• Basic Research •

Effects of high-intensity light on early lens-induced myopia in chicks: optical coherence tomography assessment

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Abstract

● **AIM:** To evaluate the impact of high-intensity light on ocular length and structural thickness in chick model of the early lens-induced myopia (LIM) by optical coherence tomography (OCT).

● **METHODS:** Four-day-old chicks were monocularly covered with −10 D lenses for a period of 2d, exposed to either high-intensity light (HL, 10 000 lx) or normal-intensity light (NL, 500 lx) for 12h per day. A two-dimensional visualized swept-source OCT system was used to measure anterior chamber depth (ACD), lens thickness, vitreous chamber depth, axial length, retinal thickness and choroidal thickness. The refractive error was measured with retinoscopy.

● **RESULTS:** After 2d of treatment, the LIM eyes under NL condition experienced a greater myopic shift (P=0.028) and greater axial elongation (P=0.033) than those exposed to HL. ACD significantly increased in LIM eyes under NL condition when compared with baseline, while no statistical difference of ACD was found between day 2 and day 0 in LIM eyes under HL condition. LIM eyes all experienced the thinning of retinal thickness and choroidal thickness (all P<0.05), with no statistically significant differences between NL and HL group.

● **CONCLUSION:** HL could reduce the development of LIM at an early stage. Under NL, hyperopic defocus appears to induce a deepening of ACD at the early stage; this effect could potentially be mitigated by HL. At an early stage, HL may have no significant effects on the changes in retinal and choroidal thickness in LIM eyes.

● **KEYWORDS:** high intensity light; lens-induced myopia; chick; optical coherence tomography; retinal thickness; choroidal thickness

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INTRODUCTION

With the increasing myopia prevalence worldwide, myopia has become an important public health concern[1-2]. As a common refractive error, myopia, mainly caused by excessive axial elongation, could induce blurring of distance vision. Different levels of myopia could increase the risk of retinopathy[3-5], like macular degeneration and retinal detachment, and high myopia carries the highest risk of complications, resulting in severe visual impairment.

Previous studies[6-12] investigating experimental myopia model mostly used the contact A-scan ultrasonography to measure the biometric parameters. Several studies[13-15] started to use the non-contact optical coherence tomography (OCT) to obtain anterior and posterior segment parameters, as it also provides great repeatability of the measurement results[16-17]. Moreover, the high-resolution OCT, as a powerful imaging tool, could generate small animal images of different ocular structures and better distinctly differentiate the true border between different layers[17], thereby giving the more accurate measuring orientation.

Numerous studies[18-21] have shown outdoor activity has a protective effect against myopia onset and development.

While the underlying mechanisms are complex[22], the high-intensity light (HL) appears to be a compelling factor. Several studies[8,23-26] have demonstrated that exposure to HL could retard the experimental myopia in different animal models, like chick, guinea pig and rhesus monkey. Studies focused on chick model[7-8,13,27] showed that HL could significantly reduce lens-induced myopia (LIM) shift, mostly after three days of exposure (5–6h per day), but no statistically significant protective effect of HL was found in the earlier time. In addition, some previous studies[28-29] suggested that spending more time outdoors may prevent the onset of myopia or potentially reduce the development of myopia in children, which require further researches. Therefore, it is worth exploring whether increasing the exposure time of HL (*e.g.*, 12h per day, longer than previous chick studies[8,13]) could retard or even prevent experimental myopic development at an earlier stage. And it might serve as a biometric reference for exploring the early signal molecules associated with the protective effect of HL on myopia.

It is known that choroidal thickness (ChT) decreased in myopic animal models[10,30], and HL could reduce choroid thinning in the LIM eyes of chicks on day 8[7], while few studies showed the impact of HL on ChT in a chick model of early-stage LIM. There were also few studies having reported the changes in retinal thickness (RT) of myopia animals under HL condition.

