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The Effects of Complex Optical Environments on the Development, Progression and Control of Myopia

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The Effects of Complex Optical Environments on the Development, Progression and Control of Myopia

By

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Committee in charge:

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Abstract

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on the Development, Progression and Control of Myopia

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Doctor of Philosophy in Vision Science

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Myopia or nearsightedness is a condition in which the axial length of the eye is too long relative to its optical focal length. This condition is reaching epidemic levels worldwide, and has become a tremendous public health burden. Myopia is one of the leading causes of vision loss and high myopia significantly increases the risk of permanent blindness. Consequently, myopia cannot be considered as a benign condition and early interventions aimed at slowing down or even stopping the progression of myopia rather than merely correcting the associated optical focusing error is of great importance.

The consistent evidence from animal model studies showing that imposed hyperopic defocus, if sustained, comprises an effective myopogenic stimulus, accelerating eye growth, while imposed myopic defocus slows ocular elongation, has motivated clinical studies using bifocal and progressive addition spectacles for myopia control. While these studies, reviewed in Chapter 2 of this dissertation, failed to provide convincing evidence for a clinically significant treatment effect, i.e., slowed myopia progression; smaller scale, non-randomized studies using multifocal soft contact lenses and orthokeratology lenses have shown much more promising effects in terms of slowing myopia progression. However, the mechanism(s) underlying the latter anti-myopia treatment effects are poorly understood, thereby limiting their further refinement. In addition to the aforementioned systematic review of relevant clinical evidence for optical interventions for myopia control, this dissertation described 3 other studies using chick model, representing efforts to further understand how multifocal optical environments affect normal ocular development, specifically the process of emmetropization. These manipulations have also been used as a tool to investigate the mechanism underlying the myopia controlling effect of the novel contact lens applications referred above.

Chapter 2 describes a systematic review and meta-analysis performed on randomized controlled trials investigating the effects of three traditional optical interventions, bifocal and progressive addition spectacles, and rigid contact lenses, all believed to control myopia progression, based on anecdotal evidence. The overall treatment effect was estimated to be only small and clinically insignificant. A number of reasons were proposed to explain the discrepancy between the results of related animal studies and these clinical trials - strong support for optical control of myopia from the former studies

and inconclusive results from the latter studies. Poor compliance to the spectacle corrections in clinical studies, inaccurate measurement of adherence to the treatments, inadequate and potentially, ambiguous classification systems for clinical myopia, and potential flaws in the optical designs are likely to have contributed to the discouraging results from the clinical trials.

Chapter 3 describes the first of three studies in which the chick model was used to test the effects on ocular growth of a series of custom-designed 2-zone multifocal "spectacle" lenses. This and the two follow-up studies using this model have the following merits; they made use of standardized experimental paradigms and objective methods of measurement to track ocular changes. The first study applied the lenses in a simple experimental paradigm, in which monocular lenses were attached to the normal eyes of young chickens. The 2-zone lenses included a plano zone, either in the center or peripheral surround, with either positive or negative power (+5 or -5 D) incorporated into the other zone. The size of the central zone was also allowed to vary, to control the size of the unifocal central and peripheral retinal areas, and intermediate multifocal zone, as was the placement of the powered zone, i.e. in the center or periphery of the lenses. Single vision lenses were included as a control treatment. Two important observations from this study were that 1) peripheral optical defocus can influence both peripheral (off-axis) and central (on-axis) refractive error development and 2) the inhibitory effect on axial ocular growth of myopic defocus imposed using 2-zone lenses (positive zones) could exceed that induced by single vision lenses of the same power. These results suggested complex interactions between adjacent retinal regions, with the peripheral retina apparently able to decode optical defocus, as well as complex interactions between the eyes own optical aberrations and those of the 2-zone lenses, which introduced large amounts of spherical aberration.

Chapter 4 described a closely related study in which subsets of the same 2-zone lenses were tested on eyes that had undergone surgical manipulations (sectioning of the ciliary nerve, CNX or iridectomy, ID) to investigate the influence of the pupil size of the eye as well as accommodation on the effects of the 2-zone lenses. Both were uncontrolled in the first study; yet create a dynamic optical system on which the multifocal optical environment was imposed. The ID surgery produced a fixed dilated pupil without any effect on accommodation (confirmed in another study, reported in Appendix I), while the CNX surgery produced a similarly enlarged pupil while also eliminating accommodation. This study revealed pupil size to be a critical factor in the treatment effects of 2-zone lenses, likely to reflect at least in part, its influence on the optical experience of various retinal zones (center to periphery), and also suggested a significant role of accommodation in the decoding of imposed optical defocus stimuli, in this case, complex multifocal optical stimuli.

Chapter 5 described a third study which attempted to develop a clinically more relevant scenario; specifically, 2-zone lenses that incorporated two different negative powers (-5 & -10 D) in two optical zones (center & periphery or vice versa), were tested on both normal eyes and eyes made myopic before being fitted with one of the 2-zone lenses. The latter combination was intended to simulate the ocular conditions created when concentric multifocal contact lenses, i.e. with a near addition, are prescribed to human

myopes, one of the novel, myopia control treatments currently being explored. When the 2-zone lenses were fitted to normal eyes, they induced myopia, of a magnitude falling between the values expected, had single vision lenses of the same powers been used. However, on myopic eyes, the lenses had a strong myopia inhibiting effect with the already induced myopia undergoing substantial regression. This result supports the further investigation of appropriately designed concentric multifocal contact lenses for the control of myopia progression.

In summary, the studies reported in this dissertation indicate complex interactions between central and peripheral retinal regions in decoding and responding to complex defocus signals as well as critical influences of pupil size and accommodation on these processes. The strong and consistent inhibitory effects on ocular growth of concentric 2-zone lenses incorporating a zone of either positive power or a near addition, 2-zone designs lend plausibility to the notion of using custom-designed novel optical treatments for the control of myopia progression.

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LIST OF ABBREVIATIONS

ACD	anterior chamber depth
ANOVA	analysis of variance
BF	bifocal
CI	confidence interval
COAS	complete ophthalmic analysis system
COMET	correction of myopia evaluation trial
CNX	cililary nerve section
CRT	corneal reshaping technology
CT	choroidal thickness
CZD	center zone diameter
D	diopter
HOA	high order aberrations
ID	iridectomy
IOL	intraocular lens
LASIK	laser-assisted in situ keratomileusis
LORIC	longitudinal orthokeratology research in children
LT	lens thickness
M	mean
MD	mean difference
MF	multifocal
Ortho-K	orthokeratology
PAL	progressive addition lens
PL	plano
PRK	photorefractive keratectomy
PZD	peripheral zone diameter
RCT	randomized controlled trials
RGPCL	rigid gas permeable contact lens
RK	radial keratotomy
RPR	relative peripheral refraction
SA	spherical aberration
SCL	soft contact lens
SD	standard deviation
SER	spherical equivalent refraction
SV	single vision
VCD	vitreous chamber depth
VD	vertex distance

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Chapter 1

Epidemiology, Clinical Management of Myopia, and Research Background

Abstract

Myopia is one of the most common causes of visual impairment and should not be considered a benign condition because of the potential risks of permanent vision loss. Current mainstream clinical management of myopia focuses on the correction rather than the control of this progressing condition and clinical trials using optical interventions on myopia control failed to provide clear guideline for practice. Results from animal studies have consistently demonstrated that emmetropization is an active rather than a passive process and both the development of refractive error and ocular growth can be manipulated by imposing defocus signals to either the central and/or the peripheral regions of the retina. A better understanding of how complex optical environment affects the process of emmetropization; and the potential interactions between the defocus stimuli and the optics of the eye such as pupil sizes and accommodation can provide substantial insight into the design of optical interventions for clinical myopia control.

1.1 Epidemiology of Myopia

Myopia, also referred as nearsightedness, is a disorder caused by the mismatch of the refracting power of the eye and its axial length. Specifically, parallel light rays entering a myopic eye are brought to focus in front of the retina. In other words, a myopic eye is too long for its converging power. The major symptoms are blurred vision at distance, as well as at near for those with moderate to high myopia. Myopia is a common disorder, both domestically and worldwide. In the United States approximately 40% of the population is myopic.[1] Prevalence figures vary with socioeconomic status and ethnicity, although the reasons behind these differences remain unclear.[2] Because of the dramatic increases in the prevalence of myopia in recent years, and the serious complications associated with high myopia, it has become one of the most important and urgent topics to be addressed by modern ophthalmic research.

Myopia is often considered as a condition of low ocular morbidity that can be solved simply by the prescription of spectacles or contact lenses. However this viewpoint is misleading. In the US, high myopia is the seventh leading cause of blindness.[3] Furthermore, the average age of onset of its complications is much earlier than other leading causes of blindness such as diabetic retinopathy and age-related macular degeneration. Some of the common complications related to high myopia include retinal detachment, choroidal neovascularization, macular degeneration, cataract, and glaucoma.[4] Because the risk of developing such vision-threatening complications increases with the degree of myopia, it is also of relevance that the prevalence of high myopia is increasing and that many of these complications result in permanent vision loss. For these reasons alone, it is without question that researches into methods of preventing or controlling myopia should be given a high priority.

1.2 Clinical Management of Myopia

The World Health Organization describes uncorrected refractive errors as the second most common cause of visual impairment globally, only behind cataract.[3] Nonetheless, for those with access to eye care and low-to-moderate degrees of myopia, most can achieve normal or dramatically improved vision with standard optical appliances – spectacles and contact lenses – which have improved in quality with the development of technologies such as improved anti-reflective coating, high index lens materials, aspheric lens designs, and high oxygen-permeable contact lens materials etc. There are also now surgical alternatives for the correction of myopia, including LASIK (Laser-Assisted In Situ Keratomileusis), PRK (Photorefractive Keratectomy), anterior chamber IOL (Intraocular Lens) implant, and RK (Radial Keratotomy). These procedures effect changes to the total refracting power of the eye. However, these solutions have significant limitations. First of all, regardless of the method used to correct myopia, this disorder has a significant socioeconomic impact worldwide. In the United States, the annual estimate of expenditure on eyewear and surgical procedures is $15 billions, imposing a huge burden on American's health care spending.[3] Second, while these "treatment methods" are generally effective in relieving the symptom of blurred vision, myopia progression rates and so the risks of serious visual complications are not affected.

Recent research using primates and other animal models for myopia lend credibility to

the notion that myopia progression may be controlled by appropriate optical intervention. These studies describe myopic changes in response to manipulations that degrade the retinal image without imposing defocus.[5-8] Other studies have shown that optical defocus imposed on young animals induces refractive error changes, with myopia and hyperopia (farsightedness) occurring in response to minus and plus lenses, respectively.[9-11] These refractive changes in both cases reflected altered eye growth, myopia being the product of increased axial elongation. These observations open the possibility that visual conditions that result in hyperopic defocus (image positioned behind the retina) in children and young adults might also induce myopia.

Writings describing procedures intended to slow myopia progression date back to the beginning of the last century.[4] Since that time, there have been many clinical trials published, involving both pharmacological- and optically-based treatment regimens. An overview of this body of research is presented in the next chapter, with the emphasis on optical manipulations because of their relevance to the dissertation research.

1.3 Peripheral Refractive Errors, Aberrations and Implications for Myopia Progression and Retardation

Most recent myopia research has emphasized the role of the fovea in the regulation of refractive errors, the fovea long being considered the major source of signals regulating both accommodation and emmetropization. However, direct studies of eye shape have demonstrated that myopic eyes are more prolate in contour (i.e., having a longer axial length than equatorial diameter) in contour than emmetropic and hyperopic eyes.[12, 13] As a result, images falling on the peripheral retina will always be out-of-focus in myopes wearing traditional optical corrections (spectacles or contact lenses) that fully correct their central (on axis) myopia. Specifically, peripheral image falls behind the retina (i.e., relatively hyperopic defocused) and moreover, this relative peripheral hyperopia will increase during accommodation.[14] In animal studies, such hyperopic errors are known to stimulate eye growth, i.e., increase myopia.[5, 15, 16]

Can peripheral hyperopic errors result in an overall increase in myopia? This could happen if homeostatic signals from the central retina directing the eye to elongate less (the result of the myopic central refractive error) are overridden by signals from the peripheral retina directing it to elongate more. Despite the lower density of neurons in the periphery, the total number of cells representing the peripheral retina is still larger compared to those in central retina due to the much larger total surface area of the retinal periphery. As a result, if signals from these respective areas are each integrated, i.e., myopic center and the hyperopic periphery, the "peripheral" signal is likely to dominate. The prediction is that eyes will continue to elongate, until peripheral hyperopic errors are sufficiently attenuated and the central error, sufficiently myopic, such that the "center" inhibitory "stop" signal is strong enough to counter the peripheral "grow" signal.

1.4 Dissertation Outline

In this dissertation, the influence of complex optical stimulus to both central and peripheral retina introduced by a series of custom designed 2-zone lenses on refractive error and ocular development is investigated. Additionally, the influence of potential

interactions between the multifocal defocus stimulus and pupil sizes as well as accommodation of the eye is also closely studied.

1.4.1 Specific Aims

This dissertation research focused on the influences on refractive error and ocular development of complex optical stimuli, introduced by a series of custom-designed 2-zone lenses to both central and peripheral retinal regions. The potential interactions between imposed multifocal defocus stimuli and the eye's pupil size and accommodation were also explored.

1.4.1 Specific Aims

Chapters 2, 3, 4 and 5 of the dissertation are ordered according to the specific aims as follows:

1. To systematically compile the current evidence from relevant randomized controlled trials (RCT) evaluating the effectiveness of optical interventions to retard the progression of myopia. Eligible trials include interventions involving bifocal (BF) lenses and progressive additional lenses (PALs), single vision (SV) soft contact lenses (SCL) and rigid gas permeable contact lenses (RGPCL).

2. To characterize the effects of manipulating peripheral retinal defocus on ocular growth in the chick model, using a series of 2-zone lens designs that varied in both defocusing power (+10 & -10 D combined with plano), and diameter of respective zones. The primary goal was to simulate the bifocal soft contact lenses trialed for myopia control in humans to promising effect. Monocular defocusing lenses were worn for 5 days from 17 days of age. A minimum of 6 birds was included in each lens group. The lens design and the imposed multifocal defocus profile when the eye is at the central gaze is shown schematically in Figure 1-1. Treatment assignments are shown in Figure 1-2. Baseline refractive errors and axial ocular dimensions were measured immediately before the start of lens treatments. Ocular dimensional measurements were the repeated every other day, and refraction was repeated once, at the end of the lens treatment. Refractive error measurements were made on-axis as well as 30 degrees nasally and temporally. The work was published in Investigative Ophthalmology and Visual Science (Liu & Wildsoet, 2010).

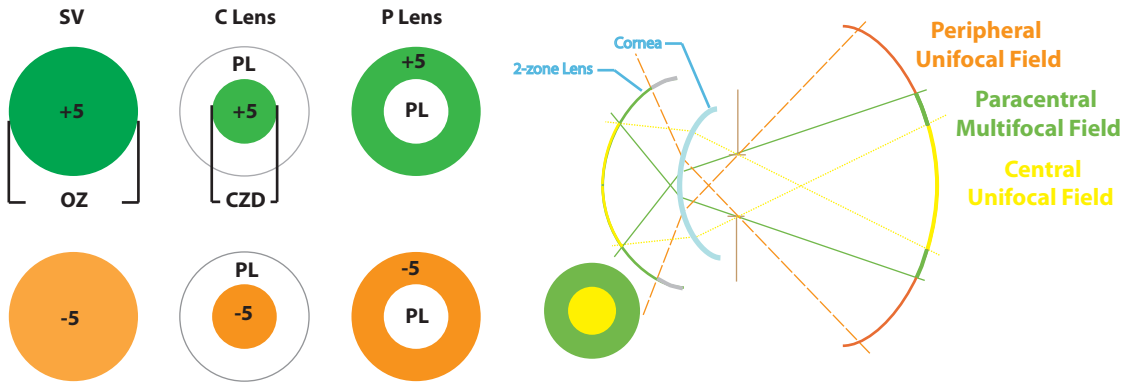


Figure 1-1. Schematic of the lens design (left) and projected visual fields (right) of the 2-zone concentric lenses.

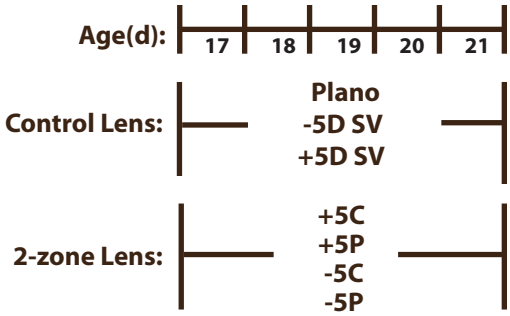


Figure 1-2. Schematic of the lens treatment assignment in Chapter 3.

3. To investigate the effects of ciliary nerve section (CNX) and iridectomy (ID) on the 2-zone lens-induced refractive error development and eye growth in young chicks. CNX eliminates accommodation and produces a fixed dilated pupil, while the effect of ID is limited to the latter. In this study, we sought to answer the following questions: 1) are the effects induced by the 2-zone lenses pupil size-dependent and if yes, 2) is the pupil-size dependence linked to the on-axis HOA introduced by the lenses or its impact on the multifocal optics imposed on the peripheral retina, and 3) what is the role of accommodation in the 2-zone lens-induced ocular growth. Seven birds were included in each treatment group and the treatment regimens were illustrated in Figure 1-3. Ocular dimensions and refractions were measured the same way as in Chapter 2.

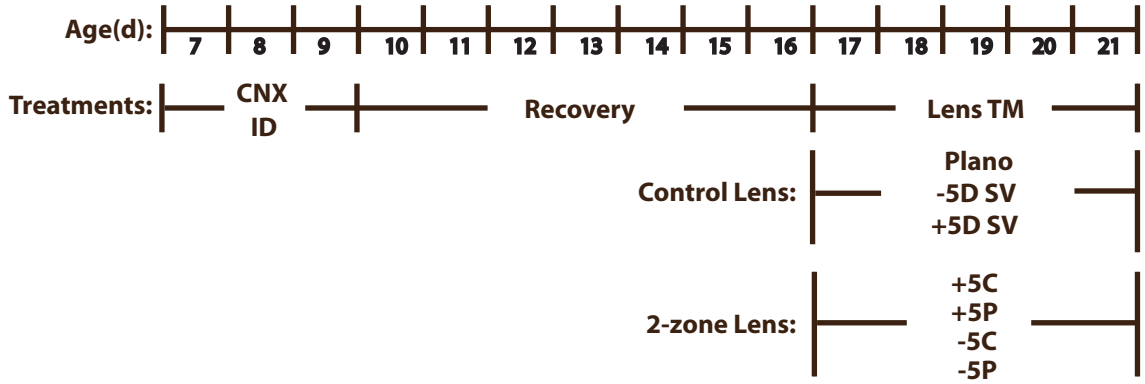


Figure 1-3. Schematic summary of the surgery and lens treatment paradigms used in Chapter 4.

4. To investigate the effects on refractive development and ocular growth of 2-zone concentric lenses with negative powers in both optical zones. We tested the lenses on both normal eyes and eyes that had been made myopic, to simulate the conditions experienced when multifocal soft contact lenses are used to treat myopia. This study sought to address the following questions: 1) what are the relative contributions of peripheral and central retinal regions in guiding emmetropization, 2) does the control of myopia progression require reductions in hyperopic defocus over the entire retina or are treatments targeting the central or peripheral retinal regions alone sufficient to slow progression. Sample size is also 7 in each group. The study design is shown schematically in Figure 1-4.

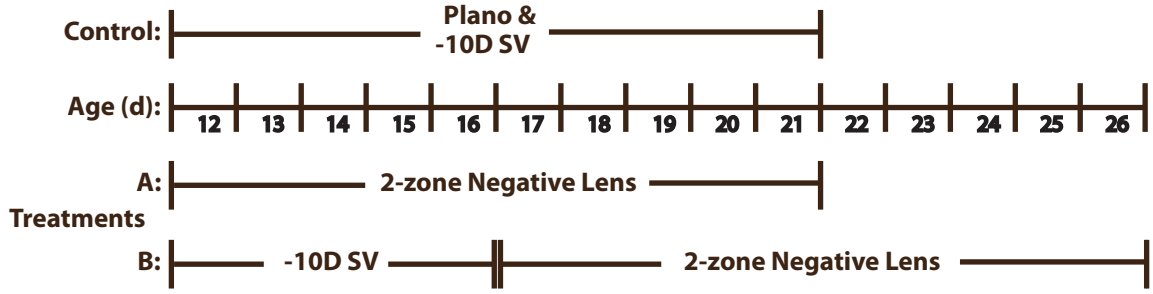


Figure 1-4. Schematic summary of the two lens treatment paradigms (A & B) used Chapter 5; single vision (SV) lenses were included either as a comparison control treatment (A), or as an initial myopia-inducing treatment (B).

These individual specific aims are elaborated in greater detail in chapters 2-5. A further validation study covering the use of iridectomy as a method for producing a fixed, enlarged pupil without affecting accommodation is presented in Supplement I, which has also been published in Investigative Ophthalmology and Visual Science (Ostrin, Liu, *et al.*, 2011). In chapter 6, the major findings of studies described in this dissertation are summarized, their clinical implications discussed and future research directions outlined.

Chapter 2

The effectiveness of optical interventions in

myopia retardation---a systematic review

Abstract

Purpose: To compare the effectiveness of optical interventions such as bifocal (BF) glasses and progressive additional lenses (PALs) as well as soft contact lenses (SCLs) and rigid gas permeable contact lenses (RGPCLs) in controlling the progression of myopia.
Data sources: Trials published in English were identified from the Cochrane Library (2008, issue 1), MEDLINE (1970 to January 2008), and EMBASE (1980 to January 2008). The reference lists of the studies and the Science Citation Index were also searched.
Selection criteria: Only randomized clinical trials conducted in myopic children with their primary objective as the prevention of the progression of myopia were included in this review. Trials with interventions other than optical only corrections such as pharmaceutical agents, used alone or in combination with spectacles, were not included in this review.
Data extraction and synthesis: The guidelines from the Cochrane Handbook for Systematic Reviews of Intervention were used to assess the quality of the trials. Data were summarized using mean differences and were combined using a fixed-effects model.
Main results: This review included seven randomized controlled trials involving a total of 1385 subjects, of which 641 children in treatment groups and 744 children in control groups completed the study. Overall the amount of myopia progression was statistically significantly slower in the treatment group (Mean Difference -0.20 D, 95% Confidence Interval -0.21 to -0.19 D, $p< 0.0005$). Subgroup analyses by gender, age, and baseline binocular eye posture indicated that treatments were statistically but not clinically more effective in boys, older children, and esophores at baseline.
Conclusion: There is insufficient evidence to provide clear guidelines for prescribing the tested optical appliances to control myopia progression, as the magnitude of the treatment effect is small and clinically insignificant. Custom designed contact lenses, which are now emerging onto the market, are likely to replace more traditional optical treatments as the predominant anti-myopia interventions, although published well-designed, large scale clinical trials are currently lacking.

2.1 Objective

The objective of this study was to systematically compile the current evidence from relevant randomized clinical trials evaluating the effectiveness of optical interventions in retarding the progression of myopia.

2.2 Methods

2.2.1 Selecting studies for the review

Only randomized clinical trials conducted in myopic children with their primary objective as the prevention of the progression of myopia were included in this review. Publications covering retrospective studies, nonrandomized trials, non-controlled trials, short-term trials with duration less than 1 year, and those in which the primary purpose of the trial was not myopia retardation were excluded. Trials with interventions other than optical corrections such as pharmaceutical interventions, used alone or in combination with spectacles, were also excluded from this review, due to the dramatic differences in study designs and allocation concealment.

2.2.2 Search methods for identification of studies

Only trials published in English were identified from the Cochrane Library, MEDLINE, and EMBASE using the following strategies:

#1	myopia
#2	myopia prophylaxis
#3	myopia NEAR prevent*
#4	myopia NEAR progression
#5	#2 OR #3 OR #4
#6	short NEXT sight* OR shortsight*
#7	near-sight* OR nearsight*
#8	#6 OR #7
#9	myopia NEAR control
#10	#9 AND #5

The publication type is restricted to clinical trials.

The titles and abstracts were assessed and copies of papers covering all potentially or definitely relevant studies were obtained to determine whether the studies met the criteria for inclusion in this review. References of all included publications were also reviewed.

2.2.3 Validity assessment

The validity of each trial included in the review was assessed using the following four parameters, listed in Section 6 of the Cochrane Handbook for Systematic Reviews of Interventions:

- selection bias
- performance bias
- attrition bias
- detection bias.

Each parameter of trial quality was graded using the following scale: A, low risk of bias; B, moderate risk of bias; or C, high risk of bias. Sensitivity analysis was performed to

examine the effect on the results of including or excluding trials attracting the poorest score, C.

2.2.4 Data extraction and synthesis

Information on patients and characteristics of the 9 studies that were initially included are summarized in Table 2-1.

As primary outcomes, mean changes in refractive error from baseline, specified as the mean spherical equivalent refractive error in diopter (D), were derived from published data. To compare the differences in the results between the treatment and control groups, the nonstandardized mean difference (MD) was used, as all included trials used the same absolute scale (D) in reporting their data. The intention-to-treat analysis was neither applied in individual study nor in the meta-analysis, as there was no complete outcome data for all randomized subjects, due to dropouts.

Data analyses were performed according to the Cochrane Eyes and Vision Group statistical guidelines. Initial analyses included data from all studies, which was then stratified by gender, age, and baseline binocular eye posture (heterophoria). The decision to perform subgroup analysis in this way is consistent with findings from previously published epidemiological evidence in this area.

Heterogeneity was examined by assessing the overlap of the confidence intervals for the results of individual studies. Significant heterogeneity was considered to be present when there was little or no overlap between the 95% CI of the estimated outcome measures. Nonetheless, a fixed effect meta-analysis was performed, regardless of whether the evidence suggested significant heterogeneity, as refractive error measurement techniques are highly standardized worldwide and it is reasonable to assume that the true effect of the treatments under review is fixed across studies, in both magnitude and direction.

Publication bias and other reporting biases were addressed by performing sensitivity analysis and funnel plots.

2.3 Description of studies

2.3.1 Finding the trials

The original electronic searches identified 78 potentially relevant abstracts. Twenty-five potentially relevant publications were retrieved for further evaluation, of which 12 were subsequently excluded. Specific information covering the number of included and excluded studies is presented in a QUOROM statement flow diagram (Figure 2-1). Ultimately, 12 publications covering 9 randomized clinical trials that met the inclusion criteria were identified. Below is a summary of the characteristics of the included trials. Further details are summarized in Table 2-1. One additional trial (Grosvenor 1987[17]) was excluded from the meta-analysis because the outcome measures were presented without any error estimates, leaving a total number of 8 trials included in this study.[18-26]

2.3.2 Included studies

Participants in all trials were children, aged from 6 to 12 years, with low to moderate myopia. Exclusion criteria included any ocular pathology, previous ocular surgery, manifest strabismus, amblyopia and astigmatism higher than 2 D.

