EFFECTS OF DIETARY L-ARGININE ON OCULAR DIMENSIONS IN CHICKENS

A thesis presented to the graduate faculty of New England College of Optometry in partial fulfillment of the requirements for the degree of Master of Science.

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This manuscript has been read and accepted by the Thesis Committee in satisfaction of the thesis requirement for the degree of Master of Science

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Abstract

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New England College of Optometry, 2023

Purpose:

L-arginine is a pre-cursor to nitric oxide easily found in green leafy vegetables, and beetroot. In animal models nitric oxide has been shown to modulate choroidal thickness, resulting in alterations of axial growth. In this study, we aim to explore the effects of dietary L-arginine on the ocular dimensions in chicks exposed to lens-induced myopia, hyperopia, and form deprivation with high frequency A-scan ultrasound.

Methods:

Two-week old White Leghorn chicks (Gallus gallus domesticus) were divided into two groups and fed either 5% total arginine food, or regular chicken chow S-G supplied by TestDiet. In all experiments, the right eye was treated as the experimental eye and the left eye was untreated and utilized as the control eye. -10D, +10D, and frosted diffuser lenses were mounted on Velcro rings and attached to the right eye. Changes in ocular dimensions were measured using high frequency A-scan ultrasound. Refraction was measured in the untreated group using a Hartinger refractometer. Statistical analyses between groups used two-sample paired T tests (alpha level = 0.05). Inter-ocular differences were analyzed with one-sample paired T tests. Similar data analyses were applied to anterior chamber depth, vitreous chamber depth, choroid thickness, and axial length differences.

Results:

Birds exposed to L-arginine displayed transient choroidal thickening between days 1-14, and followed by rapid choroidal thinning ($p<0.05$).

Conclusions:

L-arginine is found to transiently thicken the choroids of otherwise untreated eyes, and result in subsequent axial growth inhibition mainly through the inhibition of anterior chamber growth. In recovering eyes, axial growth was inhibited relative to (no drug) controls, but there was no effect on fellow eyes. There was also no effect on choroidal thickness. In contrast, there was no effect on axial growth in either negative or positive lens-wearing eyes, and no effect on choroidal thickness. However, an interesting yoking phenomenon was witnessed in the fellow eyes. The fellow eyes in the experimental negative lens group showed growth stimulation relative to no-drug fellow eyes, while the opposite was true for the positive lens group.

Acknowledgements

This endeavor would not have been possible without my graduate faculty advisor, Dr. Nickla. I would like to thank her for her patience, encouragement, and feedback throughout this process. She's an incredible mentor and I'm extremely grateful to have been able to work on this project under her guidance. I'm also deeply grateful to Dr. Panorgias for his compassion and encouragement throughout this program. I would not have been able to finish this project without his support. Thank you for believing in me.

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1. Introduction

1.1 Myopia: A Global Epidemic

Myopia is a global public health problem affecting about 30% of the population in the United States and as high as 80% in East Asia (Pan et al.; 2012). It is projected to affect 4.758 billion people worldwide by 2050 (Holden et al, 2016). Additionally, most individuals develop myopia during childhood (Morgan et al., 2012). This early onset of myopia usually results in higher degrees of myopia in life (Chua et al., 2016). As the severity of myopia increases, excessive axial elongation of the eye increases the risk of pathological changes such as staphyloma, optic disc changes, and myopic macular degeneration (MMD) (Saw et al., 2005). The complications of MMD can include chorio-retinal atrophy, lacquer cracks, choroidal neovascularization (CNV), fuchs spot, and tessellated fundus (Spaide et al., 2014). Despite the tremendous global impact of myopia, its etiology is still unclear.

It is well understood that myopia is a state of refraction in which parallel rays of light are brought into focus in front of the retina (Curtin, 1985). Many factors have been implicated to increase the risk of myopia incidence and progression. These factors are both genetic and environmental and include family history, socio-economic factors, and near-work and outdoor activity (Foster and Jiang, 2014). A strong association has been shown between the number of myopic parents and the increased prevalence of myopia in children (Ip et al., 2007). Compared with children without myopic parents, those with one myopic parent are twice as likely to be myopic, and those with two parents myopic are three times more likely to by myopic themselves (Xiang et al., 2012). Several population-based prevalence studies have also showed increased prevalence of myopia in households with higher levels of

education, better housing, and higher individual monthly income (Wong et al., 2003). However, this correlation is debated. Many studies believe increased near work and less time outdoors to be the true cause of the increased rate of myopia seen in households with higher levels of education (Pan et al., 2012). Near-work activities, such as reading, writing, computer use, and playing video games may be responsible for the remarkable increase in the prevalence of myopia (Pan et al., 2012). A cohort study in Australian school-children showed that those with incident myopia performed significantly more near work (French et al., 2013). On the other hand, several recent epidemiological studies suggest greater time spent outdoors may be associated with reduced prevalence of myopia (Rose et al., 2008). Given the increasing prevalence, economic impact, and associated risks it's important to investigate different modes of myopia treatment.

Current therapeutic efforts to reduce myopia progression have limited success. Treatments utilize optical control such as spectacles or contact lenses, specialty contact lenses such as orthokeratology, and pharmacological treatments such as atropine and 7-methylxanthine (Vasudevan et al, 2014). Additionally, there have been efforts to increase time outdoors in children to protect against the onset of myopia (Vasudevan et al, 2014). Unfortunately, the mechanisms behind these methods and emmetropization are not completely understood.

1.2 Emmetropization in the Chick Model

Emmetropization refers to the developmental process that matches the eye's optical power to its axial length so that the unaccommodated eye is focused at distance (Troilo et al., 2019). From an evolutionary perspective, this adjustment of neo-natal refractive error to yield good vision is advantageous. Therefore, we expect similar mechanisms to exist in not only humans, but other species as well. Emmetropization has been extensively studied in various animal models including chicks, guinea pigs, cats, and monkeys (Troilo et al., 2019). Through these animal models researchers have established the existence of visual regulation of eye growth and refractive development as well as local retinal control of eye growth (Troilo et al., 2019). Additionally, they have also revealed various biochemical signaling cascades within the retina which signal changes to the retinal pigment epithelium (RPE), choroid, and eventually sclera, resulting in altered eye growth and changes in refractive states (Troilo et al., 2019). The findings of these animal studies help provide us with a framework for development of optical and pharmacologic treatments to effectively reduce the prevalence and progression of myopia.

Similar to the eyes of primates and humans, chick neonates often exhibit substantial hyperopic errors that over time develop in such a manner to decrease the degree of hyperopia (Troilo et al., 2019). Chick eyes also have the capability of developing an environmentally induced myopia (Wallman et al., 1978). This is a phenomenon that parallels the myopia hypothesis in humans. The prior qualities in addition to high availability, low cost, and rapid response to experimental manipulation allow chicks to be a primary animal model in emmetropization research.

1.3 Regulation of Ocular Growth

The ability to induce refractive error in animal models while having control over many environmental conditions provides a unique opportunity to study emmetropization. Several techniques have been utilized to obtain a better understanding of emmetropization. Some of the most notable experiments involve plus and minus lens compensation, and form deprivation experiments in chicks. These experiments provide strong evidence for active matching of axial length to the focal plane during the emmetropization process. Placing a concave (minus-power) lens in front of an emmetropic eye shifts the focal plane posteriorly to be behind the retina creating hyperopic defocus. When a minus lens is placed in front of the eyes of a normal developing chick, this produces a compensatory increase in the axial elongation of the eye (Wallman et al., 1978). Over a period of days, the axial length of the treated eye increases until the retinal location has shifted by an amount that approximately matches the shift of the focal plane (Schaeffel et al., 1990). Compensation to a minus power lens is accurate, and quite rapid in chicks under 2 weeks of age (Schaeffel et al., 1990). On the other hand, placing a convex (plus-power) lens in front of an emmetropic eye shifts the focal plane towards the plane of the cornea creating myopic defocus. When a plus lens is placed in front of the eyes of a normal developing chick, this produces a decrease in the axial elongation rate (Wallman et al., 1978). As lens wear continues, the axial length growth of the eye slows resulting in a shorter than normal eye and hyperopia when the lens is removed (Schaeffel et al., 1990). In addition to the above lens induced techniques, another common experiment is form deprivation. In form deprivation experiments, visual images are obscured either by surgical restriction of the eye or the use of translucent diffusers (Norton, 1990). In

these experiments, animals consistently developed myopia, however, the amount of myopia varies between individual animals (Schaeffel et al., 1990). Through these experiments, it's evident that the visual environment plays an active role in the precise regulation of axial length. However, the specific neural pathways and the retinal neurons critically involved are not fully understood.

Additional experiments have further investigated the biomechanical mechanisms behind these axial length changes. It is hypothesized that the mechanism for axial length elongation is both retinal mediated and spatially local. In previous studies where the ganglion cell output to central structures is disrupted either surgically via optic nerve section, or functionally, the eyes of chicks, can continue to detect the presence of form deprivation and elongate to become myopic (Troilo et al., 1987). Therefore, it's believed axial elongation signals must be of retinal origin. The signals must reach the sclera without leaving the eye. This locally-mediated process is believed to include a series of steps in which the visual signals from the retina travel through the retinal pigment epithelium (RPE), to the choroid, and eventually the sclera. In addition to being retinal-mediated, the mechanism is also spatially local. Chicks exposed to translucent diffusers or to minus lenses that cover only half of the visual field, leaving the other half unaffected, become elongated and myopic in only the treated hemifield (Diether and Schaeffel, 1997). Although it is not fully understood how these mechanisms are regulated at a molecular level, it's evident that the signals are influenced by circadian rhythms, dopaminergic systems, and various other biochemical systems within the retina and choroid (Devadas and Morgan, 1996; Gottlieb et al., 1992; Nickla et al., 1998; Weiss and Schaeffel, 1993; Bartmann et al., 1994; Schaeffel et al., 1994).

