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Synchronous Myopia Development Induced by Bilateral Form Deprivation in Chicks

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Abstract

Form deprivation (FD) is a widely employed experimental paradigm, typically used to induce unilateral myopia in animal models. This model is weakened by potential influence upon the FD eye from vision in the freely-viewing contralateral eye, which could be eliminated by imposing FD in both eyes; but while a few previous studies have explored the feasibility of inducing bilateral FD in chicks, substantial discrepancies in treatment outcomes were noted. Consequently, this study aimed to establish a bilateral FD myopia model in chicks, with validation by investigating the associated ocular growth patterns, feeding, and social behavior. Six-day-old chicks were treated with bilateral (n= 21) or unilateral (n= 10) FD for 12 days; the fellow untreated eyes in the unilateral FD group served as controls. Refractive error, corneal power, and ocular axial dimensions were measured at 4-day intervals after the onset of form deprivation, with a Hartinger refractometer, a custom-made videokeratography system, and a high-resolution A-scan ultrasonographer, respectively. Body weight was monitored to assess the chick's physical development. Our results showed that birds treated with bilateral FD grew as robustly as the unilaterally form-deprived chicks, with similar or slightly heavier body weights and mortalities. Unilateral FD induced significantly higher myopia in the treated eye, with stronger corneal power, deeper anterior and vitreous chambers, and longer axial length. Moreover, either bilaterally or unilaterally FD eyes developed similar refractive error (bilateral FD, left: –28.03±9.06 D, right: –28.44±9.45 D; unilateral FD: –29.48±8.26 D) and ocular biometric changes; but choroidal thickness was thicker in bilaterally FD eyes, rather than thinner as in unilaterally FD eyes. In addition to the highly synchronized (symmetrical, parallel) development reported previously in bilateral FD, we found in this study that the correlations between bilaterally form-deprived eyes were highest for ocular biometric parameters directly contributing to myopia development, including corneal power (r = 0.74 to 0.93), anterior chamber depth (r = 0.60 to 0.85), vitreous chamber depth (r = 0.92 to 0.94), and axial length (r = 0.90 to 0.96). The remarkably synchronized growth pattern confirmed the feasibility of the bilateral FD paradigm for future research on myopia.

Keywords: Myopia; Bilateral form deprivation myopia; Synchronous myopia development; Refractive development; Animal models

1. Introduction

Form deprivation (FD) is a widely used method for studying myopia development in animal models, involving techniques such as suturing the eyelids shut or imposing a translucent diffuser. These techniques result in a constant degradation of retinal image quality, eliminating spatiotemporal features that are essential for emmetropization and causing unrestricted eyeball elongation. Consequently, a significant amount of myopia is developed until the eye loses developmental plasticity (Schaeffel and Howland, 1991; Thomson *et al.*, 2020; Troilo *et al.*, 2019). This form deprivation myopia (FDM) was first discovered in the lid-sutured monkey model in 1977 (Wiesel and Raviola, 1977) and was later demonstrated in a diverse range of animal species, including birds (Troilo *et al.*, 1987; Wallman *et al.*, 1978b), fish (Shen and Sivak, 2007; Shen *et al.*, 2005), mammals (Howlett and McFadden, 2006; Tkatchenko *et al.*, 2010), and non-human primates (Smith *et al.*, 1987; Troilo and Judge, 1993; Wiesel and Raviola, 1977). Clinically, FD manifests in infants with uncorrected congenital cataracts, corneal opacity, or blepharoptosis, leading to axial myopia similar to that in experimental animal models when left untreated early in life (Calossi, 1994; Gee and Tabbara, 1988; Rabin *et al.*, 1981).

Negative lens-induced myopia (LIM), another type of experimental myopia paradigm, operates through a *closed-loop* mechanism – prompting the eye to grow towards the displaced hyperopic focal plane, with the magnitude of the induced myopia closely matching the power of the inducing lens (Howlett and McFadden, 2009; Hung *et al.*, 1995; Schaeffel *et al.*, 1988; Shaikh *et al.*, 1999). While LIM is advantageous for inducing a specific magnitude of myopia, it is constrained by the limited range of defocus compensation. For instance, chicks, which exhibit the greatest flexibility of defocus compensation among animal models used in myopia research, can only compensate for up to −10 D of hyperopic defocus (Irving *et al.*, 2015) (for a review of other animal species, see (Troilo *et al.*, 2019)). This limitation makes LIM unsuitable for investigating severe myopia.

On the other hand, FDM is proposed to develop through an *open-loop* mechanism, which rapidly induces myopia to as much as −25 D within a week in chicks (Kang *et al.*, 2018), thus offering the advantage of studying severe myopia progression and the efficacy of potential interventions. Additionally, visual input can be better controlled in the FD paradigm, as the degraded retinal image quality remains relatively stable throughout the experimental period – regardless of ocular accommodation, eye growth, or tear film quality, which could affect the optical defocus received

by the eyes in the LIM paradigm. However, the use of FD in myopia research is hindered by its significant variability, with induced myopia ranging from −12 D to −45 D after 7 days of treatment (in chicks (Kang *et al.*, 2018)). The extreme unpredictability of refractive error development in the chick FD model creates challenges in evaluating intervention effects and necessitates large sample sizes to account for the variability. In this study, we investigated whether a bilateral FD paradigm combined with a controlled environment could lead to synchronized eye growth in both eyes.

Early research conducted in the 1980-90s found that bilaterally form-deprived chicks exhibited equal degrees of refractive error and axial length in the two eyes (Fujikado *et al.*, 1997; Schaeffel and Howland, 1991; Sivak *et al.*, 1989). Interestingly, although they all observed synchronization of eye growth, these studies reported vastly different final refractive errors. Contrary to the myopia typically observed following unilateral FD, Schaeffel and Howland found that bilateral FD, on average, induced hyperopia (+2.46±3.93 D) in chicks after 8 days of treatment (Schaeffel and Howland, 1991). In contrast, Sivak, Barrie, and Weerheim reported significant myopia development (−27.33±4.50 D) in bilaterally form-deprived eyes, with the magnitude of myopia being nearly double than that in the eyes treated with unilateral FD (−14.33±1.50 D) following 14 days of treatment (Sivak *et al.*, 1989). A third study, not primarily focused on the effects of bilateral FD, found that bilateral FD produced high and equal degrees of FDM in both eyes (approximately −15 D) after 6 days of treatment, but less than reported by Sivak, Barrie, and Weerheim (Fujikado *et al.*, 1997). Given the considerable inconsistencies in these findings, further validation is warranted before employing this paradigm in myopia research.

Additionally, it is pertinent to consider whether animals raised under such conditions can grow normally, because bilateral FD with full-field diffusers allows only light-perception (with very limited form discrimination) in both eyes. Indeed, previous studies reported that genetically blinded chicks had difficulty finding food, leading to maldevelopment and a higher mortality rate than with visually healthy chicks (Collins *et al.*, 2011; Hocking and Guggenheim, 2013). If subnormal body growth occurs in bilaterally form-deprived chicks, it could interfere with eye growth and result in inconsistent degrees of refractive error development. However, previous studies on bilateral FD did not report chicks' physical health, such as their body weights, mortality rates, and behavior (Fujikado *et al.*, 1997; Schaeffel and Howland, 1991; Sivak *et al.*, 1989).