To reiterate, trials involving only optical interventions were included in this review. All subjects randomized to treatment groups received either spectacle lenses incorporating near additions, such as bifocal (BF) lenses or progressive additional lenses (PAL), or contact lenses, such as soft contact lenses (SCL) or rigid gas permeable contact lenses (RGPCL). All but one study used single vision (SV) distant spectacle correction as the control "treatment". The one exception was the Walline 2004 study[27], which used SV SCLs as the control treatment, to improve compliance and minimize differential dropouts across the two treatment groups.

All included trials defined the primary outcome as myopia progression, specified as the change in mean equivalent spherical refractive error (D). Eyes were cyclopleged prior to refraction measurements in all but one study, that of Horner 1999.[25]

2.3.3 Methodological quality of included studies

Selection bias (allocation concealment):

All included studies were graded A, indicating a low risk of bias. In all of the included studies, neither the participants nor the recruiters were aware of the next assignment in the sequence until after the decision about eligibility had been made. The approaches used to ensure adequate concealment were centralized randomization, in Gwiazda 2003 (COMET) study,[22] and sequentially numbered, sealed, opaque envelopes in other included studies. Furthermore, a minimal number of subjects requested to alter their assignments across all included studies.

A family history of myopia is a potential confounder in the association between the optical interventions and the progression of myopia. Myopic parents tend to be more aware of the development of refraction errors and any reduction in eyesight of their children, and are so more concerned with myopia progression. Consequently they are more motivated to have their children participate in studies aimed at retarding myopia progression. Furthermore, parental myopia has been consistently demonstrated to be positively associated with the development and progression of myopia in children. However, the potential for bias in the characteristics of study participants, which may not have been truly representational of the overall population of myopic children, does not affect the internal validity of the studies, as the baseline characteristics of the participants in both treatment and control groups should have been similar, with proper randomization.

Performance bias: masking of participants and/or care providers

Due to the different optical properties and comfort associated with each type of optical intervention, it was impossible for either the participants or their care providers to be masked from details of the assigned treatment. Thus this information was not used as a parameter for assessing the methodological quality of studies.

Detection bias: a) outcome assessment
Generally speaking, the potential for detection bias in studies investigating the progression of myopia is unlikely to be significant, as all of the outcomes were measured quantitatively and objectively. In all of the included studies, standardized refraction protocols were rigidly applied in measuring both the experimental and the control groups. All except 2 studies, by Horner 1999,[25] and by Katz 2003,[26] were awarded a grade of A, indicating a low risk of bias. In most studies, the potential for detection bias was further reduced by ensuring that those responsible for data collection were not provided with information about allocated interventions. The Katz 2003 study was graded B, corresponding to a moderate risk of bias, as it was not clear whether data collectors were masked or whether any of efforts were made to reduce this bias. The Horner 1999 study was graded C, corresponding to high risk of bias, as refractions were not performed under cycloplegia, which minimizes the risk of overestimating myopic refractive errors in children and thus considered the gold standard for refracting children. The direction of the bias could also not be predicted for the latter study, as the degree of measurement error cannot be accurately estimated, for either the treatment or the control group.
b) Selective reporting (eyes analyzed/reported: right, left, worse, or average of two eyes)
All studies were awarded a grade of A, i.e., with a low risk of bias, as the eyes analyzed/reported were consistent between treatment and control groups, in all studies.

Attrition bias: rates of follow-up
Three studies (Parssinen 1989,[19] Gwiazda 2003,[22] and Walline 2004[27]) were awarded a grade of A (low risk of bias), as losses to follow-up were low and similar between treatment and control groups. Four studies (Fulk 1996,[28] Fulk 2000,[23] Edwards 2002,[29] and Horner 1999,[25] Katz 2003[26]), were awarded a grade of B (moderate risk of bias), either because of significant losses to follow-up or a differential dropout rates between treatment and control groups. Only one study (Grosvenor 1987)[17] was awarded a grade of C (high risk of bias), due to both significant losses to follow-up and a differential dropout rates between treatment and control groups.

2.4 Results

The study by Horner 1999 was excluded from initial analysis as it had at high risk of bias. The sensitivity to its inclusion of the results of statistical analyses was then examined. All results reported below are for the fixed-effect model. As mentioned earlier, the available case, instead of the intention-to-treat, analysis was used in this review because the means and standard deviations of outcome measures for the dropouts were not available.

2.4.1 Mean difference of myopia progression

2.4.1.1 All participants (Analysis 1, Figure 2-2)
Data pooled from 7 trials (Parssinen 1989;[19] Fulk 1996;[28] Fulk 2000;[23] Edwards 2002;[29] Gwiazda 2003;[22] Katz 2003;[26] Walline 2004[27]) were used in analyses. The data covered a total of 1385 myopic children, with the treatment group receiving either BF lenses or PALs (n = 129, 356 respectively) or RGPCLs (n=156), and the control group, SV lenses

(n=744). Analysis of the data showed that the amount of myopia progression was statistically significantly slower in the treatment group (MD -0.20 D, 95% CI -0.21 to -0.19 D, $p < 0.0005$). However, the treatment effect was not considered clinically significant, as it was smaller than the variability in objective refractions measured using commercially available autorefractors.

Sensitivity of results

The statistical significance of the results was not affected by the inclusion of Horner 1999 study; however the mean difference showed a smaller treatment effect (MD -0.14 D, 95% CI -0.15 to -0.13 D, $p< 0.0005$) with Horner 1999 study included.

To investigate whether the optical interventions varied in their effectiveness across the different subgroups, two-sample t-tests were performed on mean differences to examine whether any of the differences in the treatment effect of the various subgroups reached statistical significance.

2.4.1.2 Stratified by gender (Analysis 2, Figure 2-3)

Mean differences, stratified by gender, were available for a total of 229 girls and 217 boys, from three trials (Parssinen 1989; Edwards 200223; Gwiazda 2003). The dropout rates were assumed to be similar for girls and boys, when there was no data describing the numbers of boys and girls completing the studies. This analysis indicated that the treatment effect was statistically more effective in boys than girls (ΔMD, 0.021 D, 95% CI, 0.019 to 0.0233 D). However, here also, the gender-related difference of treatment effects was deemed to be clinically insignificant.

2.4.1.3 Stratified by age (Analysis 3, Figure 2-4)

Mean differences, stratified by age, were available from three trials for a total of 311 young children (< 10 yr in Fulk 2000[23] & Gwiazda 2003;[22] <10.6 yr in Walline 2004),[27] and 349 older children. This analysis showed that the treatment effect was statistically more effective in older children than younger children (ΔMD, 0.011 D, 95% CI, 0.0090 to 0.014 D). However, this age-related difference of the treatment effects was not clinically significant.

2.4.1.4 Stratified by baseline binocular eye posture (heterophoria) (Analysis 4, Figure 2-5)

Mean differences, stratified by baseline heterophoria, were available for a total of 147 esophoric children from three trials (Fulk 1996;[28] Fulk 2000;[23] Gwiazda 2003).[22] The test of heterogeneity here showed a much smaller test statistics, suggesting better consistency in results across the three trials. This analysis showed the treatment effect to be statistically more effective in esophoric children than all subjects combined (ΔMD, 0.024 D, 95% CI 0.0022 to 0.026 D).

2.4.2 Examine the influences of individual studies (Analysis 5, Figure 2-6)

The meta-analysis is dominated by the Gwiazda 2003 study, and omission of other studies made little difference to the overall combined treatment effect estimate.

Nonetheless, when Gwiazda 2003 was omitted, a smaller but still statistically significant treatment effect was recorded (MD -0.17 D, 95% CI, -0.25 to -0.09 D).

2.4.3 Assessing the publication bias (Analysis 6, Figure 2-7)
The funnel plot appeared relatively symmetric, and there was no evidence of publication bias, using either the Egger method (p=0.88) or the Begg method (p=0.72).[30-33]

2.5 Discussion

2.5.1 Strength of evidence
Despite strong and consistent evidence from animal studies for the modulatory influence of optical defocus on emmetropization and myopia development, the best available evidence from randomized clinical trials of optical devices for myopia control in children is inconclusive, the magnitude of treatment effects being only small and clinically insignificant, albeit consistent. All of the studies included in the meta-analysis reported here were randomized clinical trials. Such studies are generally relatively free of biases. However, it would have been impossible to institute double-masking effectively, to eliminate performance bias, due to the nature of the interventions involved. For example, the near additions of BF lenses have visible edges, and distortions in the immediate surround of the near additions are evident in PALs. Performance biases may have contributed to the discrepancy between the strong evidence from animal studies and inconclusive results from clinical trials in the following ways. First, unlike in the animal studies, where the compliance to lens treatment can be rigorously monitored, the adherence to the lens-wearing protocols is generally extremely poor among children for MF spectacle lenses. Importantly, self-reported compliance to lens-wear has been suggested to be highly inaccurate, although this information was used to monitor compliance in at least some of the studies. Second, human myopia appears to have multiple etiologies, being the product of complex genetic and environmental interactions. Thus there are likely to be subgroups of myopes, with different genetic susceptibilities and environmental risk factors. This situation contrasts with animal models of induced myopia, in which both the genetic background and environmental exposures tend to be well controlled and standardized. Thus while treatment interventions produce predictable outcomes in animal studies, the application of a standardized treatment to a heterogeneous population of human myopes, encompassing different subtypes of myopia, may be expected to mask the true treatment effect relevant for one or several subgroups.

2.5.2 Applicability
2.5.2.1 Variation in baseline characteristics
As mentioned earlier, the characteristics of the participants in published myopia intervention studies may not adequately represent the population of myopic children, given that myopic parents tend to pay more attention to such clinical studies and are thus more likely to have their children participate. For this reason, the results from this review might not be generally applicable to the general population of myopic children.

2.5.2.2 Variation in adherence

Variation in the adherence of the participants to prescribed treatment regimens may also limit the general applicability of results. Several differences in compliance between treatment and control groups are predicted. First, due to the unavoidable visual distortions, especially in the peripheral visual fields, associated with wearing BF lenses or PALs, the adherence to prescribed treatment regimens (lens-wearing schedules), are likely to be much lower for experimental groups than for control groups, which were consistently assigned SV distant corrections in studies involving spectacle lenses. BF lenses are also cosmetically unacceptable to some children. Likewise, due to the discomfort associated with wearing rigid contact lenses, the adherence to the prescribed treatment regimen of RGPCL group is likely to be lower than that of the control, SCL-wearing group. These inter-group differences in compliance, specifically, the tendency of treatment groups to wear their appliances less, is predicted to lead to an underestimation of the treatment effect.

2.6 Conclusion

2.6.1 Implication for practice

The optical interventions included in this review fall into to category 2B, according to Section 9 of the Cochrane Handbook for Systematic Reviews of Interventions. There is insufficient evidence to provide clear guidelines for clinical practice, as the magnitudes of effect of tested optical interventions in controlling myopia are small and clinically insignificant. Nonetheless, the treatments investigated may require further attention, as there are plausible reasons to expect better effects from them, at least in subgroups of myopic children.

2.6.2 Implication for research

The optical distortions inherent in BF lenses and PALs, the cosmetic drawback of BFs, and the practical inconvenience of spectacle wear for more active children; remain major drawbacks to these optical interventions for myopia control. Furthermore, without an effective measure of true compliance in spectacle wear, an accurate estimation of the effectiveness of these appliances will remain elusive. On the other hand, the rapid advances in the material used in and design of contact lenses, hold promise of improved comfort and optical quality of contact lenses, with risks of contact lens-induced corneal hypoxia, inflammation and infection being reduced to a minimal level with proper lens care procedures. In addition, MF contact lenses outperform spectacle corrections in terms of cosmesis, and associated optical distortions are less readily detected. Thus the main reasons for poor compliance with MF spectacles and likely high dropout rates in myopia control studies are resolved. Consequently, specially-designed contact lenses, making use of advances in our understanding of how optical defocus influences refractive development through animal model studies, seem likely to replace spectacle interventions as primary treatments for myopia control. Indeed, promising small-scale clinical trials of both MF soft contact lenses and rigid corneal reshaping (CRT) lenses have already published. While further clinical trials will be required to assess their efficacy, the efficiency of such studies will likely to be much improved over the spectacle intervention trials described here, as both the differential performance of treatment and control groups,

in terms of adherence to treatment, and dropout rates, are expected to be significantly reduced.

2.6.3 Limitations of this review

This review has several limitations. First, the author herself was in charge of both identifying qualified publications and assigning scores to each included study for its methodological validity. Thus the objectivity of this systematic review could be challenged. Second, due to time constraints, no effort was made to contact the investigators of individual studies, to either clarify ambiguities in data presentations in the original publications or to acquire copies of raw data, to improve the efficiency of data usage in the meta-analysis. Finally, although this systematic review included a total of 9 studies, with 7 studies included in the meta-analysis, the estimate of the treatment effect largely reflected the treatment effect of one study, that by Gwiazda 2003, due to its very large sample size and the small standard errors associated with reported treatment effect estimates. Thus this trial attracted major weighting. With this study omitted from the meta-analysis, the combined treatment effect would have been even smaller than that reported.

Table 2-1. Characteristics of Included Studies

Study	Participants	Methods	Interventions	Outcomes
Spectacle Study				
Grosvenor 1987	Country: US Numbers randomized: 207 Age: range 6 to 15 years Gender: 50% girls Baseline refractive error: (range -0.35 to -3.0D)	Randomization: age, gender and baseline RE stratified block random number table. Masking: yes for outcome measurement. Exclusion after randomization: None. Losses to follow up: 83 (30 from SVL, 28 from +1D BF and 25 from +2D BF group) Duration/frequency of follow-up of the study: 1.5/0.5yrs	+1D BF +12 BF SVL*	1.5-yr myopia progression: +2D BF: 0.48D +1D BF: 0.51D SVL: 0.45D
Parssinen 1989	Country: Finland Numbers randomized: 240 Age: 10.9 (range 8.8 to 12.8 years 0 Gender: 50% girls Baseline refractive error: (range -0.35 to -3.0D)	Randomization: gender-stratified random permuted block design, sealed envelop. Masking: yes for outcome measurement. Exclusion after randomization: 1. Losses to follow up: 2 (2 from SVL continuous use and 1 from BF group) Duration/frequency of follow-up of the study: 3/1yrs	+1.75D BF SVL distant use SVL continuous use*	3-yr myopia progression: BF: 1.50±1.1D SVL distant use: 1.44±1.1D SVL continuous use: 1.35±1.2D
Fulk 1996	Country: US Numbers randomized: 32 Age: range 6 to 14 years Gender: unclear Baseline refractive error: -2.12D	Randomization: gender-stratified random permuted block design, sealed envelop. Masking: yes for outcome measurement. Exclusion after randomization: none. Losses to follow up: 4 (2from SVL group and 2 from BF group) Duration/frequency of follow-up of the study: 1.5/0.5yrs	+1.25D BF SVL*	1.5-yr myopia progression: BF: 0.585±0.180D SVL: 0.855±0.165D
Fulk 2000	Country: US Numbers randomized: 82 Age: range 6 to 13 years (10.65±1.41) Gender: 48% girls Baseline refractive error: -2.32±1.40D.	Randomization: gender-stratified random permuted block design, sealed envelop. Masking: yes for outcome measurement. Exclusion after randomization: none. Losses to follow up: 7 (6 from BF and 1 from SVL group) Duration/frequency of follow-up of the study: 2.5/0.5yrs	+1.5D BF SVL*	2.5-yr myopia progression: BF: 0.99±0.68D SVL: 1.24±0.65D
Edwards 2002	Country: Hong Kong, China Numbers randomized: 298 Age: 9.05 (range 7 to 10.5 years) Gender: 52.2% girls	Randomization: predetermined random sequence Masking: yes for outcome measurement. Exclusion after randomization: none.	+2D PALs SVLs *	Adjusted 2-yr myopia progression: PAL: 1.12±0.67D

	Baseline refractive error: -2.87±0.99D.	Losses to follow up: 44 (17from PAL and 27from SVL group) Duration/frequency of follow-up of the study: 2/0.5yrs		SVL: 1.26±0.74D
Gwiazda (COMET) 2003	Country: US Numbers randomized: 469 Age: range 6 to 11 years (9.35±1.30) Gender: 52.5% girls Baseline refractive error: -2.39±0.84D.	Randomization: center-stratified random permuted block design. Masking: yes for outcome measurement Exclusion after randomization: none. Losses to follow up: 7 (6 from PAL and 1 from SVL group) Duration/frequency of follow-up of the study: 3/0.5yrs	+2D PALs SVLs *	Adjusted 3-yr myopia progression: PAL: 1.28±0.06D SVL: 1.48±0.06D
Contact Lens Study				
Horner 1999	Country: US Numbers randomized: 175 Age: 8.4±1.4 (range 6 to 12 years Gender: 55.1% girls Baseline refractive error: -2.84±0.83D (non-cyclopleged) .	Randomization: permuted block design, sealed envelop Masking: yes for outcome measurement. Exclusion after randomization: none. Losses to follow up: 33 (12 from SCL and 21 from SVL group) Duration/frequency of follow-up of the study: 3/0.5yrs	SCLs SVLs *	3-yr myopia progression: SCL: 1.066±0.102D SVL: 0.909±0.096D
Katz 2003	Country: Singapore Numbers randomized: 564 Age: range 6 to 11 years (8.4±1.5) Gender: 46.7% girls Baseline refractive error: -2.39±0.84D.	Randomization: permuted block design, sealed envelop Masking: none Exclusion after randomization: 181. Losses to follow up: 86 (53 from RGP and 33 from SVL group) Duration/frequency of follow-up of the study: 2/0.5yrs	RGP SVLs *	Adjusted 2-yr myopia progression: SCL: 1.33±0.84D SVL: 1.28±0.78D
Walline (CLAMP) 2004	Country: US Numbers randomized: 116 Age: range 8 to 11 years (10.7±1.1) Gender: 59.5% girls Baseline refractive error: -2.40±0.90D.	Randomization: gender-stratified random permuted block design. Masking: yes for outcome measurement. Exclusion after randomization: none. Run-in period: yes, 65.7±33.1days Losses to follow up: 0 Duration/frequency of follow-up of the study: 3/1yrs	RGP SCLs *	Adjusted 3-yr myopia progression: RGP:1.56±0.95D SCL: 2.19±0.89D

* Control group D=diopters

Figure 2-1. The QUOROM statement flow diagram

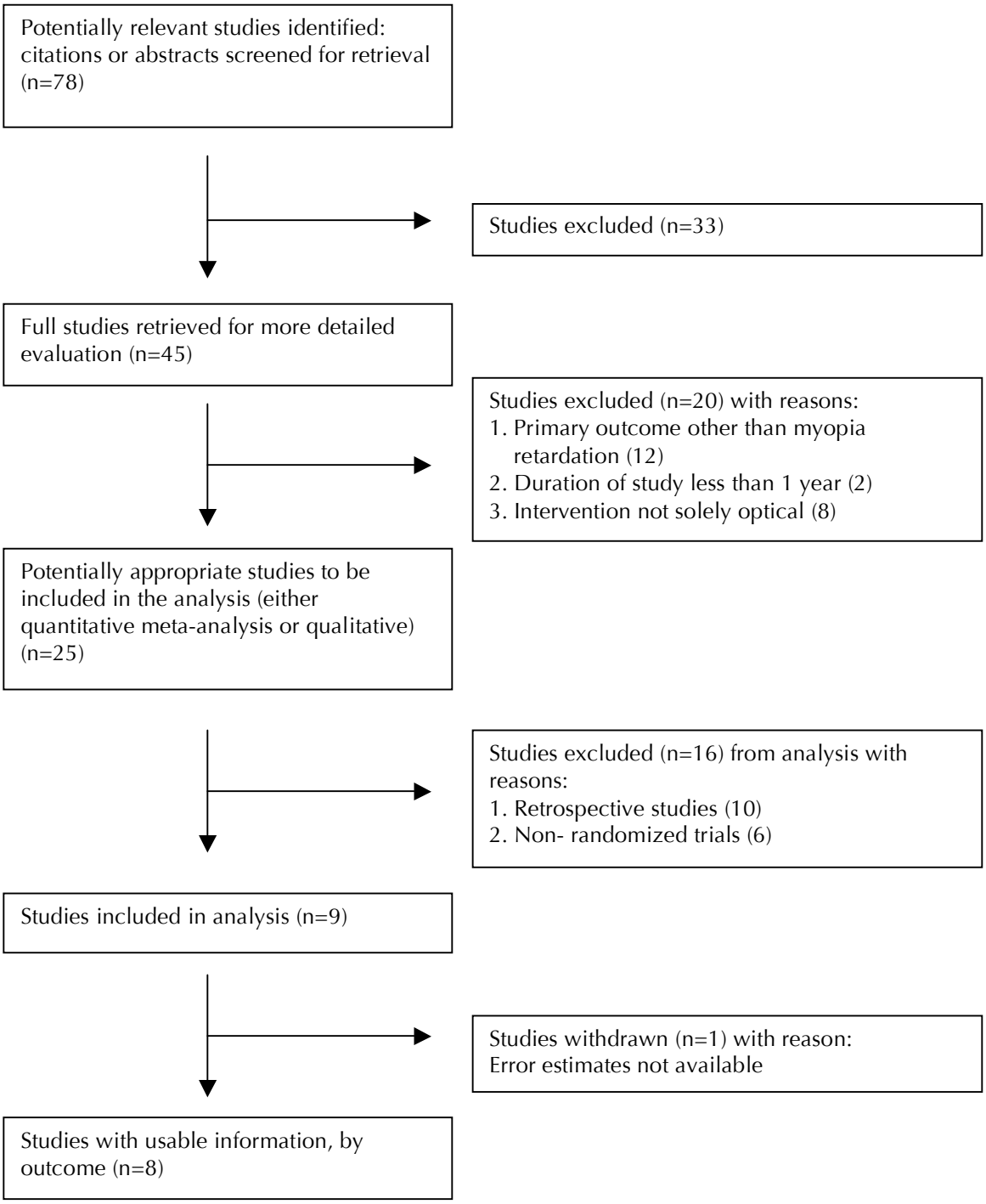
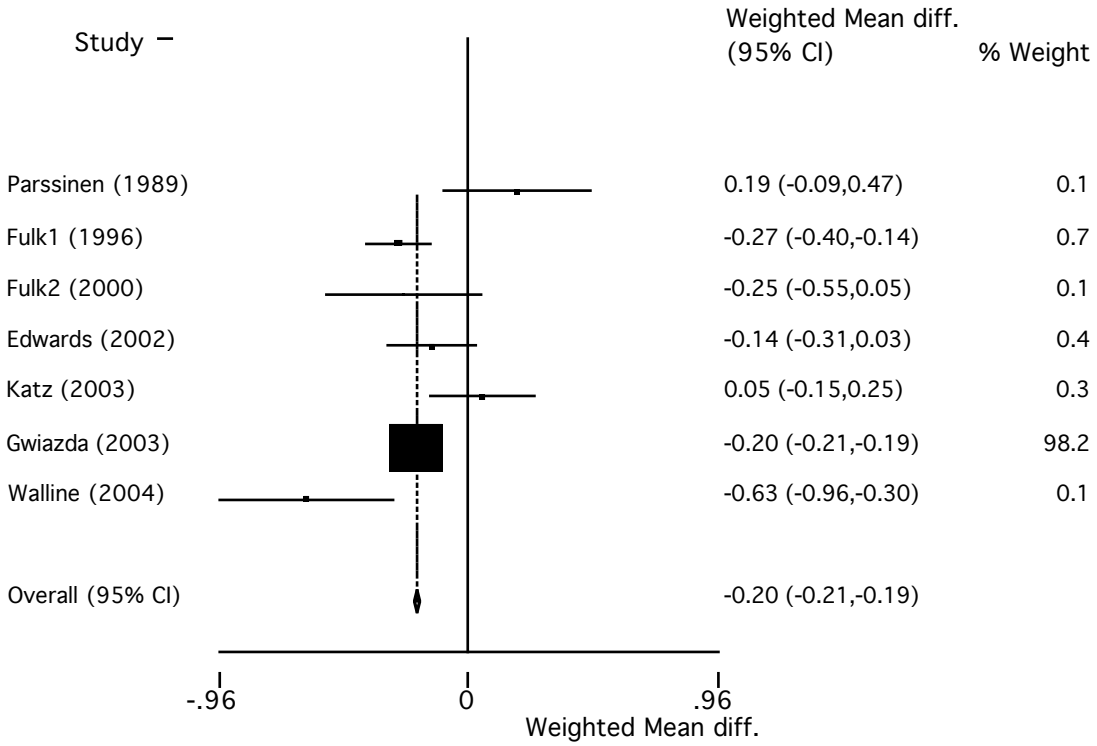


Figure 2-2. Mean difference of myopia progression--- all participants



Study	**WMD**	**[95% Conf. Interval]**		**% Weight**
Parssinen (1989)	.19	-.09	.47	.15
Fulk1 (1996)	-.27	-.40	-.14	.71
Fulk2 (2000)	-.25	-.55	.05	.13
Edwards (2002)	-.14	-.31	.03	.39
Katz (2003)	.05	-.15	.25	.29
Gwiazda (2003)	-.20	-.21	-.19	98.24
Walline (2004)	-.63	-.96	-.30	.10
I-V pooled WMD	**-.20**	**-.21**	**-.19**	

Heterogeneity chi-squared = 21.40 (d.f. = 6) p = 0.002
Test of WMD=0 : z= 36.32 p = 0.000