1.4 The Role of the Choroid

The choroid of the eye is primarily a vascular structure supplying the outer retina with oxygen and nutrients. It's hypothesized to play a pivotal role in the regulation of the emmetropization pathway. It is known that chick eyes are capable of swift compensation to lens wear. This compensatory response is due to changes in two components, ocular length, and choroidal thickness (Wildsoet and Wallman, 1995). The ocular length changes mediated by the scleral growth response is much slower compared to the choroidal response which occurs within several hours of lens wear (Nickla and Wildsoet, 2004). When a convex (plus-power) lens is placed in front of a normal developing chick eye to produce lens-induced myopic defocus, the choroid immediately thickens to bring the retina closer to the focal plane (Wildsoet and Wallman, 1995). Additionally, the eyes exposed to myopic defocus experience a decrease in axial length elongation rate (Nickla et al., 2001). In contrast, when a concave (minus-power) lens is placed in front of the eye to produce lens-induced hyperopia, the choroid immediately thins to reduce the degree of defocus (Wildsoet and Wallman, 1995). Additionally, the eyes exposed to hyperopic defocus experience an increase in axial length elongation rate (Nickla et al., 2001). Hence, thick choroids are associated with slower growing eyes and thin ones with faster growing eyes, suggesting that perhaps choroidal thickness might be mechanistically-associated with growth changes. Both responses are local, in that if only one half of the retina experiences defocus, only the choroid underlying that part will thicken or thin (Nickla and Wallman, 2010). The mechanisms underlying these choroidal thickness changes are currently being researched. Current hypotheses include changes in one or several of the following: choroidal proteoglycan synthesis and hydration,

capillary fenestration, fluid exchange between the anterior chamber and uvea, choroidal blood flow, or the tonus of non-vascular smooth muscle (Nickla and Wildsoet, 2004). The gaseous messenger molecule nitric oxide is suspected to be involved in this biochemical pathway due to its rapid action and ubiquitous presence within the retina (Nickla and Wildsoet, 2004).

1.5 The Role of the Nitric Oxide

Nitric oxide (NO) is a freely diffusible gaseous messenger molecule known for its vasodilatory effects on vascular smooth muscle through stimulation of guanylyl cyclase and cGMP production (Nickla and Wildsoet, 2004). In the body, it's synthesized by many cell types from its pre-cursor L-arginine (Neilly et al., 1994). This reaction to form NO requires L-arginine and oxygen, and is catalyzed by the enzyme nitric oxide synthase (NOS) (Flam et al., 2007). In mammals and birds, three isoforms of NOS exist: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Shepherd et al., 2022; Nilsson, 1996). These isoforms are responsible for catalyzing NO formation in response to various external and internal stimuli such as stress, immune, or inflammatory processes (Shepherd et al., 2022). nNOS and eNOS are known as the constitutive forms assigned to produce a steady-state NO level whereas iNOS is regulated in response to an inflammatory signal (Forstermann and Sessa, 2012). The production of NO can also be promoted through oral supplementation of nitrate found in green leafy vegetables and beetroot or L-arginine

supplement formulations and subsequent conversion through the L-arginine/nitric oxide pathway (Neilly et al., 2010; Lidder and Webb, 2012).

In the eye NO is produced by many different cell types including amacrine cells, ganglion cells, Muller cells, and cells in the outer and inner plexiform layers (Fischer and Stell, 1999; Goldstein et al., 1996). Its synthesis is regulated by various neurotransmitters, and illumination (Vielma et al., 2014; Fujii et al., 1998). It's also found in many structures including the retinal pigment epithelium (RPE), choroid, sclera, and possibly ciliary ganglion (Nickla and Wildsoet, 2004; Goldstein et al., 1996; Sun et al., 1994). The presence of NO among numerous cell types and structures suggest NO modulates multiple ocular functions. NO also appears to play a vital role in the vasodilation of many tissues such as the iris, ciliary body, and choroid (Nilsson, 1996; Goldstein et al., 1996). In the choroid, NO regulation of blood flow is mediated by its enzyme nitric oxide synthase (NOS) released by the vessel endothelium and neural NOS released by the parasympathetic neurons (Nickla and Wildsoet, 2004; Goldstein et al., 1996). Additionally, recent studies in both birds and primates, have shown existence of clusters of NO-sensitive neurons in the choroid with unknown function (Nickla and Wildsoet, 2004). These non-vascular NO-sensitive structures suggest NO has additional function in the choroid unrelated to vasodilation (Nickla and Wildsoet, 2004).

Early studies utilizing nitric oxide synthase (NOS) inhibitor L-NAME have shown that reduction of NO prevents choroidal thickening in myopic defocus eyes, resulting in a disinhibition of axial growth (Nickla and Wallman, 2010). This effect is transient, lasting about 24 hours, and dose dependent (Nickla and Wildsoet, 2004). In these experiments, myopic defocus was induced in chicks using two different paradigms: form deprivation, or

positive lens (Nickla and Wildsoet, 2004). The chicks were then either injected with L-NAME, a non-specific NOS inhibitor, or saline (Nickla and Wildsoet, 2004). A-Scan ultrasound measurements were then taken at various time intervals post-injection (Nickla and Wildsoet, 2004). The experiment found that L-NAME injected eyes experienced very little choroidal thickening relative to saline injected eyed (Nickla and Wildsoet, 2004). Effectively, L-NAME inhibited the choroidal thickening response. Additionally, L-NAME injected eyes experienced a much greater increase in axial growth than saline injected eyes (Nickla and Wildsoet, 2004). The same effect is also seen with another non-specific NOS inhibitor, L-NMMA (Nickla et al., 2009). Additionally, if the NOS inhibitor was injected several hours after the defocus-induced thickening of the choroid had taken place, there was no effect on the rate of ocular elongation, linking the choroidal and axial responses (Nickla et al., 2006). Interestingly, if L-NAME was injected prior to giving form-deprived eyes brief daily periods of unrestricted vision over the course of several days preventing the transient daily increase in choroid thickness, the eyes did develop axial myopia (Nickla et al., 2006). The close temporal association between the effect of L-NAME on choroidal thickening and its subsequent effect on ocular growth would suggest that either NO participates in the choroidal thickening, resulting in inhibition of ocular growth, or that NO participates in the retinal processing of myopic defocus (Nickla and Wallman, 2010).

1.6 Purpose

The purpose of this project is to study the effects of dietary L-arginine on both ocular dimension and growth in chicks exposed to lens-induced myopia, hyperopia, and form deprivation. Specifically, it will determine if a significant choroidal and axial response can be induced through oral administration of a nitric oxide precursor. We further hypothesize choroidal thickening is part of a signaling cascade mediating ocular growth inhibition. Another aim of this study is to determine whether the regulatory mechanism involving NO may differ between the two forms of induced myopia – negative lens induced and form deprivation. If dietary-administered pre-cursors to NO are effective, this would be much less invasive than intravitreal injections and may lead to new therapies for myopia in humans.

2. Methods

2.1 Animals

White Leghorn chickens (Gallus gallus domesticus) were hatched in an incubator and raised in temperature-controlled brooders. The light cycle used was 12L/12D for all experiments meaning the chicks were exposed to 12 hours of light, followed by 12 hours of darkness. Food and water were supplied ad libitum. All experiments started at age 10-12 days. The chicks were randomly selected, and in experiments using ocular devices (lens or diffuser), the right eye was treated as the experimental eye and the contralateral left eye was the untreated control eye. The purpose of the experiments was to evaluate the effects on ocular growth parameters of supplemental dietary L-Arginine, hence experimental groups

were fed a diet with 5% total L-arginine (supplied by TestDiet; Richmond, IN) while control groups were fed the standard chicken chow S-G (supplied by TestDiet; Richmond, IN) throughout the entire experiment. This concentration of L-arginine selected was the only concentration available.

Ocular dimensions were measured using high frequency (30 MHz) A-scan ultrasound at the start of the experiment and at various intervals after (see below). Chicks were anesthetized with inhalation of 1.5% isoflurane in oxygen for all measurements. For each measurement, the animals were placed in an adjustable multi-axis holder with their heads in an upright position. A lid retractor was used to keep one eye open at a time. The internal ocular dimensions measured using this technique were anterior chamber depth, lens thickness, vitreous chamber depth (posterior lens to retinal inner limiting membrane), retina, choroid, and scleral thickness. Figure 1 (Wallman and Wildsoet, 1995) displays a sample A-scan ultrasound trace. The arrows indicate selection of the peaks corresponding to the cornea, anterior lens, posterior lens, retina, choroid, and sclera respectively. The scan to the right shows an expanded view of the posterior structures. Additionally, inbetween each peak, the respective sections are labeled. Axial length is defined as the sum of the components from the cornea to the front of the sclera. The accuracy of these measurements depends on the consistency of the various waveforms and the ability to assign appropriate peaks. It should be noted that chicks were excluded if the lenses were dislodged for more than 16 hours.

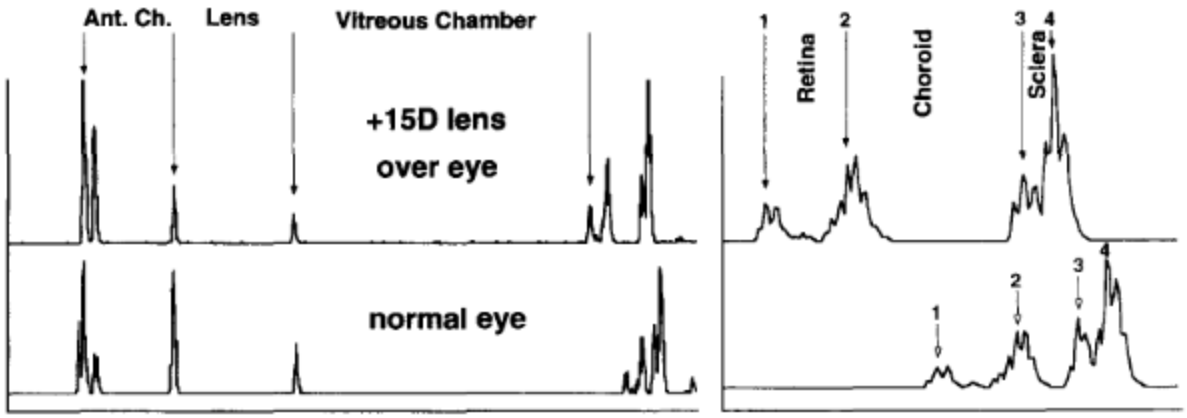

Figure 1: Sample traces of ultrasound biometry, depicting how retinal and choroidal echoes can be clearly distinguished. The trace on the right represents an expanded view of the posterior eye wall. Adapted from **"**Choroidal and Scleral Mechanisms of Compensation for Spectacle Lenses in Chicks," by J. Wallman and C. Wildsoet, 1995, *Vis.Res*. 35, 1175-94.