This study aimed to investigate the impact of HL (10 000 lx) on monocular LIM chicks for a short period of two days, with long daily exposure time of 12h, to provide a reference for further exploring early-stage signal molecules or biomarker. In addition, ocular biometric measurement was performed with a high-resolution two-dimensional visualized OCT system.

MATERIALS AND METHODS

Ethical Approval As part of a study investigating the optical effects of peripheral defocus visual stimulation, the experimental procedures in this study were approved by the Animal Research and Ethics Committee of the Eye and ENT Hospital of Fudan University (IACUC-DWZX-2022-018), and the experiments conformed to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Four-day-old, healthy White Leghorn chickens were obtained from the Shanghai Academy of Agriculture Sciences, and were raised under a 12-h light/dark cycle, with lights on at 8 *a.m.* and off at 8 *p.m.* Unrestricted food and water were provided.

Experimental Design and Light Conditions The chicks were all treated monocularly with −10 D lenses on right eyes to induce myopia, and were randomly assigned to two groups: HL (10 000 lx, *n*=5) and normal-intensity light (NL, 500 lx, *n*=5). There were four subgroups: LIM eyes under HL condition (HL+LIM), LIM eyes under NL condition (NL+LIM), contralateral control eyes under HL condition (HL+C), and contralateral control eyes under NL condition (NL+C). The HL and NL illumination was provided by white dimmable light emitting diode (LED) lighting tubes (12 W, 6000–6500 K, 365–795 nm; RuiGaoXiang Light & Electronic Co. Shanghai, China). The chicks were kept in specially designed cages, with four tubes situated above the cage to provide high light level of 10 000 lx and one tube situated to provide normal light of 500 lx. The viewing distance in the room was roughly 1.5 m. The animals were exposed to light of 10 000 lx or 500 lx for 12h per day.

The −10 D lenses were fixed onto a Velcro ring, and the whole Velcro ring was glued to the feathers around the right eyes of chicks. As contralateral control groups, the left eyes remained untouched. The lenses were cleaned twice daily during the process of the experiment.

Refraction and Ocular Biometry Measurement All measurements were performed at baseline [before the start of treatments including hyperopic defocus of −10 D and light exposure (day 0)], and on the second day after the treatments (day 2).

Before the measurement, the chicks were anesthetized by 2% isoflurane. Retinoscopy was performed in a dark room by two experienced observers with a streak retinoscope (66 Vision Tech, Jiangsu Province, China). Recorded as spherical equivalent (SE) error, the refraction results were calculated as the mean values of the horizontal and vertical meridians.

Measurements of ocular biometric parameters, including axial length (AL), anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), RT, and ChT, were performed with a high-resolution visualized swept-source OCT (CASIA2, Tomey Corporation, Nagoya, Japan). The detailed measurement operations and calculations of all parameters have been described previously[16,31]. AL was defined as the distance between the anterior surface of the cornea and the retina. The identification of choroid and retina in OCT images referred to a previous study[17]. The RT in this study was equal to the retinal neurosensory layer thickness plus retinal pigment epithelium thickness.

Statistical Analysis SPSS software (SPSS version 26.0, Chicago, IL, USA) was used for the statistical analyses. Results are presented as the mean±standard deviation (SD). Normal distribution was tested with the Shapiro-Wilk (S-W) tests. Two-way repeated-measures analysis of variance (ANOVA) followed by post hoc testing was used to compare the SE and biometric parameter values between the two time points and between the two light condition groups. The changes in SE and biometric parameter values (day 2 minus day 0) between LIM eyes and the contralateral eyes were compared with the paired *t*-tests or Wilcoxon tests. Independent *t*-tests or Mann-Whitney

Table 1 Comparison of ocular parameters between HL and NL groups on day 0 and day 2 mean±SD