The present study aimed to characterize ocular growth patterns in form-deprived chick eyes, by measuring

refractive error and ocular biometric parameters longitudinally every four days from T0 (baseline; post-hatching day 6) to T12. Unlike previous studies, which measured only refractive error and axial length (Fujikado *et al.*, 1997; Schaeffel and Howland, 1991; Sivak *et al.*, 1989), this study measured the axial biometric parameters of individual ocular components, from corneal to scleral thickness, using a high-frequency A-scan ultrasonography. The corneal power was also measured using a custom-built videokeratograph for small eyes (Chu *et al.*, 2014; Kang *et al.*, 2018). Furthermore, we also assessed chicks' physical development by measuring body weight and reporting the mortality rate, and we documented their feeding and social activity. Finally, we discussed the technical considerations that researchers should consider when employing this FD paradigm in future experiments.

2. Material and Methods

2.1. Animal Husbandry & Treatment Design

Thirty-one White Leghorn chicks (*Gallus gallus domesticus*) were obtained from the Centralized Animal Facility of The Hong Kong Polytechnic University and were housed in a temperature- (25 °C) and light-controlled room (12h:12h light-dark cycle from 7 AM to 7 PM) provided with food and water *ad libitum*. To induce unilateral and bilateral FD, dome-shaped translucent diffusers were custom-made by molding white polyvinyl chloride plastic sheets. Each diffuser has a similar thickness (0.5 mm), diameter (12 mm), and spectral transmittance, which is uniformly about 35 % across the visible spectrum (400 nm – 700 nm) and nearly 0 % in the ultraviolet range (< 400 nm), as determined by a Lambda 650S Spectrophotometer (PerkinElmer, U.S.). At baseline (T0; post-hatching day 6), diffusers were attached with Velcro to the periorbital feathers of chicks (see Figure 1A), and they were removed for daily cleaning (< 1 min) and ocular biometry measurements (< 15 mins, every 4 days) throughout the study period.

Chicks were randomly assigned to receive bilateral (n = 21, BFD) or unilateral (n = 10, UFD) FD for 12 days. Their behavior in the holding cage was closely monitored using an Internet Protocol camera (Arlo Technologies, U.S.) installed in the cage; this was particularly important for chicks treated with bilateral FD, to verify that they could and did access food and water (see Supplementary Video S1 & S2). Chicks' body weight was recorded every 4 days from the baseline, no bird showed a drop in body weight throughout the study. All experiments were conducted in accordance

with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocols were approved by the Animal Subject Experiment Subcommittee of the Hong Kong Polytechnic University (ASESC#20-21/284).

2.2. Refractive Error and Ocular Biometry Measurements

Corneal power, refractive state, and ocular axial dimensions were measured longitudinally at 4-day intervals from T0 to T12. All measurements started at 08:00 AM and were completed by 11:00 AM to minimize the potential effects of circadian rhythm on the outcome measurements (Nickla *et al.*, 1998b; Nickla *et al.*, 2001). The instruments used in this study and the measurement protocols have been reported in previous studies (Chu *et al.*, 2014; Kang *et al.*, 2018; Kee and Deng, 2008; Vyas and Kee, 2021), and a brief description of each method follows.

2.2.1. Corneal Power Measurement

Corneal power was measured using a custom-built videokeratography system (Chu *et al.*, 2014; Kang *et al.*, 2018; Vyas and Kee, 2021) when chicks were awake, prior to refractive and ocular axial dimension measurements that required anesthesia. In brief, the chicks' pupillary center was first aligned with the Placido rings of the system, and the images reflected from the corneal surface were then recorded continually using a charge-coupled device camera. At least three images per eye were manually selected on the basis of the following image selection criteria (see Supplementary Figure S1):

1) all of the captured Placido ring images were sharply focused
2) the central Placido rings were aligned with the pupil center
3) the Placido rings were not constricted, i.e., there was no sign of corneal accommodation

This rigorous image selection process controlled for potential confounding factors, such as optical misalignment and corneal accommodation (Chu *et al.*, 2014). Screened images were then analyzed using a custom-written MATLAB algorithm to extract the mean corneal power (Chu *et al.*, 2014).

2.2.2. Refractive Error Measurement

Following the corneal power measurement, chicks were anesthetized by isoflurane inhalation (1 to 1.5 % in oxygen, depending on age and weight) to stabilize accommodation (Kee and Deng, 2008). Refractive error was then measured along the pupillary axis using a modified Hartinger coincidence refractometer (Model 110, Carl Zeiss Meditec, Germany). Spherical-equivalent refractive error (SE) was calculated from the average of three repeated measurements per eye (Thibos *et al.*, 1997).

2.2.3. Ocular Axial Dimensions Measurement

A high-frequency A-scan ultrasonography system equipped with a 50 MHz transducer (PVDF; PI50-2-R0.50; GE Panametrics, U.S.) was used to measure on-axis ocular axial dimensions while chicks were anesthetized. A drop of phosphate buffer saline was applied to the cornea to reduce irritation caused by ultrasonograph-interfacing gel (Aquasonic; Parker Laboratories, U.S.). Thirty datasets per eye were collected, and the distances between the amplitude spikes of the returning echoes were analyzed using a custom-written algorithm to identify the depth or thickness of seven ocular components (Nickla *et al.*, 1998a; Troilo *et al.*, 2000): central corneal thickness, anterior chamber depth, crystalline lens thickness, vitreous chamber depth, retinal thickness, choroidal thickness, and scleral thickness.

2.3. Data Analysis

All statistical analyses were conducted using IBM SPSS (version 21.0.0, IBM, U.S.). After testing data normality (Shapiro-Wilk) and homogeneity of variance (Levene's) to confirm that the conditions for parametric statistics were met, the treatment-induced changes in body weight and ocular biometrics over time were tested using a repeated two-way ANOVA (within-group factor: time & laterality, factor 2: treatment) followed by Bonferroni's post-hoc multiple comparison tests. Greenhouse-Geisser (if $\varepsilon > 0.75$) or Huynh-Feldt (if $\varepsilon < 0.75$) correction was applied to test the effects of the two factors, based on the data sphericity determined by Mauchly's test. Correlations between ocular biometrics

at different time points were tested using Pearson's correlation analysis. All results were represented in mean ± standard deviation. The significance level for all tests was set at $P < 0.05$.

3. Results

3.1. Body weight and mortality rate

Chicks' body weight and mortality rate were used for objective evaluation of the bilateral FD paradigm for extra-ocular effects due to poor nutrition. At baseline (T0), the body weights of animals in the bilateral and unilateral FD groups were statistically indistinguishable (Bonferroni post-hoc test: 43.57±1.16 g vs 43.79±1.68 g, respectively; p = 0.91). Throughout the treatment period, the body weights of chicks in both groups increased significantly with time (Figure 1B, two-way repeated ANOVA, Time effects: $F = 1468$, $p < 0.001$). Interestingly, chicks treated with bilateral FD were about 10 % heavier than those treated with unilateral FD (Treatment effects: $F = 3.34$, $p = 0.07$; Time × Treatment interaction: $F = 3.24$, $p = 0.04$) at T4 (Bonferroni post-hoc test: 66.38±8.79 g vs 59.63±3.07 g, $p = 0.02$) and T8 (93.87±10.60 g vs 86.13±4.22 g, $p = 0.03$), but they weighed about the same at T12 (133.84±11.11 g vs 128.34±8.84 g, $p = 0.18$). Despite their lack of form vision, chicks treated with bilateral FD could still quickly identify the location of food and water (see Supplementary Videos S1 & S2), and none of the chicks treated with bilateral or unilateral FD died during the treatment period (mortality rate = 0 %). Our results indicated that bilateral FD is a feasible paradigm, and that chicks could grow normally with normal food intake despite having bilateral FD.