Figure 2-3. Mean difference of myopia progression--- girls compared to boys

Figure 2-3a. Mean difference of myopia progression--- girls

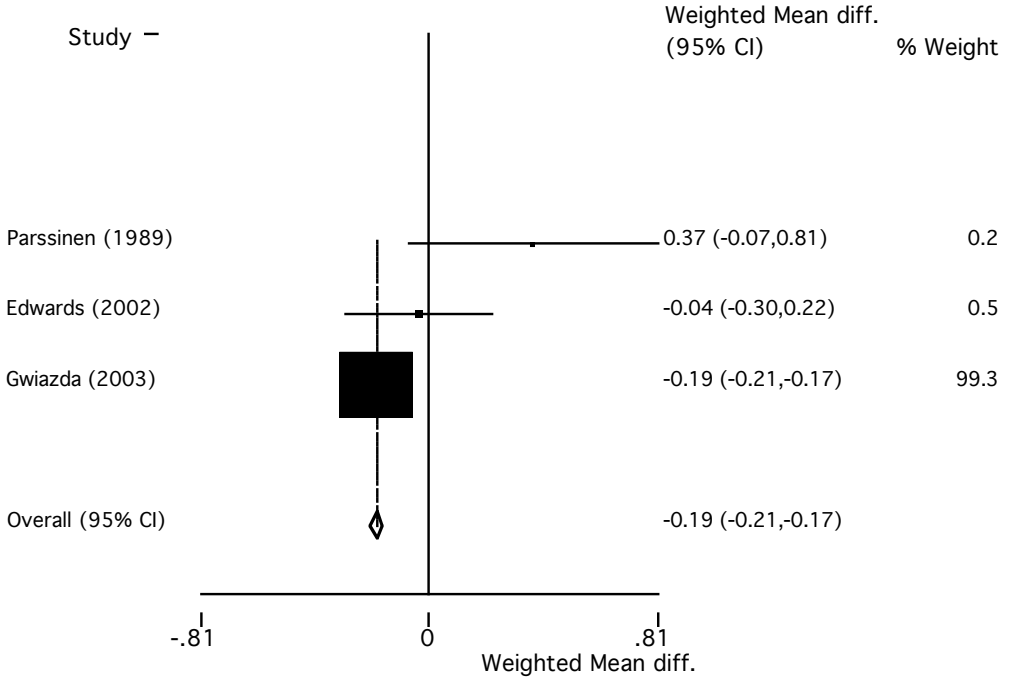
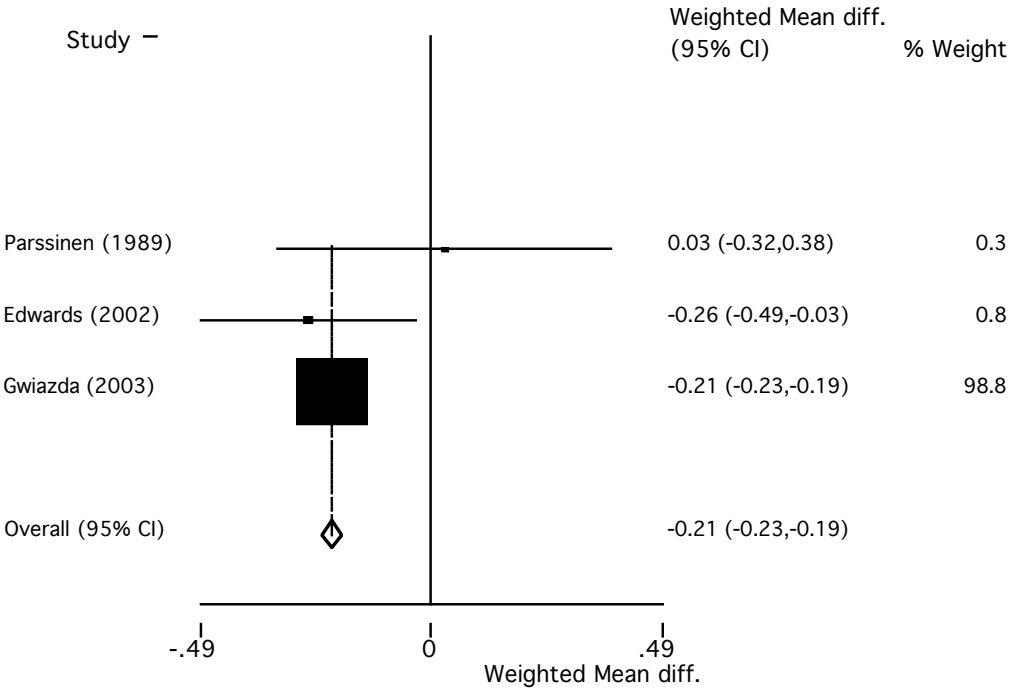


Figure 2-3b. Mean difference of myopia progression--- boys



Study--- Girls	**WMD**	**[95% Conf. Interval]**		**% Weight**
Parssinen (1989)	.37	-.07	.81	.18
Edwards (2002)	-.04	-.30	.22	.52
Gwiazda (2003)	-.19	-.21	-.17	99.30
I-V pooled WMD	**-.19**	**-.21**	**-.17**	

Heterogeneity chi-squared = 7.36 (d.f. = 2) p = 0.025
Test of WMD=0 : z= 19.72 p = 0.000

Study--- Boys	**WMD**	**[95% Conf. Interval]**		**% Weight**
Parssinen (1989)	.03	-.32	.38	.35
Edwards (2002)	-.26	-.49	-.03	.83
Gwiazda (2003)	-.21	-.23	-.19	98.82
I-V pooled WMD	**-.21**	**-.23**	**-.19**	

Heterogeneity chi-squared = 1.95 (d.f. = 2) p = 0.376
Test of WMD=0 : z= 19.68 p = 0.000

Testing the significance of the difference of treatment effects stratified by gender:

	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Girls	221	-.19	.00064	.0095	-.189	-.187
Boys	214	-.21	.00073	.011	-.211	-.208
combined	435	-.20	.00071	.015	-.200	-.197
diff		.021	.00097		.019	.023

diff = mean(x) - mean(y) t = 22.0289
Ho: diff = 0 degrees of freedom = 433
Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 1.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 0.0000

Figure 2-4. Mean difference of myopia progression--- young vs. old children
Figure 2-4a. Mean difference of myopia progression--- young children

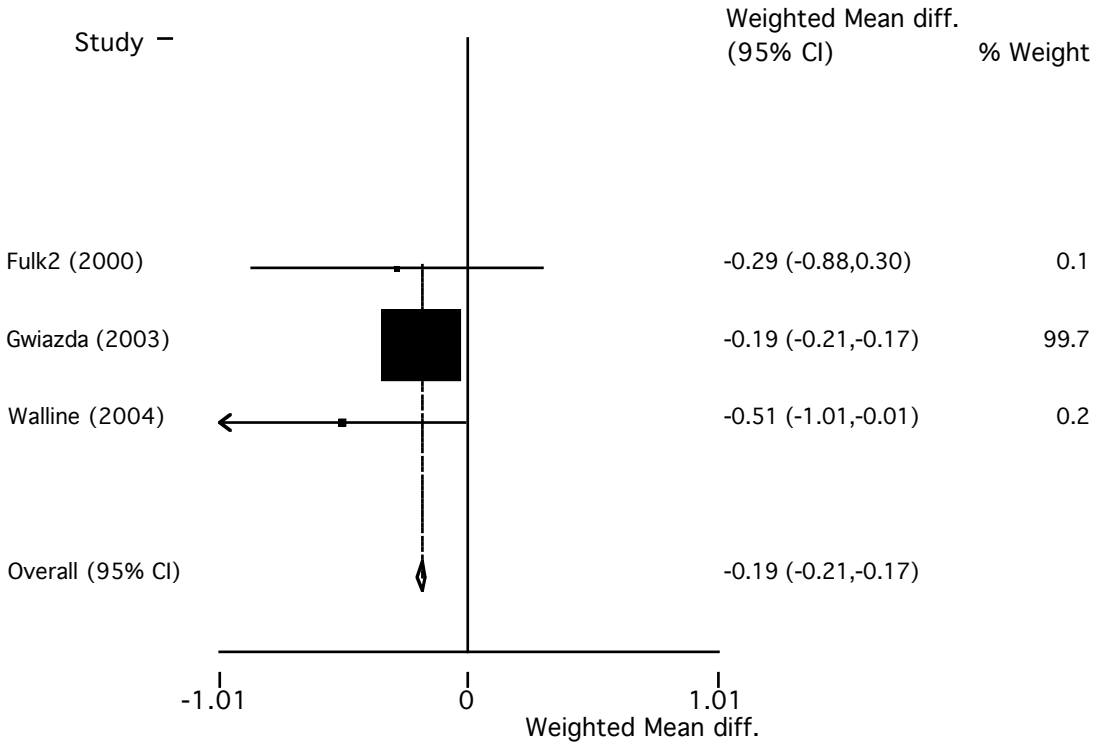
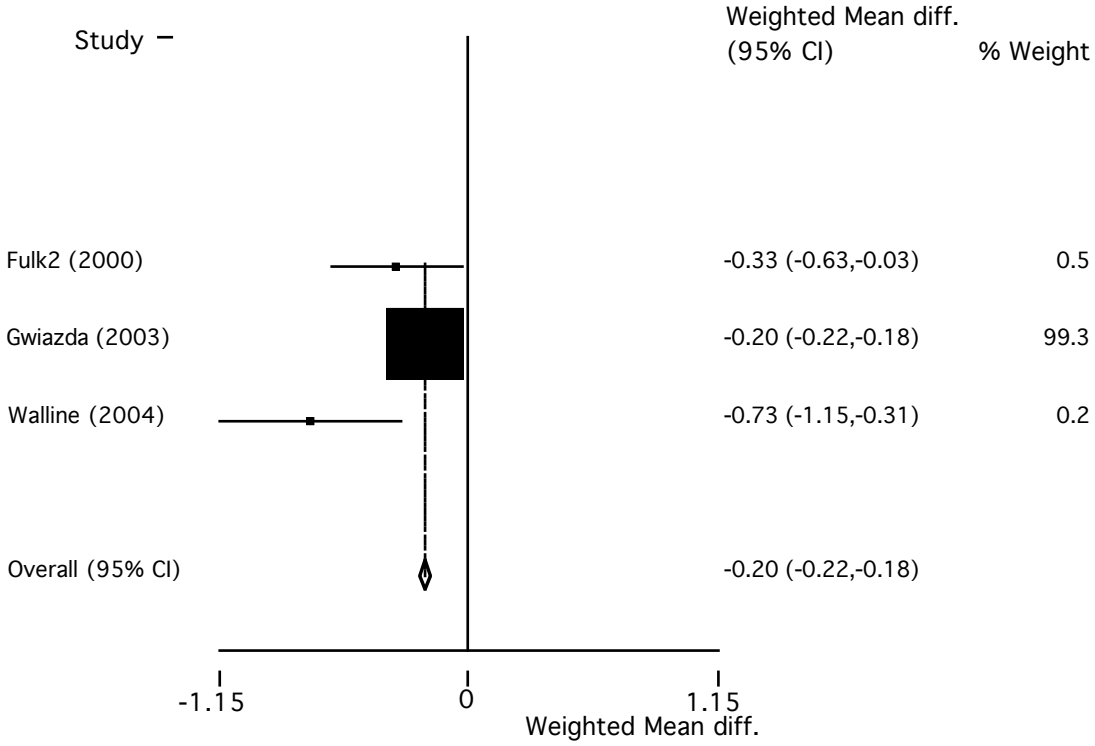


Figure 2-4b. Mean difference of myopia progression--- old children



Study--- Young	**WMD**	**[95% Conf. Interval]**		**% Weight**
Fulk2 (2000)	-.29	-.88	.30	.12
Gwiazda (2003)	-.19	-.21	-.17	99.71
Walline (2004)	-.51	-1.01	-.011	.17
I-V pooled WMD	**-.19**	**-.21**	**-.17**	

Heterogeneity chi-squared = 1.69 (d.f. = 2) p = 0.429
Test of WMD=0 : z= 18.17 p = 0.000

Study--- Old	**WMD**	**[95% Conf. Interval]**		**% Weight**
Fulk2 (2000)	-.33	-.63	-.031	.46
Gwiazda (2003)	-.20	-.22	-.18	99.31
Walline (2004)	-.73	-1.15	-.31	.23
I-V pooled WMD	**-.20**	**-.22**	**-.18**	

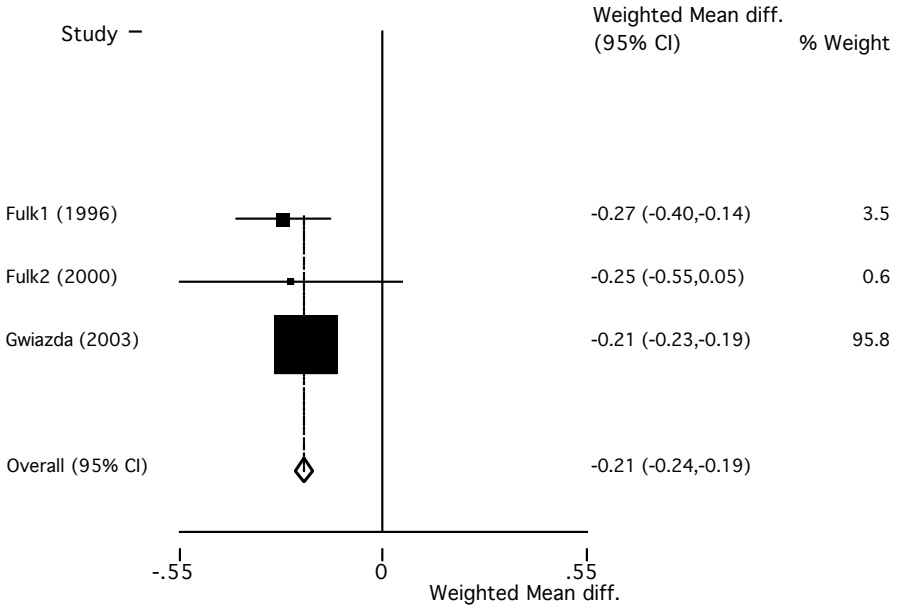
Heterogeneity chi-squared = 6.78 (d.f. = 2) p = 0.034
Test of WMD=0 : z= 19.48 p = 0.000

Testing the significance of the difference of treatment effects stratified by age:

	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Young	161	-.19	.00083	.010	-.192	-.189
Old	169	-.20	.00080	.010	-.203	-.200
combined	330	-.20	.00065	.012	-.198	-.195
diff		**.011**	**.0011**		**.00895**	**.0134**

diff = mean(x) - mean(y) t = 9.7553
Ho: diff = 0 degrees of freedom = 328
Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 1.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 0.0000

Figure 2-5. Mean difference of myopia progression--- baseline esophoric children



Study	**WMD**	**[95% Conf. Interval]**		**% Weight**
Fulk1 (1996)	-.27	-.40	-.14	3.53
Fulk2 (2000)	-.25	-.55	.052	.63
Gwiazda (2003)	-.21	-.23	-.19	95.83
I-V pooled WMD	**-.21**	**-.24**	**-.19**	

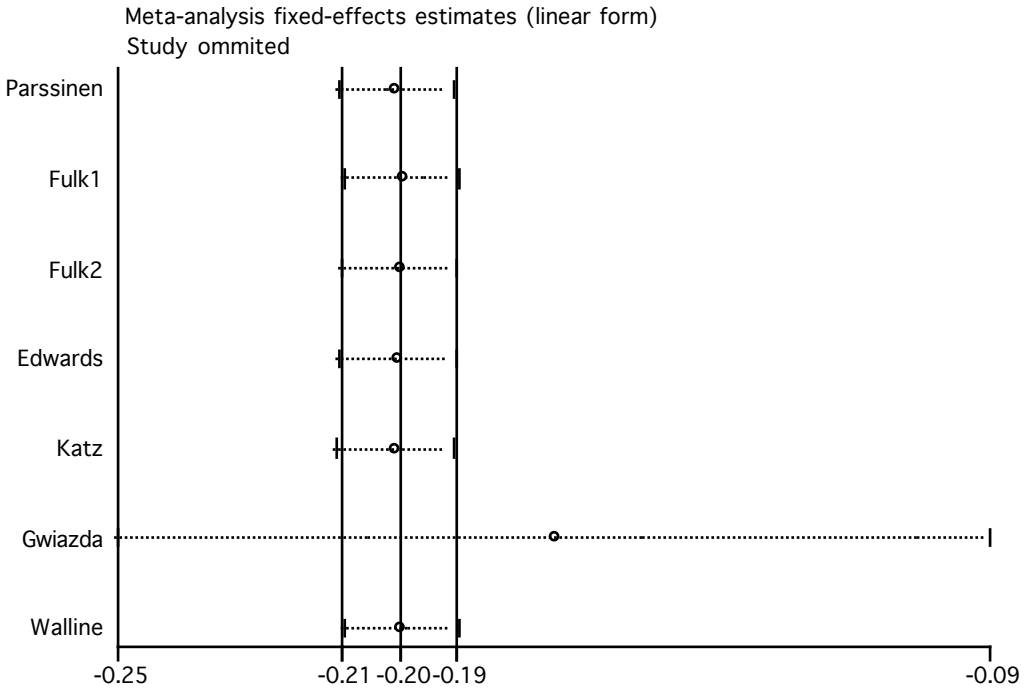
Heterogeneity chi-squared = 0.88 (d.f. = 2) p = 0.646
Test of WMD=0 : z= 17.31 p = 0.000

Testing the significance of the difference of treatment effects esophoric vs. overall

	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Overall	641	-.19	.00038	.0096	-.189	-.187
Esophoric	147	-.21	.0010	.012	-.214	-.210
combined	788	-.19	.00049	.014	-.194	-.192
diff		**.024**	**.00092**		**.022**	**.026**

diff = mean(x) - mean(y) t = 26.1233
Ho: diff = 0 degrees of freedom = 786
Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = 1.0000 Pr(|T| > |t|) = 0.0000 Pr(T > t) = 0.0000

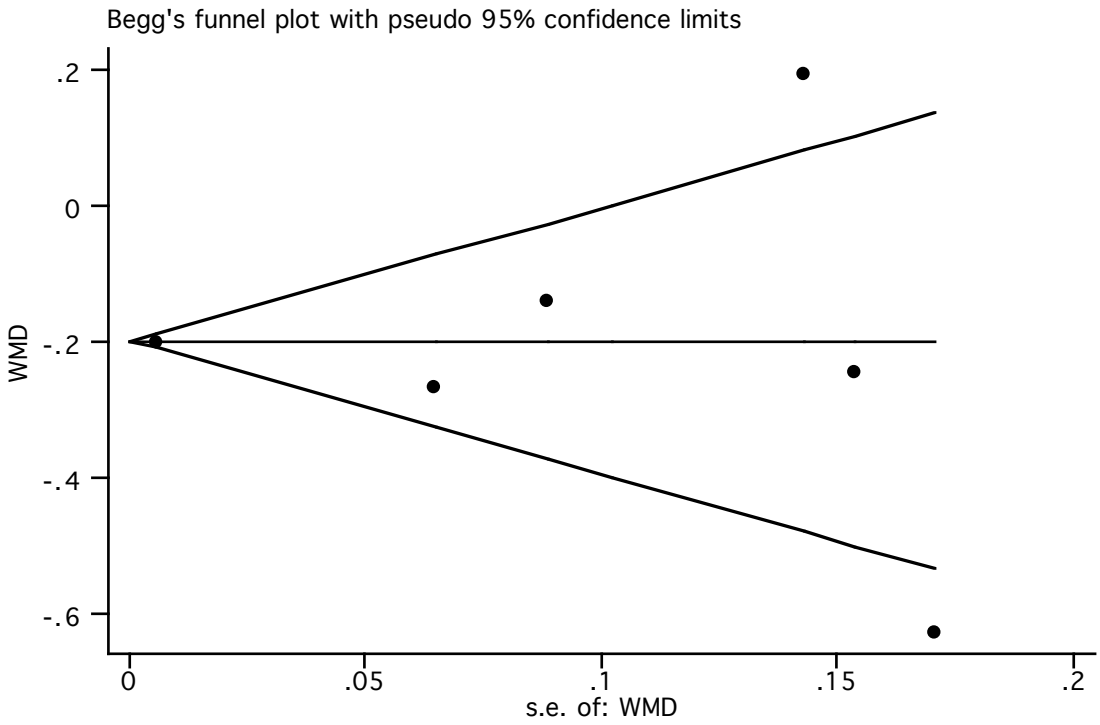
Figure 2-6. Influence of individual studies



Study	WMD	[95% Conf. Interval]		% Weight
Parssinen (1989)	.19	-.091	.47	.15
Fulk1 (1996)	-.27	-.40	-.14	.71
Fulk2 (2000)	-.25	-.55	.052	.13
Edwards (2002)	-.14	-.31	.033	.39
Katz (2003)	.05	-.15	.25	.29
Gwiazda (2003)	-.20	-.21	-.19	98.24
Walline (2004)	-.63	-.96	-.30	.10
I-V pooled WMD	**-.209**	**-.210**	**-.199**	

Heterogeneity chi-squared = 21.40 (d.f. = 6) p = 0.002
Test of WMD=0 : z= 36.32 p = 0.000

Figure 2-7. Assessing publication bias



Begg's Test

adj. Kendall's Score (P-Q)	=	1
Std. Dev. of Score	=	6.66
Number of Studies	=	7
z	=	0.15
Pr > |z|	=	**0.881**
z	=	0.00 (continuity corrected)
Pr > |z|	=	1.000 (continuity corrected)

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.20	.013	-15.71	0.000	-.23	-.17
bias	.34	.88	0.38	**0.717**	-1.93	2.61

Chapter 3
The Effect of 2-zone Concentric Bifocal Spectacle Lenses on Refractive Error Development & Eye Growth in Young Chicks

Abstract

Purpose: To characterize the effects on refractive error development and eye growth in young chicks of 2-zone concentric lens designs, which differentially affect the defocus experiences of central and peripheral retinal regions.
Methods: Monocular defocusing lenses were worn for 5 days from 17 days of age. Four 2-zone concentric lens designs (overall optical zone diameter of 10 mm) combining plano with either -5 or +5 D powers were used. Lens designs were as follows: (i) +5 D center (+5C), (ii) +5 D periphery (+5P), (iii) -5 D center (-5C), (iv) -5 D peripheral (-5P), with plano in periphery for all C-designs and in the center for P-designs. Five central zone diameters (CZD) were tested, ranging from 2.5 to 6.5 mm in 1 mm increments. Plano, +5 D and -5 D single vision (SV) lenses were used as controls. A minimum of 6 birds was included in each lens group.
Results: For the 2-zone lenses, the P-designs, i.e., peripheral defocus, had greater effects than the C-designs, i.e., central defocus, on both on-axis eye growth and refractions. All but the 6.5 mm CZD +5P lens induced larger changes than the +5SV lens. The +5C lenses with CZD less than 5.5 mm had little effect. The 2-zone -5 D lenses had less effect than the -5SV lens, and only the 6.5 mm CZD lens of the -5C series had a significant effect.
Conclusion: The results demonstrate that peripheral defocus can influence both peripheral and central refractive development. The inhibitory effect on axial eye growth of the +5P lenses opens the possibility that appropriately designed concentric lens designs may control progression of human myopia.

3.1 Introduction

Myopia, once considered as a serious public health condition limited to East Asia,[34-36] is reaching epidemic levels in North America.[1] High myopia is one of the leading causes of vision loss worldwide.[3, 37, 38] The management of myopia remains largely limited to optical devices such as spectacles and contact lenses, prescribed to correct the refractive errors, and ophthalmic surgical procedures for the same purpose. Nonetheless, novel applications of existing contact lens designs for the control of myopia progression have recently been explored, with promising early results.[39-41]

While the success of optical treatments for myopia rest on the assumption that there are environmental as well as genetic influences on the development and progression of myopia, their relative contributions remain the subject of ongoing debate. Results from twin and family studies suggest a strong genetic contribution to myopia,[42-44] yet the dramatic increase in the prevalence and severity of myopia reported in recent large-scale population studies strongly suggests a significant environmental influence, being too rapid to be explained by genetic variation alone.[36, 45, 46]

Altered emmetropization in response to specific visual manipulations is a consistent finding for a diverse range of animals including chicken,[9, 47] tree shrew,[48, 49] guinea pig,[10, 50] and monkey,[11, 51] with myopia being the product of both spatial form deprivation and imposed hyperopic defocus. The early finding that the eyes of young chicks will adjust their growth to compensate for imposed optical defocus has since been generalized to other animal models. With imposed hyperopic defocus (negative lenses), eyes increase their axial length and with myopia defocus (positive lenses), eyes slow their elongation.[9-11, 49] Evidence for a role of the peripheral retina in eye growth regulation also comes from early studies in chick, followed by recent studies in monkey. In chicks, form deprivation treatments restricted to one half of the visual field cause excessive eye growth only in the affected field,[5] and similar field-dependent changes occur when optical defocus is limited in extensity.[52] In monkeys, it has been shown using isolated central (foveal) laser lesioning, that eyes can recover from induced myopia,[16] implying that the peripheral retina alone is capable of guiding emmetropization. In other more recent studies, limiting either form deprivation or optical defocus to a hemifield was reported to induce asymmetric eye growth, consistent to appropriately localized responses. [53, 54] However, despite the converging evidence for a role for the peripheral retina in guiding eye growth, the nature of the interactions between peripheral and central retinal regions as determinants of eye shape and their respective influences on central (on-axis) refractive errors remain inconclusive.

In human myopia, two examples of hyperopic defocus offer plausible links to the finding of defocus induced myopia in animals: 1) relatively prolate eye shapes, which have been linked to myopia, introduce relative hyperopic defocus at peripheral retinal locations,[12, 55, 56] and 2) lags of accommodation introduce hyperopic defocus centrally, and are reported to be increased in some studies of myopia.[57-59] In the context of myopia control, previously explored bifocal and spectacle lens treatments are based on the premise that lags of accommodation underlie myopia progression. Although clinical trials of the same report disappointing efficacy,[22, 23, 60] it is perhaps noteworthy that their effect on peripheral (off-axis) refractive errors is also likely to be variable, between lens designs

and as a function of eye position. On the other hand, 2 of the contact lens treatments showing promise as myopia control therapies, i.e. corneal reshaping therapy and concentric, distance center multifocal soft contact lenses, are predicted to reduce peripheral hyperopic refractive errors,[39-41] as well as accommodative lags.

Animal studies using multifocal lenses have been so far limited to two studies in chicks. Both involved Fresnel lens designs, each incorporating 2 defocusing powers in alternative narrow rings (zones).[61, 62] These studies reported similar outcomes for otherwise normal eyes; when positive lens power was incorporated into the lens design, its effect dominated, whether or not the alternate rings included plano (zero) or negative lens power. Similar domination of myopic defocus over hyperopic defocus has been reported in other types of experiments. For example, when myopic and hyperopic defocus were set in competition in a cone imaging system that also restricted the visual experience to 2 planes of defocus, eyes showed reduced elongation, as expected with imposed myopic defocus.[63] In other studies in which appropriate single vision lenses with powers in opposite signs were interchanged, myopic defocus was shown to be about five times more effective than hyperopic defocus in inducing axial growth changes.[64] Moreover, interrupting hyperopic defocus with several brief periods of myopic defocus, spaced across the day, completely negated the ocular response to hyperopic defocus but not myopic defocus.[65]

In the study reported here, we sought to better characterize the effects of manipulating peripheral retinal defocus on ocular growth in the chick model, using a series of 2-zone lens designs that varied in both defocusing power and diameter of respective zones. Our intention was to simulate the bifocal soft contact lenses trialed for myopia control in humans to promising effect.

3.2 Methods

Animals: White-Leghorn chicks, obtained as day-old chicks from a commercial hatchery (Privett Hatchery, New Mexico), were used in this study. They were reared in a normal diurnal lighting environment (12 hr on/12 hr off), with food and water freely available. Spectacle lenses were fitted monocularly to 17-day-old chicks and worn for 5 days. A total of 185 birds were used in this study. All animal care and treatments in this study conform to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Experimental protocols were approved by the Animal Care and Use Committee of the University of California Berkeley.

Lens treatments: All lenses had a total optical zone diameter of 10 mm and overall lens diameter of 12.2 mm. Four 2-zone concentric lens designs combining plano with either -5 or +5 D were used to differentially expose the central and peripheral retina to defocus. Lens designs were as follows: (i) +5 D center (+5C), (ii) +5 D periphery (+5P), (iii) -5 D center (-5C), (iv) -5 D peripheral (-5P), with plano in periphery for all C-designs and in the center for P-designs. Five central zone diameters (CZD) were tested, ranging from 2.5 to 6.5 mm in 1 mm increments. Because of eye movements, only limited regions of the central and peripheral visual fields will experience unifocal (single vision) effects with the 2-zone lenses; the remaining, paracentral visual field will receive optical input from

both the central and peripheral lens zones. The dimensions of the "multifocal" central field and the unifocal peripheral field also depend on the interactions between the sizes of the CZD of the lenses, the entrance pupil of the eye, and the vertex distance of the lenses. As the CZD is increased, the multifocal central field will expand while the unifocal peripheral field will narrow. Table 1 summarizes the estimated dimensions of these visual field zones for the various 2-zone lens designs, based on a vertex distance (VD) of 3.3mm.[15, 66] The latter was measured with high frequency A-scan ultrasonography, after filling the space between the lens and the cornea with ultrasound gel; VD was defined as the distance between the peaks corresponding to the back surface of the lens and the front surface of the cornea. Three single vision (SV) lenses (plano, +5 & -5 D) were used as control treatments. These lenses also had optical zone diameters of 10 mm. Chickens were randomly assigned to one of sixteen treatment groups, with repeated runs of experiments achieving final group numbers of between 6 and 11 chickens. Lenses were cleaned and inspected at least 3 times daily to ensure their optical centers remained approximately aligned with the pupil centers of the eyes of the chickens, thereby minimizing the potential confounding effect of lens decentration.