Refractions were measured using a Hartinger's refractometer (Jena Coincidence Refractometer: Carl Zeiss Meditec, GmbH, Jena, Germany). The chicks were anesthetized and positioned using the same techniques used for ultrasound measurements. All refractive error measurements were conducted without any paralytic of mydriatic ophthalmic drops. However, it should be noted that isofluorane anesthesia has a mild mydriatic effect. Refractive errors of the two principal meridians were measured along the pupillary axis. A set of four consecutive readings were recorded for each eye. These values were converted to Diopters using the following formula (Wallman & Adams, 1987): Diopters (D) = 2.09*(Horizontal or vertical reading)+20. The average of the vertical and horizontal meridians was recorded. The five readings are then averaged and used for analysis. Research

was approved by the NECO Institutional Animal Care and Use Committee and adhered to the ARVO resolution for animal use.

2.2 Protocols

2.2.1 Dietary L-Arginine Effects on Ocular Dimensions in Untreated Eyes

The chicks were divided into experimental and control groups according to the protocol above and observed for 29 days. Ocular dimensions were measured daily starting day 0 using A-scan ultrasonography. Refractions were performed on day 21, and 29 using a Hartinger refractometer (Jena Coincidence Refractometer: Carl Zeiss Meditec, GmbH, Jena, Germany) according to the previously discussed protocol. More frequent measurements were done for this group as it was unclear when the potential effects would be seen. The results of this experiment allowed us to determine measurement periods in the other paradigms. The number of subjects in each condition, ultrasound measurement days measurement days in this section and all subsequent sections will be summarized below in Table 1.

2.2.2 Dietary L-arginine Effects on Ocular Dimensions in the Presence of Translucent Diffuser Induced Form Deprivation

The chicks were divided into experimental and control groups according to the protocol above and observed for 8 days. Each chick was fitted with a translucent diffuser over the right eye using Velcro rings attached to a matching ring glued to the chicken's feathers. Ocular dimensions were measured on days 1, 3, 5, and 8. Recovery occurs when the diffuser is removed on day 5. This removal of the myopia-inducing device results in a more myopic eye which will then compensate by slowing eye growth (Wildsoet and Wallman, 1995). We wanted to determine if dietary administration of L-Arg would affect this process.

2.2.3 Dietary L-arginine Effects on Ocular Dimensions in the Presence of -10D Lens-induced Hyperopic Defocus

The chicks were divided into experimental and control groups according to the protocol above and observed for either 8 or 15 days. Each chick was fitted with a -10D lens over the right eye using Velcro rings attached to a matching ring glued to the chicken's feathers. Ocular dimensions were measured on days 1, 3, 5, and 8 or 1, 3, 5, 10, and 15. Recovery occurs when the lens is removed on either days 8 or 10.

2.2.4 Dietary L-arginine Effects on Ocular Dimensions in the Presence of +10D Lens-induced Myopic Defocus

The chicks were divided into experimental and control groups according to the protocol above and observed for either 8 or 15 days. Each chick was fitted with a +10D lens over the right eye using Velcro rings attached to a matching ring glued to the chicken's feathers. Ocular dimensions were measured on days 2, 3, and 5.

VISUAL MANIPULATION	US Measurement days	
Untreated	Daily d1-d17 (5); day 29 (5)	
	L-ARG	CONTROL
Form Deprivation	D3 (9) **D5** (9) D8 (9)	D3 (8) **D5** (7) D8 (7)
Negative Lenses	D3 (8) D5 (12) D8 (5) **D10** (14) D15 (14)	D3 (12) D5 (15) D8 (9) **D10** (8) D15 (8)
Positive Lenses	D2 (8) D3 (8) D5 (7)	D2 (8) D3 (8) D5 (8)

Table 1: Summary of the number of chicks in each condition during each ultrasound measurement period for all experiments.

2.3 Statistical Analysis

Paired one-sample t-test was used to test the inter-ocular difference between experimental right eyes with lens wear vs control left eyes without lens wear. Unpaired two-sample t-tests were used to compare between groups. The alpha level was set to 0.05. Similar data analyses were applied to anterior chamber depth, vitreous chamber depth, choroid thickness, and axial length differences. Refraction data was calculated using the methods stated previously.

3. Results

3.1 Dietary L-arginine Effects on Ocular Dimensions in Untreated Eyes

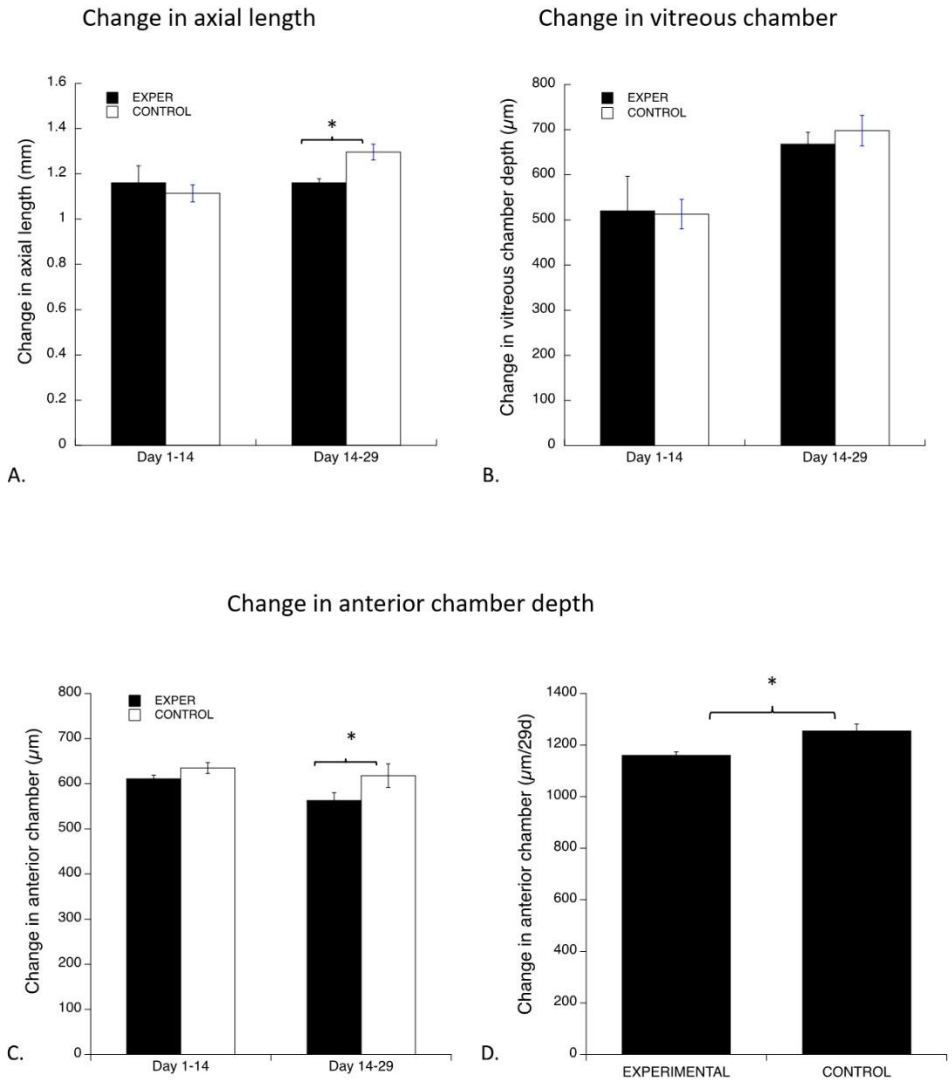

Figure 2: Figure 2A-D summarizes the change in axial parameters in experimental and control eyes without visual manipulations. Figure 2A displays the change in axial length between groups during days 1-14, and 14-29. Figure 2B displays the change in vitreous chamber dimensions between groups during days 1-14, and 14-29. Figure 2C displays the change in anterior chamber depth between groups during days 1-14, and 1-29. Figure 2D displays the change in anterior chamber depth throughout the entire 29 day period. Error bars indicate ± 1 SEM. * denotes statistically significant differences ($p<0.05$).

Axial Effects

While there was no significant difference in ocular elongation (changes in axial length) over the first 2 weeks, there was an inhibition of axial growth over the course of the following 2 weeks (fig. 2A). During days 1-14, experimental eyes experienced a 1.15mm change in axial length growth, while controls experienced a 1.114mm change. During days 14-29, experimental eyes experienced a significant 1.15mm change in axial length, whereas control eyes experienced a 1.296mm change in axial length ($p<0.05$). When looking at vitreous chamber depth (fig. 2B), although the inhibitory trend was apparent, the diifference was not significant. During days 1-14, experimental eyes experienced a 520µm increase, whereas controls experienced a 513 µm increase. During days 14-29, experimental eyes experienced a 668µm increase, whereas controls experienced a 698µm increase.

However, there was a significant inhibition in the growth of the anterior chamber over this period (fig. 2C); this difference was also significant over the entire 4 week period (fig. 2D). During days 1-14 experimental eyes experienced a 611µm increase, whereas controls experienced a 635µm increase. During days 14-29, experimental anterior chambers experienced a 563µm increase, whereas controls experienced a significant 618µm increase ($p<0.05$). Finally, over the entire period, experimental anterior chambers experienced a 1160µm change in depth, whereas controls experienced a significant 1254µm change ($p<0.05$). Therefore, the growth inhibition was mainly a result of changes in the anterior segment of the eye, and not the posterior segment.