3.2. Form deprivation induced refractive and ocular biometric changes

At baseline, refractive errors and ocular biometric parameters were similar – between the right and left eyes (mixed model ANOVA, Ocular laterality: $F < 1.69$, $p > 0.19$) and between the two FD groups (Treatment effect: $F < 2.98$, $p > 0.08$) – except that the vitreous chamber depths of unilaterally form-deprived chicks were slightly longer (+163 μm) and their retinas were thicker (+16 μm) than those of the bilaterally form-deprived chicks (Treatment effect: $F < 19.50$, $p < 0.001$). Because of the inter-animal variations of refractive errors and ocular biometric parameters, the longitudinal changes from baseline (i.e., Follow-up – Baseline) were calculated and analyzed to determine the effects

of FD treatment. The raw data are shown in Supplementary Table S1 for reference.

Figure 2 summarizes the changes in refractive errors and ocular biometrics in chicks reared with bilateral FD (Right eye: *Red* symbols and lines; Left eye: *Blue* symbols and lines) and unilateral FD (FD treated eye: *Green* symbols and line; Control eye: *Black* symbols and lines). Regardless of the treatment, eyes developed a flatter cornea, deeper anterior and vitreous chambers, and a greater axial length throughout the study period (mixed model ANOVA, Time effect: $F < 12194$, $p < 0.001$). However, the treatment effect of FD interacted significantly with the ocular laterality (i.e., unilateral or bilateral FD) during the observation period (Time × Treatment × Ocular laterality interaction: $F < 263.28$, $p < 0.01$), such that the form-deprived eyes in bilateral and unilateral FD groups differed from the untreated control eyes in developing higher myopia, stronger corneal power, deeper anterior and vitreous chambers, and longer axial length. Other ocular biometric parameters also changed significantly with age (Time effects: $F < 131.85$, $p < 0.05$), but the differences in treatment effects between bilateral and unilateral FD groups were inconsistent. The results are described below in detail.

3.2.1. Refractive State, Vitreous Chamber Depth, and Axial Length

Pairwise post-hoc tests with Bonferroni's corrections were used to compare the untreated control eyes in the unilateral FD group with form-deprived eyes in both the bilateral and unilateral FD groups. As expected, FD induced high myopia in the treated eyes, accompanied by excessive increases in vitreous chamber depth and axial length. Compared to the untreated control eye, form-deprived eyes in both FD groups developed more myopia with longer vitreous chamber and axial length from T4 to T12, i.e., by the end of the study period (Bonferroni post-hoc tests, $p < 0.001$; Figure 2). Specifically, the form-deprived eyes developed nearly −28 D more myopic refractive error (bilateral FD, left: −28.03±9.06 D, right: −28.44±9.45 D; unilateral FD: −29.48±8.26 D; Figure 2A), 1,200 μm deeper vitreous chamber (bilateral FD, left: +1696±297 μm, right: +1731±276 μm; unilateral FD: +1724±287 μm; Figure 2F), and 1,600 μm greater axial length (bilateral FD, left: +2780±508 μm, right: +2792±488 μm; unilateral FD: +2803±459 μm; Figure 2G) than untreated control eyes (Myopia: −1.20±0.55 D; Vitreous chamber depth: +552±198 μm; Axial length: +1240±202 μm) at the end of the 12-day treatment period (T12). However, no significant differences in these parameters were found among the form-deprived eyes under either bilateral or unilateral FD ($p > 0.34$).

3.2.2. Corneal Biometrics and Anterior Chamber Depth

FD caused steeper corneas (i.e., higher refractive power) and deeper anterior chambers than in untreated control eyes, with significant differences observed at T8 and T12 (Bonferroni post-hoc tests, $p < 0.05$). As shown in Supplementary Table 1, the chicks' corneas progressively flattened (i.e., exhibited a reduction in corneal power) with age. However, the rate of this flattening process was slower in the form-deprived eyes compared to the untreated control eyes. At T12, the corneal powers in form-deprived eyes were significantly steeper than those in the control eyes due to the lesser degree of flattening (bilateral FD, left: −12.76±3.80 D, right: −12.18±3.78 D; unilateral FD: −14.49±3.73 D; Figure 2B), but their anterior chambers were deeper (bilateral FD, left: +634±242 μm, right: +622±292 μm; unilateral FD: +666±276 μm; Figure 2D) than those in the untreated control eyes (corneal power: −19.41±3.70 D; anterior chamber depth: +241±62 μm). The differences between these parameters of the form-deprived eyes in bilateral and unilateral FD groups, were insignificant ($p > 0.20$).

3.2.3. Other Ocular Biometric Parameters

The change in choroidal thickness depended on whether FD was imposed unilaterally or bilaterally (Time × Treatment × Ocular laterality interaction: $F = 12.94$, $p = 0.001$). In the unilateral FD group, the choroid in the untreated control eye became thicker over time, but FD made the choroid of the treated eye thinner. These differences between unilateral FD and non-FD eyes were significant at T8 (Bonferroni post-hoc test, $p < 0.001$) and T12 ($p < 0.001$). By T12 (i.e., T12 − T0), the choroid of the untreated control eye was thickened by +51.12±21.11 μm, and that of the unilateral form-deprived eye thinned by –33.42±31.31 μm. On the other hand, the choroid in the bilateral FD group was slightly thickened. By T12, bilateral FD thickened the choroid of the form-deprived right eyes by +31.72±61.07 μm and the left eye by +23.68±48.12 μm; these effects were significantly different from those in the form-deprived eye in the unilateral FD group ($p \leq 0.043$).

On the other hand, FD had no significant effect on the thicknesses of the cornea, crystalline lens, and sclera ($F < 1.37$, $p > 0.25$), neither were there any interaction effects among the time, treatment, and ocular laterality ($F < 1.64$, p

> 0.20).

3.3. Synchronized refractive development in bilaterally form-deprived chicks

In the bilateral FD group, the right and left form-deprived eyes shared similar refractive errors and ocular biometry at all time points tested ($p > 0.12$). Figure 3 plots the correlations between each tested parameter in the two eyes, to better understand whether they developed synchronously. Had FD induced an open-loop eye growth process, the two eyes under bilateral FD might have grown independently, and the data would have been scattered randomly.

Intriguingly, despite the considerable inter-subject variation in myopia induced after the exposure to FD (range of SE: –17.10 D to –47.75 D at T12), the refractive-error data of the two eyes were clustered near the diagonal line, with strong correlations between the fellow eyes throughout the study period (Pearson's $r > 0.80$, $p < 0.001$). Similarly, ocular biometric parameters that contribute to the development of FDM (i.e., corneal power, anterior chamber depth, vitreous chamber depth, and axial length) in the right and left eyes also were strongly correlated (all Pearson's $r > 0.60$, $p < 0.001$) – in particular, the vitreous chamber depth and axial length, for which the correlation coefficients were higher than 0.90 at all timepoints tested.

On the other hand, the thicknesses of central cornea, retina, choroid, and sclera showed less agreement between the two form-deprived eyes, and the correlation coefficients were moderate or not significant (Figure 3).