Table 3-1. Dimensions of center and peripheral optical zones (CZD, PZD) of the five 2-zone lens designs used and corresponding dimensions of the unifocal central visual field, and annular multifocal paracentral and unifocal peripheral visual fields.

CZD (mm)	PZD (mm)	Unifocal Central Field (deg)	Multifocal Paracentral Hemi-field (deg)	Unifocal Peripheral Hemi-field (deg)
2.5	3.75	-	34	20
3.5	3.25	-	38	16
4.5	2.75	12	35	13
5.5	2.25	34	27	10
6.5	1.75	54	19	8

#Calculations based on anterior chamber depth (ACD) of 1.56 mm and vertex distance (VD) of 3.3 mm (means for 185 chickens tested), and estimated entrance pupil diameter of 4 mm, which is 0.2 mm in front of the iris based on an assumed total corneal power of 96 D.[67]

Measurements: Baseline refractive errors and axial ocular dimensions were measured immediately before the start of lens treatments using static retinoscopy and high frequency A-scan ultrasonography respectively, under gaseous anesthesia (1.5% isoflurane in oxygen). Ultrasonography measurements were repeated every other day, around the same time of day (early afternoon), and retinoscopy was repeated once, after 5 days of lens treatment. Refractive error measurements were made on-axis (centrally) as well as 30 degrees nasally and temporally. Angular estimations were made with reference to a protractor, fitted to a custom-built head holder used to deliver isoflurane.

Statistical analyses: Both central (C) and peripheral (P) refractive errors are represented as spherical equivalent refraction (SER; averages between the refractions of two principal meridians), as there was no significant increase in astigmatism with increased eccentricity, either before or after the lens treatments. Off-axis refractive errors were normalized with respect to the on-axis values (i.e. P-C), by way of identifying differential effects on these regions induced by the lenses. This parameter is referred to as the relative

peripheral refraction (RPR). Although A-scan ultrasonography allowed measurements of all ocular components, including the layers making up the posterior wall of the eye (retina, choroid and sclera), changes in axial length, calculated as the sum of anterior chamber depth, lens thickness, and vitreous chamber depth, largely accounted for the changes in central refractive error, and thus only these data are presented. Initial analyses found no group-related differences in untreated contralateral (fellow) eyes, either in terms of refractive errors or axial lengths. Thus only changes over the treatment period in both refractive errors and axial dimensions for treated eyes are reported as the primary outcome measures of the treatment effects.

Descriptive analyses were performed and the normality of the distributions of the changes in refractive error and axial dimensions were verified prior to statistical modeling. Factorial ANOVAs were performed using STATA (STATA Corp. TX), with the primary outcome measures of interest being changes in the on-axis refractive error and axial length over treatment period, as well as endpoint RPR, controlling for baseline interocular difference. These outcome measures were modeled as a function of lens types and CZD, as well as the interaction between these two factors. Differences between groups were assessed using 2-sample t-tests. To keep the family-wise error rate for the entire set of tests equal to 0.05, the Hochberg step-up procedure was used; thus *p* values were adjusted to take into account the number of tests undertaken. This procedure has more power to detect differences than the simpler Bonferroni adjustment. Results are graphically illustrated as box plots, which show medians as well as the distribution of the data; whisker lengths represent either 1.5 times the interquartile range or the distance to the extreme, whichever is shorter.

3.3 Results

3.3.1 Effects of lens design on central (on-axis) refractive errors

On-axis refractive error changes over the treatment period were generally consistent with the sign of the lens power incorporated in all or part of the lens, i.e. eyes wearing positive lenses exhibited hyperopic shifts in refractive error while eyes wearing negative lenses exhibited myopic shifts. These data are summarized by lens type and CZD in Table 2. The effect of lens type was highly significant ($F_{6,22} = 87.83$, $p<0.0001$), but not the effect of CZD alone ($F_{4,22} = 2.03$, $p=0.09$), although there was a significant interaction between CZD and lens type ($F_{12,22} = 5.64$, $p<0.0001$).

Table 3-2. Central (on-axis) refractive changes over treatment period (Mean±SD, D), organized by lens type and CZD (sample size in brackets).

CZD (mm)	Lens Type			
	+5C	+5P	-5C	-5P
2.5	+0.58±0.88 (6)	+4.25±0.35 (6)	+0.11±1.98 (9)	-3.71±3.35 (6)
3.5	+0.68±1.09 (10)	+5.35±0.93 (10)	-0.53±1.63 (8)	-2.86±2.24 (7)
4.5	+1.77±1.91 (10)	+4.48±1.79 (11)	+0.68±1.14 (10)	-2.05±2.28 (10)
5.5	+2.75±1.97 (6)	+4.43±1.25 (11)	-0.25±1.36 (6)	-1.89±1.37 (11)
6.5	+4.54±1.11 (6)	+1.71±1.23 (6)	-1.79±1.41 (6)	-0.83±0.92 (6)
10 (SV)	+3.36±0.43 (7)		-5.84±0.50 (8)	

Equivalent values for the plano lens are +0.19±1.14 D (n=9).

Hyperopic changes induced by 2-zone and SV positive lenses (Figure 3-1, top panel): As expected, the +5SV lens induced a significant hyperopic shift in refractive error (+3.36±0.43 D) by the end of the treatment period. Although the endpoint refractive errors (+4.73±1.10 D) closely matched the imposed defocus (+5 D), the mean hyperopic change was less than the imposed defocus, this discrepancy reflecting the low hyperopia at baseline. In contrast, the plano lens-wearing eyes showed minimal change and thus the difference between the 2 groups was highly significant (p<0.0001). For 2-zone lenses with positive power in their centers (+5C lenses), lenses with CZD of less than 4.5 mm had little effect, while lenses with CZDs above this threshold induced increasing hyperopia. Thus while the lens with a 4.5 mm CZD induced only a small hyperopic shift (+1.77±1.91 D, p=0.09), lenses with CZDs of 5.5 mm or larger induced much larger shifts (p=0.02), with the change by the 6.5 mm CZD being slightly greater than that of +5SV lenses (+4.54±1.11 D; p=0.06, Figure 3-1a). The latter trend was also observed with the +5P lenses (Figure 3-1b). Specifically, all of the latter lens designs induced larger hyperopic shifts than the +5SV lens (p<0.05 for CZD of 2.5, 3.5, 5.5, p=0.06 for CZD of 4.5), except for the 6.5 mm CZD, for which the hyperopic shift in refractive error dropped to less than half of what was seen with the 5.5 mm CZD (p<0.01 cf. both 5.5 mm CZD +5P & +5SV lenses).

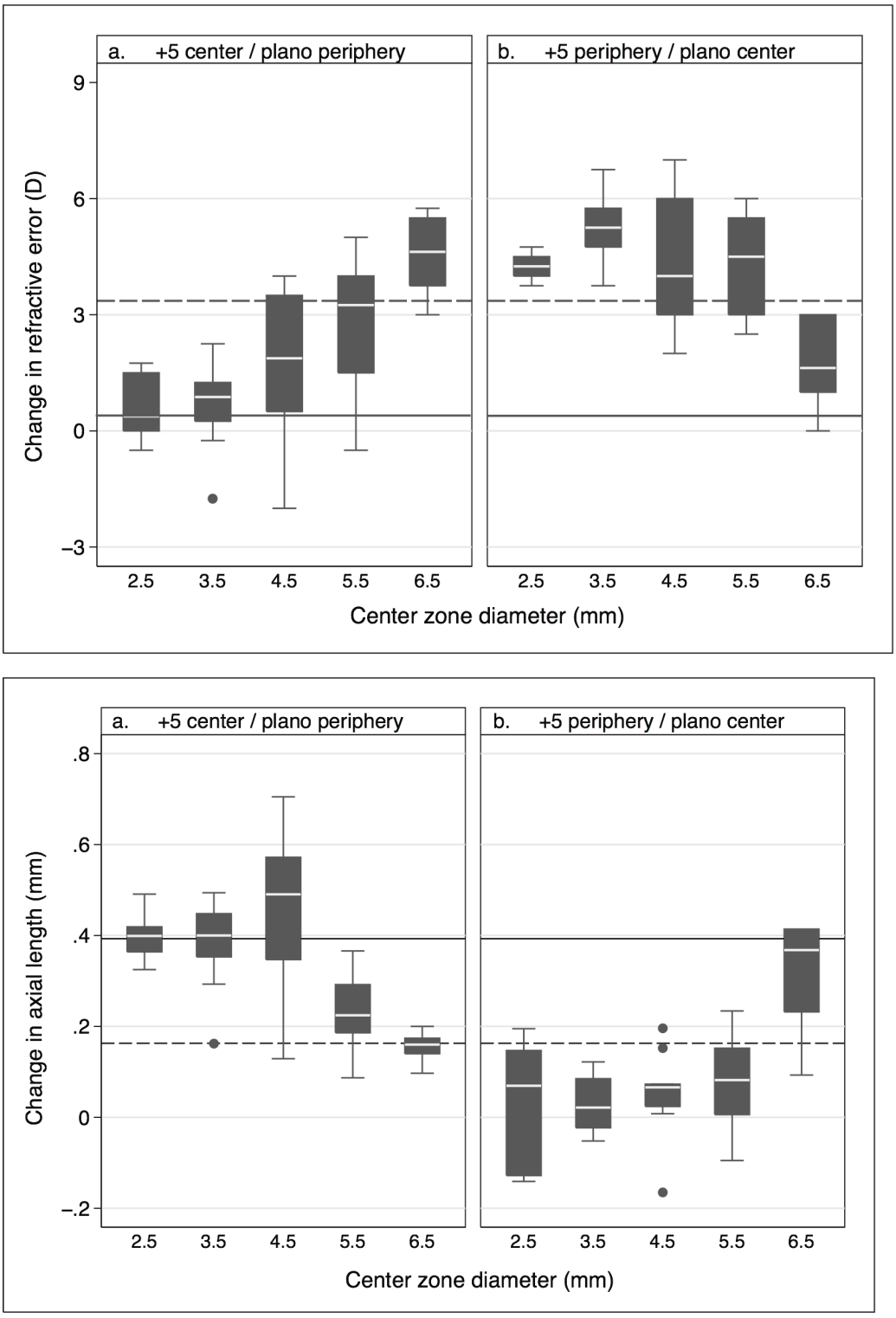


Figure 3-1. Boxplots of central refractive error changes (top panel), and corresponding axial length changes (bottom panel) over the treatment period for (a) the +5C lens designs, (b) the +5P lens designs. Central zone diameters (CZD, on X-axis) ranged from 2.5 to 6.5 mm. Dashed reference lines denote the mean changes induced by +5SV lens; and solid lines denote the mean change induced by plano lens. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme.

Myopic changes induced by 2-zone and SV negative lenses (Figure3-2, top panel): The -5SV lens induced nearly complete compensation over the treatment period, with the observed myopic shift in refractive error being comparable to the imposed defocus, and significantly different from the change in control (plano lens-wearing) eyes (-5.84±0.50 D, $p<0.0001$). The myopic shifts in refractive errors induced by the 2-zone negative lenses were all much smaller than that recorded with the SV lens, contrasting with the exaggerated responses seen with some of the 2-zone positive lenses. Nonetheless, increasing the CZD for the -5C designs tended to increase the myopic shift in refractive error, with the change reaching statistical significance for the 6.5 mm CZD lens ($p=0.01$, cf. plano lens, Figure 3-2a). For the -5P designs, significant myopic changes were seen with all CZDs compared to the plano lens-wearing control ($p<0.05$ for all, Figure 3-2b).

3.3.2 Effect of lens design on ocular axial growth

Choroid thickening was significantly associated with the development of hyperopia ($R^2=0.17$, $p<0.0001$); however changes in choroidal thickness did not fully account for either the refractive error changes or changes of axial length.

The axial length changes in lens-wearing eyes over the treatment period were consistent with observed changes in on-axis refractive errors ($R^2=0.61$, $p<0.0001$), with the largest changes in negative lens-wearing eyes showing the largest myopic shifts in refractive error, and smallest changes in positive lens-wearing eyes showing the largest hyperopic shifts. These data are summarized by lens type and CZD in Table 3. As with the changes in refractive error, axial length changes were influenced significantly by lens type ($F_{6,22} = 49.60$, $p<0.0001$), and the interaction between lens type and CZD ($F_{12,22} = 6.47$, $p<0.0001$). The effect of CZD alone was of borderline significance ($F_{4,22} = 3.22$, $p=0.01$).

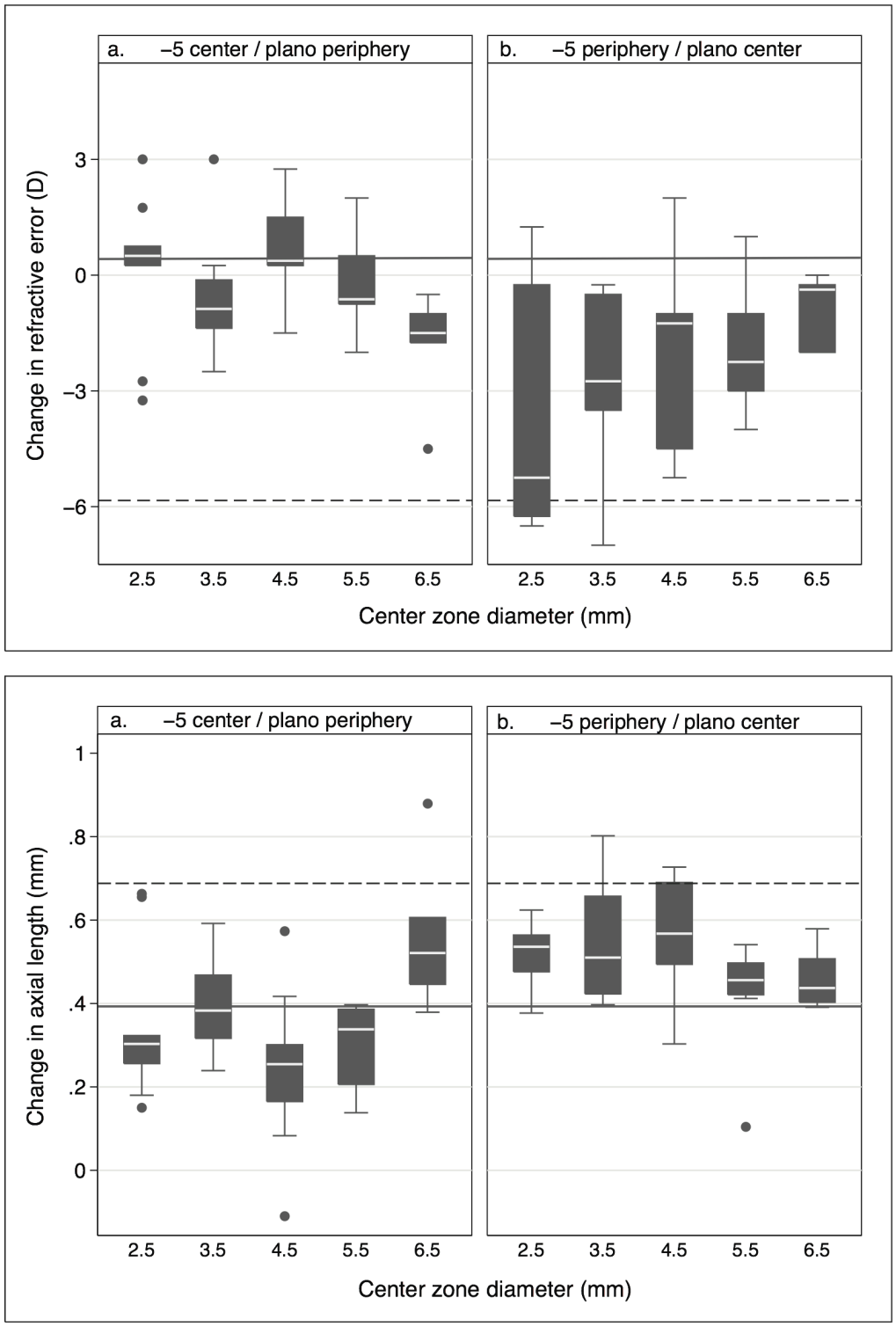


Figure 3-2. Boxplots of central refractive error changes (top panel) and axial length changes (bottom panel) over the treatment period for (a) the -5C 2-zone lens designs, (b) the -5P 2-zone lens designs. CZDs ranged from 2.5 to 6.5 mm. Dashed reference line denotes the mean change induced by the -5SV lens and solid lines denote the mean change induced by plano lens.

Table 3-3. Changes of axial length over treatment period (Mean±SD, mm), organized by lens type and CZD.

CZD (mm)	Lens Type			
	+5C	+5P	-5C	-5P
2.5	0.40±0.056	0.035±0.14	0.35±0.19	0.52±0.086
3.5	0.38±0.095	0.027±0.062	0.40±0.12	0.55±0.14
4.5	0.45±0.19	0.053±0.091	0.24±0.18	0.56±0.14
5.5	0.23±0.096	0.071±0.095	0.30±0.11	0.44±0.12
6.5	0.16±0.036	0.32±0.13	0.56±0.18	0.46±0.071
10 (SV)	0.16±0.074		0.69±0.061	

Equivalent values for the plano lens are 0.39±0.10 mm.

Axial length changes induced by 2-zone positive lenses (Figure 3-1, bottom panel): Compared to eyes wearing plano lenses, those wearing +5SV lenses showed significantly less axial elongation over the treatment period (0.16±0.074 mm, p=0.0001), consistent with their increased hyperopia. With the +5C lenses, axial length changes were similar to those of plano lens-wearing eyes for CZDs of less than 5.5 mm, but significantly smaller for both 5.5 and 6.5 mm CZDs (p=0.009 & p=0.0001 respectively), these trends being consistent with described CZD-dependent changes in refractive error. Furthermore, the mean change in axial length with the 6.5 mm CZD lens design was similar to, and not significantly different from, that seen with the +5SV lens (p=0.41). With the +5P lenses, changes in axial length were significantly smaller than that seen with the plano lens (p<0.0001 for 2.5 to 5.5 mm CZD), and the change with the 4.5 mm CZD design was also significantly smaller than that seen with the +5SV lens (p=0.01, Figure 3-1). These results are consistent with the large hyperopic shifts seen with all but the largest (6.5 mm) CZD for this +5P design.

Axial length changes induced by 2-zone negative lenses (Figure 3-2, bottom panel): As expected, the myopic shift in refractive error observed with the -5SV lens was linked to a significant increase in axial elongation compared to that seen with the plano lens (0.69±0.061 mm, p<0.0001). Among the -5C lenses, only the lens with CZD of 6.5 mm induced greater elongation than that seen with the plano lens, and this change was of borderline significance (p=0.04). In contrast, with the -5P series of lenses, there was a consistent trend of increased axial elongation with all CZDs, with the difference from the plano lens effect reaching statistical significance for the 2.5, 3.5 and 4.5 mm CZDs (p<0.05 for all).

3.3.3 Effect of lens design on relative peripheral refractive errors (RPR)

Because there was no evidence of nasal-temporal asymmetries in peripheral refractive errors in either treatment or control groups, the relative peripheral refractive errors derived for the nasal and temporal fields were averaged for use in analyses of the influences of lens design and CZD. These data are summarized in Table 4.

Table 3-4. Relative peripheral refractive error (RPR, Mean±SD, D), organized by lens type and CZD.

CZD (mm)	Lens Type			
	+5C	+5P	-5C	-5P
2.5	-0.40±0.83	-1.15±0.85	-0.39±0.90	+0.79±0.98
3.5	-0.45±1.08	-0.96±0.69	-0.17±0.25	0.00±2.03
4.5	-1.08±1.04	+0.32±1.77	+0.38±1.36	-0.80±1.64
5.5	-0.85±1.19	-0.58±1.22	-0.042±0.30	+0.32±1.63
6.5	-1.98±1.50	+0.29±1.15	-0.008±1.46	+0.083±0.63
10 (SV)	+0.14±1.12		+0.83±1.24	

Equivalent values for the plano lens are -0.083±0.63 D.

The RPR data were subjected to a factorial ANOVA, with on-axis refractive error change, lens type and CZD as factors. The main effect of on-axis refractive error change proved highly significant ($F_{1, 23} = 50.78$, $p<0.0001$). The origin of this effect is revealed in Figure 3-3 in which RPRs are plotted against changes in on-axis refractive error for all four 2-zone lens designs; in all cases, increasing on-axis hyperopic changes are associated with increasing relative peripheral myopia and vice versa. Lens design was also found to significantly influence RPR ($F_{6,23} = 5.71$, $p<0.0001$), with the effect being greater for the positive lens designs, as reflected in the steeper slopes for the +5C and +5P data compared to -5C and -5P data, after adjusting for all other variables in the model (Figure 3-3). Note that despite a moderate level of overlap in the data representing each group, the RPR distributions show design-dependent differences, resulting in similar on-axis refractive error changes being coupled to different RPRs, and implying differences in eye shape. Although the CZD itself had no significant effect on RPR ($F_{4,23} = 0.82$, $p=0.51$), its interaction with lens design was significant, albeit borderline ($F_{16,23} = 1.76$, $p=0.041$).

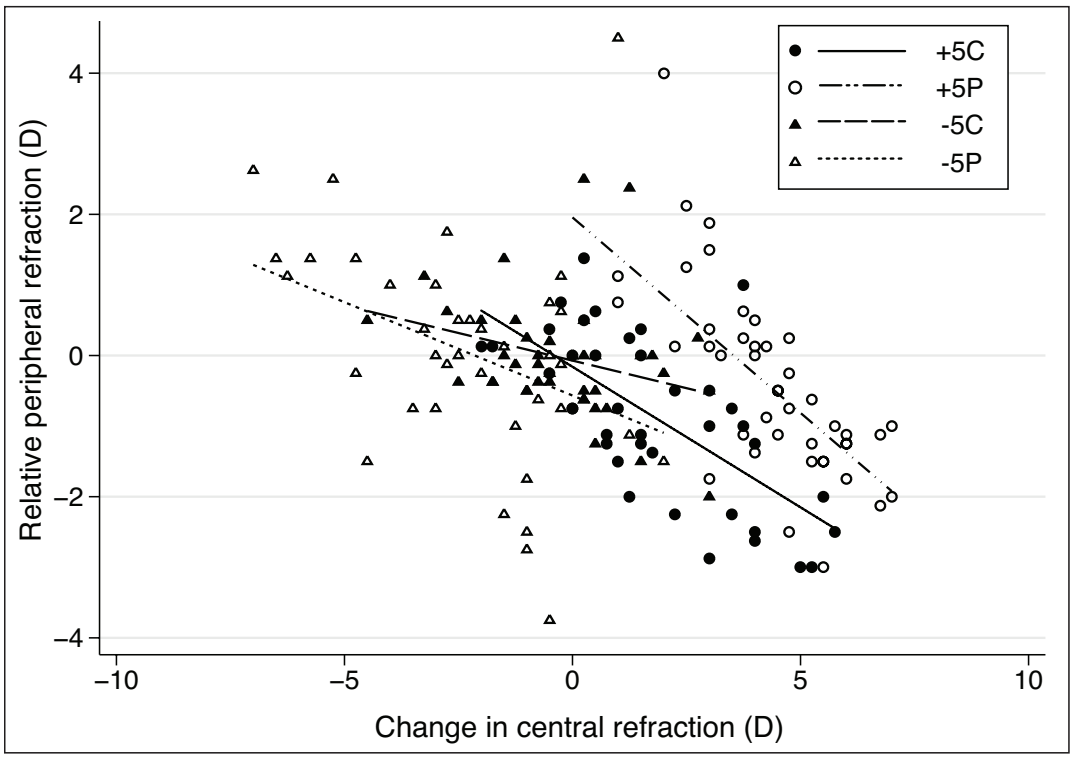


Figure 3-3. RPRs plotted as a function of changes in on-axis refractive error for each of the four 2-zone lens types (solid circle: +5C, open circle: +5P, solid triangle: -5C, open triangle: -5P.

3.4 Discussion

The study reported here used 2-zone lenses to differentially titrate the optical defocus experience of central and peripheral retinal regions. Both our center power (C) and peripheral power (P) designs induced changes in the central (on-axis) refractive errors and axial length of the growing eyes of the young chicks. The peripheral power design tended to have greater effects than the central power design, for both positive and negative lenses, and for the former design, the effect sometimes exceeded that observed with a single vision lens of the same power. While we also observed changes in peripheral refractive errors, they cannot be fully explained in terms of local retinal responses to imposed defocus. Below we considered possible explanations for our results and compare them with a closely related study that used lenses with central apertures,[68] instead of plano zones.

Any explanation for our results must take into account the eye movements of our animal subjects. For lenses in which defocusing power was restricted to the lens periphery, even the central retinal region would have experienced defocus, at least intermittently. The defocus experience of midperipheral retinal regions would also have varied over time. Thus one would expect the peripheral retina and adjacent sclera to be most affected, and central (axial) regions least affected, with the accumulated "dose" of defocus experienced by the central region decreasing as the size of the central plano zone was increased. The opposite would be true for lenses with power limited to the central zone. However, the

effect of CZD does not show a clear dose effect, for any of the lens series used in the current study. Except for the largest CZD of 6.5 mm, which reduced the width of the unifocal peripheral field of view to 8 degrees, the P series of lenses induced significant changes in on-axis refractive errors that reflected changes in axial elongation, with these effects being more robust with the +5 series compared to -5 series of lenses.

Our finding that the changes induced by the -5C lenses were generally weak, irrespective of the size of the CZD, is consistent with results of previous studies exploring the temporal integration of defocus-induced signals in chick, assuming that it is a major determinant of the on-axis response for these lenses. For example, interruption of lens wear with 3 hours of normal vision is sufficient to prevent the development of myopia in response to -10 D lenses in chicks.[69] The equivalent experiment with +10 D lenses showed the response to imposed myopic defocus to be more robust. In another study, it was noted that the choroidal thinning induced by short-term exposure to negative lenses was less enduring than the thickening induced by positive lenses.[70] With the C series of lenses in the current study, we also found the responses to imposed myopia (+5 D) to be more robust than the responses to imposed hyperopia (-5 D). Lateral interactions between adjacent retinal regions are also likely to influence the response of local retinal regions, either dampening or enhancing the signal generated by the local defocus experience.

The response pattern recorded with the +5P series of lenses is striking in that the induced on-axis refractive error changes are greater than that induced by the +5 D SV lens, with one exception, even though the central retina would have had a more consistent defocus experience with the latter lens. The above model cannot fully explain this outcome. A potential alternative explanation relates to interactions between the higher order aberrations (HOA) introduced by the 2-zone lenses and those of the eye, which may alter the target plane of best focus for emmetropization. In studies involving concentric bifocal contact lenses in humans, it has been shown that interactions between lens and ocular aberrations, especially spherical aberration (SA), may alter the position of plane of best focus with respect to the retina.[71] SA would also have been most affected by our 2-zone lens design. Although we did not measure the optical aberrations of the chicks in the current study, a transition from negative SA at week 2 to positive SA at week 5 was observed in a previous study of the same strain of chicks.[72] It is also known from *in vivo* studies in humans[71] and *in vitro* studies in chicks[73] that spherical aberration becomes increasingly negative with accommodation. However, while this explanation seems plausible for the results with the +5P lenses, a similarly exaggerated response was recorded with the largest CZD +5C lens, which would impose the opposite type of SA, implying that other factors are at play.