Groups	AL (mm)		SE (D)		ACD (mm)		LT (mm)	
	day 0	day 2	day 0	day 2	day 0	day 2	day 0	day 2
HL+LIM	7.985±0.066	8.313±0.065[b]	+6.850±0.548	6.050±1.110	1.052±0.063	1.094±0.083[b]	1.724±0.047	1.815±0.043[b]
NL+LIM	8.074±0.136	8.500±0.165[b]	+5.750±1.436	0.725±3.499[b]	1.083±0.034	1.160±0.031[b]	1.735±0.054	1.834±0.064[b]
P[a]	0.226	0.048	0.148	0.012	0.366	0.139	0.726	0.607
HL+C	7.944±0.052	8.268±0.047[b]	+5.100±1.207	6.525±1.448[b]	1.060±0.064	1.085±0.067[b]	1.726±0.045	1.814±0.055[b]
NL+C	8.063±0.129	8.367±0.110[b]	+6.675±2.449	6.250±2.257	1.104±0.039	1.124±0.040[b]	1.725±0.039	1.849±0.060[b]
P[a]	0.092	0.101	0.233	0.824	0.223	0.302	0.974	0.367

AL: Axial length; SE: Spherical equivalent; ACD: Anterior chamber depth; LT: Lens thickness; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia; C: Control group; SD: Standard deriation; AVOVA: Analysis of variance. [a]*P* value was obtained by using two-way repeated-measures ANOVA followed by post hoc testing; [b]*P*<0.05 in comparison to the values on day 0 by using two-way repeated-measures ANOVA followed by post hoc testing.

Table 2 Comparison of changes in ocular parameters between HL and NL groups mean±SD, mm

Parameters	HL+LIM	NL+LIM	*P*[a]	HL+C	NL+C	*P*[a]
AL	0.328±0.051	0.424±0.066	0.033	0.324±0.054	0.304±0.062	0.598
SE (D)	−0.800±1.242	−5.025±4.520	0.028	1.425±0.779[b]	−0.425±1.525[b]	0.042
ACD	0.042±0.040	0.077±0.007	0.094	0.026±0.010	0.020±0.024[b]	0.637
LT	0.092±0.007	0.098±0.013	0.337	0.088±0.019	0.124±0.028	0.049
VCD	0.187±0.030	0.238±0.079	0.219	0.201±0.043	0.147±0.057	0.128
ChT	−0.043±0.033	−0.082±0.059	0.235	−0.053±0.026	−0.047±0.039	0.771
RT	−0.010±0.003	−0.013±0.009	0.365	−0.009±0.005	−0.012±0.003	0.281

Change values were computed as day 2 measurement minus baseline. AL: Axial length; SE: Spherical equivalent; ACD: Anterior chamber depth; LT: Lens thickness; VCD: Vitreous chamber depth; RT: Retinal thickness; ChT: Choroidal thickness; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia; C: Control group; SD: Standard deriation. [a]*P* value was obtained by independent *t*-tests or Mann-Whitney *U* tests; [b]*P*<0.05 in comparison to the values in HL+LIM group or NL+LIM group by using paired *t*-tests or Wilcoxon tests.

U tests were used to compare the data between HL and NL groups. Values of $P<0.05$ were considered to be statistically significant.

RESULTS

Before the treatments (day 0), there were no differences in the mean SE, AL, ACD, LT between HL and NL groups (HL+LIM *vs* NL+LIM, HL+C *vs* NL+C, all $P>0.05$; Table 1). After 2d of −10 D lens wearing, the LIM eyes under NL displayed a myopic shift in refraction when compared to the untreated contralateral control eyes (changes in SE: −5.025±4.520 D *vs* −0.425±1.525 D, $P=0.043$; Table 2). The OCT results revealed that although axial elongation of LIM eyes under NL condition was greater than the contralateral eyes, the significance was borderline ($P=0.062$). The LIM eyes under NL condition had the more myopic refraction ($P=0.012$) and longer AL ($P=0.048$) than those under HL condition on day 2 (Table 1). Figure 1 shows the OCT images of the entire eyes of two chicks from NL+LIM and HL+LIM groups, respectively. High light level of 10 000 lx reduced the myopia shift and axial elongation induced by LIM (HL+LIM *vs* NL+LIM: changes in SE: −0.800±1.242 D *vs* −5.025±4.520 D, $P=0.028$; changes in AL: 0.328±0.051 mm *vs* 0.424±0.066 mm, $P=0.033$; Figure 2, Table 2).