4. Discussion

In accordance with previous research, this study observed that FD induced a broad range of myopia, a steep cornea, an increased anterior chamber, and an expanded vitreous chamber depth (Gottlieb *et al.*, 1987; Kang *et al.*, 2021; Kang *et al.*, 2018; Napper *et al.*, 1995; Troilo *et al.*, 1995; Wallman *et al.*, 1978a). Notably, our findings further revealed that the growth of the two eyes in bilaterally form-deprived chicks was strongly linked (or coupled), particularly for the main ocular components directly involved in refractive development (i.e., corneal power, anterior chamber depth, vitreous chamber depth, and axial length). These results suggest that genetically determined susceptibility to form vision

may regulate the open-loop condition of FDM in individual chicks (Schaeffel and Howland, 1991), resulting in coupled growth of the two eyes. In agreement with our observations, Chen et al. demonstrated that the susceptibility to FDM in chicks is strongly influenced by genetics (Chen *et al.*, 2011). By selectively breeding chicks under the two extreme tails of susceptibility to FDM (i.e., those developed high and low myopia after being reared with unilateral FD), they found that offspring from highly myopic chickens were more prone to develop myopia as a result of FD than offspring from low-myopic chickens (Chen *et al.*, 2011). Evidence from this and previous studies indicates that myopia-predisposing gene variants might exist to determine the magnitude of environmentally induced myopia in chicks.

The results of the current study appear to contradict those of Schaeffel and Howland, who found *form deprivation hyperopia* instead of myopia when rearing chicks under bilateral FD with full-field translucent diffusers (Schaeffel and Howland, 1991). On the other hand, when repeating the experiment by opening a notch in the diffuser to allow normal frontal vision, they found that both eyes of bilaterally FD chicks developed myopia (Schaeffel and Howland, 1991), as usually observed in unilateral FD treatment. Thus, they speculated that FDM development might be suppressed when both retinas received only diffuse illumination, devoid of spatial and temporal image details. In contrast, in the present study we found myopia in form-deprived eyes treated with full-field translucent diffusers, regardless of whether FD was imposed unilaterally or bilaterally. It is reasonable to suppose that the differences in animal strains, diffuser properties, and experimental designs might explain the variation of the treatment outcomes. However, we speculate that chicks' living behavior during bilateral FD treatment might also contribute to their refractive development. In the current study, we raised chicks receiving bilateral and unilateral FD in the same cage and observed that chicks in both groups were active in the daytime (see Supplementary Video S1). In an on-going pilot study to develop this bilateral FD model, we further reared bilaterally form-deprived chicks separately from unilaterally form-deprived chicks in different cages. Although the same treatment design was adopted, this batch of isolated bilaterally form-deprived chicks ($n = 8$) developed much lower myopia after 12 days of treatment (around −6 D, see Supplementary Figure S2) than did those raised together in the same cages with a mix of bilaterally and unilaterally FD chicks. According to our observations, these isolated bilaterally form-deprived chicks were not as active as those kept together with unilaterally FD chicks: they distanced themselves socially from other birds and spent most of their time sitting alone. Thus, we speculate that the low myopia developed was somehow related to inactive lifestyle caused by social distance, indicating that animals' behavior should also be considered when designing experiments using the bilateral FD paradigm.

Ocular growth in chicks is believed to be controlled locally by the retina according to the blur signals received (Troilo *et al.*, 2019). A series of experiments confirmed this local mechanism for control of eye growth, showing that FDM could still develop even after disconnecting the brain and eyes surgically (McFadden and Wildsoet, 2020; Troilo *et al.*, 1987; Wildsoet and Pettigrew, 1988) or pharmacologically (Mcbrien *et al.*, 1995; Norton *et al.*, 1994). However, Sivak, Barrie, and Weerheim (Sivak *et al.*, 1989) argued the existence of information transfer between eyes and showed that bilaterally form-deprived eyes developed an average –13 D more myopia than when treated unilaterally (calculated from Table 1 of Sivak et al. (Sivak *et al.*, 1989)). In fact, they adopted a different method to impose bilateral FD than we did in the current study. In contrast to using the same translucent diffuser for both eyes, they treated one eye with a translucent diffuser and the fellow eye with an opaque occluder; i.e., both eyes received different visual inputs. On the other hand, the current study showed that the bilateral and unilateral FD induced by translucent diffusers resulted in similar magnitude and incidence of myopia (48 % and 50 % of bilateral and unilateral form-deprived eyes, respectively, develop > –30 D). Our results appear to favor the hypothesis that the bilaterally synchronized eye growth in form-deprived eyes is genetically regulated by local eye growth mechanism instead of interocular transfer.

However, this study still cannot fully rule out the possibility that the highly coordinated refractive and ocular biometric changes in both treated eyes were due to some sort of interocular interaction. Previous studies on unilateral lens-induced myopia and hyperopia revealed that even though the fellow eyes were untreated, they grew differently from the eyes of control birds (Wildsoet and Wallman, 1995; Zhu and McFadden, 2017; Zhu and Wallman, 2009). It has been suggested that neurotransmitters regulating eye growth can diffuse from one eye to the fellow eye, because the sclerae of chick eyes are closely apposed and separated only by a thin septum (Smith *et al.*, 2002; Wildsoet and Wallman, 1995). Nevertheless, we believe that such interocular interaction, if any, could only partially explain our results. As shown consistently in myopic chick models, the magnitude of refractive errors developed in the fellow untreated eye was always minor compared to what would be expected from the treated eye (e.g., –1 D was induced in the fellow untreated eye when the treated eye developed –4.8 D of myopia after exposure to –6.0 D hyperopic defocus (Wildsoet and Wallman, 1995)), suggesting that the interocular transfer is minimal in chicks. Furthermore, the eye growth pattern of the fellow untreated eye relative to the treated eye is controversial. Some studies found that the fellow untreated eye follows the same eye growth pattern as the treated eye, i.e., accelerating eye growth with hyperopic defocus and slowing eye growth with myopic defocus (yoking effect (Bitzer and Schaeffel, 2002; Fischer *et al.*, 1999; Ohngemach *et al.*,

2001; Wildsoet and Wallman, 1995; Zhu and McFadden, 2017; Zhu and Wallman, 2009)), while other studies indicated the opposite eye growth pattern (anti-yoking effect (Rucker and Wallman, 2008; Schmid and Wildsoet, 1996; Zhu and McFadden, 2017; Zhu and Wallman, 2009)). It has been speculated that interocular interaction has an alternative pathway to neuronal connection because yoking still exists after disconnecting eyes and brain through optic nerve section (Wildsoet and Wallman, 1995). In particular, it was hypothesized that bilaterally distributed choroid, innervated by autonomic nervous system, is a possible channel – supported by studies showing that monocular light stimulation or form deprivation causes choroidal blood flow changes in both eyes (Reiner *et al.*, 1983; Shih *et al.*, 1993a). More studies are needed to understand whether and how the interaction between eyes contributes to bilateral FDM development.

One unexpected result of the current study is the slight increase of choroidal thickness in the bilateral FD-treated chicks. Our study showed that compared to baseline (T0), around 80 % of bilaterally form-deprived chicks had thick choroid (right eyes: 31.72±61.07 μm and left eyes: 23.68±61.07 μm) after 12 days of treatment (T12), whereas 90 % of unilaterally form-deprived chicks had significantly thin choroid by −33.42±31.31 μm (see Figure 2I and Supplementary Table S2). The choroid has been considered to play a crucial role in myopia development because of its bidirectional changes in thickness and blood flow prior to myopic and hyperopic eye growth (Hung *et al.*, 2000; Nickla and Totonelly, 2015; Troilo *et al.*, 2000; Wallman *et al.*, 1995; Wildsoet and Wallman, 1995): the choroidal thickness was decreased upon fast eye-growth stimulus (i.e., hyperopic defocus or FD) but increased upon slow eye-growth stimulus (i.e., myopic defocus) (Fitzgerald *et al.*, 2002; Shih *et al.*, 1993b). Thus, a decreased choroidal thickness is expected in form-deprived eyes (Fitzgerald *et al.*, 2002; Kang *et al.*, 2018; Yan *et al.*, 2021). It is unclear why choroidal thickness was reduced only when treated with the unilateral FD but not the bilateral FD, even though, on average, form-deprived eyes in both groups developed a similar magnitude of myopia. Whether clear vision of at least one eye is needed to regulate the blood flow and thickness of the choroid, and how the choroid plays a role in the synchronized eye growth in bilateral FD treatment, requires further investigation.

