | | | |
| Age (yrs) | 0.054 (−0.12 to 0.23) | 0.548 | | | | |
| NSE (D) | **0.148 (0.074 to 0.223)** | **<0.001** | **0.137 (0.062 to 0.212)** | **<0.001** | | |
| nCP (D) | −0.012 (−0.089 to 0.066) | 0.763 | | | | |
| nCCT (μm) | −0.001 (−0.006 to 0.005) | 0.827 | | | | |
| nACD (mm) | 0.645 (−0.43 to 1.72) | 0.237 | | | | |
| nLT (mm) | −0.064 (−1.323 to 1.196) | 0.921 | | | | |
| nAL (mm) | −0.092 (−0.346 to 0.163) | 0.478 | | | | |
| CSE (D) | **-0.088 (−0.167 to −0.008)** | **0.031** | | | **-0.093 (−0.173 to −0.013)** | **<0.001** |
| cCP (D) | **0.078 (0.009 to 0.146)** | **0.026** | 0.009 (−0.064 to 0.082) | 0.803 | −0.035 (−0.11 to 0.041) | 0.364 |
| cCCT (μm) | <0.001 (−0.005 to 0.005) | 0.997 | | | | |
| cACD (mm) | 0.36 (−0.649 to 1.369) | 0.482 | | | | |
| cLT (mm) | 0.589 (−0.625 to 1.804) | 0.339 | | | | |
| cAL (mm) | −0.087 (−0.341 to 0.168) | 0.502 | | | | |
| dCP (D) | **-0.286 (−0.403 to −0.17)** | **<0.001** | **-0.308 (−0.43 to −0.186)** | **<0.001** | **-0.308 (−0.434 to −0.182)** | **<0.001** |
| dCCT (μm) | −0.002 (−0.011 to 0.007) | 0.706 | | | | |
| dACD (mm) | **4.186 (−0.331 to 8.704)** | **0.069** | −3.351 (−8.503 to 1.801) | 0.2 | −2.316 (−7.642 to 3.01) | 0.391 |
| dLT (mm) | **-7.574 (−11.551 to −3.596)** | **<0.001** | **-7.692 (−12.638 to −2.746)** | **0.003** | **-10.345 (−15.329 to −5.36)** | **<0.001** |
| dAL (mm) | −0.71 (−3.806 to 2.386) | 0.651 | | | | |

Abbreviations: SE, spherical equivalent; NSE, noncycloplegic spherical equivalent; CSE, cycloplegic spherical equivalent; CP, corneal power; CCT, central corneal thickness; ACD, anterior chamber depth; LT, lens thickness; AL, axial length; CI, confidence interval. * The prefix 'c' denotes cycloplegic measurements, and the prefix 'd' indicates differences in measurements before and after cycloplegia. † Male sex was the reference. P values <0.05 were considered to indicate statistical significance (bold font).

Table 4. Univariable and multivariate linear mixed analysis of factors correlated with the cycloplegic spherical equivalent.

| Variables* | Univariate model | | Multivariate model | |
|---|---|---|---|---|
| | Coefficient, 95% CI | P value | Coefficient, 95% CI | P value |
| Sex† | −0.18 (−0.81 to 0.45) | 0.57 | | |
| Age (yearsrs) | **-0.369 (−0.722 to −0.017)** | **0.04** | 0.189 (−0.073 to 0.451) | 0.155 |
| cCP (D) | **-0.216 (−0.351 to −0.081)** | **0.002** | **-0.769 (−0.902 to −0.635)** | **<0.001** |
| cCCT (μm) | 0.001 (−0.008 to 0.01) | 0.852 | | |
| cACD (mm) | **-4.007 (−5.934 to −2.079)** | **<0.001** | 1.319 (−0.454 to 3.091) | 0.143 |
| cLT (mm) | **2.694 (0.281 to 5.108)** | **0.029** | **-1.994 (−3.857 to −0.131)** | **0.036** |
| cAL (mm) | **-1.54 (−1.988 to −1.093)** | **<0.001** | **-3.474 (−4.013 to −2.936)** | **<0.001** |
| DSE (D) | **-0.356 (−0.679 to −0.033)** | **0.031** | **-0.418 (−0.596 to −0.239)** | **<0.001** |
| dCP (D) | **0.361 (0.115 to 0.607)** | **0.004** | **-0.372 (−0.541 to −0.202)** | **<0.001** |
| dCCT (μm) | 0.001 (−0.018 to 0.019) | 0.919 | | |
| dACD (mm) | **9.921 (0.863 to 18.979)** | **0.032** | 3.046 (−3.628 to 9.719) | 0.368 |
| dLT (mm) | **-7.76 (−16.051 to 0.531)** | **0.066** | 0.868 (−5.818 to 7.553) | 0.798 |
| dAL (mm) | 4.405 (−1.793 to 10.603) | 0.162 | | |

Abbreviations: CP, corneal power; CCT, central corneal thickness; ACD, anterior chamber depth; LT, lens thickness; AL, axial length; DSE, differences in spherical equivalent; CI, confidence interval. * The prefix ‘c’ denotes cycloplegic measurements, and the prefix ‘d’ indicates differences in measurements before and after cycloplegia. † Male sex was the reference. P values <0.05 were considered to indicate statistical significance (bold font).

cycloplegia. Furthermore, cycloplegia induced distinct refractive and biometric changes in pseudomyopic eyes, similar to those observed in true-myopic eyes.