In regard to control eyes, the refractive error of the uncovered contralateral eyes exposed to HL was more hyperopic on day 2 when compared with the baseline (day 2 *vs* day 0: +6.525±1.448 D *vs* +5.100±1.207 D, $P=0.015$; Table 1). Differently, the uncovered eyes under NL condition showed no difference in SE between baseline and 2d after treatment ($P=0.567$). Significant difference of the changes in SE was found between HL+C and NL+C group ($P=0.042$; Figure 2A), while there was no difference of the changes in AL between HL+C and NL+C group (Table 2).

The ACD of the LIM eyes under NL condition increased over the 2d period ($P<0.001$), and the increase was greater than that of the contralateral eyes (changes in ACD: 0.077±0.007 *vs* 0.020±0.024 mm, $P=0.008$; Figure 3A). Differently, no statistical difference of the change in ACD was found between the LIM and the contralateral eyes under HL condition ($P=0.424$). However, difference of changes in ACD between the HL+LIM and NL+LIM group did not reach statistical significance (0.042±0.04 *vs* 0.077±0.007 mm, $P=0.094$).

The LT of the four subgroups all increased over the 2d period (all $P<0.05$). No statistical differences of the changes in LT were found between HL+LIM and NL+LIM group, or between LIM and contralateral eyes under HL or NL condition, while

the LT in NL+C group increased more than that in HL+C group (P=0.049; Figure 3B).

After 2d of treatment, the VCD all increased when compared to the VCD at baseline in all subgroups (all P<0.05; Figure 4A). There were no significant differences of the changes in VCD between the HL+LIM and NL+LIM group, the HL+C and NL+C group, or between treated and contralateral eyes under HL or NL condition (all P>0.05).

The eyes from four subgroups all exhibited decreased trend in RT (all P<0.05; Figure 4B). Regarding the changes in RT, no significant differences were found between the HL and NL groups (change in RT: HL+LIM *vs* NL+LIM: −0.010±0.003 mm *vs* −0.013±0.009 mm, P=0.365; HL+C *vs* NL+C: −0.009±0.005 mm *vs* −0.012±0.003 mm, P=0.281). Differences between LIM and contralateral eyes under HL or NL condition also did not reach statistical significance (all P>0.05).

Over the 2d period, ChT showed a significant decrease from baseline in three subgroups (P<0.05), but not in the HL+LIM group (P=0.077; Figure 4C). ChT in HL+LIM group did not differ from that in NL+LIM group (P<0.05). The ChT decreased by 0.082±0.059 mm in the NL+LIM group and decreased by 0.043±0.033 mm in HL+LIM group, while the difference of changes in ChT between the two groups did not reach statistical significance (P=0.235).

DISCUSSION

The present study demonstrated that after a short period of 2d, exposure to HL (10 000 lx) for 12h daily could significantly reduce the myopic shift in refraction induced by the hyperopic defocus (−10 D), but it could not prevent this development. The protective effects of HL on the experimental myopia in previous chick studies[6-8,13,27,32] mostly occurred after 3d of treatment. A study[8] observed that exposed to HL (15 000 lx) for 5h per day, chicks fitted with −7 D lenses displayed less myopic shift beginning at day 3. Biswas *et al*[13] demonstrated that daily exposure to 6h of HL (15 000 lx) reduced lens-induced (−10 D) myopic refraction on day 4. In the current study, the significant retardation of myopia appeared in the earlier time (2d), which may be due to the longer exposure time of 12h per day, even though HL could not fully halt the myopic shift. This result was consistent with the finding in the previous study[13] that showed exposure of HL reduced LIM in a duration-dependent manner in chicks. Moreover, previous longitudinal studies[18,33] observed that the more outdoor time was associated with the less myopia progression in children. Therefore, our finding, from the perspective of animal research, provides a valuable supplement for previous pediatric epidemiological studies and randomized controlled trials. More specifically, the result in current study and previous findings indicate that extending outdoor time appropriately for younger children might give the earlier and the more protective effect on myopia. Furthermore, the finding that exposure to HL for long time per day significantly retarded LIM in the earlier time could serve as a biometric basis for further investigation