This study confirmed that malnutrition is unlikely to explain the refractive error development in bilaterally form-deprived chicks, given the similar body weight in chicks treated FD unilaterally or bilaterally (Figure 1B and Supplementary Table S1). Like other avian species, chickens rely heavily on visual cues for daily living (Prescott *et al.*, 2003). Thus, it is legitimate to suspect that impairing chicks' vision bilaterally would affect their eating and drinking. Indeed, immediately after bilateral FD treatment, chicks showed a clear sign of distress (e.g., excessive preening (Collins

et al., 2011)), but they adapted to the impaired vision quickly and started navigating around the cage to search for food after around 1 to 2 hours of treatment, probably based on low spatial frequency visual cues. After a day of bilateral FD, all chicks could locate sustenance precisely (see the Supplementary Video S1 and S2). Our observation appears to disagree with studies using genetically blinded chicks, which were unable to find food and had lighter body weight and higher mortality rates than visually healthy chicks (Collins *et al.*, 2011; Hocking and Guggenheim, 2013). However, unlike the genetically modified chicks blinded since hatching (Collins *et al.*, 2011), the bilaterally form-deprived chicks spent the first 5 post-hatching days in the cage, with unobstructed vision in both eyes. It is known that chicks have an inherited ability to discriminate whether food and water are safe for consumption, on the bases of both visual and olfactory cues (viz., whether the food has a familiar appearance and smell (Marples and Roper, 1996)). Thus, these 5 days of clear vision might have habituated the chicks to the available food, water, and living environment, so that they might use the odor cue for survival during bilateral FD treatment.

5. Conclusion

This study showed that bilaterally form-deprived eyes grew synchronously, with similar rates of refractive and ocular biometric changes, throughout the 12-day treatment period. Our results indicated that FDM is developed through an open-loop condition but might be also regulated by genetic or physiological factors shared by the two eyes. The normal body weight and zero mortality rate also suggest that bilateral FD is a feasible paradigm in chicks, as they can find food and water even when clear vision is restricted by bilateral diffuser wear. Given that FD is effective in inducing high myopia (on average around −25 D in some chick models after a week of treatment and to more than −40 D if treated longer (Kang *et al.*, 2018)), this bilateral FD paradigm can be very useful for future studies on high myopia, such as genetic contribution to the pathogenesis of high myopia-related ocular complications. However, one should be aware of the behavioural changes of chicks when adopting the bilateral FD paradigm.

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References

Bitzer, M., Schaeffel, F., 2002. Defocus-induced changes in ZENK expression in the chicken retina. Invest Ophth Vis Sci 43, 246-252.

Calossi, A., 1994. Increase of Ocular Axial Length in Infantile Traumatic Cataract. Optometry Vision Sci 71, 386-391.

Chen, Y.P., Hocking, P.M., Wang, L., Povazay, B., Prashar, A., To, C.H., Erichsen, J.T., Feldkaemper, M., Hofer, B., Drexler, W., Schaeffel, F., Guggenheim, J.A., 2011. Selective Breeding for Susceptibility to Myopia Reveals a Gene-Environment Interaction. Invest Ophth Vis Sci 52, 4003-4011.

Chu, C.-h.G., Zhou, Y., Zheng, Y., Kee, C.-s., 2014. Bi-directional corneal accommodation in alert chicks with experimentally-induced astigmatism. Vision Res 98, 26-34.

Collins, S., Forkman, B., Kristensen, H.H., Sandoe, P., Hocking, P.M., 2011. Investigating the importance of vision in poultry: Comparing the behaviour of blind and sighted chickens. Appl Anim Behav Sci 133, 60-69.

Fischer, A.J., McGuire, J.J., Schaeffel, F., Stell, W.K., 1999. Light- and focus-dependent expression of the transcription factor ZENK in the chick retina. Nat Neurosci 2, 706-712.

Fitzgerald, M.E.C., Wildsoet, C.F., Reiner, A., 2002. Temporal relationship of choroidal blood flow and thickness changes during recovery from form deprivation myopia in chicks. Exp Eye Res 74, 561-570.

Fujikado, T., Kawasaki, Y., Fujii, J., Taniguchi, N., Okada, M., Suzuki, A., Ohmi, G., Tano, Y., 1997. The effect of nitric oxide synthase inhibitor on form-deprivation myopia. Curr Eye Res 16, 992-996.

Gee, S.S., Tabbara, K.F., 1988. Increase in Ocular Axial Length in Patients with Corneal Opacification. Ophthalmology 95, 1276-1278.

Gottlieb, M.D., Fugate-Wentzek, L.A., Wallman, J., 1987. Different visual deprivations produce different ametropias and different eye shapes. Invest Ophthalmol Vis Sci 28, 1225-1235.

Hocking, P.M., Guggenheim, J.A., 2013. The chick as an animal model of eye disease. Drug Discovery Today: Disease Models 10, e225-e230.

Howlett, M.H.C., McFadden, S.A., 2006. Form-deprivation myopia in the guinea pig (Cavia porcellus). Vision Res 46, 267-283.

Howlett, M.H.C., McFadden, S.A., 2009. Spectacle lens compensation in the pigmented guinea pig. Vision Res 49, 219-227.
Hung, L.F., Crawford, M.L.J., Smith, E.L., 1995. Spectacle Lenses Alter Eye Growth and the Refractive Status of Young Monkeys. Nat Med 1, 761-765.
Hung, L.F., Wallman, J., Smith, E.L., 2000. Vision-dependent changes in the choroidal thickness of macaque monkeys. Invest Ophth Vis Sci 41, 1259-1269.
Irving, E.L., Sivak, J.G., Callender, M.G., 2015. Refractive plasticity of the developing chick eye: a summary and update. Ophthal Physl Opt 35, 600-606.
Kang, B.S., Lam, T.C., Cheung, J.K.W., Li, K.K., Kee, C.S., 2021. Corneal proteome and differentially expressed corneal proteins in highly myopic chicks using a label-free SWATH-MS quantification approach. Sci Rep-Uk 11.
Kang, B.S., Wang, L.K., Zheng, Y.P., Guggenheim, J.A., Stell, W.K., Kee, C.S., 2018. High myopia induced by form deprivation is associated with altered corneal biomechanical properties in chicks. Plos One 13.
Kee, C.-s., Deng, L., 2008. Astigmatism associated with experimentally induced myopia or hyperopia in chickens. Invest Ophthalmol Vis Sci 49, 858-867.
Marples, N.M., Roper, T.J., 1996. Effects of novel colour and smell on the response of naive chicks towards food and water. Anim Behav 51, 1417-1424.
Mcbrien, N.A., Moghaddam, H.O., Cottriall, C.L., Leech, E.M., Cornell, L.M., 1995. The Effects of Blockade of Retinal Cell-Action Potentials on Ocular Growth, Emmetropization and Form Deprivation Myopia in Young Chicks. Vision Res 35, 1141-1152.
McFadden, S.A., Wildsoet, C., 2020. The effect of optic nerve section on form deprivation myopia in the guinea pig. J Comp Neurol 528, 2874-2887.
Napper, G.A., Brennan, N.A., Barrington, M., Squires, M.A., Vessey, G.A., Vingrys, A.J., 1995. The duration of normal visual exposure necessary to prevent form deprivation myopia in chicks. Vision Res 35, 1337-1344.
Nickla, D.L., Totonelly, K., 2015. Choroidal thickness predicts ocular growth in normal chicks but not in eyes with experimentally altered growth. Clin Exp Optom 98, 564-570.
Nickla, D.L., Wildsoet, C., Wallman, J., 1998a. The circadian rhythm in intraocular pressure and its relation to diurnal ocular growth changes in chicks. Exp Eye Res 66, 183-193.
Nickla, D.L., Wildsoet, C., Wallman, J., 1998b. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. Exp Eye Res 66, 163-181.
Nickla, D.L., Wildsoet, C.F., Troilo, D., 2001. Endogenous rhythms in axial length and choroidal thickness in chicks: Implications for ocular growth regulation. Invest Ophth Vis Sci 42, 584-588.
Norton, T.T., Essinger, J.A., Mcbrien, N.A., 1994. Lid-Suture Myopia in Tree Shrews with Retinal Ganglion-Cell Blockade. Visual Neurosci 11, 143-153.
Ohngemach, S., Feldkaemper, M., Schaeffel, F., 2001. Pineal control of the dopamine D-2-receptor gene and dopamine release in the retina of the chicken and their possible relation to growth rhythms of the eye. J Pineal Res 31, 145-154.
Prescott, N.B., Wathes, C.M., Jarvis, J.R., 2003. Light, vision and the welfare of poultry. Anim Welfare 12, 269-288.
Rabin, J., Vansluyters, R.C., Malach, R., 1981. Emmetropization - a Vision-Dependent Phenomenon. Invest Ophth Vis