*Characteristics of pseudomyopia in cynomolgus macaques:* The prevalence of pseudomyopia in our cynomolgus population (23.49%) is comparable to that in 6-year-old children, reported as 24.1% [29]. The median pre-post cycloplegia difference in pseudomyopic eyes was −1.42 (−2.25, −0.92) D, comparable to the median pseudomyopia of 1.13 D (0.63, −1.63) in children aged 6 years [29].

Few studies have explored the relationship between pseudomyopia and biometric changes. Our results indicated distinct pre-post cycloplegia differences in biometric parameters among pseudomyopic, true-myopic, and non-myopic eyes. Before cycloplegia, pseudomyopic eyes exhibited greater LT and smaller ACD compared to those in non-myopic and true-myopic eyes, suggesting an active accommodative status in pseudomyopic eyes. After cycloplegia, we observed significant increases in ACD and decreases in LT across all groups. True-myopic eyes exhibited the most pronounced ACD increases followed by pseudomyopic and non-myopic eyes, whereas the LT decreases were greater in pseudomyopic and true-myopic eyes than in non-myopic eyes. This finding suggests that accommodative changes in pseudomyopic eyes are more similar to those in true-myopic eyes than to those in non-myopic eyes and may contribute to the flattening and movement of the lens. Prior studies have reported that chronic retinal defocus at nearby objects occurs due to a lag in accommodative response and is more frequent and often greater in myopic eyes [21].

When ocular accommodation was fully relaxed, non-myopic and pseudomyopic eyes demonstrated similar biometric parameters and distinct differences with the biometric parameters in true-myopic eyes. Pseudomyopia refers to myopia induced by excessive accommodation that disappears after the induction of cycloplegia without associated structural changes in the eye indicative of true myopia [11]. Prolonged periods of near work, which require sustained accommodative effort, could contribute to the lag of accommodation in pseudomyopic eyes. The visual system may respond to blur and defocus signals by initiating a series of biochemical events that lead to scleral remodeling and axial elongation to improve image clarity [30]. Longitudinal studies have proven that greater DSE is associated with refractive error progression among children with myopia [13,29,31].

*Factors associated with DSE:* In this study, a greater hyperopic shift was related to a higher hyperopic NSE, greater LT, and CP decrease, which is consistent with previously reported findings in humans [31-36]. Li et al. reported a DSE for hyperopia, emmetropia, and myopia of 0.97±0.75 D, 0.49±0.68 D, and 0.28±0.58 D, respectively, in 6-year-old children [34], while Lin et al. found comparable DSE of 1.26±0.93 D, 0.55±0.61 D, and 0.23±0.40 D in 6−17-year-old children and adolescents [31].

While we observed no significant correlation with age, human studies have suggested that DSE decreases as age increases. In the study of Fotedar et al., the DSE was 1.18 D (0.05−1.30) in 6-year-old children and 0.84 D (0.81−0.87) in 12-year-old children [37]. Similarly, Fotouhi et al. reported a DSE of 0.71 D in the 5−10 years age group, 0.40 D in the 16−20 years age group, and 0.14 in the 71−75 years age group [38].

Our findings align with those of human studies indicating a negative correlation between LT and DSE, attributed to lens flattening induced by cycloplegic agents [39]. Although we observed mild but significant changes in CP after cycloplegia and a negative correlation between dCP and DSE, the exact role of dCP in DSE remains unclear. While most studies have reported insignificant changes in CP after cycloplegia, contributing minimally to refractive changes [40-42], one study reported a flattened change after cycloplegia with tropicamide [43].

Other factors, such as the type of cycloplegic agents used and the method of refractive measurements employed, could potentially influence the observed cycloplegic changes. In our study, the administration of 1% cyclopentolate resulted in a median hyperopic shift of 0.75 D (−1.38 to 0.02) after cycloplegia. Similarly, Ostrin et al. found that 2% pirenzepine, a relatively selective muscarinic (M1) antagonist, induced a hyperopic shift of 1.07±0.23 D in rhesus monkeys [44]. Conversely, Hung et al. reported that subjective refractions were minimally affected by 1% tropicamide in rhesus macaques of similar ages [45].