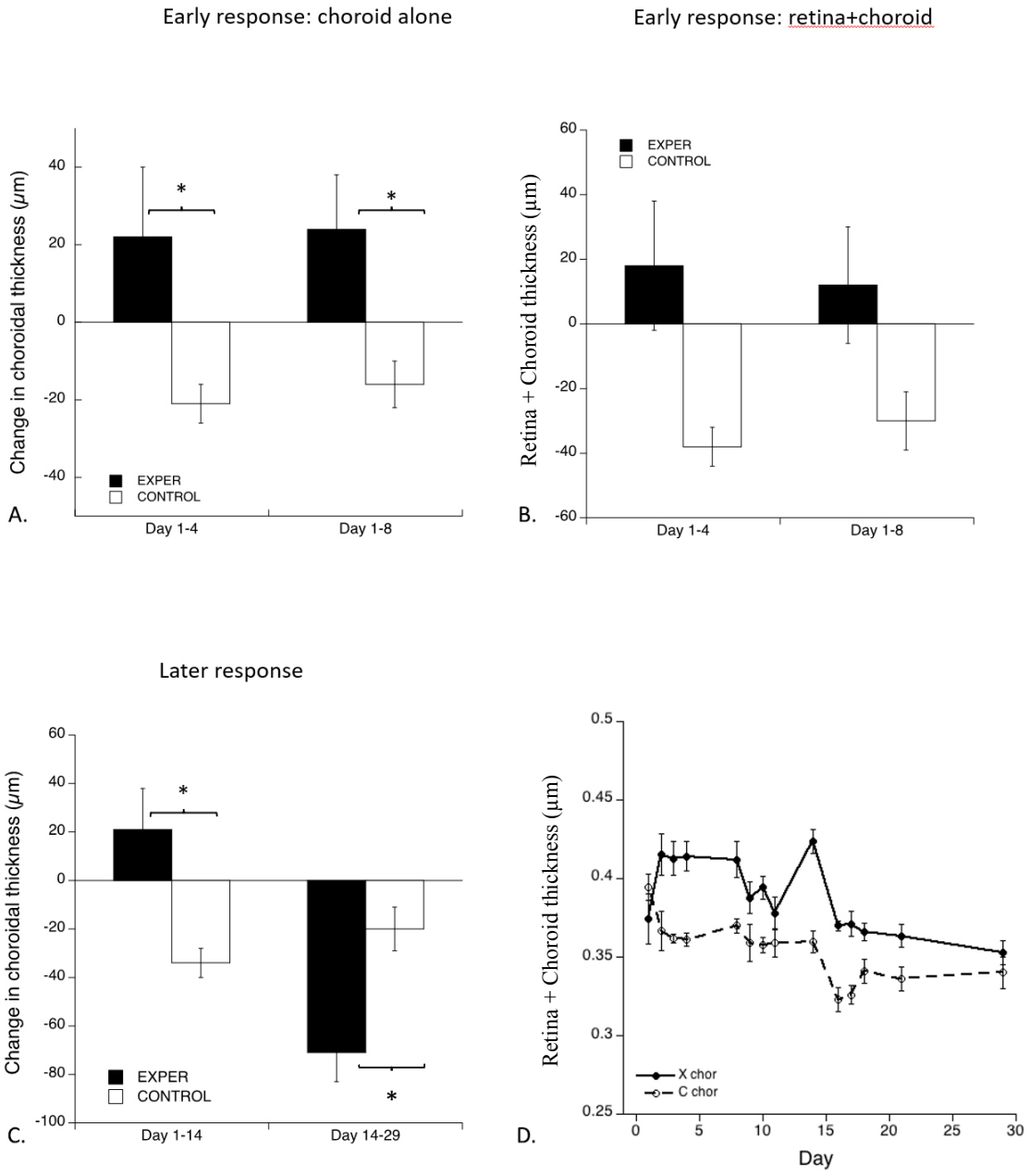

Figure 3: Figure 3A-D summarizes the choroidal response in experimental and control eyes without visual manipulation. Figure 3A displays the change in choroidal thickness between groups during days 1-4, and 1-8. Figure 3B displays the change in thickness of the retina and choroid between groups. Figure 3C displays the change in choroidal thickness between groups during days 1-14, and 14-29. Figure 3D displays the combined retina and choroid thickness between groups throughout the 29 day experiment. Error bars indicate ± 1 SEM. * denotes statistically significant differences ($p<0.05$).

Choroidal Effects

L-arginine resulted in significant choroidal thickening over the initial 2 weeks of treatment (fig. 3). During days 1-4 experimental eyes experienced a significant 22µm increase, whereas controls experienced a -21µm decrease in choroidal thickness (fig. 3A, $p<0.05$). During days 1-8 experimental eyes experienced a significant 24µm increase, whereas controls experienced a -16µm decrease in choroidal thickness (fig. 3A, $p<0.05$). In figure 3C, during days 1-14, experimental eyes experienced a significant 21µm increase, whereas controls experienced a -34µm decrease in choroidal thickness ($p<0.05$).

In figure 3B, during days 1-4 experimental eyes experienced an 18 µm increase, whereas controls experienced a -38µm decrease in retina+choroid thickness. During days 1-8 experimental eyes experienced a 12µm increase, whereas controls experienced a -30µm decrease in retina+choroid thickness. Although, statistical significance was not reached in either period. However, following this period, from day 14-29, experimental choroids thinned relative to those of control choroids. Figure 3D shows the data in longitudinal form.

3.2 Dietary L-arginine Effects on Ocular Dimensions in the Presence of Translucent Diffuser Induced Form Deprivation and Recovery

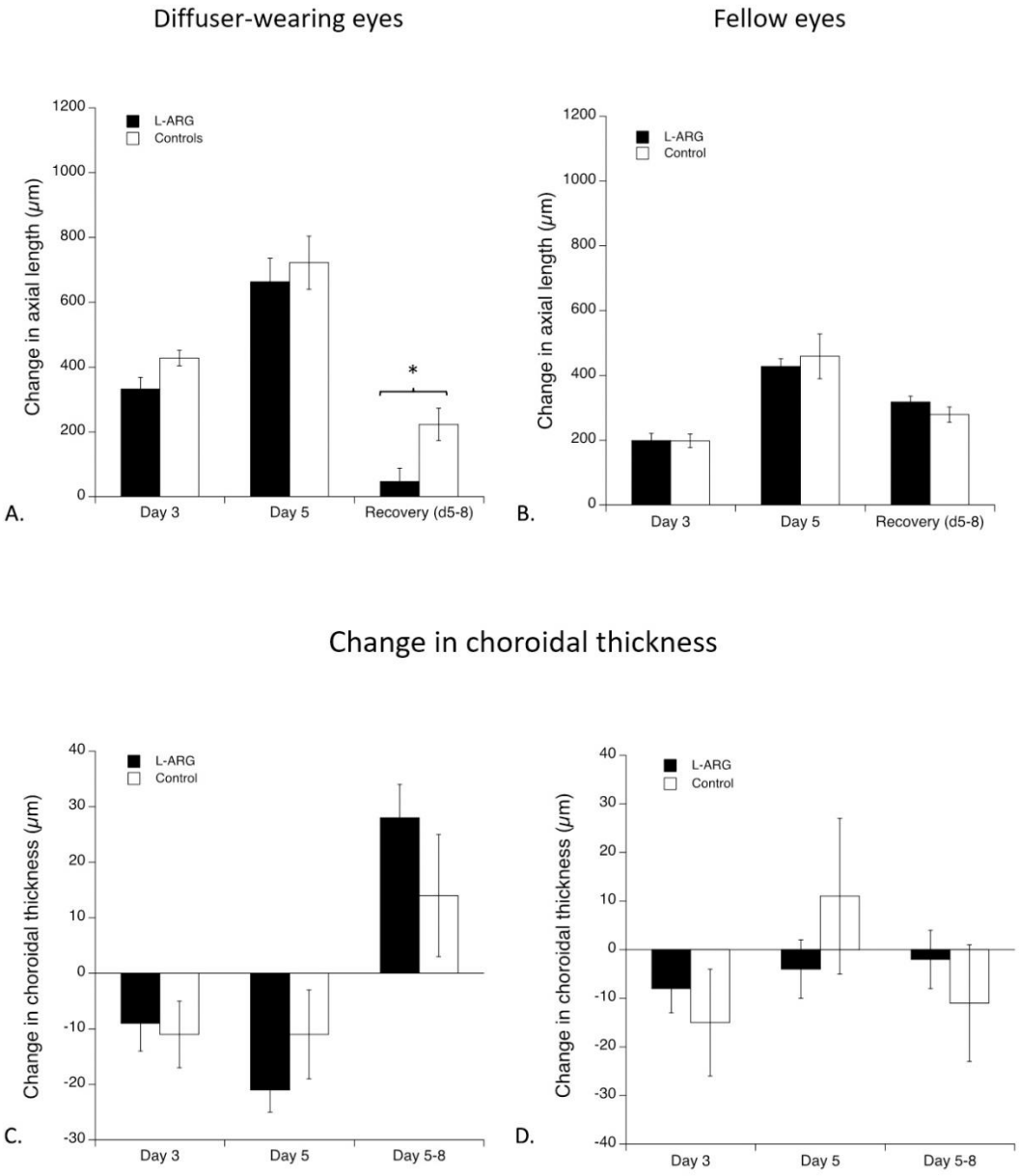

Figure 4: Figure 4A-B summarizes the axial length changes in eyes exposed to form deprivation. Figure 4A displays the axial length change in diffuser-wearing eyes during days 3, 5, and recovery (days 5-8). Figure 4B displays the axial length change in the fellow eyes during days 3, 5, and recovery (days 5-8). Figure 4C-D summarizes the choroidal thickness

changes in eyes exposed to form deprivation. Figure 4C displays the choroidal thickness change in diffuser-wearing eyes during days 3, 5, and recovery (days 5-8). Figure 4D displays the choroidal thickness change in the fellow eyes during days 3, 5, and recovery (days 5-8). Statistical significance was not reached during any period. Error bars indicate ± 1 SEM. * denotes statistically significant differences ($p<0.05$).

Axial Effects

There were no significant effects on ocular elongation during the 5 days of diffuser-wear, however, eye growth was significantly inhibited over the 3 days of recovery (restoration of vision) (fig. 4A, $p<0.05$). There were no significant effects on fellow eyes of either the experiment nor the control groups. In figure 4A, during day 3, control eyes were observed to grow more than experimental eyes. Experimental eyes increased by 332µm, whereas control eyes increased by 428µm. During day 5, the same effect was observed. Experimental eyes grew 663µm, whereas control eyes grew 722µm. During recovery, experimental eyes grew significantly less than control eyes. Experimental eyes experienced a 47µm change in axial length, whereas control eyes experienced a 223µm change ($p<0.05$).