Sci 20, 561-564.
Reiner, A., Karten, H.J., Gamlin, P.D.R., Erichsen, J.T., 1983. Parasympathetic Ocular Control - Functional Subdivisions and Circuitry of the Avian Nucleus of Edinger-Westphal. Trends Neurosci 6, 140-145.
Rucker, F.J., Wallman, J., 2008. Cone signals for spectacle-lens compensation: Differential responses to short and long wavelengths. Vision Res 48, 1980-1991.
Schaeffel, F., Glasser, A., Howland, H.C., 1988. Accommodation, Refractive Error and Eye Growth in Chickens. Vision Res 28, 639-&.
Schaeffel, F., Howland, H.C., 1991. Properties of the Feedback Loops Controlling Eye Growth and Refractive State in the Chicken. Vision Res 31, 717-734.
Schmid, K.L., Wildsoet, C.F., 1996. Effects on the compensatory responses to positive and negative lenses of intermittent lens wear and ciliary nerve section in chicks. Vision Res 36, 1023-1036.
Shaikh, A.W., Siegwart, J.T., Norton, T.T., 1999. Effect of interrupted lens wear on compensation for a minus lens in tree shrews. Optometry Vision Sci 76, 308-315.
Shen, W., Sivak, J.G., 2007. Eyes of a lower vertebrate are susceptible to the visual environment. Invest Ophth Vis Sci 48, 4829-4837.
Shen, W., Vijayan, M., Sivak, J.G., 2005. Inducing form-deprivation myopia in fish. Invest Ophth Vis Sci 46, 1797-1803.
Shih, Y.F., Fitzgerald, M.E., Norton, T.T., Gamlin, P.D., Hodos, W., Reiner, A., 1993a. Reduction in choroidal blood flow occurs in chicks wearing goggles that induce eye growth toward myopia. Curr Eye Res 12, 219-227.
Shih, Y.F., Fitzgerald, M.E.C., Norton, T.T., Gamlin, P.D.R., Hodos, W., Reiner, A., 1993b. Reduction in Choroidal Blood-Flow Occurs in Chicks Wearing Goggles That Induce Eye Growth toward Myopia. Curr Eye Res 12, 219-227.
Sivak, J.G., Barrie, D.L., Weerheim, J.A., 1989. Bilateral Experimental Myopia in Chicks. Optometry Vision Sci 66, 854-858.
Smith, E.L., Harwerth, R.S., Crawford, M.L.J., Vonnoorden, G.K., 1987. Observations on the Effects of Form Deprivation on the Refractive Status of the Monkey. Invest Ophth Vis Sci 28, 1236-1245.
Smith, E.L., Hung, L.F., Kee, C.S., Qiao, Y., 2002. Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys. Invest Ophth Vis Sci 43, 291-299.
Thibos, L.N., Wheeler, W., Horner, D., 1997. Power vectors: an application of Fourier analysis to the description and statistical analysis of refractive error. Optom Vis Sci 74, 367-375.
Thomson, K., Karouta, C., Ashby, R., 2020. Form-Deprivation and Lens-Induced Myopia Are Similarly Affected by Pharmacological Manipulation of the Dopaminergic System in Chicks. Invest Ophth Vis Sci 61.
Tkatchenko, T.V., Shen, Y.M., Tkatchenko, A.V., 2010. Mouse Experimental Myopia Has Features of Primate Myopia. Invest Ophth Vis Sci 51, 1297-1303.
Troilo, D., Gottlieb, M.D., Wallman, J., 1987. Visual Deprivation Causes Myopia in Chicks with Optic-Nerve Section. Curr Eye Res 6, 993-999.
Troilo, D., Judge, S.J., 1993. Ocular Development and Visual Deprivation Myopia in the Common Marmoset (Callithrix-Jacchus). Vision Res 33, 1311-1324.

Troilo, D., Li, T., Glasser, A., Howland, H.C., 1995. Differences in eye growth and the response to visual deprivation in different strains of chicken. Vision Res 35, 1211-1216.

Troilo, D., Nickla, D.L., Wildsoet, C.F., 2000. Choroidal thickness changes during altered eye growth and refractive state in a primate. Invest Ophth Vis Sci 41, 1249-1258.

Troilo, D., Smith, E.L., Nickla, D.L., Ashby, R., Tkatchenko, A.V., Ostrin, L.A., Gawne, T.J., Pardue, M.T., Summers, J.A., Kee, C.S., Schroedl, F., Wahl, S., Jones, L., 2019. IMI - Report on Experimental Models of Emmetropization and Myopia. Invest Ophth Vis Sci 60, M31-M88.

Vyas, S.A., Kee, C.S., 2021. Early Astigmatism Can Alter Myopia Development in Chickens. Invest Ophth Vis Sci 62.

Wallman, J., Turkel, J., Trachtman, J., 1978a. Extreme myopia produced by modest change in early visual experience. Science 201, 1249-1251.

Wallman, J., Turkel, J., Trachtman, J., 1978b. Extreme Myopia Produced by Modest Change in Early Visual Experience. Science 201, 1249-1251.

Wallman, J., Wildsoet, C., Xu, A.M., Gottlieb, M.D., Nickla, D.L., Marran, L., Krebs, W., Christensen, A.M., 1995. Moving the Retina - Choroidal Modulation of Refractive State. Vision Res 35, 37-50.

Wiesel, T.N., Raviola, E., 1977. Myopia and Eye Enlargement after Neonatal Lid Fusion in Monkeys. Nature 266, 66-68.

Wildsoet, C., Wallman, J., 1995. Choroidal and Scleral Mechanisms of Compensation for Spectacle Lenses in Chicks. Vision Res 35, 1175-1194.

Wildsoet, C.F., Pettigrew, J.D., 1988. Experimental Myopia and Anomalous Eye Growth-Patterns Unaffected by Optic-Nerve Section in Chickens - Evidence for Local-Control of Eye Growth. Clin Vision Sci 3, 99-107.