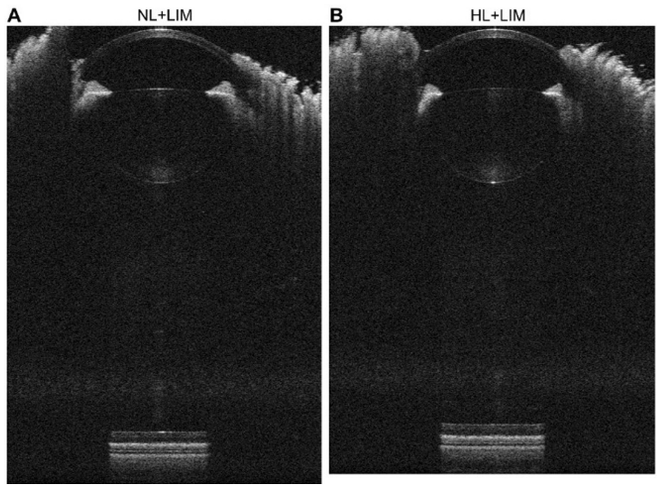

Figure 1 OCT images of the entire eyes of two chicks from NL+LIM and HL+LIM groups, respectively OCT: Optical coherence tomography; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia.

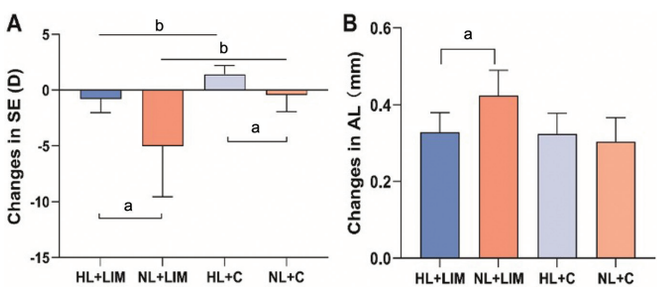

Figure 2 Changes in SE and AL of the four subgroups after 2d of treatment [a]P<0.05, independent t-tests or Mann-Whitney U tests between HL and NL groups; [b]P<0.05, paired t-tests or Wilcoxon tests between LIM eyes and contralateral eyes. AL: Axial length; SE: Spherical equivalent; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia; C: Control group.

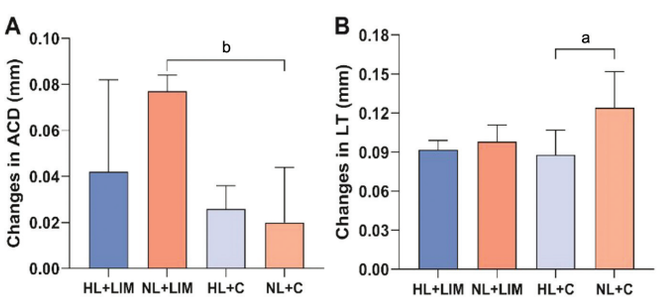

Figure 3 Changes in ACD and LT of the four subgroups after 2d of treatment [a]P<0.05, independent t-tests or Mann-Whitney U tests between HL and NL groups; [b]P<0.05, paired t-tests or Wilcoxon tests between LIM eyes and contralateral eyes. ACD: Anterior chamber depth; LT: Lens thickness; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia; C: Control group.