In figure 4B, during day 3, experimental and control eyes grew approximately the same amount. Experimental eyes experienced a 199µm increase, whereas control eyes experienced a 198µm increase. During day 5, experimental eyes grew less than control eyes. Experimental eyes experienced a 428µm increase, whereas control eyes experienced a 459µm increase. During recovery, the effect reversed. Experimental eyes experienced a 318µm increase, whereas controls experienced a 279µm increase.

Choroidal Effects

There were no significant effects on the choroids of form deprived eyes during the wearing of the diffuser (fig. 4C), or during the 3 days of recovery. Neither were there any effects on choroids of fellow eyes. In figure 4C, during day 3, control eyes experienced more thinning than experimental eyes. Experimental eyes experienced a -9µm change in choroidal thickness, whereas controls experienced a -11µm change in choroidal thickness. During day 5, the opposite effect was observed. Experimental eyes experienced a -21µm change in choroidal thickness, whereas controls experienced a -11µm change in choroidal thickness. During day 5-8, both groups displayed choroidal thickening. Experimental eyes experienced a 28µm change in choroidal thickness, and control eyes experienced a 14µm change in choroidal thickness.

In figure 4D, during day 3, experimental eyes experienced less thinning than control eyes. Experimental choroids experienced a -8µm change, whereas control choroids experienced a -15µm change. During day 5, experimental choroids thinned, while control choroids thickened. Experimental choroids experienced a -4µm change, whereas controls choroids experienced a 11µm change. During recovery, both experimental and control choroids thinned again. Experimental choroids experienced a -2µm change, whereas controls experienced a -11µm change. However, statistical significance was not reached during any period.

3.3 Dietary L-arginine Effects on Ocular Dimensions in the Presence of -10D Lens-induced Hyperopic Defocus and Recovery

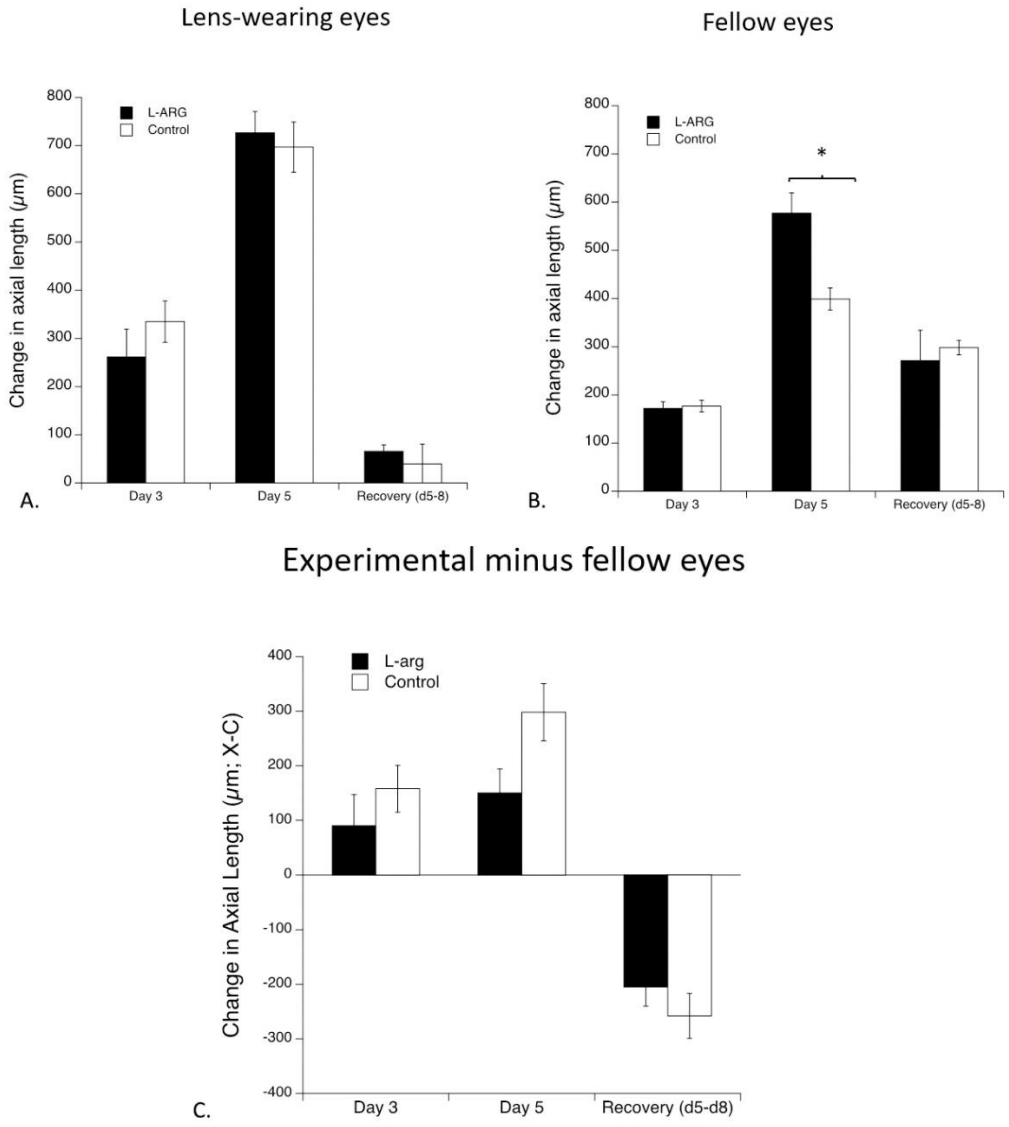

Figure 5: Figure 5A-B summarizes the axial length changes in eyes exposed to negative lens-wear. Figure 5A displays the axial length changes in lens-wearing eyes during days 3, 5, and recovery (days 5-8). Figure 5B displays the axial length change in the fellow eyes during days 3, 5, and recovery (days 5-8). Figure 5C displays net axial length change between

experimental and control fellow eyes during days 3, 5, and recovery (days 5-8). Error bars indicate ± 1 SEM. * denotes statistically significant differences ($p<0.05$).

Axial Effects

There were no significant effects on ocular elongation during the 5 days of lens-wear, nor during the 3 days of recovery (fig 5A). However, surprisingly, we found that in fellow eyes, L-arginine resulted in ocular growth stimulation (fig. 5B) over the first 5 days of treatment. There was no effect over the course of the 3 days of recovery.

In figure 5A, during day 3, control eyes grew more than experimental eyes. Experimental eyes experienced a 262µm increase, while control eyes experienced a 335µm increase. This trend reversed during day 5. Experimental eyes grew more than control eyes. Experimental eyes experienced a 727µm increase, whereas control eyes experienced a 697µm increase. The trend continued throughout the recovery period. Experimental eyes grew more than control eyes. Experimental eyes experienced a 66µm, whereas control eyes experienced a 40 µm increase. Statistical significance was not reached in any period.

In figure 5B, during day 3, experimental and control eyes grew approximately the same amount. Experimental eyes experienced a 172µm increase, whereas control eyes experienced a 177µm increase. During day 5, experimental eyes grew more than control eyes. Experimental eyes experienced a 577µm increase, whereas controls eyes only experienced a 399µm increase. This effect was statistically significant ($p<0.05$). During recovery, control eyes grew more than experimental eyes. Experimental eyes experienced a

271µm change, whereas control eyes experienced a 298µm change. However, this effect did not reach statistical significance.

Figure C shows the data for the interocular differences for both groups: the growth stimulation in the fellow eyes of the L-arginine group is apparent in the smaller interocular difference (although this did not reach significance). During day 3, experimental eyes experienced a 90µm increase, whereas control eyes experienced a 198µm increase. During day 5, the same effect is observed. Experimental eyes experienced a 150µm increase, while control eyes experienced a 298µm increase. During recovery, both eyes experienced a decrease. Experimental eyes experienced a -205µm reduction, whereas controls experienced a -258µm reduction.