The inherent complexity of ocular growth regulation is further evidenced by the refractive error profiles of lens-treated eyes. In the chick eye, which has a rigid bi-layered sclera, it is generally assumed that eye shape changes reflect the strength of local retina-derived growth modulatory signals. However, this model does not fully account for the refractive patterns observed with our 2-zone lenses. For example, while relative peripheral myopia combined with on-axis hyperopia was recorded with +5P lens series, the lenses with the smallest CZDs resulted in the largest differences and conversely, for the +5C lenses, those with the largest CZDs induced the largest differences (Table 3-4).

These influences of lens design on refractive error profiles are illustrated in Figure 3-3; for the two +5 D series of lenses, the regression line fitted to the P series data is displaced to the right of the regression line fitted to the C series data, and also steeper. Differences in the ability of peripheral retinal regions to decode defocus and/or differences in signal gain for peripheral versus central regions may contribute but cannot alone explain these response patterns. The significant negative correlation between changes in central refractive errors and relative peripheral refractive errors in our data has an intriguing parallel with human refractive error data, where the coupling of relative hyperopic peripheral refractive error with on-axis myopia has led to speculation of a causal link between the two. Follow-up studies are required to establish whether the response patterns reported here translate to mammals, before any conclusion can be drawn concerning the stimulus for myopic growth in humans.

In the current study, changes in relative peripheral refractive errors were used as a surrogate for eye shape. However, we cannot eliminate potential confounding influences related to the unique properties of the peripheral optics of the eye, which have not been characterized for the chick in any study to-date. Off-axis measurements of axial length, as recently popularized using the IOLMaster in human studies, would be an appropriate follow-up study. Nonetheless, our study demonstrates that concentric 2-zone spectacle lenses can be used to modify both on- and off-axis refractive development. Note that although there has been no comparable study in primates or humans, the possible causal relationship between myopia progression and relative peripheral hyperopia, evident when myopes are corrected with standard single vision spectacle lenses, has lead to the recent development of a radial refractive gradient spectacle lens, which is reported to reduce such peripheral errors.[74]

Our observation of a significant influence of peripheral defocus on central refractive development contrasts with the previously reported lack of effect on the latter of spectacle defocusing lenses with central apertures.[68] In this study, chicks wore lenses with central apertures of varying diameter, limiting defocus to peripheral retinal regions. Several factors may have contributed to the discrepancy in the results of our 2 studies. First, when lenses with central apertures were used, as in the earlier study, chicks may have artificially restricted their eye movements, as in a similarly designed study involving young monkeys, which were observed to limit their eye movements, to allow viewing through the central apertures of attached lenses.[15] Modified eye movements are likely with such lenses if not regularly cleaned, as the presence of a central aperture will allow dust to accumulate on the inside of the lenses, turning them into light diffusers. This potential problem was avoided with our 2-zone lens design. Second, the previous study used younger chicks than in the current study although one might expect an exaggerated response in the younger animals, i.e. larger changes in on-axis refractive errors, rather than the converse, based on previously reported age-dependent differences in the rates of response to imposed defocus.[75] On the other hand, due to the smaller eyes of younger chicks combined with possible shorter lens vertex distances (range given as 2-3 mm), the unifocal peripheral visual field introduced with the central aperture lenses are likely to have been much smaller than those imposed with the 2-zone P lenses with corresponding CZDs in the current study, offering a partial explanation for this discrepancy in study outcomes. Finally, it is possible that estimates of the visual angular

subtense of the peripheral optical zone of the lenses were inaccurate in the earlier study. Such estimates are directly dependent on the lens vertex distance and axial parameters of the experimental subjects and are prone to error, due to the small distances involved. Our estimates are based on highly accurate measurements made using high frequency ultrasonography in both cases, while approximated parameters from model eyes were used in the earlier study.

In summary, in chicks wearing 2-zone concentric spectacle lenses, responses varied according to the sign of the defocusing power and whether it was limited to either the central or peripheral zones. Lens designs with optical power limited to their periphery elicited greater responses than the opposite design and the responses to lenses incorporating positive powers were more robust than the responses to lenses incorporating negative power. The observed inhibitory effect on axial eye growth of the +5P designs has particular salience for human myopia, and is consistent with isolated reports of effective control of myopia progression with some concentric multifocal contact lens designs.[41, 76] Contact lenses, being in contact with the cornea, are likely to afford better control over the imposed optical conditions and as a consequence, may offer better control over myopia progression, than novel spectacle lens designs, which introduce centration and eye movement-related confounders. Nonetheless, further animal-based studies using multifocal contact lenses may provide additional insights into how to best optimize inhibitory effects on eye growth.

Chapter 4

The effect of 2-zone lenses on refractive and eye growth in young chicks after Ciliary Nerve Section (CNX) and Iridectomy (ID)

Abstract

Purpose: To investigate the effects of CNX (induces surgical cycloplegia and mydriasis) and ID (produces an fixed, enlarged pupil) on the refractive error development and eye growth in young chicks of 2-zone concentric lens designs.
Methods: Monocular CNX or ID was performed on 7-9 day-old chicks. All surgical eyes were subsequently fitted with SV or 2-zone lenses after recovery. Three SV lenses (plano, +5 & -5 D) and four 2-zone lenses (CZD of 3.5 and 6.5 mm) were tested. Data with nonsurgical eyes from a prior study wearing the same lenses were used as controls.
Results: With SV lenses, compensation to the +5 SV lens was reduced after both CNX and ID although neither surgery affected compensation to the -5 SV lens. The surgeries also had little affect on the responses to the 3.5 mm CZD 2-zone lenses but significantly affected the responses to the 6.5 mm CZD lenses. With the 6.5 mm CZD lenses, CNX eyes showed reduced responses to the +5 D 2-zone lenses but increased responses to the equivalent -5 D lenses, compared to those of normal and ID eyes.
Conclusion: The results suggest a critical influence of pupil size on the treatment effects of 2-zone lenses and also point to a critical role of accommodation in the decoding of ocular complex optical stimuli.

4.1 Introduction

The possibility of controlling myopia progression with optical interventions has attracted significant attention recently, largely attributable to the rapid increase in both the prevalence and severity of myopia worldwide. Although only equivocal outcomes are reported from randomized clinical trials (RCT) of conventional optical devices such as bifocal or progressive spectacle lenses for myopia, more recent clinical studies using novel contact lenses that incorporate concentric multifocal designs, as well as orthokeratology, have shown consistent and clinically significant myopia controlling effects.[39-41, 77] Despite these promising findings, the exact mechanism underlying these controlling effects remains poorly understood. There is also little information available concerning the design features of newly developed myopia-control lenses, so research has largely been limited to already approved multifocal lenses being used off-label for myopia control, instead of presbyopia correction.

Some progress in understanding the complex optical influences on refractive development and myopia progression has been made through optical defocus studies using animal models to investigate the effects of multifocal lenses.[61, 69] A key finding from such studies is that imposed myopic defocus dominates over the hyperopic defocus in ocular development, when myopic and hyperopic defocus are presented simultaneous across the retina, as with Fresnel lenses; studies using a dual-focus cone paradigm also lead to the same conclusion. In both paradigms, "multifocality" is experienced by the same or adjacent retinal areas. In some other studies, lens designs with 2 distinctive central and peripheral optical zones have been used to present retinal eccentricity-dependent differences in optical defocus. In our recently published study using the chick model, we systematically tested a series of such 2-zone lens designs, by varying the center zone diameter of the lenses to derive a dose-response relationship between the central area of imposed defocus and the magnitude of induced ocular growth changes. Perhaps not surprisingly, 2-zone positive lenses, which had positive power in one optical zone and plano power in the other, significantly slowed ocular growth, with corresponding hyperopic shifts in refractions. However, an intriguing aspect of our findings is that the induced hyperopic shifts in refraction were significantly greater with 2-zone positive lens designs than that with single vision lenses of the same positive power, which provide a uniform myopic defocus experience over the entire retina.[78]

Two hypotheses were proposed in attempts to explain this interesting finding. First, it is possible that lateral interactions between adjacent retinal regions influenced the response of local retinal areas, either dampening or enhancing the signal generated in response to the local defocus experience. Second, interactions between higher order aberrations (HOA), especially spherical aberration (SA), introduced by the 2-zone lens design and those of the eye, may have altered the position of the plane of best focus with respect to the retina, similar to the effects described for multifocal soft contact lenses in human studies.

Another important finding from our 2-zone lens study in chicks related to the induced changes in relative peripheral (off-axis) refraction (RPR). Although consistent with human population data, we found a negative correlation between the central (on-axis) refractive errors and RPRs generated by the 2-zone lenses in our chick model, the RPRs

also showed a strong lens design-dependent pattern, with the peripheral optics of the lenses proving to be an important influence on RPRs.

In the normal eye, there were two important ocular variables that also influence the optical experience of the retina, created with our 2-zone lenses, namely, pupil size and accommodation, which represent important confounders in our previous 2-zone lens study. In the study reported here, we further examined the effects on ocular development of 2-zone lenses by controlling each of these variables through surgical interventions. In one procedure, ciliary nerve section (CNX) was used to control (eliminate) accommodation. However, because the pupil and accommodation share a common neural motor pathway, the effect of the surgery was both cycloplegia and mydriasis. In a second procedure, an iridectomy (ID) was performed to remove the iris, producing a fixed and enlarged pupil with near normal accommodation. That accommodation was minimally affected as confirmed in an independent study.[79] By testing the same 2-zone lenses used in our previous study on CNX and ID eyes, we were able to address several critical questions: 1) are the effects induced by the 2-zone lenses pupil size-dependent, and if so 2) is this dependency associated with its influence on on-axis HOA introduced by the lenses or its impact on the imposed multifocal optics experienced by the peripheral retina, and finally 3) what is the role of accommodation in the 2-zone lens-induced ocular growth changes.

4.2 Methods

Animals: Day-old White-Leghorn chicks, obtained from a commercial hatchery (Privett Hatchery, New Mexico), were used in this study. They were reared in a normal diurnal lighting environment (12 hr on/12 hr off), with food and water available ad libitum. A total of 154 birds were used in this study. All animal care and treatments in this study conform to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Experimental protocols were approved by the Animal Care and Use Committee of the University of California Berkeley.

Surgical treatments: Unilateral CNX or ID was performed under gaseous anesthesia (2% isofluorane in oxygen), on chicks aged between 7-9 days. Both surgical procedures are described in detail elsewhere.[79, 80] In brief, in CNX, an inferior temporal skin incision was made and the portion of the ciliary nerve adjacent to the ganglion was exposed and sectioned. Antibiotic ointment was applied to the skin lesion after the suture. For ID, the iris tissue was removed from its root with microsurgical forceps through a limbal incision. Topical steroid/antibiotics were applied for four days after surgery and slit lamp exam was performed on both CNX and ID eyes to verify at the baseline of lens treatment: 1) enlarged pupil and lack of accommodative miosis after CNX, indicating a complete section of ciliary nerve, and 2) no residual inflammation or secondary cataract post ID. The contralateral fellow eyes were not treated.

Lens treatments: All surgical eyes were subsequently fitted with various single vision (SV) or 2-zone lenses after a recovery period of 6-8 days, i.e. at 17 days of age, with the lenses being left on for 5 days. Chickens were randomly assigned to one of 11 treatment

groups (Figure 4-1.), with repeated runs of experiments achieving final sample sizes of 7 for each surgery/lens treatment combination. Lenses were cleaned and inspected at least 3 times daily to ensure their optical centers remained approximately aligned with the pupil centers of the eyes of the chickens, thereby minimizing the potential confounding effect of lens decentration.

All lenses had a total optical zone diameter of 10 mm and overall lens diameter of 12.2 mm. Three SV lenses (plano, +5 & -5 D) and four 2-zone lenses were tested, and for the 2-zone lenses, two central zone diameters (CZD), 3.5 and 6.5 mm, and two power combinations, plano/+5 D and plano/-5 D, were tested. The specifications of the 2-zone lenses used in this study are described in more detail elsewhere (Fig. 4-1).[78] Data from our previous study involving normal eyes (i.e., no surgery controls) wearing the same lenses were used in comparative analyses (see Chapter 3). Note however that because of surgically-induced mydriasis with both procedures, most of the visual field of lesioned eyes will receive multifocal inputs with the 2-zone lenses, with only the far peripheral visual fields receiving unifocal input, corresponding to the peripheral lens zone (Table 4-1).

Table 4-1. Dimensions of center and peripheral optical zones (CZD, PZD) of the two 2-zone lens designs used and corresponding dimensions of the multifocal central visual field, and annular unifocal peripheral visual fields for lesioned eyes; data for control (no surgery) eyes in parenthesis. Method of calculation described in Chapter 3.[78]

CZD (mm)	PZD (mm)	Unifocal Central Field (deg)	Multifocal Paracentral Field (deg)	Unifocal Peripheral Hemi-field (deg)
3.5	3.25	- (-)	92 (72)	8 (16)
6.5	1.75	- (54)	106 (19)	2 (8)

#Calculations based on estimated entrance pupil diameter of 6.5 mm for CNX and ID eyes, and 4 mm for normal (control) eyes.

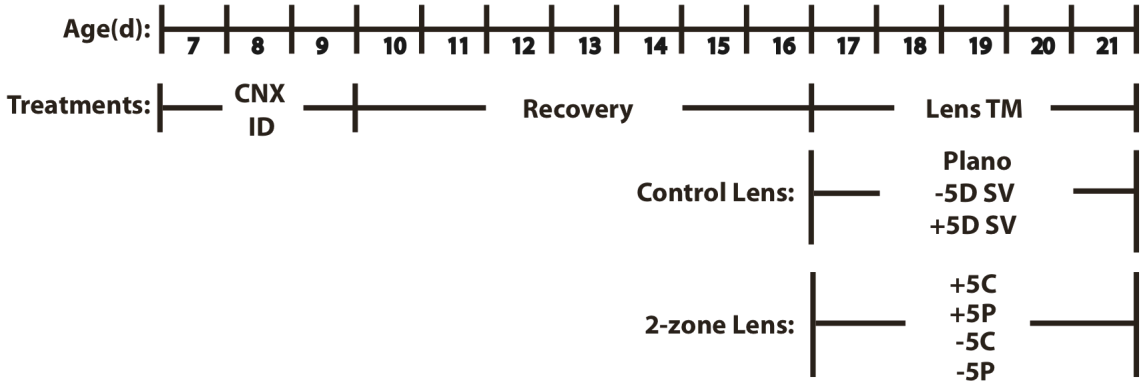


Figure 4-1. Schematic summary of the details and timelines for lesioning surgeries and lens treatments used in this study. Two-zone lenses combined plano with either +5 D or -5 D power in either the lens center (+5C, -5C) or periphery (+5P, -5P).

Measurements: Baseline refractive errors and axial ocular dimensions were measured prior to but on the day of attachment of the lenses (baseline), using static retinoscopy and high frequency A-scan ultrasonography respectively, under gaseous anesthesia (1.5% isoflurane in oxygen). Ultrasonography measurements were repeated every other day, around the same time of the day (early afternoon), and retinoscopy was repeated once, at the end of the lens treatment period. Refractive error measurements were made on-axis, as well as 30 degrees nasally and temporally.

Statistical analyses: Both central (on-axis, C) and peripheral (off-axis, P) refractive errors are represented as spherical equivalent refraction (SER). Relative peripheral refractions (RPRs) represent the differences between peripheral and central refractions (i.e. P-C). To avoid potential confounding effects of the surgeries, changes over the lens treatment period in both refractive errors and axial dimensions for treated eyes are used as primary outcome measures of the lens treatment effects.

Data were analyzed using the same approach as in our previous study.[78] Differences between groups were assessed using 2-sample t-tests. To keep the family-wise error rate for the entire set of tests equal to 0.05, the Hochberg step-up procedure was used; thus *p* values were adjusted to take into account the number of tests undertaken. Results are graphically illustrated as box plots, which showed medians as well as the distribution of the data; whisker lengths represent either 1.5 times the interquartile range or the distance to the extreme, whichever is shorter.

4.3 Results

4.3.1 Effects of CNX and ID surgeries on ocular development

Summary statistics for ocular dimensions and refractive errors recorded approximately 7 days after the surgery, immediately prior to the start of lens treatments (i.e. baseline values), are listed in Table 4-2. In general, both ID and CNX increased the variability in both axial dimensions and refractions, compared to that recorded in normal eyes. CNX eyes had significantly thinner lenses, thicker choroids, and shorter vitreous chambers, resulting in a hyperopic shift in refractive error ($p<0.001$ for all). ID eyes shared some of the same features of CNX eyes; they had thicker choroids, shorter vitreous chambers and were more hyperopic than normal, consistent with the reduction in VCD in both cases. However, ID eyes showed lens thickening instead of thinning as well as shallower than normal anterior chambers, both changes reaching statistical significance ($p<0.001$ for both).

Table 4-2. Baseline axial dimensions and refractions by surgery group (* denotes statistical significance compared to normal (control) group).

Ocular Component (mm)	Control Eye	CNX	ID
Anterior chamber depth (ACD)	1.56 ± 0.05	1.56± 0.08	1.40± 0.11*
Lens thickness (LT)	2.34 ± 0.05	2.27± 0.08*	2.38± 0.10*
Vitreous chamber depth (VCD)	5.72 ±0.20	5.60± 0.30*	5.56± 0.32*
Retinal thickness (RT)	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
Choroidal thickness (CT)	0.20 ± 0.03	0.33± 0.15*	0.29 ± 0.10*
Refractive error (D)	1.89± +0.54	2.41± 1.18*	2.30± 0.93*

4.3.2 Effects of CNX and ID surgeries on central (on-axis) refractive error changes induced by single vision lenses

Central refractive error changes over the lens treatment period are summarized by surgery, lens type and CZD in Table 4-3. With the plano lens, both the CNX and ID groups showed minimal change in refractive error over the treatment period. In contrast, with the +5 SV lens, compensation to the imposed myopic defocus, defined as the change in refraction over the treatment period, was significantly less for both the CNX and ID groups compared to the control group (+1.55±0.77 D, p=0.0001 & +2.79±0.66 D, p=0.04 respectively, vs. +3.36±0.43 D, normal eyes). However, when lens treatment effects were compared using endpoint refractions as outcome measures, there was no significant difference between the ID and control (normal) groups (+5.04±0.58 D vs. +4.86±0.54 D for +5 D lens, p=0.28). This difference in statistical outcome is attributable to the hyperopic shift in baseline refractions post-surgery, evident in both CNX and ID groups. On the other hand, with the -5 SV lens, compensation to the imposed hyperopic defocus was not significantly different among surgical groups (p=0.54, Figure 4-2b).

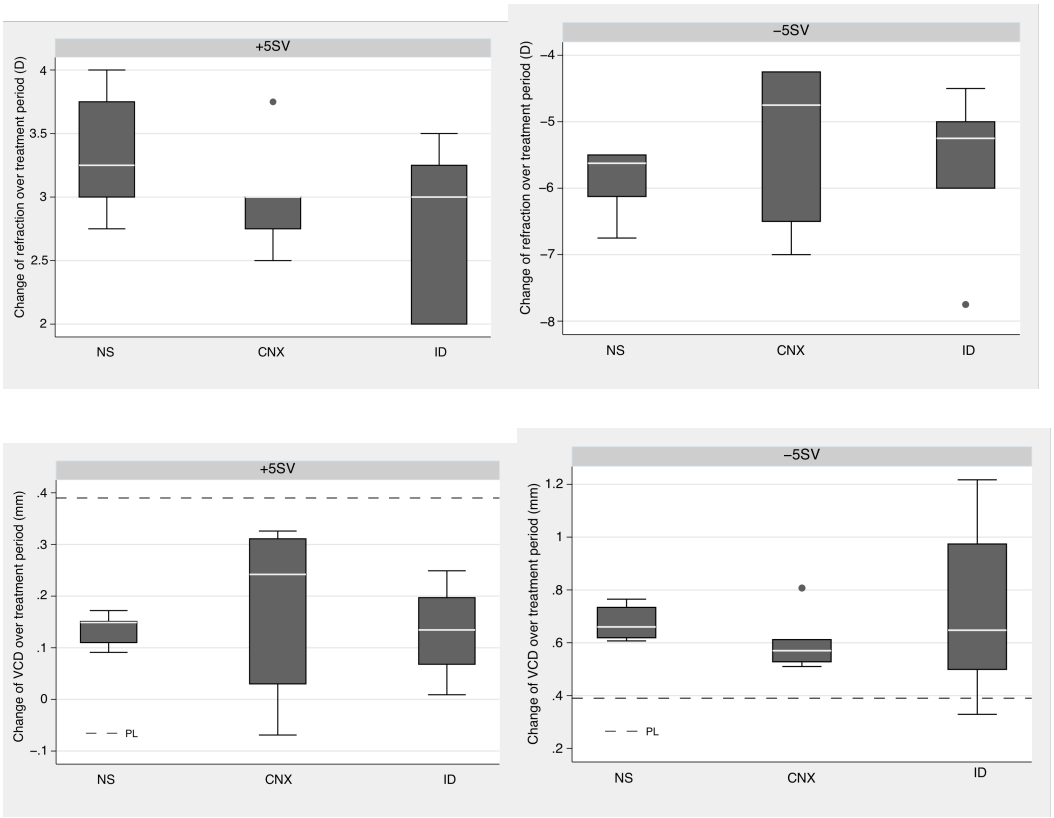


Figure 4-2. Boxplots of central refractive error changes (top panel), and corresponding changes of vitreous chamber depth (bottom panel) over the treatment period for (left) the +5 SV lens, (right) -5 SV lens, with 3 surgical groups. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme. Dashed reference line denotes the change by plano lens.

Table 4-3. Central (on-axis) endpoint refractive error data (Mean±SD, D), corresponding to a 5 day lens treatment period (change from baseline in parenthesis), organized by surgery, lens type (single vision (SV, PL) or 2-zone (+5C, -5C, +5P, -5P)) and CZD (n=7 in each cell).

Lens Type	CZD (mm)	Surgery		
		Normal (no surgery)	CNX	ID
+5C	3.5	+2.65±1.20 (+0.68±1.09)	+2.58±0.92 (+0.63±1.44)	+2.34±0.95 (+0.53±1.79)
	6.5	+6.79±1.23 (+4.54±1.11)	+4.96±0.46 (+2.63±1.10)	+6.67±0.89 (+4.42± 0.88)
+5P	3.5	+7.73±0.86 (+5.35±0.93)	+6.71±0.90 (+4.33±1.53)	+6.25±0.94 (+4.07±1.11)
	6.5	+3.96± 1.25 (1.71±1.23)	+2.92±1.30 (+0.25±1.28)	+3.50±0.87 (+1.25±0.76)
-5C	3.5	+1.47±1.98 (-0.53±1.63)	+1.54±0.53 (-0.75±0.63)	+1.67±0.68 (-0.63±0.52)
	6.5	0.42±1.28 (-1.79±1.41)	+0.21±1.75 (-2.58±1.63)	-0.15±1.84 (-3.00±1.83)
-5P	3.5	-0.57±2.15 (-2.86±2.24)	-0.50±1.28 (-1.46±1.32)	-0.67± 1.27 (-1.63±1.49)
	6.5	1.79±0.84 (-0.83±0.92)	-1.29±0.83 (-4.54±1.03)	-0.04±1.03 (-3.21±0.78)
+5SV	10	+4.86±0.54 (+3.36±0.43)	+3.92±0.92 (+1.55±0.77)	+5.04± 0.58 (+2.79±0.66)
-5SV	10	-3.84± 0.57 (-5.84±0.50)	-2.83±1.03 (-5.25±1.23)	-2.46±0.77 (-5.63±1.15)
Plano	10	+1.46±0.74 (-0.39±1.13)	+1.89±1.14 (-0.48±1.56)	+1.95±1.50 (-0.33±1.11)

4.3.3 Effects of CNX and ID surgeries on central (on-axis) refractive error changes induced by 2-zone lenses

Overall, the effect of lens type was highly significant ($F_{6,22} = 158.02$, $p<0.0001$), as well as the effect of CZD ($F_{1,22} = 11.63$, $p=0.0009$), and there was also a significant interaction between CZD and lens type ($F_{3,22} = 52.09$, $p<0.0001$). The effect of surgery was only borderline significant ($F_{2,22} = 3.29$, $p=0.04$), and there was no significant interaction between surgery and lens type ($F_{10,22} = 0.78$, $p=0.65$) for on-axis changes in refractive error.

Hyperopic changes induced by 2-zone positive lenses by surgical group (Figure 4-3, top panel): For the 2-zone lenses with positive power in their centers (+5C lenses), those with the smaller (3.5 mm) CZD had little effect; the changes were similar to those in plano lens-treated normal eyes for both surgical groups. In contrast, the +5C lenses with 6.5 mm CZD induced significant hyperopia in both CNX and ID groups. With CNX, the hyperopic shift induced by the +5C lens with 6.5 mm CZD was significant larger than the change in plano lens-treated CNX eyes (+2.63±1.10 D vs. -0.48±1.56 D, $p=0.0005$), although it was significantly less than that recorded in normal eyes with this 2-zone lens (+2.63±1.10 D vs. +4.54±1.11 D $p=0.004$). With ID, the hyperopic shift induced by the +5C lens with 6.5 mm CZD was also significantly larger than that induced by the +5SV lens (+4.42±0.88 D vs. +2.79±0.66 D, $p<0.0001$), similar to the hyperopic overshoot observed in normal eyes. For the lenses with positive power in the periphery (+5P lenses), the lenses with the smaller CZD had strong and similar effects in both CNX and ID groups, which both showed hyperopic overshoot (i.e., effect greater than that induced by +5SV lens) here (+4.33±1.53 D vs.+1.55±0.77 D, $p=0.0005$, CNX; +4.07±1.11 D vs. +2.79±0.66 D, $p=0.01$, ID). Compared to the effect of the 3.5 mm CZD +5P lens, the 6.5 mm CZD +5P lens had less effect, with the change for the CNX group also being significantly smaller than that recorded in normal eyes (+0.25±1.28 D vs. +1.17±1.23 D, $p=0.03$).