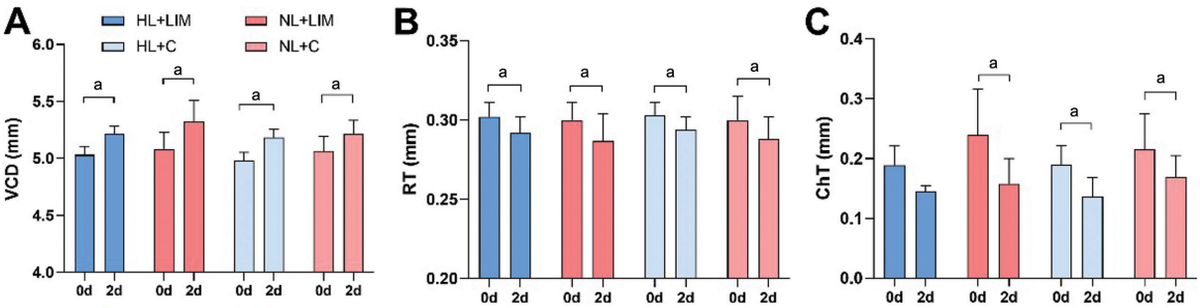

Figure 4 The VCD, RT, and ChT of four subgroups on day 0 and day 2 [a]*P*<0.05, two-way repeated-measures ANOVA followed by post hoc testing, between day 2 and day 0. VCD: Vitreous chamber depth; RT: Retinal thickness; ChT: Choroidal thickness; HL: High-intensity light; NL: Normal-intensity light; LIM: Lens-induced myopia; C: Control group; ANOVA: Analysis of variance.

of early signal molecules or biomarkers associated with the protective effects of HL.

The current study used chick model of myopia and found that HL retarded the early lens induced myopic development. The chick model is one of the common myopic animal models because of its advantages such as sensitive response to visual signals, its relatively large eyeball, and the similar thinning trend in the fibrous sclera during myopic development[34]. Although several structural differences in chick eyes from human such as an extra cartilaginous layer in the sclera and different accommodation process limit the applicability of such model to human myopia, the chick model still could provide important findings for the studies on mechanisms of myopia and emmetropization[34]. Karouta and Ashby[23] demonstrated that in chicks, increasing the light intensity (10 000–40 000 lx) could enhance the protection of HL from the development of form-deprivation myopia, which showed the reasonable illuminance for further animal studies and clinical trials. The current finding that in chicks, long HL exposure time of 12h per day delayed the myopic development in the early time could provide good reference for further studies to optimize the outdoor time or duration of exposure to reduce the myopic progression in children during the early visual development period.

In this study, exposure to HL over 2d induced hyperopic shift in the control eyes, which is similar to the results from other studies[25,35]. However, HL had no significant effect on the axial elongation of the control eyes in the early time, as the AL of HL control eyes was still elongated and no statistical difference of change in AL was found between HL+C and NL+C groups. Therefore, we speculate that the hyperopic shift might be attributable to flattened corneal curvature caused by HL, according to a previous study[35]. This topic merits further exploration.

No differences of the changes in LT were found between NL+LIM and NL+C group, or between NL+LIM and HL+LIM group. These results indicated that HL might have no effect on LT of LIM eyes. After 2d of treatment, the ACD of LIM eyes exposed to NL increased more than the contralateral eyes, which is similar to several previous studies[36-37] showing that anterior chamber deepened in some experimental myopic models. Differently, the LIM eyes under HL condition exhibited the similar change in ACD compared to the contralateral eyes. However, there was no significant difference of the change in ACD between the NL+LIM and HL+LIM group. Although no statistical difference was found between the two light groups, a numerical trend in the hypothesized direction (slower ACD deepening under HL) was observed. More studies are needed to determine whether HL can delay the progression of ACD deepening in LIM eyes. A study[7] has found that the ACD of chicks fitted with the multizone dual-power lenses (pl/−10 D, 50:50 area) under HL was significantly shorter than those reared under control light after 6d, and the LT was not influenced by HL. The shallower ACD could relatively reduce the peripheral hyperopic defocus[38-39], which may further enhance the effect of HL on slowing the development of myopia. Further studies targeting the effects of HL on the long-term changes in ACD and LT are required.