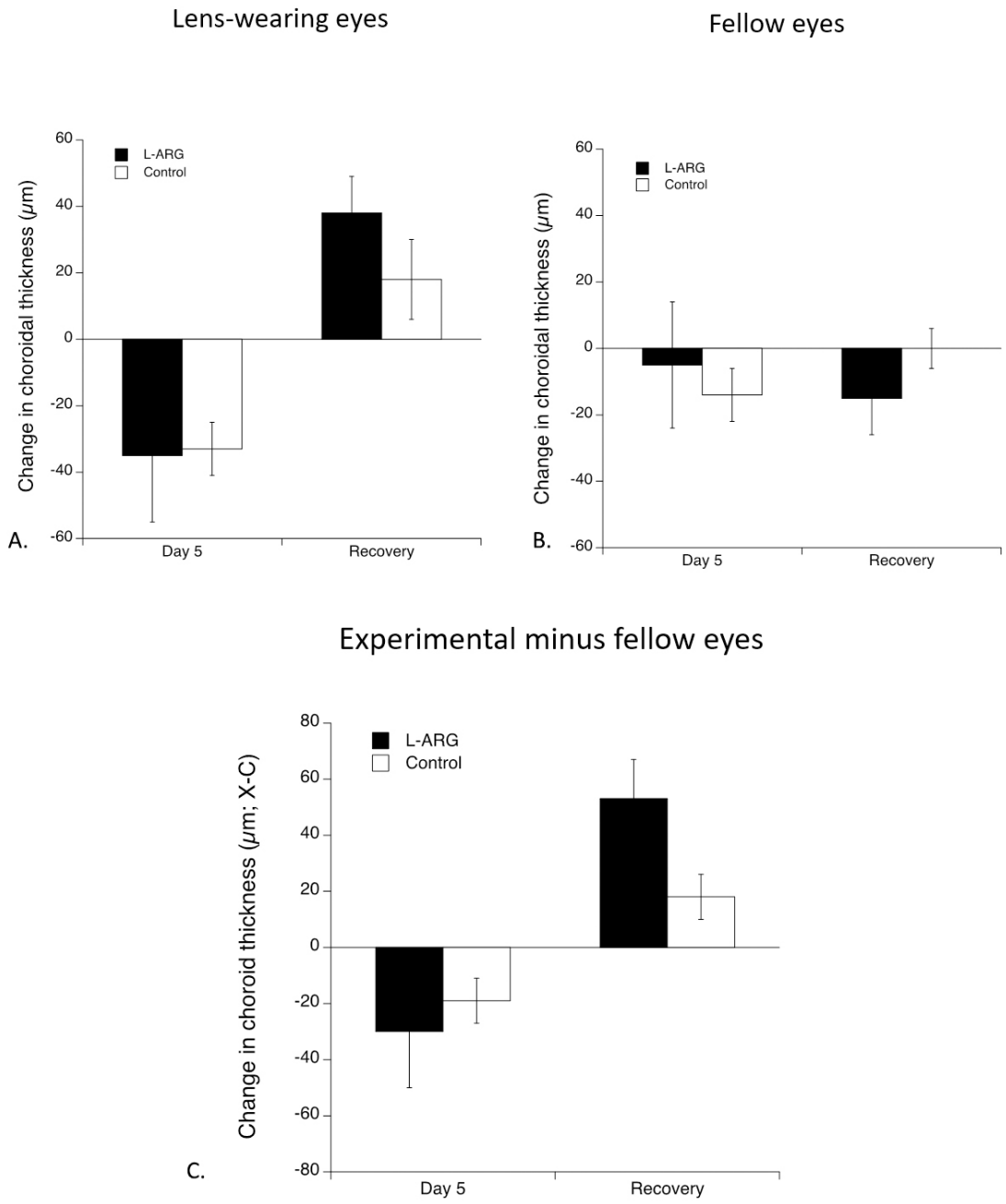

Figure 6: Figure 6A-B summarizes the choroidal thickness changes in eyes exposed to negative lens-wear. Figure 6A displays the choroidal thickness changes in lens-wearing eyes during days 5, and recovery. Figure 6B displays the choroidal thickness change in the fellow eyes during day 5, and recovery. Figure 6C displays the net choroidal thickness change between experimental and control fellow eyes during day 5, and recovery. Error bars indicate ± 1 SEM. * denote statistically significant differences ($p<0.05$).

Choroidal Effects

There were no significant effects on the choroids of experimental eyes during the wearing of the lenses (fig. 6A), or during the 3 days of recovery. Choroids of the lens-wearing eyes thinned (in both groups) over the 5 days of lens-wear and thickened (not significant) during recovery. In figure 6A, during day 5, experimental eyes experienced a -35µm decrease, whereas controls experienced a -33µm decrease. During recovery, experimental eyes experienced a 38µm increase, whereas control eyes experienced a 18µm increase. Statistical significance was not reached in any period.

There were no effects on choroids of fellow eyes of either group. In figure 6B, during day 5, experimental fellow eyes experienced a -5µm decrease, whereas controls experienced a -15µm decrease. During recovery, experimental eyes experienced a -15µm decrease, but control eyes experienced no change. In figure 6C, during day 5, both experimental eyes experienced a -30 µm decrease, whereas control eyes experienced a -19µm decrease. During recovery, the opposite effect was observed. Experimental eyes experienced a 53µm increase, whereas controls experienced a 18µm increase. Statistical significance was again not reached in any period.

3.4 Dietary L-arginine Effects on Ocular Dimensions in the presence of +10D Lens-induced Myopic Defocus

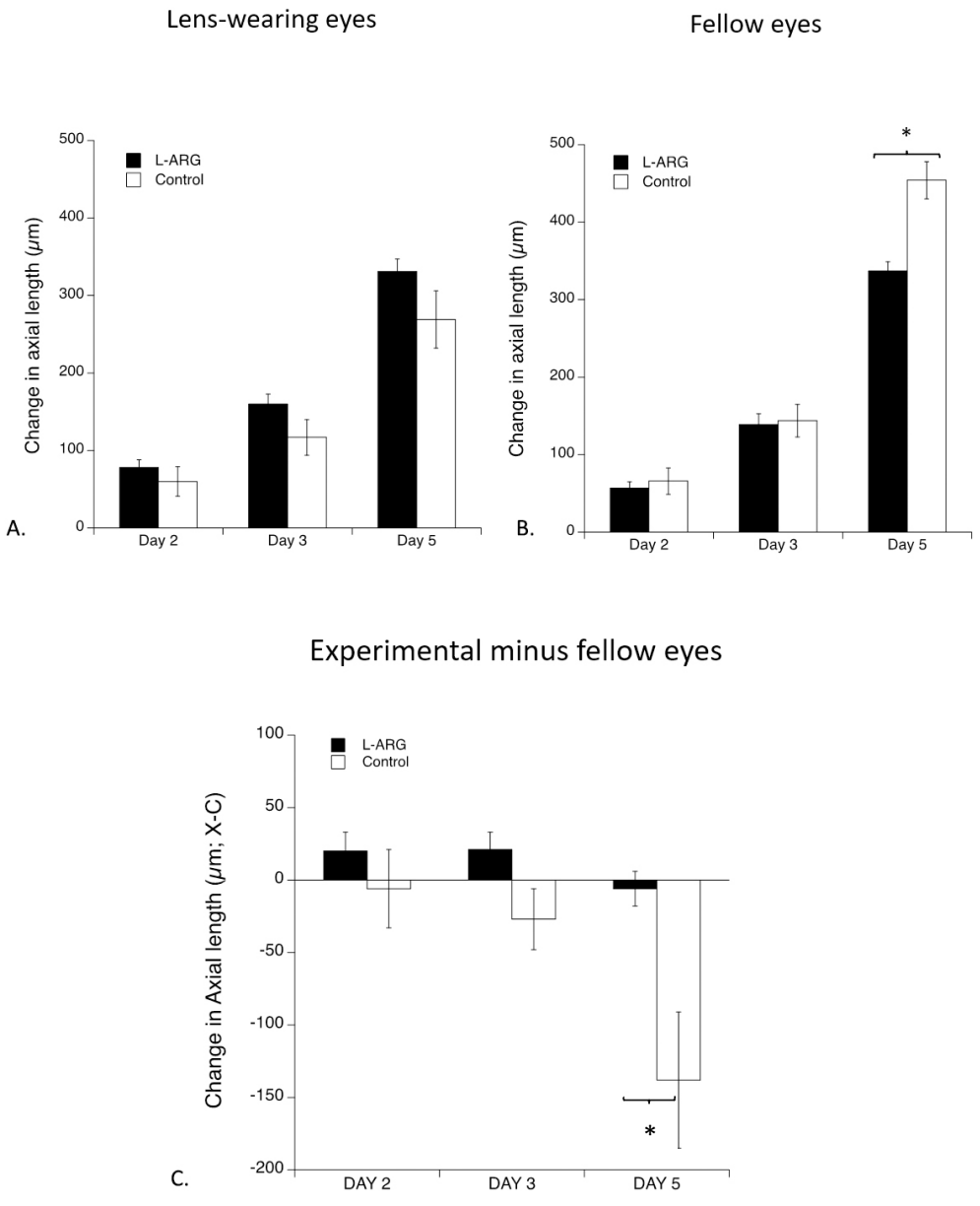

Figure 7: Figure 7A-B summarizes the axial length changes in eyes exposed to positive lens-wear. Figure 7A displays the axial length changes in lens-wearing eyes during days 2, 3, and

5. Figure 7B displays the axial length change in the fellow eyes during days 2, 3, and 5. Figure 7C displays the net axial length change between experimental and control fellow eyes during days 2, 3, and 5. Error bars indicate ± 1 SEM. * denote statistically significant differences ($p<0.05$).

Axial Effects

There were no significant effects on ocular elongation during the 5 days of lens-wear (fig 7A). However, similar to that seen in the negative lens paradigm, there was a significant effect on the fellow eyes of the L-arginine group (fig. 7B). In this condition, fellow eyes showed ocular growth *inhibition* relative to that of fellow eyes of controls. This is also apparent in the graph showing the interocular differences, where the 2 eyes of the experimental group grew at a similar rate while the lens-wearing eyes of the controls were significantly inhibited relative to fellows (fig. 7C). So, in both conditions of defocus (but not form deprivation), L-arginine appears to "yoke" the response of the fellow eye to that of the contralateral lens-wearing eye, stimulating them in the case of the hyperopic defocus, and inhibiting them in the case of the myopic defocus.

In figure 7A, it's observed during day 2, experimental eyes experienced a 78µm increase, while control eyes experienced a 60µm increase. The same trend is seen during day 3 where experimental eyes experienced a 160µm increase, and controls experienced a 117µm increase. The trend continues with day 5. Experimental eyes experienced a 331µm increase, and control eyes experienced a 269µm increase. However, statistical significance was not reached in any period.

In figure 7B, during all periods control fellow eyes grew more than experimental eyes. During day 2, experimental eyes experienced a 57µm increase, whereas controls experienced a 66µm increase. During day 3, experimental eyes experienced a 139µm increase, while controls experienced a 144µm increase. Statistical significance was not reached in either period. However, during day 5, control eyes grew significantly more than experimental eyes. Experimental eyes experienced a 337µm increase, while controls experienced a significant 454µm increase ($p<0.05$).

In figure 7C, we can see controls eyes experienced less growth than experimental eyes in all periods. During day 2, experimental eyes experienced a 20µm increase, while controls experienced a -6µm decrease. During day 3, experimental eyes experienced a 21µm increase, while controls experienced a -27µm decrease. During day 5, experimental eyes experienced a -6µm decrease, while controls experienced a significant -138µm decrease ($p<0.05$).