Yan, F., Wang, C., Wilson, J.A., O'Connell, M., Ton, S., Davidson, N., Sibichan, M., Chambers, K., Ahmed, A., Summers, J., Tang, Q.G., 2021. Visually guided chick ocular length and structural thickness variations assessed by swept-source optical coherence tomography. Biomedical Optics Express 12, 6864-6881.

Zhu, X.Y., McFadden, S.A., 2017. Interactions between paired eyes during normal growth and lens compensation in chicks. Invest Ophth Vis Sci 58.

Zhu, X.Y., Wallman, J., 2009. Temporal Properties of Compensation for Positive and Negative Spectacle Lenses in Chicks. Invest Ophth Vis Sci 50, 37-46.

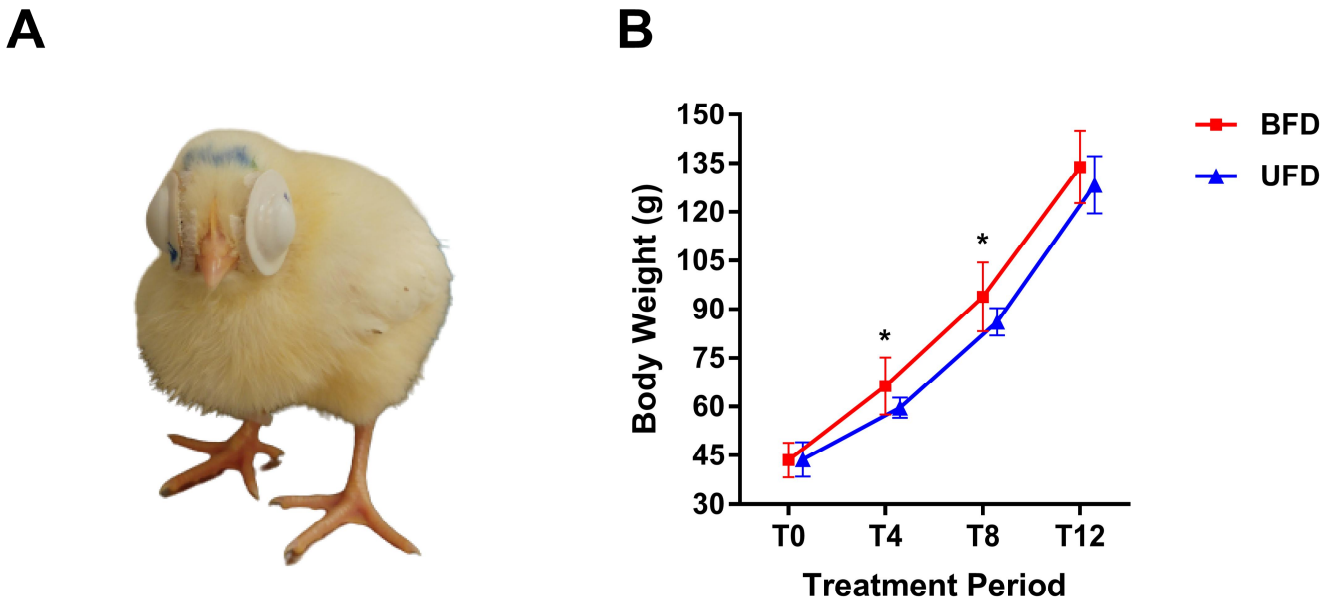
Figure Legends

Figure 1. (A) A chick wearing full-field diffusers bilaterally. (B) Body weight of chicks treated with bilateral (BFD; *Red* symbols) and unilateral form-deprivation (UFD; *Blue* symbols) from T0 (baseline; post-hatching day 6) to T12. Two-way ANOVA with Bonferroni-adjusted pairwise comparisons: * $p < 0.05$. Symbols and error bars represent the mean and standard deviations, respectively.

Figure 2. Longitudinal changes in refractive and ocular biometric parameters from baseline (i.e., Follow-up – Baseline) for chicks receiving bilateral (BFD; n = 21) and unilateral form deprivation (UFD; n = 10): (A) Spherical-equivalent refractive error, (B) corneal power, (C) central corneal thickness, (D) anterior chamber depth, (E) lens thickness, (F) vitreous chamber depth, (G) axial length, (H) retinal thickness, (I) choroidal thickness, and (J) scleral thickness. *Red* symbols: right eyes of chicks in the BFD group (BFD_R); *Blue* symbols: left eyes of chicks in the BFD group (BFD_L); *Green* symbols: right eyes of chicks in the UFD group (UFD_R); *Black* symbols: contralateral untreated control eyes of chicks in the UFD group (UFD_L). Two-way ANOVA with Bonferroni-adjusted pairwise comparisons (UFD_R vs. UFD_L): * $p < 0.05$, *** $p < 0.001$. Symbols and error bars represent the mean and standard deviations, respectively.

Figure 3. Correlations of ocular biometric parameters between right and left eyes of BFD treated chicks measured at different time points: (A) Spherical-equivalent refractive error **(SE)**, (B) corneal power **(CP)**, (C) central corneal thickness **(CCT)**, (D) anterior chamber depth **(ACD)**, (E) lens thickness **(LT)**, (F) vitreous chamber depth **(VCD)**, (G) axial length **(AXL)**, (H) retinal thickness **(RT)**, (I) choroidal thickness **(CT)**, and (J) scleral thickness **(ST)**. *Black* symbols: T0 (baseline); *Red* symbols: T4; *Green* symbols: T8; *Blue* symbols: T12. Pearson's correlations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