*Refractive and biometric characteristics of young cynomolgus macaques:* The median CSE in our population was 1.00 D (0.25 to 1.50 D), with a mean AL of 17.38±0.61 mm, indicating greater hyperopic refraction and shorter AL compared with those in older cynomolgus macaques and same-aged rhesus macaques (Table 5). Similar to that in humans [46,47], the refractive status in our cohort of cynomolgus macaques was mainly determined by the cAL (−3.474 (−4.013 to −2.936)), cLT (−1.994 (−3.857 to −0.131)), and cCP (−0.769 (−0.902 to −0.635)), suggesting that greater AL, greater LT, and steeper corneas may contribute to higher myopia. However, in rhesus macaques with experimentally induced ametropia, no systematic relationship was found between refractive error and any parameter of the crystalline lens [48]. Therefore, it is reasonable to infer that the main

Table 5. Summary of refractive and biometric measurements in cynomolgus macaques and rhesus macaques.

| Ref | Cynomolgus macaques | | | | | Rhesus macaques | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Current study | Choi, 2021 [52] | Xue, 2023 [28] | Chen, 2023 [26] | Zeng, 2021 [25] | Qiao, 2007 [24] | She, 2022 [53] | Lin, 2021 [54] | Ma, 2023 [27] | Fernandes, 2023 [55] |
| Age (yrs) | 3–6.7 | 3.8–8.3 | 9–25 | 5–26 | 12–17.7 | 19–39 days | 20–348 days | 0.7–29 | 8–21 | 0–29 |
| Sample (N) | 88 | 11 | 90 | 263 | 15 | 204 | 163 | 114 | 219 | 120 |
| SR (D) | 1.00 (0.25, 1.50) | NA | NA | 0.42 (0.83, 1.33) | NA | NA | NA | NA | NA | 1.19±1.77 |
| CR (D) | −1.25 (−2.17, −0.75) | NA | NA | −1.42 (−2.42, −0.69) | NA | NA | NA | NA | NA | −1.79±1.53 |
| SEM (D) | 0.13 (−0.50, 0.90) | NA | NA | −0.25 (−1.78, 0.67) | −8.75±6.49 | 4.17±1.52 | NA | NA | −1.25 (−4.88, −0.04) | 0.30±1.70 |
| KM (D/mm) | 58.95±2.26 | NA | 5.77±0.17 | 5.70 (5.56, 5.83) | NA | 61.83±1.87 | NA | NA | NA | 52.41±2.74 |
| CCT (μm) | 454.92±33.93 | NA | 445±29 | 453 (431, 469) | NA | NA | NA | NA | NA | 473.06±32.16 |
| ACD (mm) | 2.70±0.16 | 3.09±0.21 | 3.14±0.22 | 3.23 (3.07, 3.41) | NA | 2.58±0.28 | 3.04±0.25 | NA | NA | 3.45±0.24 |
| LT (mm) | 3.02±0.13 | 3.51±0.52 | 3.19±0.25 | 3.31 (3.15, 3.51) | NA | 3.34±0.20 | 3.78±0.13 | 4.24±0.41 | NA | 3.31±0.25 |
| AL (mm) | 17.38±0.61 | 18.41±0.52 | 18.40±1.03 | 18.44 (18.11, 18.9) | 20.30±1.62 | 14.49±0.47 | 16.55±0.77 | 0–4 years: 18.89±0.83 25–30 years: 20.28±0.97 | 8–9 years: 17.9±0.81 10–13 years: 19.07±1.09 14–16 years: 18.87±1.10 17–19 years: 18.84±1.02 20–21 years: 20.24±1.93 | 19.46±0.56 |

Abbreviation: NA, not available; SR, spherical refraction; CR, cylinder refraction; SE, spherical equivalent; CP, corneal power; CCT, central corneal thickness; ACD, anterior chamber depth; LT, lens thickness; AL, axial length. Data are presented as mean ± standard deviation or median (interquartile range).

difference between naturally occurring and induced myopia lies in lens development. Abnormal visual signals may cause an increase in the vitreous chamber depth but fail to reshape the morphology of the lens. In human studies, the lens has been found to play a crucial role in the development of myopia, with decrease in thickness, flattening of the curvature, and loss of lens power occurring before the onset of myopia [49-51].

*Limitations:* First, it would be beneficial to include data from infant cynomolgus macaques (0−2 years old) in order to expand the current data set. Second, the refractive errors observed in our study population were predominantly mild, which may limit the generalizability of our findings to macaques with more severe refractive errors. Third, the sample size was not large enough to detect subtle variations in CSE and AL within each age group. Finally, it is important to consider environmental factors (such as outdoor conditions) as a control. Future longitudinal studies are needed to examine the emmetropic process and myopic development in nonhuman primates. Addressing these limitations in future studies would provide a more comprehensive understanding of pseudomyopia and cycloplegic changes in cynomolgus macaques.

*Conclusion:* The findings of our study provide valuable insights into the relationship between pseudomyopia, non-myopia, and true myopia in 3−6-year-old cynomolgus macaques, shedding light on the potential role of accommodation in myopia development. Despite resembling non-myopic eyes under relaxed conditions, pseudomyopic eyes exhibited active accommodation before cycloplegia. The administration of 1% cyclopentolate resulted in distinct refractive and biometric changes in pseudomyopic eyes, similar to those observed in true-myopic eyes. These findings underscore the importance of considering the role of the lens in investigations involving naturally occurring myopia animal models.

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