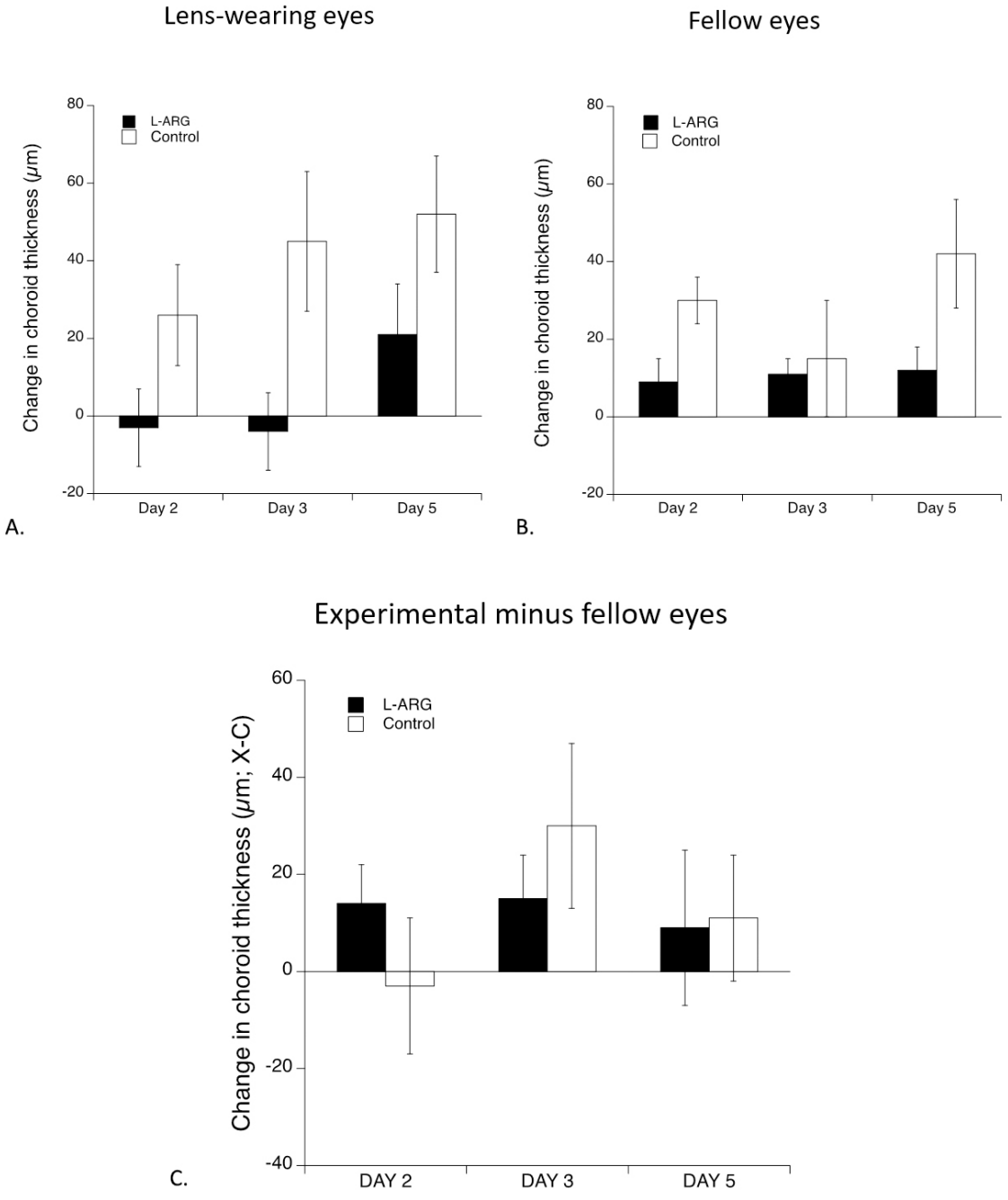

Figure 8: Figure 8A-B summarizes the change in choroidal thickness in eyes exposed to positive lens-wear. Figure 8A displays the change in choroidal thickness in lens-wearing eyes during days 2, 3, and 5. Figure 8B displays the change in choroidal thickness in fellow eyes during days 2, 3, and 5. Figure 8C displays the net choroidal thickness change between experimental and control fellow eyes during the same period. Error bars indicate ± 1 SEM. * denote statistically significant differences ($p<0.05$)

Choroidal Effects

There were no significant effects on the choroids of experimental eyes during the course of the lens-wear (fig. 8A-C). Choroids of lens-wearing controls thickened, as expected; choroids of the L-arg group did not thicken as much, but the difference was not significant (fig. 8A). During day 2, experimental eyes experienced a -3 μm decrease, while control eyes experienced a 26 μm increase. During day 3, experimental eyes experienced a -4 μm decrease, while control eyes experienced a 45 μm increase. During day 5, experimental eyes experienced a 21 μm increase, while control eyes experienced a 52 μm increase.

There were no effects on choroids of fellow eyes of either group (fig. 8B). During day 2, experimental choroids experienced a 9 μm increase, while controls experienced a 30 μm increase. During day 3, experimental choroids experienced a 11 μm increase, while controls experienced a 15 μm increase. During day 5, experimental choroids experienced a 12 μm increase, while control choroids experienced a 42 μm increase. Statistical significance was not reached in any period.

This is also shown in the interocular difference data (fig. 8C). During day 2, experimental eyes experienced a 14 μm increase, while control eyes experienced a -3 decrease. The effect reverses for days 3, and 5. During day 3, experimental eyes experienced a 15 μm increase, while control eyes experience a 30 μm increase. During day 5, experimental eyes experienced a 9 μm increase, and control eyes experienced a 11 μm increase. Again, statistical significance was not reached in any period.

4. Discussion

In this study, we studied the effects on ocular parameters of chicks undergoing experimental visual manipulations whose diet was supplemented with L-arginine, an essential amino acid. L-arginine is involved in the synthesis of nitric oxide, a gaseous neurotransmitter involved in signal pathways; it is crucial to mediating vasodilation. NO has also been investigated as a signal molecule involved in emmetropization, the visual regulation of ocular growth. Specifically, it is thought to be one of the signals involved in the compensatory choroidal thickening response.

In normal (non-visually-manipulated) eyes, we found that L-arg thickened the choroids of otherwise untreated (normal) eyes, but the effect was transient, lasting around two weeks, after which it thinned to baseline. Immediately subsequent to this, axial growth was inhibited, lasting over the 4-week period. This effect was mainly due to an inhibition of anterior chamber growth. In eyes recovering from form deprivation, axial growth was inhibited relative to the no- drug controls. There were no effects on any parameters for the fellow eyes. By contrast, there was no effect on axial growth in either negative or positive lens-wearing eyes, and no effect on choroidal thickness. Surprisingly, however, the fellow eyes in the experimental (drug) negative lens group showed growth stimulation relative to no-drug fellow eyes, while the opposite was true for the positive lens group, i.e. fellow eyes were inhibited relative to no-drug fellow eyes. In other words, the L-ARG, while having no effect on the lens-wearing eyes appeared to have “yoked” the fellow contralateral eyes to the defocus effect of the lenses relative to a normal (basal?) growth rate.

4.1 Nitric Oxide and the Compensatory Choroidal Response

As discussed in the introduction, ocular compensatory changes to plus and minus lenses, and form deprivation are well documented. Placing a convex (plus-power) lens in front of an emmetropic eye shifts the focal plane towards the plane of the cornea creating myopic defocus. When a plus lens is placed in front of the eyes of a normal developing chick, this produces choroidal thickening to bring the retina closer to the focal plane (Wildsoet and Wallman, 1995). This is followed by a decrease in the axial elongation rate (Irving et al., 1995). As lens wear continues, the axial length growth of the eye slows resulting in a shorter than normal eye and hyperopia when the lens is removed (Schaeffel et al. 1990). Placing a concave (minus-power) lens in front of an emmetropic eye shifts the focal plane further away from the plane of the retina creating hyperopic defocus. When a minus lens is placed in front of the eyes of a normal developing chick, this produces choroidal thinning to bring the retina closer to the focal plane (Wildsoet and Wallman, 1995). This is followed by an increase in the axial elongation rate (Irving et al., 1995). Over a period of days, the axial length of the treated eye increases until the retinal location has shifted by an amount that approximately matches the shift of the focal plane resulting in a longer than normal eye and myopia when the lens is removed (Schaeffel et al. 1990). When eyes of a normal developing chick are exposed to form deprivation an “open-loop” condition is created as there is no “target” for the retina (Zhang et al. 2019). This results in choroidal thinning, followed by an increase in the axial elongation rate (Wildsoet and Wallman, 1995). Although these effects are well documented the mechanism is not fully understood.

NO has been hypothesized as a likely mediator of this effect as it plays many roles in the eye. In the retina, it is involved in visual transduction, synaptic transmission and horizontal cell coupling (Koistinaho and Sagar, 1996). In the choroid, it has a well-established role in blood flow regulation (Deussen et al., 1993, Mann et al., 1995, Zagvazdin et al., 1996). There is also growing evidence that it mediates the choroidal changes in thickness that occur in response to myopic defocus (Nickla and Wildsoet, 2004). It is possible that these changes are mediated by changes in tonus of the non-vascular smooth muscle spanning the choroid (Wallman et al., 1995). These cells are contacted by NO-positive terminals (Poukens et al., 1998) and NO is a potent relaxant of smooth muscle.

Nitric Oxide Synthase (NOS) is the enzyme that catalyzes the synthesis of nitric oxide from L-arginine (Nickla et al., 2009). It exists in three isoforms, all of which are found in the eye (Nickla et al., 2009). nNOS has been found in the pterygopalatine ganglion (Cuthbertson et al., 1997), the ciliary neurons (Sun et al., 1994), and their terminals in the choroid. iNOS activity has been found in the retina, in macrophages and neutrophils (Goldstein et al., 1996), outer segments of the photoreceptors, the RPE and choroid (Fujii et al., 1998) and in Muller cells (Goureau et al., 1998). eNOS is not found in the retina but is present in the vascular endothelium of the choroid (Nickla et al., 2009). In previous experiments, it has been shown that injections of L-NAME, a non-specific NOS inhibitor in chicks experiencing myopic defocus blocks both the increase in choroidal thickness and the subsequent inhibition of axial elongation (Nickla and Wildsoet, 2004). The same effect is also observed with N^{ω}-propyl-L-arginine, a highly selective nNOS inhibitor (Nickla et al., 2009). However, when promotors of NO production are introduced, such as L-arginine and sodium nitroprusside the opposite is

seen, choroidal thickening is promoted and subsequent inhibition of axial elongation occurs (Carr and Stell, 2016).