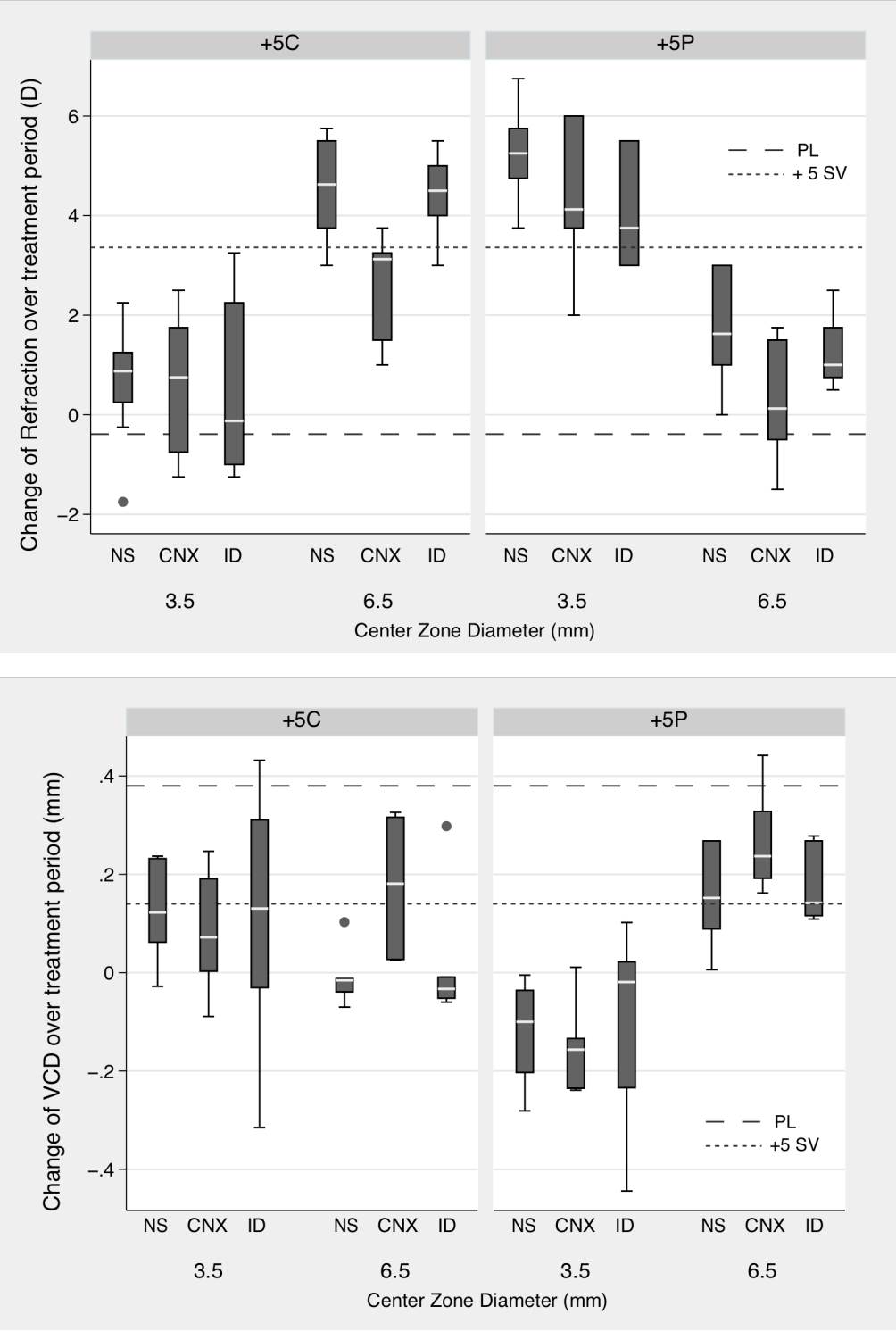


Figure 4-3. Boxplots of central (on-axis) refractive error changes (top panel), and corresponding changes of vitreous chamber depth (bottom panel) over the lens treatment period for the +5C lens (left), +5P lens (right), and 3 groups, normal (NS), ciliary nerve section (CNX), and iridectomy (ID groups. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme.

Myopic changes induced by 2-zone lenses by surgical groups (Figure 4-4, top panel): The myopic shifts in refractive errors induced by the 2-zone lenses incorporating negative power were all much smaller than that recorded with the SV lens, contrasting with the strong responses seen with some of the 2-zone positive lenses. Indeed, the -5C lens with smaller, 3.5 mm CZD had little effect, although the larger 6.5 mm CZD -5C lens induced significant myopic shifts in refractive error in all groups, with the effects observed in both CNX and ID groups tending to be larger than that in normal eyes. Likewise, the -5P lens with the 3.5 mm CZD had apparently little effect in all 3 groups, as indicated by endpoint refractive errors although significant myopic shifts in refractive error were recorded in both surgical groups (-4.54±1.03 D and -3.21±0.78 D respectively vs. -0.83±0.92 D, normal group, $p<0.001$ for both). There was also significant difference between the myopic changes in CNX and ID group, being larger in the former group (-4.54±1.03 vs. -3.21±0.78 D, $p=0.01$).

4.3.4 Effects of CNX and ID surgeries on SV and 2-zone lens-induced ocular growth

As lens treatment-induced changes were limited to changes in choroidal thickness and vitreous chamber depth, only changes in these two parameters are reported here. Specifically, changes in the VCDs of lens-wearing eyes over the treatment period were better correlated with observed changes in on-axis refractive errors than changes in axial length ($R^2=0.66$ vs. $R^2=0.61$ for axial length). Hence changes in VCD are used as the primary outcome variable describing ocular growth. These data were summarized by surgical group, lens type and CZD in Table 4-3. As with the changes in refractive error, changes of VCD were influenced significantly by lens type ($F_{6,12} = 78.40$, $p<0.0001$), the CZD ($F_{1,12} = 17.66$, $p<0.0001$), and interaction between lens type and CZD ($F_{3,12} = 6.27$, $p=0.0004$). The effect of the surgery was of borderline significance ($F_{2,12} = 2.87$, $p=0.06$).

The change of choroid thickness (CT) was significantly associated with induced hyperopic shifts in refractive error for lenses incorporating positive power ($R^2= 0.18$, $p<0.0001$), although changes in CT did not fully account for either the refractive error changes or changes of VCD.

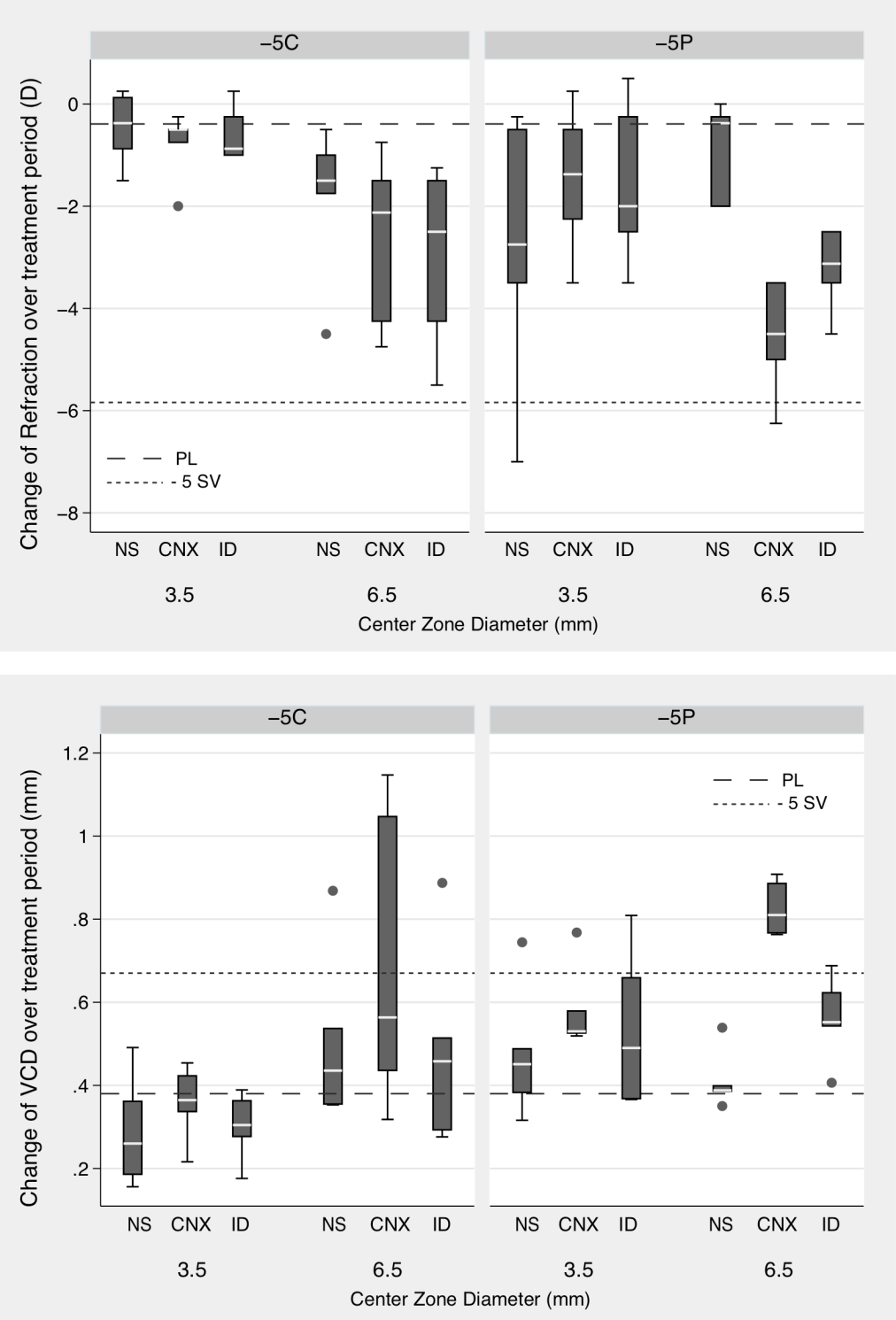


Figure 4-4. Boxplots of central refractive error changes (top panel), and corresponding changes of vitreous chamber depth (bottom panel) over the treatment period for (left) the -5C lens, (right) -5P lens, with 3 surgical groups. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme.

Table 4-4. Changes of VCD (Mean±SD, mm) over treatment period, organized by surgery, lens type (single vision (SV, PL) or 2-zone (+5C, -5C, +5P, -5P)) and CZD (n=7 in each cell).

Lens Type	CZD (mm)	Surgery		
		Normal (no surgery)	CNX	ID
+5C	3.5	0.13± 0.09	0.083±0.012	0.12±0.25
	6.5	-0.0082± 0.059	0.18±0.15	0.018±0.14
+5P	3.5	-0.12±0.099	-0.15±0.092	-0.11±0.20
	6.5	0.16±0.10	0.27±0.11	0.18±0.077
-5C	3.5	0.28±0.11	0.36±0.084	0.30±0.076
	6.5	0.50±0.20	0.68±0.34	0.49±0.25
-5P	3.5	0.47±0.13	0.58±0.097	0.53±0.19
	6.5	0.41±0.066	0.82±0.064	0.56±0.094
+5SV	10	0.14±0.28	0.18±0.16	0.13±0.090
-5SV	10	0.67±0.062	0.60±0.11	0.72±0.32
PL	10	0.38±0.081	0.42±0.18	0.32±0.22

VCD changes induced by SV lenses (Figure 4-2, bottom panel): While both the +5SV and -5SV lens treatments induced changes in VCD, these effects were not significantly affected by the surgery (p=0.55 & 0.69 respectively), and thus the data for the CNX and ID groups were pooled for comparisons of the effects of these lenses than that of the plano lens. Compared to eyes wearing plano lenses, those wearing +5SV lenses showed significantly less VCD elongation over the treatment period (diff. -0.23±0.048 mm, p=0.0002), consistent with observed hyperopic shifts in refractive error. On the other hand, those wearing -5SV lenses showed significantly greater VCD elongation (diff. 0.29±0.078 mm, p=0.002), with observed myopic shifts in refractive error.

VCD changes induced by 2-zone positive lenses (Figure 4-3, bottom panel): Here, the size of the CZD affected the outcome for both of the 2-zone positive lenses. Specifically, with the smaller 3.5 mm CZD lenses, the change in VCD was not significantly affected

by the surgery but the surgery did affect the outcome for the larger 6.5 mm CZD lenses (p=0.82 & 0.04 respectively). Although +5C lens with 3.5 mm CZD had minimal effect on the refractive errors of any of the three groups, the induced VCD change proved to be significantly less than that in plano lens-treated eyes (p<0.0001). Consistent with the larger changes in refractive errors with the +5C 6.5 mm CZD lens, changes in VCD were also larger than for the 3.5 mm CZD lens; the former were only slightly smaller than that seen with the +5SV lens for ID group (p=0.05), similar to the changes in normal eyes, while the changes in CNX group were significantly larger (p=0.005) than those in normal eyes, which also showed a smaller hyperopic shift in refractive error. For the +5P lenses, the mean change in VCD with the 3.5 mm CZD lenses was significantly less than that seen with the +5SV lens for all groups (p<0.0001), consistent with the larger hyperopic shift in refraction observed in the +5SV lens group. Similar to what was observed in +5C lenses, the change of VCD was significantly different in CNX group (p=0.04).

VCD changes induced by 2-zone negative lenses (Figure 4-4, bottom panel): With one exception, lens-induced changes in VCD were not significantly affected by surgery, the exception being the -5P lens with 6.5mm CZD (p<0.0001). Of the two -5C lenses, the lens with 3.5 mm CZD had little effect on VCD elongation; however, the lens with a 6.5 mm CZD induced greater elongation than the plano lens (p=0.003). For the -5P lens with the smaller 3.5 mm CZD, all three groups induced similar, and statistically significant increases in VCD compared to that seen in the plano group (p=0.0002), although the changes were significantly smaller than that induced by -5SV lens (p=0.004). Interestingly, for the -5P lens with the larger, 6.5mm CZD, the increase in VCD was significantly greater in both surgical groups (p<0.001 for both) compared to that in normal group, which is consistent with the larger myopic shifts in refraction recorded for both CNX and ID groups. There was also significant difference between the VCD changes in CNX and ID group, being larger in the former group (0.82±0.064 vs. 0.56±0.094 mm, p<0.001).

4.3.5 Effects of surgeries on changes in relative peripheral refractions (RPRs) induced by 2-zone lenses

The RPRs for the nasal and temporal fields were averaged for use in the analyses as there was no evidence of nasal-temporal asymmetries in peripheral refractive errors in either surgical or control groups. These data are summarized by lens type, CZD and surgery in Table 4-4.

The RPR data were subjected to a factorial ANOVA, with on-axis refractive error change, lens type, CZD and surgery as factors. The main effect of on-axis refractive error change proved highly significant ($F_{1, 24}$ = 13.04, p=0.0004), with increasing on-axis hyperopia associated with increasing relative peripheral myopia and vice versa. Lens design was found to be borderline significant as a determinant of RPR ($F_{6,24}$ = 2.08, p=0.06). Neither CZD and surgery nor their interactions with lens type significantly affected RPR ($F_{1,24}$ = 0.02, p=0.88; $F_{2,24}$ = 0.36, p=0.70 for CZD & surgery; $F_{3,24}$ = 0.30, p=0.82; $F_{9,24}$ = 0.80, p=0.62) for interactions between CZD & surgery with lens type respectively).

Table 4-5. Peripheral (off-axis) refractive changes over treatment period (Mean±SD, D), organized by surgery, lens type (single vision (SV) or 2-zone (+5C, -5C, +5P, -5P) and CZD (n=7 in each cell).

Lens Type	CZD (mm)	Surgery		
		Normal (No surgery)	CNX	ID
+5C	3.5	-0.45±1.08	-0.79±1.78	-0.16±0.48
	6.5	-1.98±1.50	-1.08±1.50	-1.44±1.12
+5P	3.5	-0.96±0.69	0.13±0.98	-1.30±1.44
	6.5	0.29±1.15	0.44±1.00	3.50±0.87
-5C	3.5	-0.17±0.25	-1.06±1.57	-0.71±1.38
	6.5	-0.01±1.46	0.15±1.41	0.25±1.33
-5P	3.5	0.00±2.03	0.31± 0.85	0.40±1.15
	6.5	0.08±0.63	0.56±1.44	0.62±1.20
+5SV	10	0.14±1.12	-0.15±0.37	-0.26±1.16
-5SV	10	0.83±1.24	-0.60±1.23	0.42±1.26

4.4 Discussion

The study reported here combined 2-zone lenses with 2 surgical manipulations to better understand the potential influence of pupil size and/or accommodation as well as their possible interactions with lens designs on the 2-zone lens induced effect. The 2-zone lenses allow the differential titration of the optical defocus experience of central and peripheral retinal regions. While surgical removal of iris induced a fixed, dilated pupil in our chicks, the test animal, ciliary nerve section provided surgical cycloplegia in addition to a similarly enlarged pupil in lesioned eyes. By comparing the effects of the 2-zone lenses in eyes subject to these surgeries with each other and those recorded in normal eyes, we hoped to be able to obtain insight into the influence of pupil size and accommodation, both of which were uncontrolled and thus potential confounders in our first, already published study. The effect of enlarging the pupil is to enlarge the area of central retina receiving multifocal stimuli with the 2-zone lenses in place. Additionally, we have been able to confirm that ID eyes retain near normal accommodation, despite the

removal of most of their iris,[79] while CNX eyes have no residual accommodation. Thus the 2-zone lens data from CNX and ID eyes allows us to not only investigate accommodation as an independent factor, influencing the ocular growth effects of the 2-zone lenses, but also to gain insight into the interactions between accommodation and imposed multifocal defocus stimuli on the retina.

The surgeries on their own influenced ocular growth and refractive development in young chicks, with both similarities and differences between the effects of CNX and ID noted. In both cases, choroidal thickening was observed, partly accounting for both the reduced VCDs and hyperopic shifts in refraction recorded after these surgeries. Similar changes have been reported for CNX previously.[72, 80] The similarity of the changes between the two groups suggests a shared underlying mechanism. Assuming that the primary effect is on the choroid, the most parsimonious explanation for the increase in choroidal thickness is increased uveoscleral flow, in one case resulting from reducing ciliary muscle tone with loss of its neuronal input, and in the other case being a product of removing an anatomical barrier to flow. The major differences between the surgeries - shallower than normal ACDs in ID eyes and thinner than normal lenses in CNX eyes, are consistent with the described role of the iris as a retraining influence on the crystalline lens in the former case, and the loss of ciliary muscle tone in the latter case. The removal of the iris would likely allow the lens to move forward towards the cornea, as reflected in the reduction of the ACD, while the latter effect would reduce basal accommodative tone, as reflected in the observed lens. Because ID has previously been reported to impair accommodation in the chick, we also sought confirmation that ID eyes were able to reach maximum accommodative levels, similar to those of normal eyes, in a separate study using topical nicotine (see Supplement I for details). The onset and amplitude of topical nicotine-induced accommodation were similar for ID and normal eyes. This result predicts near normal physiological accommodation in ID eyes, because the neural innervation was left intact. In contrast, the lesioning of the ciliary nerve in CNX eyes eliminates any possibility of physiological accommodation. For this reason, they were not included in the latter nicotine study.

With SV lens treatments, a smaller than normal hyperopic shift in refractive error was observed with the +5 SV lens in both CNX and ID groups, although the end point refractive errors for both groups were more consistent with compensation to the magnitude of imposed optical defocus. This difference likely reflects the hyperopic shift in baseline refractive error after the surgery. Unfortunately the only other much earlier study involving CNX eyes,[69] reported results in terms of endpoint interocular differences and so does not provide any additional insight into whether compensation is impaired by CNX surgery. Nonetheless, the results of the latter study are consistent with the current findings; interocular differences showed a relative increase in hyperopia in CNX eyes wearing +10 D lenses. With the -5SV lens, the magnitudes of myopic changes in refractive error were similar for CNX, ID and normal eyes, which is also consistently with previously reported findings in relation to the effects of CNX.[69, 80] Note that both CNX and ID eyes wearing plano lenses instead of powered lenses over the lens treatment period showed little difference in the changes in their refractions and axial ocular

dimensions compared to normal eyes wearing plano lenses, suggesting that the lesioned eyes had fully recovered from the surgeries prior to the start of lens treatments.

The effects of the surgeries on 2-zone lens induced ocular changes were more complicated. In general, surgical treatments had more impact on the lens-induced changes with 6.5 mm CZD lenses, but there were also differences related to whether the imposed defocus was myopic (positive lenses) or hyperopic (negative lenses). With the 2-zone +5 D lenses and both C and P designs, CNX eyes showed a significantly reduced hyperopic shift in refraction as well as correspondingly reduced VCD changes comparing to both ID and normal eyes, suggesting a critical role for accommodation in the responses to these 2-zone positive lenses. While the baseline hyperopic shift in refraction after CNX represents a confounding factor, possibility contributing to the reduced response to the 6.5 mm CZD 2-zone +5 D lenses, the more robust response to the 3.5 mm CZD +5P lens in CNX eyes argues against this interpretation. Also both CNX and ID eyes showed similar baseline hyperopic shifts in refractive error, yet the responses of ID eyes to all four of the 2-zone positive lens/CZD combinations were similar in magnitude to those of normal eyes. The lack of a difference here also implies that pupil size in eyes with intact accommodation is not critical to the response to multifocal environments in which myopic defocus dominates. The different results for CNX eyes, which had similarly enlarged pupils to ID eyes, argue that dynamic accommodation is required for accurate decoding of multifocal stimuli, at least when myopic defocus is included. Results from two other studies involving CNX eyes, both involving competing defocus stimuli of opposite sign, in one case presented in a cone-imaging device,[81] and in the other case via Fresnel lenses,[81] reached a similar conclusion, although the nature of this accommodative effect remains unresolved.

With 2-zone negative lenses, the effects of the surgeries were limited to the 6.5 mm CZD -5P lens, with which both CNX and ID eyes showed significantly greater myopic shifts in refractions as well as greater VCD elongation comparing to the equivalent changes in normal eyes. In the 6.5 mm CZD -5P lens design, the zone of hyperopic defocus is limited to a narrow peripheral zone of the lens, and thus it is perhaps not surprising that the responses were more robust in the lesioned eyes as their enlarged pupils would have increased the overall area of retina exposed to hyperopic defocus. While this pupil size effect was not seen with the positive lenses, the more enduring nature of responses to imposed myopic defocus may reduce the effect of area; under these conditions, eye movements under the lenses may serve the same role of expanding the area exposed to myopic defocus, albeit intermittently. The significant differences in the responses of CNX and ID groups are also noteworthy, once again pointing to a critical role for accommodation in decoding the multifocal environment. Interestingly, here CNX eyes showed significantly larger responses than ID eyes, the opposite trend to that described with positive lenses. The less enduring nature of the response to imposed hyperopic defocus compared to imposed myopic defocus, offers at least a partial explanation for the differences between CNX and ID exposed to this type of defocus; with accommodation removed, the CNX eyes would have experienced a larger “cumulative dose” of defocus than ID eyes, even allowing for eye movements, which would have exposed some retinal areas to the plano lens zones, at least some of the time.

Overall, the impact of the CNX and ID surgeries on the responses to the 2-zone lenses was small when the CZD was small. An analysis of the effects of the surgeries on the patterns of retinal defocus produced by these lenses reveals the likely explanation. When the CZD is small, there is no central unifocal field, corresponding to the central optical zone of the lens, even for normal eyes that are centered behind the lens, i.e., in primary gaze. Instead there is a central multifocal field, which will decrease in size with increasing pupil size, with a corresponding increase in the size of peripheral unifocal field, the projection of the peripheral zone of the lens (Table 4-1). Such pupil-size dependent differences will be significantly dampened with only minimal eye movement, thereby reducing the differences in the optical defocus experience and thus responses of lesioned and normal eyes. Contrasting with the small differences in projected retinal fields for lenses with the smaller, 3.5 mm CZD, between normal and lesioned eyes, enlarging the pupil has a significant impact on the projected fields for the larger 6.5 mm CZD lens. Thus the large unifocal central field, corresponding to the projection of the central zone in normal eyes, is replaced in CNX and ID eyes by a large multifocal central field, which is influenced by both the central and peripheral optics of the lens, as well as of the eye. Specifically, the on-axis HOA, especially SA, increases significantly with pupil enlargement in CNX eyes,[72] and similar changes are expected in ID eyes. These differences between lesioned and normal eyes may at least partly contribute to the differences observed with the larger CZD lenses. However, the only small differences seen in treatment effects across groups with the smaller CZD lenses rule out a major influence of such altered ocular aberrations, at least for these lenses. These results may have clinical ramifications, in decisions about the size of the optical zones incorporated into multifocal contact lenses intended for myopia control, given that accommodation-driven pupil size changes are known to vary with age.[9]

Despite the significant impact of the surgeries on on-axis refractive changes for certain lens/CZD combinations, neither the surgeries nor interactions between the surgeries and lens treatments reached statistical significance for induced RPR changes with the 2-zone lenses. Furthermore, analysis of pooled data from the two surgical and normal groups, revealed on-axis refractive error to be the strongest factor influencing RPRs, with the influence of lens type on RPR reduced to only borderline significance comparing to that in the model with only nonsurgical eyes included in the previous study. This outcome implies correlated growth of on- and off-axis regions of the eye, suggesting robust interactions between central and peripheral retinal areas, and/or the operation of an integrator operating over large retinal areas to average out their respective optical defocus experiences, perhaps facilitated by eye movements. This model also serves to explain the results of another chick study presenting mosaic of competing defocus stimuli across the retina using a cone imaging system; although only on-axis refractive errors were measured, the results are consistent with some type of integrator that is more strongly influenced by imposed myopic defocus.

In summary, both CNX and ID induced small but significant changes of ocular components as well as hyperopic shifts in refractive errors. With SV lens treatments, compensation to the +5 SV lens was reduced after both CNX and ID, likely due in part to the surgically-induced shift in baseline refractive error; these surgeries did not affect

compensation to the -5 SV lens. The surgeries also had little affect on the responses to the smaller CZD 2-zone lenses but significantly affected the responses to the larger CZD lenses. CNX eyes showed reduced responses to the positive 2-zone lenses but increased responses to the equivalent negative lenses, compared to those of normal and ID eyes. These results suggest a critical influence of pupil size on the treatment effects of 2-zone lenses and also point to a critical role of accommodation in the decoding of ocular complex optical stimuli.

Chapter 5

The Effect of 2-zone Concentric Negative Bifocal Spectacle Lenses on Refractive Error Development & Eye Growth in Young Chicks

Abstract

Purpose: To investigate the effects on refractive development and ocular growth of 2-zone concentric lenses with different negative powers in each of the optical zones, in both normal and myopic eyes. The study represents an attempt to simulate the ocular optical conditions in which concentric multifocal contact lenses are applied for myopia control in humans.

Methods: Monocular defocusing lenses were worn for 10 to 15 days from 12 days of age. Two 2-zone concentric lens designs combined -5 and -10 D powers (-10C/-5P or -5P/-10C), with CZDs ranging from 4.5 to 7.5 mm were tested. One group of chickens wore 2-zone negative lenses from 12 days of age for 10 days, without any previous lens treatment. A second group of 12 day-old chickens were treated, initially with -10SV lenses for 5 days to induce myopia, followed by 2-zone lenses for another 10 days. Plano and -10SV lenses were used as control treatments. Each group comprised 7 chickens.

Results: With the 2-zone negative lens treatment alone, the magnitude of on-axis induced myopia fell between that expected for two negative powers presented in SV lens format, while for eyes first made myopic by pretreatment with -10SV lenses, the 2-zone negative lenses caused regression of the induced myopia due to inhibitory effects on axial ocular growth, with the greatest effects observed in eyes with higher baseline myopia. RPRs were negatively associated with the magnitude of on-axis myopia and highly dependant on the lens design.

Conclusion: The results from this study in the chick provide further evidence for decoding of defocus signals by the peripheral retina, thereby contributing to ocular growth regulation, independent of the central retina. They also lend weight to recent clinical observations suggesting that appropriately designed concentric multifocal contact lenses can control myopia progression and argue for further clinical studies of these lenses.