539 **Figure 1**



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542 **Figure 2**

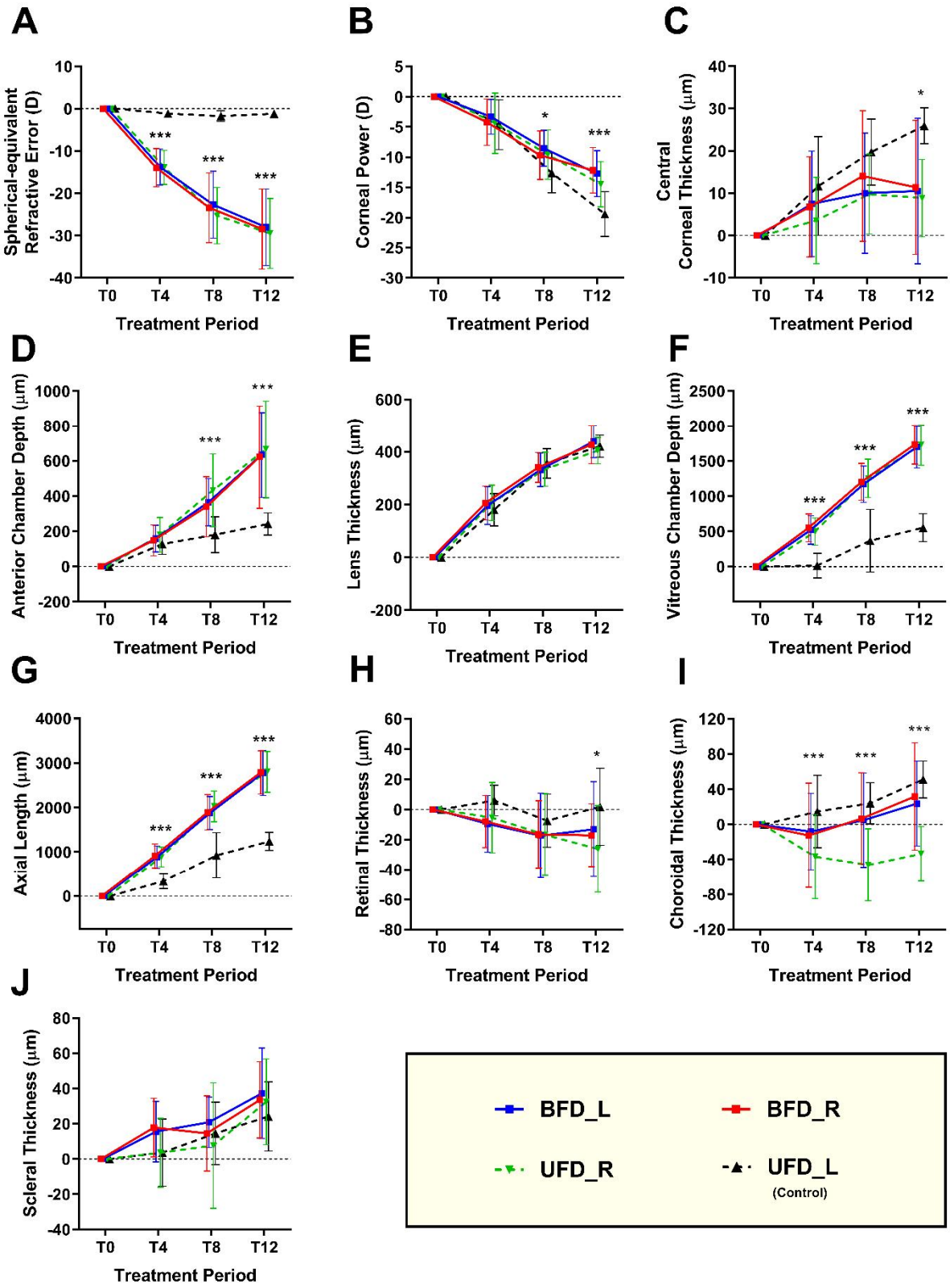

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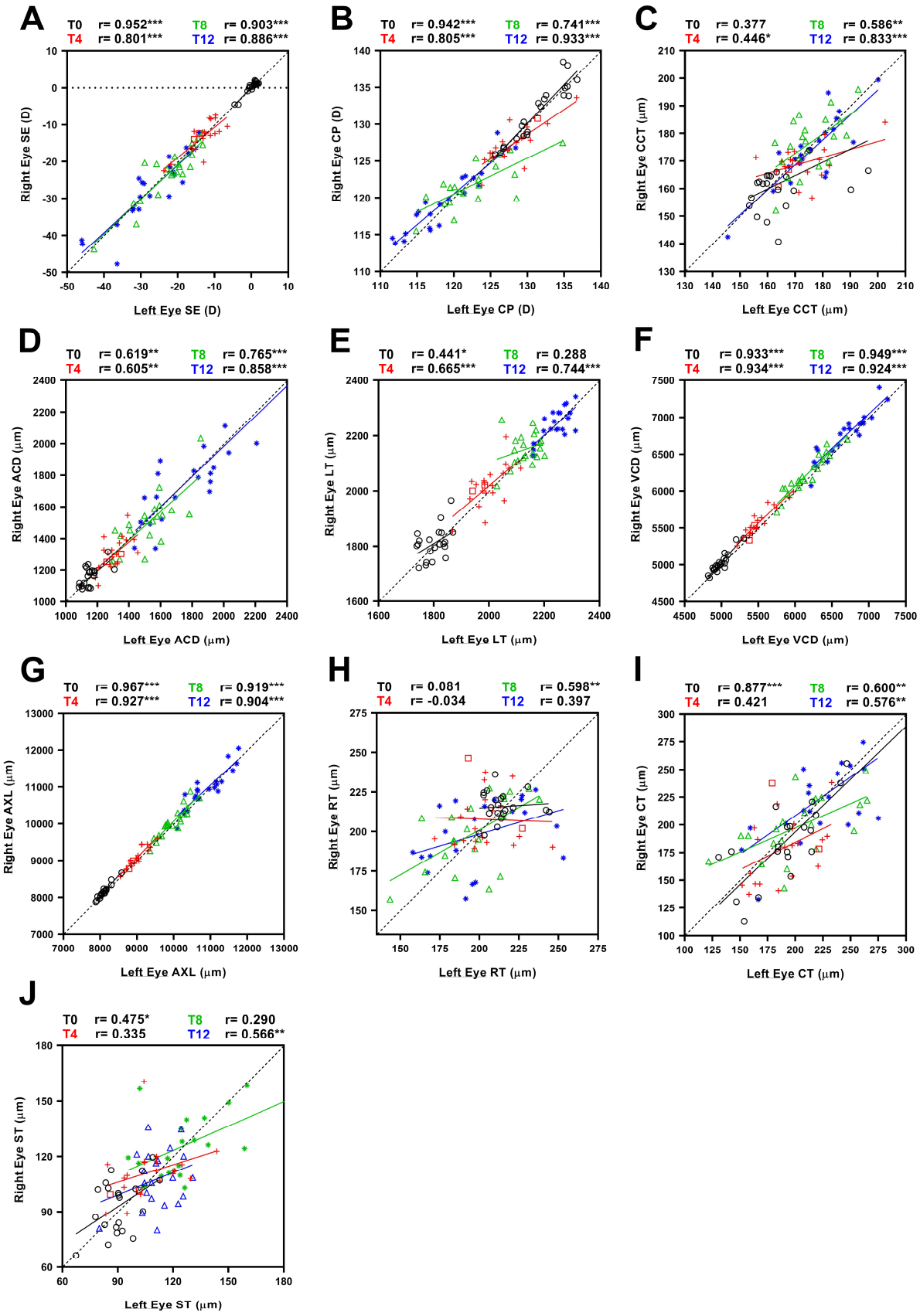
Figure 3


A
T0 r= 0.952*** T8 r= 0.903***
T4 r= 0.801*** T12 r= 0.886***
Right Eye SE (D)
Left Eye SE (D)
B
T0 r= 0.942*** T8 r= 0.741***
T4 r= 0.805*** T12 r= 0.933***
Right Eye CP (D)
Left Eye CP (D)
C
T0 r= 0.377 T8 r= 0.586**
T4 r= 0.446* T12 r= 0.833***
Right Eye CCT (µm)
Left Eye CCT (µm)
D
T0 r= 0.619** T8 r= 0.765***
T4 r= 0.605** T12 r= 0.858***
Right Eye ACD (µm)
Left Eye ACD (µm)
E
T0 r= 0.441* T8 r= 0.288
T4 r= 0.665*** T12 r= 0.744***
Right Eye LT (µm)
Left Eye LT (µm)
F
T0 r= 0.933*** T8 r= 0.949***
T4 r= 0.934*** T12 r= 0.924***
Right Eye VCD (µm)
Left Eye VCD (µm)
G
T0 r= 0.967*** T8 r= 0.919***
T4 r= 0.927*** T12 r= 0.904***
Right Eye AXL (µm)
Left Eye AXL (µm)
H
T0 r= 0.081 T8 r= 0.598**
T4 r= -0.034 T12 r= 0.397
Right Eye RT (µm)
Left Eye RT (µm)
I
T0 r= 0.877*** T8 r= 0.600**
T4 r= 0.421 T12 r= 0.576**
Right Eye CT (µm)
Left Eye CT (µm)
J
T0 r= 0.475* T8 r= 0.290
T4 r= 0.335 T12 r= 0.566**
Right Eye ST (µm)
Left Eye ST (µm)