4.2. Dietary L-arginine Effects on Ocular Dimensions in Untreated Eyes

Dietary supplements and their influence on ocular growth have been explored previously. One study demonstrated dietary supplementation of retinoic acid administered via gavage resulted in ocular dimension changes in chicks (McFadden et al., 2006). Additionally, a series of studies have been conducted on healthy human subjects to evaluate the effects of caffeine on choroidal thickness. A study researching the choroidal effects of ingestion of caffeine containing energy drinks found a significant reduction in subfoveal choroidal thickness (-14um, $p<0.0001$) occurred after 1h of Redbull intake with an even more significant effect (-20.14um, $p<0.0001$) in individuals with thick choroids (>395um) (Arej et al., 2021). However, measurements at 4h were comparable to baseline (Arej et al., 2021). Another study observed the choroidal effects of oral ingestion of a 200mg capsule of caffeine on the choroid and found that oral caffeine intake caused a significant reduction in choroidal thickness, but confirmed the results are no longer significant after 4 hours (Dervisogullari et al., 2015). Finally, a study measuring the choroidal thickness changes 5 minutes, 30 minutes, 1 hour, 2 hour, 3 hour, 4 hour, and 24 hours after coffee consumption demonstrated choroidal thickness decreased up to 4 hours after ingestion (Vural et al., 2014). In each study, caffeine was administered through ingestion of a capsule, or caffeine containing beverage such as an energy drink or coffee (Arej et al., 2021; Dervisogullari et al., 2015; Vural et al., 2014). All

of these studies found dietary caffeine ingestion significantly reduces choroidal thickness up to 4 hours after oral ingestion (Arej et al., 2021; Dervisogullari et al., 2015; Vural et al., 2014).

As expected if NO was increased by dietary L-arg; transient choroidal thickening was observed in the eyes of untreated chicks introduced to dietary L-arginine. L-arginine once ingested is absorbed and enters the L-arginine/nitric oxide pathway where it's catalyzed by NOS to form NO (Neilly et al., 1994; Lidder and Webb, 2012). This effect lasted approximately 2 weeks before returning to baseline. Subsequently, axial growth inhibition followed for a period of 4 weeks. The transient nature of this effect has been documented before. In the previously mentioned study involving L-NAME, the induced choroidal effect was documented as transient, lasting about 24 hours, and was dose-dependent (Nickla and Wildsoet, 2004). The longer effect window witnessed in our experiment may be due to the continuous consumption of dietary L-arginine compared to the periodic injections used in previous studies. Interestingly, the inhibition of axial growth by L-arginine appears to be mostly due to an inhibition of anterior chamber growth. However, past studies have shown that L-NAME, a NOS inhibitor, is responsible for inhibition of anterior chamber growth (Nickla et al., 2006).

4.3. Dietary L-arginine Effects on Ocular Dimensions in the Presence of Diffuser-wear, and Plus and Minus Lens-Wear

There were no effects on either growth rate or choroidal thickness in eyes responding to form deprivation: growth was stimulated similar to controls, and choroids thinned as expected. However, after diffuser-removal (recovering eyes with myopic defocus), axial growth was inhibited relative to (no drug) controls; there was no effect on fellow eyes. It is possible that L-arg is only effective on eyes growing normally (above) or on eyes whose growth is being inhibited, however, this hypothesis is precluded because L-arg had no effect on eyes exposed to myopic defocus by spectacle lens-wear (below).

Similar to form-deprived eyes, there were also no effects on either eye growth or choroid thickness in eyes exposed to lens-induced myopic or hyperopic defocus. However, there were significant effects on ocular growth in the contralateral fellow eyes of both groups: fellow eyes of eyes wearing positive lenses (myopic defocus) grew slower than controls, while fellow eyes of eyes wearing negative lenses (hyperopic defocus) grew faster than controls. We interpret this to exhibit a kind of "yoking", whereby the growth state of the experimentally-manipulated eyes was somehow imposed onto the contralateral eye.

Such interocular yoking effects have been reported in chicks before. In monocular treatments the contralateral eye can show response patterns similar to lens-treated eye although substantially reduced (Schmid and Wildsoet, 1995; Sivak et al., 1989). Previous studies have also noted fluctuating degrees of growth seen in fellow eyes, and occasionally no effects seen in the fellow eye. In one experiment, it was documented that the lens eye

grew 2.7% greater than the fellow eye when evaluating lens to sclera distance after wearing a -6D lens for a 5 day period (Wallman and Wildsoet, 1995). Whereas, in a subsequent experiment, it was documented that the lens eye grew 35% greater than the fellow eye when evaluating ocular length (cornea to posterior sclera) after 14 hours of continuous wear of a -6D Lens (Wallman and Winawer, 2002). This same study noted that only 14 out of 21 chicks displayed elongation in the lens-wearing eye greater than the fellow eye when exposed to continuous -6D lens-induced defocus of 14 hours (Wallman and Winawer, 2002). Additionally, another study evaluating the yoking effects in the fellow eye in the setting of lens-induced retinal defocus finds that the change in choroidal thickness, and rate of ocular elongation in the fellow eye to typically be in the same direction, but to a smaller degree of 10-30% of the lens-wearing eyes (Zhu and Wallman, 2009). This large variability between the lens-wearing eye, and fellow eye may explain why the effect failed to reach significance in the fellow eyes. However, the true degree of variability between these experiments is unclear as there is a lack of direct comparison due to differences in experimental variables such as period of lens wear, and units of measurement.

There are also examples in the literature of "anti-yoking" where fellow eyes experience a yoking effect in the opposite direction to lens-wearing eyes. In a monocular form deprivation experiment on rhesus macaques the untreated fellow eye exhibited no sign of emmetropization and was >2SD more hyperopic than the age-matched control (Smith et al., 2007). The phenomenon was also documented in chicks wherein the choroids in the fellow eyes of negative lens-wearing eyes were thicker than those of positive lens-wearing eyes (Zhu and Wallman, 2009). The mechanism for this has not been thoroughly

investigated. However, it's believed these effects occur due to diffusion of growth factors released from treated eyes, through the thin orbital septum separating the two eyes. Cortical involvement may also be possible, but it is unlikely. Finally, it should also be noted that the lack of positive lens-induced choroidal thickening in control eyes is peculiar, and we can offer no explanation other than an unknown aberration in the batch of birds used, or in the experimental conditions at the time.

4.4. Myopic Defocus: A Dissociation Between Compensatory Responses of the Choroid and Ocular Growth Rate

We found a dissociation between the choroidal compensation and axial growth in both experimental and control eyes wearing plus lenses in that there was little to no thickening in either group despite the ocular growth inhibition. We have no clear explanation for this. However, a similar effect has been documented in previous studies. In this study evaluating the temporal constraints on lens compensation in chicks it's observed that the change in rates of ocular elongation and choroid thickness, do not always occur in tandem (Wallman and Winawer, 2002). In another study, it was found that double parasympathectomies prevented the development of form-deprivation myopia, but choroidal thinning was retained, however, when the diffuser was removed, the choroid thickened back to normal, and ocular growth rate increased which may imply that choroidal thickening is not mechanistically linked to ocular growth (Nickla and Schroedl, 2012). This suggests the choroidal response may not always be paired with an axial elongation response.

4.5. Potential Implication for Human Subjects:

Although the causal link between choroidal thickening and inhibition of axial length has not been proven, it is well established that choroidal thickness varies significantly between myopes and emmetropes in both chicks and humans (Nickla and Wallman, 2010; Xiong et al, 2017). As explored previously, this suggests that choroidal thickness plays a factor in myopia progression with thinner choroids being a risk factor, and thicker choroids being protective. To further combat the global epidemic of myopia it's important to explore additional avenues of treatment. A dietary solution utilizing a widely available NO pre-cursor such as L-arginine for modulation of choroidal thickness would be highly beneficial as it would be far less invasive, and much more accessible than potential intravitreal alternatives. It would arguably even be less invasive than current optical solutions such as orthokeratology and multifocal lenses.

L-arginine is well documented in physiological research for its ability to form NO. It's found in high concentration in seafood, watermelon juice, nuts, seeds, algae, rice proteins, meats, and soy protein in addition to being available as a supplement (Trexla et al, 2019). Past studies have also verified a significant positive association between L-arginine intake and serum NO concentration in humans (Mirmiran et al, 2016). It's often suggested to enhance exercise performance by vasodilation and increasing maximal mechanical power in working muscles through the synthesis of NO (Gonzales and Trexler, 2020). Dietary NO supplementation has also been shown to have numerous health benefits including reduced blood pressure, improved cardiovascular health, and treatment of erectile dysfunction (Wu and Meininger, 2002).

A recent study has also investigated if the oral administration of nitrate-rich beetroot juice would cause significant choroidal thickening, axial length reduction, blood pressure decrease, or contrast sensitivity enhancement in human subjects (Qiu, 2022). Unfortunately, the study found no significant differences in choroidal thickening, axial length reduction, blood pressure decrease, or contrast sensitivity enhancement (Qiu, 2022). We were unable to find any additional studies investigating dietary NO in humans. It's apparent further research is needed in this area to assess if NO can be a novel method for myopia control in humans.

4.6. Limitations and Future Directions:

Our study has several limitations. The first being, it did not quantify the amount of L-arginine consumed, or the concentration of NO within the choroid. It's important for future experiments to explore these values as we know from previous experiments the choroidal compensatory mechanism is dose-dependent. More experiments will be needed to confirm potential effects of L-arginine on eye growth and choroid thickness.

Conclusion:

In conclusion, L-arginine is found to transiently thicken the choroids of otherwise untreated eyes, and result in subsequent axial growth inhibition mainly through the inhibition of anterior chamber growth. In recovering eyes, axial growth was inhibited relative to (no drug) controls, but there was no effect on fellow eyes. There was also no effect on choroidal

thickness. In contrast, there was no effect on axial growth in either negative or positive lens-wearing eyes, and no effect on choroidal thickness. However, an interesting yoking phenomenon was witnessed in the fellow eyes. The fellow eyes in the experimental negative lens group showed growth stimulation relative to no-drug fellow eyes, while the opposite was true for the positive lens group.

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