5.1 Introduction

The prevalence of myopia is increasing rapidly worldwide, with the fastest increases in East Asian populations.[2] In addition, the age of onset of myopia is decreasing, with earlier onset linked to more rapid progression, older age of stabilization, and higher prevalence of pathological myopia.[1] Thus myopia can no longer be viewed as a benign condition, requiring only simple optical corrections for the purpose of eliminating distance blur. Rather myopia should be viewed as a progressive condition carrying a potential risk of irreversible vision loss, with early intervention aimed at controlling its progression being the primary goal of treatment.

Emmetropization is now generally accepted to be an active process rather than a byproduct of normal ocular growth, with supporting evidence coming from a variety of animal models. Of relevance to both myopia and the study reported here, sustained retinal hyperopic defocus, imposed using optical lenses, is known to be a reliable stimulus to increased eye growth, leading to myopia in a variety of animals.[5] On the other hand, imposed myopic defocus is either without effect or slows eye growth. Two examples of hyperopic defocus in human eyes offer plausible links to the finding of defocus-induced myopia in animal studies: 1) relatively prolate eye shapes, which are more commonly encountered among myopic eyes, introducing relative hyperopic defocus at peripheral retinal locations, and 2) lags of accommodation, which are reported to be increased in progressing myopes, introduce hyperopic defocus on the central retina.[71] Eyes undergoing myopia progression, whether or not they are myopic, are also reported to show greater relative peripheral hyperopia, and larger lags of accommodation.[12, 55]

The optical defocus studies in animals have renewed interest in the possibility that myopia progression can be slowed through optical intervention. Furthermore, a number of recent studies have reported promising inhibitory effects on myopia progression of bifocal and multifocal soft contact lenses (SCL)[41, 77] and orthokeratology,[39, 82] contrasting with equivocal results in earlier studies testing spectacle bifocal and progressive addition lenses (PAL) as treatments to slow human myopia progression. The underlying mechanism for these "anti-myopia" effects is yet to be elucidated although reductions in, or overcorrection of, peripheral hyperopia is consistent with the inhibitory effect on eye growth of imposed myopia in the chick, and these contact lens treatments may also reduce accommodative lags. In contrast, the effects of traditional bifocal and PAL spectacles are limited to reducing accommodative lags and thus the amount of hyperopic defocus experienced by the central retina.

Despite the increasing interests in optical treatments for myopia, and at a more general level, understanding how the multifocal optical environment influences refractive development, animal studies using multifocal lenses or conditions have been so far limited to only a small number of studies in chicks. When myopic defocus and hyperopic defocus are set in competition, as with Fresnel lenses and dual-focus cone systems, myopic defocus dominates over hyperopic defocus in terms of effects on eye growth, although these presentation modes have limited potential for translation into treatments for human myopia.[61]

In a recently published study, we tested a 2-zone lens design more similar to the soft contact lens designs showing promising anti-myopia effects in humans. The lenses incorporated either positive or negative power in one optical zone and plano power in the other.[78] In contrast to the effects of the Fresnel lenses and dual-focus cone systems, with which multifocality is imposed on the same or nearby local retinal regions, our 2-zone lens design allows retinal eccentricity-dependent differences in optical defocus to be imposed. Our 2-zone positive lenses significantly slowed eye growth, resulting in hyperopic shifts in refractive errors. An unexpected and intriguing finding was that the induced hyperopic shifts in refraction were significantly greater with 2-zone positive lenses than with single vision lens of the same positive power.

The study reported here used 2-zone lenses incorporating negative powers in both optical zones, to more closely simulate the retinal defocus conditions experienced by human eyes prior to the development of myopia. We also tested the same lenses on eyes that had been made myopic, to simulate the conditions experienced when multifocal soft contact lenses are used to treat myopia. Our study sought to address the following questions: 1) what are the relative contributions of peripheral and central retinal regions in guiding emmetropization, 2) does the control of myopia progression require reductions in hyperopic defocus over the entire retina or are treatments aimed at central or peripheral retinal regions alone sufficient to slow progression.

5.2 Methods

Animals: White-Leghorn day-old chicks, obtained from a commercial hatchery (Privett Hatchery, New Mexico), were used in this study. They were reared in a normal diurnal, photopic white lighting environment (12 hr on/12 hr off), with food and water freely available. A total of 126 birds were used in this study. All animal care and treatments in this study conformed to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Experimental protocols were approved by the Animal Care and Use Committee of the University of California-Berkeley.

Lens designs: All lenses had an overall optical diameter of 10 mm and total diameter of 12.2 mm. Two 2-zone concentric lens designs combined -10 and -5 D powers in the following ways: (i) -10 D center/ -5 D periphery (peripheral add), and (ii) -5 D center/-10 D periphery (central add). Four central zone diameters (CZD) were tested, ranging from 4.5 to 7.5 mm in 1 mm increments. Single vision (SV) -10 D and plano lenses were included as control treatments. Because of eye movements, only limited central and peripheral retinal regions would have experienced only unifocal (single vision) defocus with the 2-zone lenses; the experience of the remaining, paracentral retinal regions would have been variable, influenced by both the central and peripheral lens zones. Table 1 summarizes the estimated visual angle subtense of these various retinal zones for all lens designs; the method of calculation was described in our earlier paper.

Table 5-1. Dimensions of center and peripheral optical zones (CZD, PZD) of the four 2-zone lens designs used and corresponding dimensions of the unifocal central visual field, and annular multifocal paracentral and unifocal peripheral visual fields. Method of calculation previously reported.

CZD (mm)	PZD (mm)	Unifocal Central Field (deg)	Multifocal Paracentral Hemi-field (deg)	Unifocal Peripheral Hemi-field (deg)
4.5	2.75	12	35	13
5.5	2.25	34	27	10
6.5	1.75	54	19	8
7.5	1.25	72	14	4

Treatment assignments: Chickens were randomly assigned to one of four treatment groups, which were exposed to different monocular lens treatment regimens as summarized schematically in Figure 1. In one group (A), two-zone negative lenses were applied to 12 d old chickens without any previous lens treatment and worn for 10 days. Two control groups wore single vision lenses, either plano or -10 D (-10SV) for the same period. In a fourth group (B), 12 d old chickens were first treated for 5 days with -10SV lenses, which were then replaced with 2-zone lenses, the latter being left in place for another 10 days. Seven chickens were included in each treatment group. Lenses were cleaned and inspected at least 3 times daily to ensure their optical centers remained approximately aligned with the ocular pupil centers of the chickens, thereby minimizing the confounding effect of lens decentration.

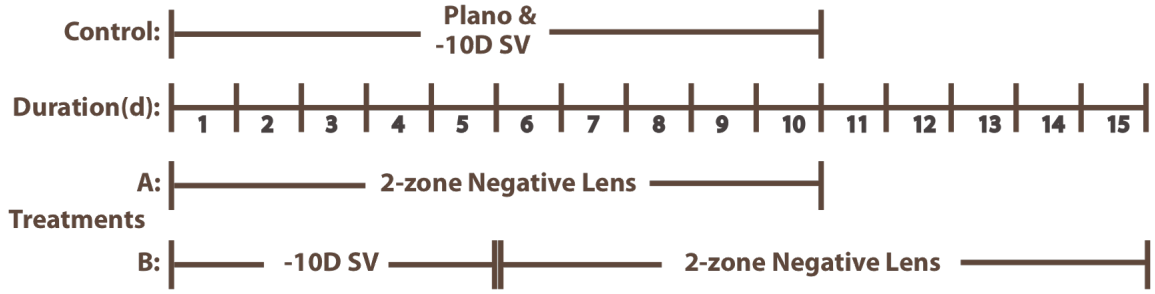


Figure 5-1. Schematic summary of the two lens treatment paradigms (A & B) used in this study; single vision (SV) lenses were included either as a comparison control treatment (A), or as an initial myopia-inducing treatment (B).

Measurements: Immediately before the start of lens treatments, baseline refractive errors and axial ocular dimensions were measured using static retinoscopy and high frequency A-scan ultrasonography respectively under gaseous anesthesia (1.5% isoflurane in oxygen). Ultrasonography measurements were repeated every other day, and retinoscopy

was repeated every 5 days. Refractive error were measured both on-axis (centrally) as well as 30 degrees off-axis nasally and temporally.

Statistical analyses: Because there was no significant increase in astigmatism at the 2 off-axis compared to on-axis locations, either before or after the lens treatments, central (C, on-axis)) and peripheral (P) refractive errors are represented as spherical equivalent refractive errors (SER; averages between the refractions for two principal meridians). Off-axis refractive errors are expressed as relative peripheral refractive errors (RPR), representing the difference between the peripheral and central values (i.e. P-C). Because changes in vitreous chamber depth (VCD) and choroidal thickness (CT), largely account for the changes in central refractive error, only these biometric changes are shown. Also, as no group-related differences in untreated contralateral (fellow) eyes were found, only changes over the treatment period in treated eyes are reported as primary outcome measures of treatment effects. Additionally, for paradigm B, as there was no significant difference between the refractive error changes recorded 5 days and 10 days after switching to the 2-zone lenses, only 5 day data were used in statistical comparisons with groups subjected to paradigm A, to avoid any confounding effect of age.

Data analyses protocols were similar to those used in our previous, closely related paper. In brief, the normality of the distributions of changes in refractive error and axial dimensions were first verified and then Factorial ANOVAs performed using STATA (STATA Corp. TX), on changes in the central refractive error and VCD over the treatment period, as well as endpoint RPR. For both central SER and VCD data, the effects of two factors - lens design and CZD, were explored, as well as the interaction between these two factors. The RPR data were subjected to a factorial ANOVA, with on-axis refractive change, lens type, CZD and the interaction term between lens design and CZD as factors. Differences between groups were assessed by 2-sample t-tests, using the Hochberg step-up procedure to keep the family-wise error rate for the entire set of tests equal to 0.05. Box plots are used to show the results graphically.

5.3 Results

5.3.1 Effects of 2-zone negative lenses on previously untreated eyes

5.3.1.1 Effects of lens design and CZD on central (on-axis) refractive errors

Changes in the on-axis refractive errors over the treatment period are summarized by lens type and CZD in Table 2, and are also shown graphically in Figure 5-2 (top panel). With the two SV control treatments, there was minimal change in refractive error with the plano lens, and with the -10SV lens, compensation to the imposed defocus was nearly complete after just 5 days of treatment. Thus refractive error changes from baseline, recorded on treatment days 5 and 10, are not significantly different (-9.61±1.25 D & -10.63±0.70 D respectively, p=0.06).

Table 5-2. Changes in central spherical equivalent refractive errors recorded on treatment day 5 and 10 (mean±SD, D), organized by lens type and CZD (n=7 in each cell).

CZD (mm)	Lens Type			
	-5C/-10P		-10C/-5P	
	day 5	day 10	day 5	day 10
4.5	-4.54±0.99	-9.17±1.07	-4.63±1.32	-6.08±1.18
5.5	-4.29±0.49	-8.67±1.95	-4.25±0.79	-6.46±1.55
6.5	-3.81±1.86	-6.03±2.22	-4.33±0.86	-7.42±1.68
7.5	-4.25±0.63	-6.67±0.61	-7.83±1.33	-9.67±1.63

Equivalent values for the -10SV lens treatment are -9.61±1.25 D, day 5, and -10.63±0.70 D, day 10; values for the plano lens treatment are -0.37± 0.24 D, day 5, and -0.54± 0.74 D, day 10.

As with the -10SV lens, all 2-zone lens designs induced significant myopic shifts in central SERs compared to values recorded with the plano lens. Significant changes were evident by day 5, and further increased by day 10, although all values were significantly less than those recorded with the -10SV lens, except for the largest CZD -10C/-5P lens, which induced similar changes to the -10SV lens (-9.67±1.63 vs. -10.63±0.70; p=0.11, day 10; p<0.05 for other designs & both time points. The changes in refraction also showed increased variability across the treatment period with the 2-zone lenses, opposite the trend with the -10D SV lens.

While lens design did not significantly influence the changes in central SERs, which were consistently in the myopic direction (there is significant overlap in the refractive errors distributions by lens type), induced myopic shifts in refraction increased in magnitude with increasing lens area devoted to -10 D power for both 2-zone lens designs, reaching statistical significance for the day 10 data (p=0.003 & <0.0001 for -5C/-10P and -10C/-5P designs respectively). For the 2-zone lenses, the ratio of day 5 and day 10 refractive error changes was also significantly affected by the lens design ($F_{1,8}$ = 8.90, p=0.005), implying that the early changes were in fact affected by the lens design.

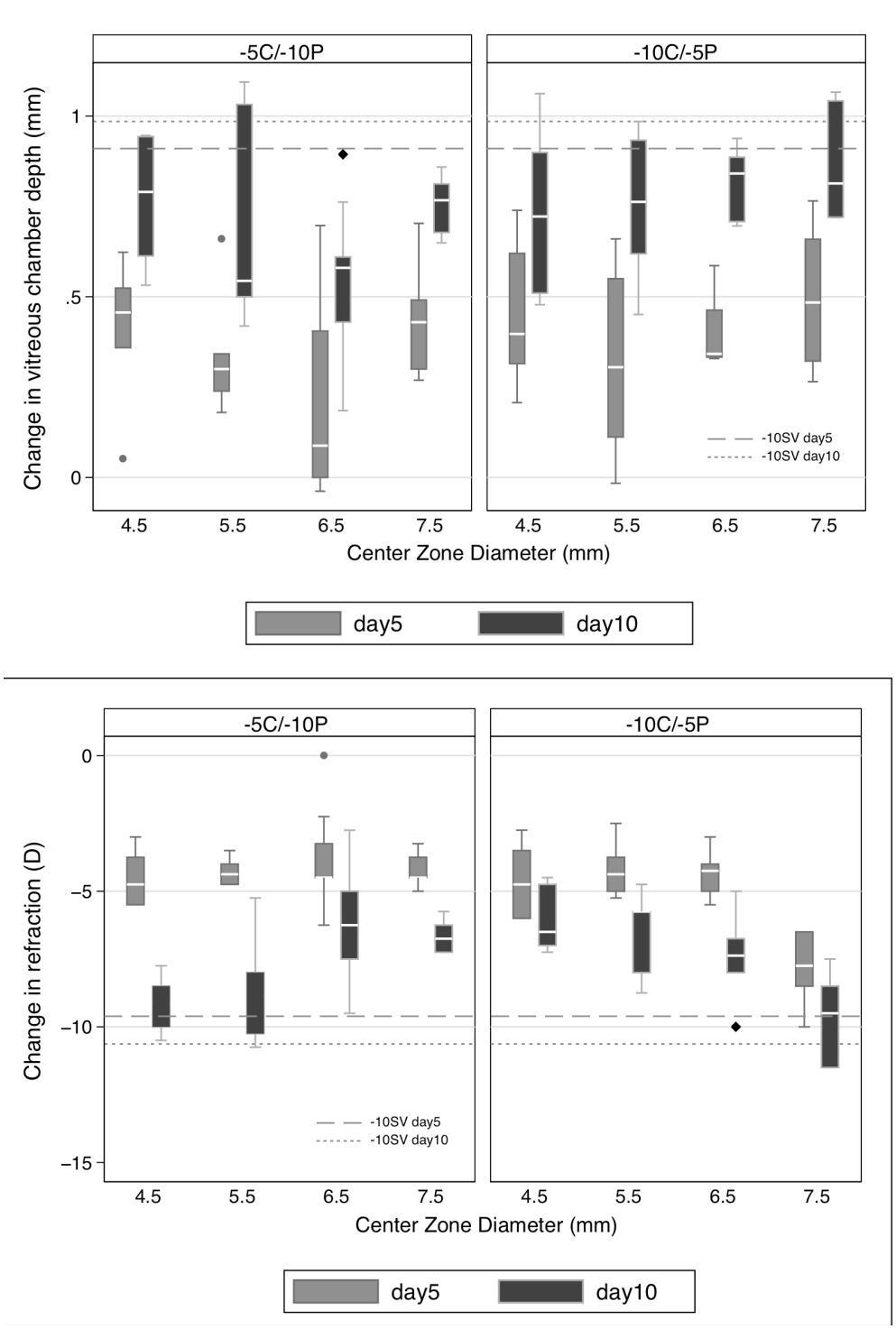


Figure 5-2. Boxplots of central refractive error changes (top panel), and corresponding changes of vitreous chamber depth (bottom panel) over the treatment period for (a) the -5C/-10P lens designs, (b) -10C/-5P lens designs, with varying central zone diameters (CZD, on X-axis) ranged from 4.5 to 7.5 mm. Dashed reference lines denote the mean changes induced by -10SV lens. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme.

5.3.1.2 Relative peripheral refractive (RPR) change induced by 2-zone negative lenses

Since there was no evidence of nasal-temporal asymmetries in RPR changes, either for the 2-zone lenses or the -10SV lens, the values derived for the nasal and temporal fields were averaged for use in analyses of the influences of lens design and CZD. Figure 5-3 shows RPRs plotted against changes on-axis refractive error for both 2-zone lens designs. The overall trend is for RPR to become more hyperopic with increasing on-axis myopia ($F_{1, 8} = 5.75$, p=0.02), although the two lens designs induced very different patterns of RPR. Specifically, exposing the peripheral retina to higher amounts of imposed hyperopic defocus than the central retina, e.g. with the -5C/-10P lens designs, resulted in more myopic RPRs, compared to the changes induced with the -10C/-5P lens designs. This design-dependent difference is reflected in the large gap between the regression lines derived for the two lens designs and was confirmed statistically ($F_{1, 8} = 31.82$, p<0.0001). Although CZD alone did not significantly affect RPR ($F_{3,8} = 2.43$, p=0.08), its interaction with lens design was significant ($F_{3,8} = 3.83$, p=0.02).

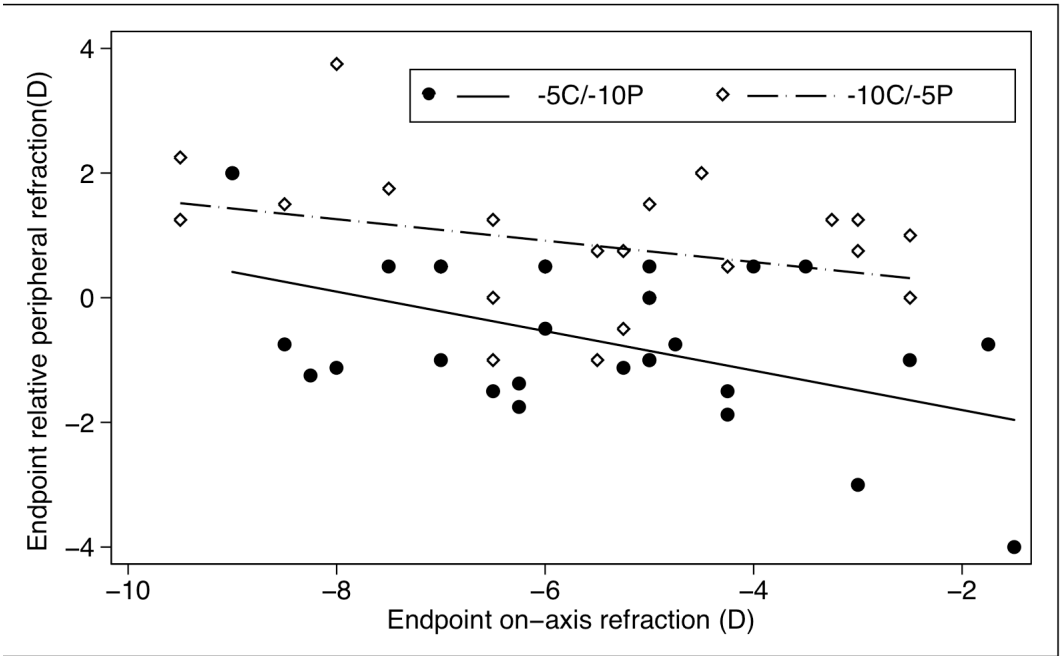


Figure 5-3. Scatter plots of endpoint relative peripheral refractive errors (RPR) as a function of endpoint on-axis refractive errors for the two lens designs (solid circle: -5C/-10P, open diamond: -10C/-5P). The mean RPR for eyes wearing -10SV and plano lenses were +1.58±0.44 D and -0.09±0.83 D and respectively.

5.3.1.3 Effects of 2-zone lenses design on central (on-axis) axial ocular dimensions

Induced changes in vitreous chamber depth (VCD) are summarized by lens type and CZD in Table 3 and Figure 2 (bottom panel). For the -10SV lens, the required compensatory increase in VCD was largely accomplished over the first 5 days of treatment, consistent with the pattern of change in central SER, and thus changes from

baseline, measured on day 5 and day 10 are similar (0.91±0.21 mm, day 5, and 0.99±0.22 mm, day 10). Increased elongation of the vitreous chamber also largely accounts for the central refractive error changes with the 2-zone lenses, with changes in these two parameters being highly correlated (R^2=0.56 & 0.30 for days 5 & 10 respectively, p<0.0001). As with the changes in SERs, significant elongation was already evident by treatment day 5, for both 2-zone lens designs and the various CZDs, with the exception of the 6.5 mm CZD, -5C/-10P lens, although all treatment groups showed greater elongation by day 10. The induced VCD changes were also significantly less than those induced by -10SV lens at both time points (p<0.05 for all groups) for all but the largest CZD -10C/-5P lens, for which the changes were indistinguishable by day 10 from those recorded with the -10SV lens. The dose-response relationship between VCD elongation and CZD was not significant for either lens design but approached significance for the -5C/-10P lens (p=0.06). All 2-zone lens groups as well as the -10SV lens group exhibited significant choroid thinning on treatment day 5 (p=0.01, treated compared to fellow eye), but these changes were not sustained and no group recorded choroidal thinning on day 10 (p=0.46).

Table 5-3. Changes in vitreous chamber depth recorded on treatment day 5 and 10 (mean±SD, mm), organized by lens type and CZD (n=7 in each cell).

CZD (mm)	Lens Type			
	-5C/-10P		-10C/-5P	
	day 5	day 10	day 5	day 10
4.5	0.41±0.20	0.77±0.17	0.45±0.20	0.73±0.23
5.5	0.34±0.17	0.69±0.29	0.32±0.26	0.75±0.21
6.5	0.20±0.26	0.55±0.21	0.40±0.10	0.82±0.10
7.5	0.44±0.16	0.76±0.08	0.50±0.19	0.86±0.16

Equivalent values for the -10SV lens treatment are 0.91±0.21 mm, day 5, and 0.99±0.22 mm, day 10; values for the plano lens treatment are 0.18±0.09 mm, day 5, and 0.41±0.12, day 10, the latter values being similar to the normal growth changes in the untreated fellow eyes (0.24±0.15 mm, day 5 and 0.45±0.11, day 10).

5.3.2 Effects of 2-zone negative lenses on already myopic eyes (pre-treated with -10SV lenses)

5.3.2.1 Effects of lens design and CZD on central (on-axis) refractive errors

Data collected at the end of the 5-day, -10SV lens treatment period confirmed that all treated eyes had become highly myopic; the mean change in central SER was -9.61±1.25 D. This induced myopia showed regression when the -10SV lenses were replaced at this time with one of the 2-zone lenses, irrespective of their design, the reduction in myopia

achieving statistical significance after both 5 and 10 days of exposure to the latter lenses ($p<0.05$ for all). These data are summarized by lens type and CZD in Table 4. The reduction in induced myopia was significantly affected by CZD ($F_{3,8} = 13.56$, $p<0.0001$), which significantly interacted with lens design ($F_{3,8} = 21.18$, $p<0.0001$), although lens design alone did not significantly affect the outcome ($F_{1,8} = 0.72$, $p=0.4$).

Further analysis of the myopia regression patterns revealed three interesting trends. First, the magnitude of the reduction in myopia with the 2-zone lenses in place was positively associated with the initial level of induced myopia ($F_{1,8} = 53.86$, $p<0.0001$), i.e., the higher the initial myopia, the larger the subsequent reduction in myopia. Second, the refractive errors recorded 5 days and 10 days after switching to 2-zone lenses were not significantly different, implying that the myopia control effect was achieved within the first 5 days, after which the lenses served to stabilize the refractive error (Table 5-5, top row). Third, endpoint refractions were generally less myopic for eyes in which a -10SV lens was switched with a 2-zone lens compared to the refractive errors of eyes wearing the same 2-zone lens for the entire treatment period (Figure 5-4, top panel), with these differences reaching statistical significance for the 5.5 mm CZD, -5C/-10P lens, as well as 4.5 and 5.5 mm CZD, -10C/-5P lenses ($p<0.05$ for all).

Table 5-4. Changes in central (on-axis) spherical equivalent refractive errors after 10 days of lens wear (mean±SD, D), organized by lens type and CZD. Eyes wore either a 2-zone lens for 10 days (2-zone alone) or for 5 days as a replacement for a -10SV lens worn for 5 days. Asterisk denotes significant differences between groups.

CZD (mm)	Lens Type			
	-5C/-10P		-10C/-5P	
	2-zone alone	-10SV pretreatment	2-zone alone	-10SV pretreatment
4.5	-9.17±1.07	-8.56±0.45	-6.08±1.18	-4.36±0.79*
5.5	-8.67±1.95	-4.63±1.10*	-6.46±1.55	-4.79±1.08*
6.5	-6.03±2.22	-4.98±1.61	-7.42±1.68	-6.98±1.24
7.5	-6.67±0.61	-5.80±1.86	-9.67±1.63	-8.21±0.53

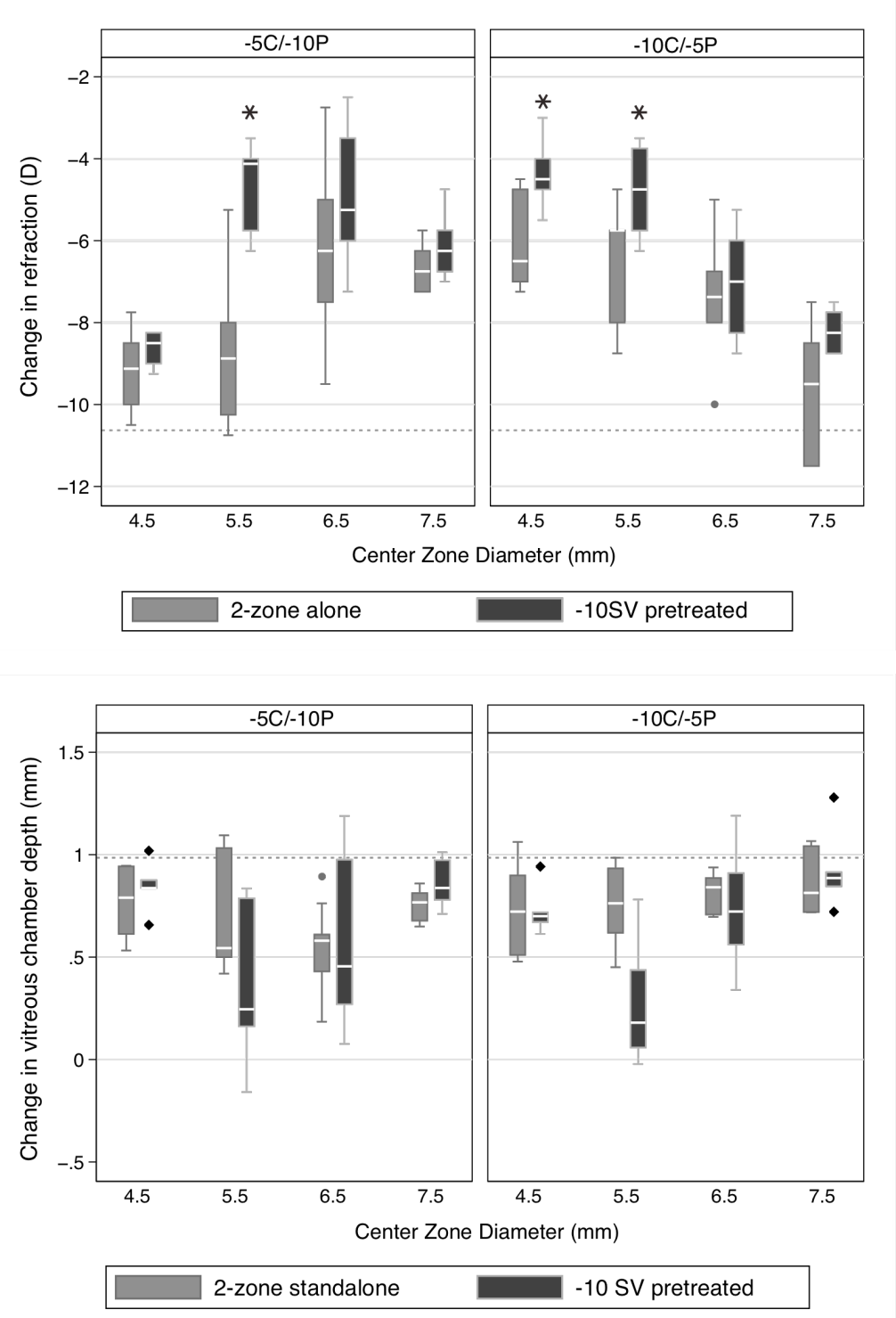


Figure 5-4. Boxplots of changes in central refractive errors (top panel), and corresponding changes in vitreous chamber depth (bottom panel) after (a) 2-zone lens treatment for 10 days (b) -10SV lens pretreatment for 5 days followed by 2-zone lens treatment for 5 days. Dashed reference lines denote the mean changes induced by -10SV lens before replacement with a 2-zone lens. Whisker length denotes the shorter of 1.5 times the interquartile range and the distance to the extreme.