This study found that HL did not influence the VCD of LIM eyes in the early time of 2d. On the day 3 after treatment in another study[8], the protective effect of HL against axial elongation manifested specifically in the VCD. A study[7] showed that chicks fitted with the multizone lenses (pl/−10 D) under HL had the shorter VCD than those reared under control light on day 6. These results indicate that in the earlier time, the effect of HL on VCD might be not apparent, and with the increase of treatment days, the VCD may exhibit significant difference between HL and NL groups.

In the current study, HL did not influence the change of ChT over the 2d period, as the ChT on day 2 in LIM eyes did not differ significantly between the HL and NL conditions. Previous studies[10,13,40] showed the hyperopic defocus could induce thinning of choroid, and Lan *et al*[14] found that HL of 15 000 lx induced choroidal thickening in untreated chicks when compared to the NL. One study[13] showed the HL for

6h per day prevented choroid thinning in the LIM eyes on day 8, while on day 4, differences of ChT in LIM eyes between HL and NL group did not reach statistical significance. These results indicate that in the earlier time, the impact of HL on the choroid of LIM eyes might be not obvious. In addition, although the sample size was determined to be reasonable according to an a priori power calculation, the relatively small sample size in this study may have still influenced the results to some extent. Furthermore study are required to investigate the change in ChT in the early time.

Over the 2d of treatment, the decreases in RT were not affected by HL or negative lens. A study[41] focused on LIM guinea pigs found that retinal thickness of LIM and the fellow eyes experienced similar decreases over the treatment period of 6wk, which is similar to the result in current study. The changes in RT under the hyperopic defocus were complicated. Kee *et al*[10] reported no significant changes in RT over the 3d of the negative lens treatment in chicks. A study[42] demonstrated that LIM eyes in marmosets experienced a retinal thickening after 22wk of treatment, while the thickening degree was less than that in untreated eyes. The less thickening mainly was attributable to thickness changes in different layers in the inner neuroretina, and thinning of the ganglion cell+inner plexiform layer was observed. In another study[43], thinning of the retinal nerve fiber layer and the retinal ganglion cell layer occurred between day 3 to 5 of treatment in LIM chicks. Few studies reported the effect of HL on the retinal thickness of LIM eyes. A study[44] showed the outdoor environment induced total RT thickening in adults when compared to indoor condition. Therefore, further studies are required to investigate the effect of HL on RT, especially on the different retinal layers in myopic animal model.

The non-contact high-resolution OCT provides visualization results of the anatomical structure of eyeball with a fast image acquisition speed, and gives the accurate measuring orientation[16,31]. Therefore, it can serve as an effective *in vivo* tool to measure the anterior segments parameters, and posterior ocular structure thickness and length of chicks[16-17].

There are some limitations of our study. First, the sample size of this study was small despite the protective effects of HL found on the myopic chicks. Second, there is a lack of the observations for the shorter time of one day. Further studies would be performed focused on these issues.

In summary, exposure to HL for 12h per day could retard the myopic shift over a short term of 2d, which could provide a biometric reference for exploring early-stage signal molecules related to the effects of HL on myopic eye. The LIM eyes exposed to normal light level had a longer ACD than their contralateral eyes at the early stage; and HL could potentially mitigate the deepening of ACD, which requires further studies. The RT and ChT all decreased over 2d in LIM eyes and the fellow eyes. HL did not affect the thinning of RT and ChT in the early time. Further studies are required to investigate the impact of HL on ACD, RT and ChT in myopic models. Visualized OCT system is a valuable tool in measuring ocular biometric parameters in chick model of myopia.

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