5.3.2.2 Relative peripheral refractive (RPR) change induced by 2-zone negative lenses

There was no significant difference between the RPRs recorded 5 and 10 days after switching from a -10SV lens to a 2-zone lens. These data are summarized in Table 5-5. The day 5 data were further analyzed statistically. While there was greater variability in the RPRs of eyes switched from a -10SV lens to a 2-zone lens than in the RPRs of eyes wearing only a 2-zone lens, lens design remained highly significant as a determinant of RPRs ($F_{1,8} = 28.40$, $p<0.0001$)(Fig. 5), and the influence of CZD and its interaction with lens design also reached statistical significant ($F_{3,8} = 2.98$, $p=0.04$ and $F_{3,8} = 2.80$, $p=0.05$, respectively). Here, as in eyes wearing 2-zone lenses for the entire treatment period (see section 1b above), those with greater on-axis myopia for which lenses were switched tended to show greater relative peripheral hyperopia; however this association did not reach statistical significance ($F_{1,8} = 2.53$, $p=0.12$).

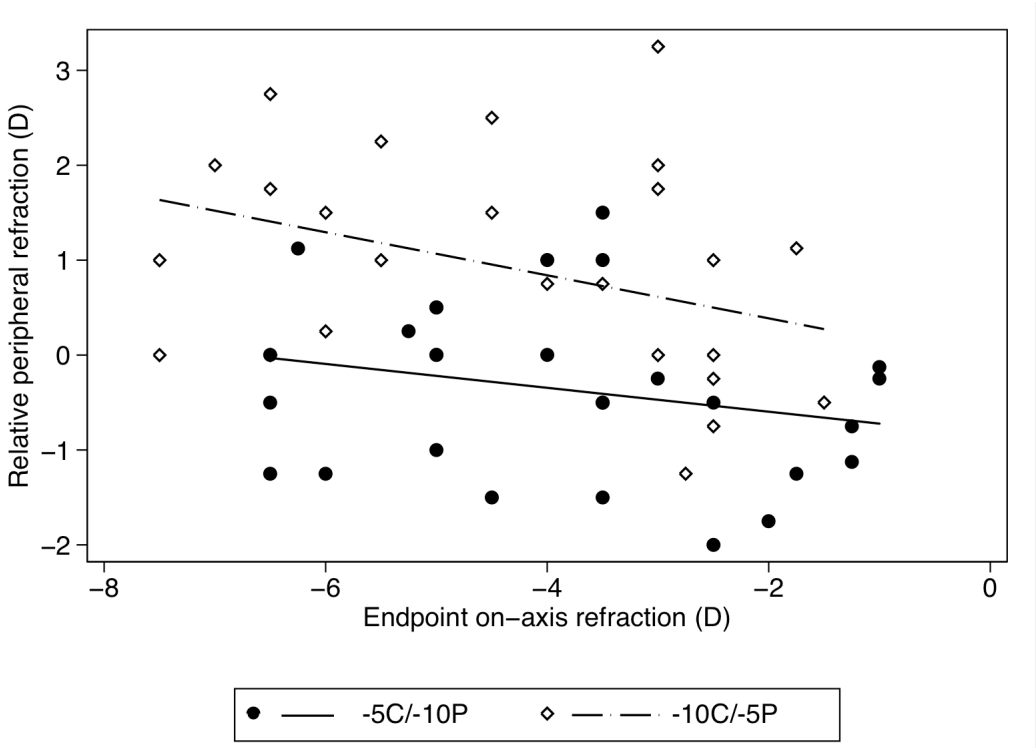


Figure 5-5. Scatter plots of relative peripheral refractive errors (RPR) as a function of on-axis refractive errors for eyes wearing a 2-zone lens for 5 days after pretreatment with a -10SV lens for 5 days (solid circle: -5C/-10P, open diamond: -10C/-5P) (bottom panel). The mean RPR induced by -10SV lens worn for 10 days was 1.58±0.44 D.

Table 5-5. Changes in central (top row) and peripheral spherical equivalent refractive errors (bottom row) of treated eyes, recorded 5 and 10 days after a -10SV lens worn for 5 days was switched to a 2-zone lens (mean±SD, D), organized by lens type and CZD.

CZD (mm)	Lens Type			
	-5C/-10P		-10C/-5P	
	-10SV pretreatment 2-zone, 5 d	-10SV pretreatment 2-zone, 10 d	-10SV pretreatment 2-zone, 5 d	-10SV pretreatment 2-zone, 10 d
4.5	-8.56±0.45 0.84±0.13	-8.86±0.70 0.89±0.17	-4.36±0.79 0.72±0.10	-4.73±0.89 0.75±0.10
5.5	-4.63±1.10 0.35±0.39	-5.13±1.41 0.39±0.30	-4.79±1.08 0.27±0.29	-5.02±0.44 0.33±0.21
6.5	-4.98±1.61 0.56±0.38	-4.72±2.54 0.55±0.44	-6.98±1.24 0.72±0.27	-6.75±1.57 0.74±0.30
7.5	-5.80±1.86 0.86±0.12	-6.45±1.24 0.93±0.10	-8.21±0.53 0.92±0.19	-8.84±1.01 0.95±0.24

5.3.2.3 Effects of 2-zone lenses design on central (on-axis) axial ocular dimensions

The changes in VCD underlying the refractive changes described above, are summarized in Table 5-6, and shown graphically in Figure 5-4 (bottom panel). The early regression in myopia seen after -10SV lenses were replaced with 2-zone lenses can be attributed to reductions in VCD (-0.14±0.26 mm;), coupled to significant choroidal thickening (0.14±0.14 mm, p=0.0001). These changes were evident at the first, day 5 measurement time point after the lenses were switched, and both changes in choroidal thickness and VCD correlated well with the reduction in induced myopia (R^2=0.59, p<0.0001). After a further 5 days (day 10 of 2-zone lens treatment), the choroids had returned to baseline thickness values (p>0.54 for all). VCDs also elongated, rather than shrinking, over the latter period, although the changes were less than the changes in the untreated fellow eyes. As with the changes in refractive error during the 2-zone lens treatment period, changes in VCD were influenced significantly by the VCD recorded at the beginning of this period, i.e. the end of the -10SV lens treatment period ($F_{1,8}$ = 7.0, p=0.01), CZD ($F_{3,8}$ = 8.70, p=0.0001), with significant interaction between lens type and CZD ($F_{3.8}$ = 3.38, p=0.03). The effect of lens design alone was not significant ($F_{1,8}$ = 0.54, p=0.5). There is a further parallel with the refractive error data in that for the 5.5 mm CZD, -10C/-5P lens, the change in VCD was significantly less than that induced by the same 2-zone lens worn for the entire treatment period (p=0.01). Data collected with the 5.5 mm CZD, -5C/-10P lens show a similar trend although the difference is of borderline significance (p=0.045).

Table 5-6. Changes in VCD of treated eyes (mean±SD, D), organized by lens type and CZD. Eyes wore either a 2-zone lens for the entire treatment period (2-zone alone) or as a replacement for a -10SV lens for the same period. VCD change after an additional 5 days of 2-zone lens treatment is shown in the parenthesis. Mean VCD growth in untreated fellow eyes from days 10 to 15 is 0.21±0.14mm.

CZD (mm)	Lens Type			
	-5C/-10P		-10C/-5P	
	2-zone alone	-10SV pretreatment 2-zone, 5 d (2-zone,10d-5d)	2-zone alone	-10SV pretreatment 2-zone, 5 d (2-zone,10d-5d)
4.5	0.77±0.17	0.84±0.13 (0.15±0.17)	0.73±0.23	0.72±0.10 (0.13±0.10)
5.5	0.69±0.29	0.35±0.39* (0.19±0.30)	0.75±0.21	0.27±0.29* (0.16±0.21)
6.5	0.55±0.21	0.56±0.38 (0.16±0.44)	0.82±0.10	0.72±0.27 (0.12±0.30)
7.5	0.76±0.08	0.86±0.12 (0.17±0.10)	0.86±0.16	0.92±0.19 (0.13±0.24)

5.4 Discussion

The study reported here was an extension of the recently published study described in Chapter 3, in which 2-zone lenses combining zones of either positive or negative power with a plano power zone were used to differentially titrate the optical defocus experience of central and peripheral retinal regions.[78] The current study made use of two different experimental paradigms and 2-zone lenses that incorporated negative powers, differing in magnitude (-5 or -10 D), in central and peripheral zones. Thus when the lenses were fitted to previously untreated eyes, the first of the experimental paradigms tested, all retinal regions were exposed to hyperopic (negative) defocus, albeit of different magnitudes for central and peripheral regions. These lens treatments induced less myopia than observed with single vision -10 D lenses, although more than expected for single vision -5 D lenses, with changes in peripheral refractions tending to more directly reflect the imposed defocus. In the second paradigm, the same lenses were substituted for single vision -10 D lenses, which had been left in place for 5 days, inducing myopia of a similar magnitude; the net effect on retinal defocus with the 2-zone lenses in place was imposed myopic defocus of approximately -5 D in the retinal regions onto which the zones of lower negative power projected, and elsewhere, approximate correction of the induced myopia. Curiously, this substitution results in regression of induced myopia to levels generally less than seen with the 2-zone lenses worn for the entire period, suggesting that different processes are at play in determining endpoint refractive errors. In the following discussion, we examine our results further and speculate on their significance for the clinical management of myopia.

In eyes exposed only to the 2-zone negative lenses, both lens designs induced myopia and VCD elongation, although the changes were smaller than seen with -10 SV lenses. While the central (on-axis) endpoint refractions after 10 days of lens wear tended to lie between results expected for each of two powers presented on their own, these data also show a "dose effect", in that the amount of induced myopia was positively associated with the area of the -10 D zone. Interestingly, this dose-response effect was not evident at the earlier, day 5 measurement time point, when the magnitude of induced myopia was similar, around -4.3 D, for most of the lenses. This early pattern is consistent with eyes responding in an all-or-none way to the imposed hyperopic defocus, irrespective of the area-average defocus, consistent with results from an earlier study.[81] At this time, the amount of induced myopia approximately compensated for the lower of the two powers incorporated in the 2-zone lenses and thereafter, the hyperopic experience of eyes would have been regionally biased and lens design-dependent, limited to more central or peripheral retinal regions for the -10C/-5P and -5C/-10P lenses respectively. Thus for these regions, the stimulus for further compensatory ocular growth remained. In contrast, in the regions exposed to the lower amount of hyperopic defocus, further enhanced growth would have resulted in overcompensation, i.e. relative myopia, which in turn is expected to generate a competing inhibitory growth signal. The endpoint refractions recorded at day 10 are consistent with competing myopic and hyperopic signals, requiring only that eye movements beneath the lenses be sufficient to expose retinal regions to both signals. This model also explains why the -5C/-10P lenses with the smallest central zones and the 7.5 mm CZD -10C/-5P lens induced more myopia, although it does not explain why significantly greater myopia was also observed at day 5 with the latter lens, comparing to that observed in the rest of the lens/CZD combinations.

Consistent with the findings of our earlier 2-zone lens study,[78] we observed in eyes exposed only to 2-zone lenses, an overall weak but statistically significant negative correlation between central (on-axis) and peripheral refraction changes, in which increasing central myopia was coupled to increasing relative peripheral hyperopia. We interpreted this observation as evidence of an underlying ocular shape regulator. However the change of RPR cannot be considered as merely a byproduct of altered on-axis growth, as there were significant lens-design dependent differences in observed peripheral refraction patterns (Figure 5-2). For example, for the -5C/-10P groups, RPRs were mostly myopic, even for eyes recording the highest amounts of central myopia, suggesting active local compensation to stronger hyperopic defocus imposed on the peripheral retina. The converse is true for -10C/-5P lens groups; here RPRs were mostly hyperopic, even in eyes recording modest amounts of central myopia. These lens-design dependent differences in RPR patterns strongly argue for active, local regulation of ocular growth by peripheral retina, as suggested previously by Diether et al, using simpler hemifield lens designs.[52] This result also necessarily implies that the peripheral retina is capable of decoding the sign of optical defocus. While these results are also generally consistent with the hypothesis put forward by Smith et al, that peripheral retina plays a major role in central emmetropization,[16] important scleral structural differences between the eyes of chicks compared to those of mammals and primates may explain the apparently more localized emmetropization responses in chicks.

The second experimental paradigm applied in this study represents an attempt to more closely simulate the clinical situation, in which patients seeking myopia control interventions are typically already myopic. Thus we first made eyes myopic before fitting the 2-zone lenses. In this case, the 2-zone lens treatments simulate the situation created by novel bifocal spectacle corrections for myopia, in which the zone of lower negative power in each design represents the incorporation of a positive addition. Interestingly, all eyes showed significant reductions in myopia as well as VCD after the 2-zone lenses were substituted for the SV lenses, irrespective of the lens design and CZDs. The latter result can be explained if the "add" of the 2-zone lenses generated a net myopic defocus signal, which is predicted to inhibit further ocular expansion, and in the case of the chicks, promote choroidal thickening. Not surprisingly then, the total amount of myopia reduction was significantly affected by the level of induced myopia by -10 SV lens at the beginning of 2-zone lens treatment period.

While relative peripheral hyperopia was a consistent finding in -10SV lens-treated eyes, the RPRs resolved into two distinctive patterns after the replacement 2-zone lenses had been worn for 5 days. Specifically, the relative change in peripheral refraction was much smaller with the -5C/-10P lenses, for which the reduction of myopia was greater centrally than in the periphery, resulting a relative peripheral myopia. Furthermore, while the reduction in central (on-axis) myopia was significantly affected by the amount of induced myopia, the RPRs were not affected by either the baseline or the endpoint central refractive error but were strongly affected by the lens design, lending further support to our earlier conclusion that the peripheral retina of the chicken eye is capable of decoding and responding to defocus signals, independent of the central retina.[78]

It is noteworthy that when -10 D SV lenses were replaced by 2-zone lenses, the endpoint refractions at the end of the 2-zone lens treatment period were not only reduced relative to the myopic refractions induced by -10 D SV lens, but were also less myopic than those recorded in eyes exposed only to these 2-zone lenses for 10 days. These differences reached statistical significance for the 5.5 CZD and both lens designs, as well as the 4.5 CZD, -10C/-5P lens. For the former eyes (2-zone lenses only), the mean endpoint refractions for both lens designs and 2 middle-sized CZDs were close to -5 D, i.e., approximately matching the lower of the two negative powers incorporated into these lenses. That the mean endpoint refractions were lower for the 5.5 CZD, -5C/-10P lens as well as -10C/-5P lenses with CZDs smaller than 6.5, is equivalent to undercompensation relative to the lower of the 2 negative powers. A similar bias towards relative hyperopia was described in our earlier study in which the refractive error changes induced by our +5/plano 2-zone lenses were more hyperopic than those induced by a +5 D SV lens.[78] The surprising, albeit consistent, strong inhibitory growth response implied by these various results are presumed to reflect complex interactions between growth modulatory signals generated by central and peripheral retinal regions although we cannot easily isolate effects of 1) intermittent exposure to relative or absolute myopic defocus and 2) interactions between the imposed multifocal optical environment and the higher order aberrations (HOAs) of the eye. First, although we made every effort to keep the 2-zone lenses well centered, eye movements behind the lenses would have expanded the retinal areas exposed to "myopic" defocus. In earlier studies encompassing many different defocus paradigms in chicks, it has been shown that the effects of myopic defocus are

very enduring.[61, 81] Second, interactions between imposed and naturally occurring HOAs may alter the target plane of best focus for emmetropization, as suggested in clinical studies involving concentric bifocal contact lenses as well as orthokeratology lenses. However this mechanism is unlikely to play a dominant role in the effects induced by the 2-zone negative lenses, since reductions in myopic endpoint refractions were found with both lens designs, which are expected to have opposite effects on spherical aberration. Nonetheless, to further explore these interactions, we have undertaken further studies in which variability in pupil size and accommodation have been controlled through selective lesioning.

Our findings of significant changes on refractive errors with 2-zone negative lenses, both centrally (on-axis) and peripherally, attributable to ocular growth changes in the case of on-axis changes, as well as complex interactions between imposed patterns of defocus and background refractions, confirm the critical contributions of both peripheral optics and peripheral retina in guiding emmetropization. They also lend weight to recent clinical observations suggesting that appropriately designed concentric multifocal contact lenses can control myopia progression and argue for further clinical studies of these lenses.

Chapter 6

Dissertation Summary and Discussion

6.1 Major Findings of the Dissertation

Despite strong and consistent evidence from animal studies suggesting the dominant influence of optical defocus in the development of refraction errors, the available evidence from the best randomized clinical trials of multifocal (bifocal BF, or progressive addition, PAL) spectacles for myopia control in children is inconclusive, with the magnitudes of reported effects of such interventions being small and clinically insignificant while consistent.

In the three studies described in this dissertation, the chicken model was used to test the effects on ocular growth of a series of custom-designed 2-zone multifocal "spectacle" lenses, using standardized experimental paradigms combined with objective methods of measurement. The lenses were tested on the normal eyes of young chickens. Additional testing involved eyes that have undergone surgical manipulations (sectioning of the ciliary nerve, CNX or iridectomy, ID) to investigate the interaction between the dynamic optical system of the eye and the imposed, complex optical environment. The main findings are summarized below.

The first study (Chapter 3) used four different concentric 2-zone lens designs, with either -5 or +5 D in one optical zone combined with plano power in the other optical zone, and the powered zone located either in the center (C-designs, i.e., central defocus) or periphery (P-designs, i.e., peripheral defocus) of the lenses. Single vision (SV) lenses were included as control treatments. Overall, the P-designs had greater effects than the C-designs on both on-axis eye growth and refractions. Importantly, all but the +5P lens with the largest CZD induced larger changes than the control +5SV lens. In contrast, the +5C lenses with CZD less than 5.5 mm had little effect. The 2-zone -5 D lenses had less effect than the -5SV lens, and only the -5C lens with largest CZD significantly influenced refractive development. The relative peripheral refractions (RPR), used as a surrogate for ocular shape changes, were negatively associated with on-axis myopia and were highly dependent on lens design.

In a separate study (Chapter 4), a subset of the above lenses were tested on chicken eyes that had first undergone either CNX or ID; both surgeries produced enlarged pupils, with near normal accommodation remaining in ID eyes but completely inhibited in CNX eyes. The effects of these surgeries on the responses to the 2-zone lenses were design-dependent. Both surgeries had little effect on the responses to 3.5 CZD 2-zone lenses; however the responses to the 6.5 CZD lenses were differentially and significantly affected by the surgeries. Specifically, with 2-zone +5D lens of 6.5 CZD, the CNX group showed a reduced response (less hyperopia) compared to those of the normal and ID groups; with 6.5 CZD -5P lens, both CNX and ID showed increased responses (more myopia) compared with that of the normal group, with the response of the CNX group

being significantly greater than that of the ID group. However, neither surgery significantly altered the RPR profiles induced by the 2-zone lenses.

The third study (Chapter 5) described a clinically more relevant scenario in which a different set of 2-zone lenses incorporating two different negative powers (-5 & -10 D) in two optical zones were tested in both normal eyes as well as eyes made myopic by exposure to -10SV lenses before being exposed to a 2-zone lens treatment. In normal eyes, the magnitude of induced myopia fell between that expected in response to each of the two negative powers alone; for the -10 SV, pretreated eyes, the 2-zone negative lenses elicited anti-myopia effects, with already induced myopia undergoing regression and with larger effects in eyes with higher baseline myopia. RPR changes were similar to that observed in previous studies.

6.2 Implications

Human clinical trials of spectacle appliances for myopia control have yielded disappointingly small effects. Those included in the meta-analysis described in Chapter 2 were randomized clinical trials, which are designed to safeguard against biases. However, due to the nature of the interventions utilized, e.g., bifocal or progressive spectacle lenses compared to single vision spectacle lenses, it is impossible to use double-masked designs to eliminate performance bias. A number of reasons likely contribute to the discrepancy between the results of related animal model studies and human clinical trials - strong support for optical control of myopia from one but inconclusive results from the other. First, compliance to lens treatment can be rigorously monitored in animal studies, while adherence to the lens-wearing protocols is difficult to monitor accurately and likely to be poor among children, due to optical distortions induced by BF lenses and PALs, especially in the peripheral visual fields. Most studies also rely on self-reported compliance to lens-wear, which has been suggested to be highly inaccurate. Second, accumulating data suggest that human myopia is likely to have multiple etiologies, all involving complex genetic and environmental interactions. Thus human myopia may comprise various subtypes with different susceptibilities and risk factors, contrasting with animal models of induced myopia, which make use of a small number of well-described treatment paradigms with both genetic and environmental factors well controlled. As a result, assigning standardized treatments to different subtypes of myopia and disregarding potential differences in the underlying etiology may mask a true treatment effect in one or several subgroups of myopia. Consequently, without effective methods of improving and measuring compliance with spectacle lens wear and more precise classifications of the subgroups of myopia, an accurate estimation of the effectiveness of any type of spectacle optical intervention remains gloomy.

A much brighter picture is painted by our studies using the chicken model. Specifically, our findings of significant changes in refractive errors and ocular growth with 2-zone lenses, both centrally (on-axis) and peripherally, attributable to ocular growth changes in the case of on-axis changes, as well as of complex interactions between imposed patterns of optical defocus and background refractions, confirmed the critical contribution of the peripheral retina in guiding emmetropization. There is also potential for important

interactions between the eyes' own optical aberrations and those of attached optical devices, with effects on retinal image quality and net imposed defocus. The strong and consistent inhibitory effects on ocular growth and thus of myopic development seen with 2-zone lenses designed to include either one plano optical zone or 2 zones of differing negative powers, are consistent with accumulating reports of effective control of myopia progression with some concentric multifocal contact lens designs. Contact lenses, being in contact with the cornea, are likely to afford better control over the imposed optical conditions and as a consequence may offer better control over myopia progression than novel spectacle lens designs, which introduce both optical centration and eye movement-related confounders. Fortunately, with the recent, rapid advances in contact lens materials and designs, both the comfort and optical quality of contact lenses have improved dramatically and the risks of lens-induced corneal hypoxia, inflammation and infection can be reduced to a minimum with proper lens care procedures. Another contact lens option showing early promise as a myopia control treatment is orthokeratology, which limits lens-wear to nighttime. Animal studies, such as described here, can provide directions for refinement of the designs of such contact lenses as replacements for spectacle interventions as the primary treatment in clinical studies of myopia control. Compared to MF spectacle lens trials, compliance in CL trials is expected to be significantly better and easier to monitor, and the dropout rate is expected to be lower because of reduced optical distortions. The efficiency of trials is likely to be better, and so results to be more reliable. Large-scale clinical trials of such contact lens devices as myopia control treatments are warranted and overdue.

6.3 Future Research Directions

6.3.1 Animal-based research

1. Multifocal contact lens in chicken and guinea pig models

Our current studies using 2-zone spectacle lenses are subject to potentially important confounders resulting from eye movements behind the lenses. As contact lenses minimize such confounders, contact lenses of similar designs need to be tested to obtain better control over the imposed optical conditions. Additionally, multifocal lens studies will be expanded to mammalian models to improve the translational value of these highly promising findings.

2. Combine off-axis ocular measurements with refraction measurements.

In this dissertation, we used RPR as a surrogate for ocular shape, with relative peripheral hyperopia assumed to be coupled with a prolate ocular shape and relative peripheral myopic, equated with an oblate shape. However, inherent in this approach are assumptions about the peripheral optics of cornea and lens, which are difficult to validate. The construction of a partial coherence interferometry device for precise off-axis ocular dimensional measurements in our animal models will allow the validation of RPR as a surrogate for peripheral ocular shape and further investigations into the optical characteristics of peripheral ocular components.

3. Combine optical interventions with pharmaceutical treatments
Given our promising findings in chicks using multifocal lenses as potential myopia control treatments and the widely acknowledged myopia-retarding effect of topical atropine in humans, it is logical to design contact lenses that serve both as an optical device and a depot for sustained release of this or other anti-myopia agents. Of course, lenses of such designs would need to be first tested in animal models.

6.3.2 Human-based research

As noted above, long-term clinical trials of commercially available concentric multifocal soft contact lenses and orthokeratology lenses should be undertaken to confirm the positive findings from animal work. Among such contact lenses are ones that impose similar defocus profiles to those induced by the 2-zone lenses described in this dissertation. Additionally, our study results can be used to produce custom-designed lenses for testing in clinical studies to better understand the potential interactions of pupil size and other optical properties of the human eye, and so to refine the optical design of such lenses to maximize the efficacy of myopia control contact lens treatments.

Supplement I

The Role of Iris in Chick Accommodation

Abstract

Purpose: Peripheral defocus, higher order aberrations and accommodation interact with pupil size to influence retinal image quality and possibly eye growth. Iridectomy (ID) provides a fixed, enlarged pupil. Results from in vitro studies suggest that ID may reduce or eliminate accommodation in the chicken. Here we further investigated the effects of ID on chicken accommodation, eye growth and refractive development.
Methods: Refraction, biometry and corneal curvature were measured before and after topical instillation of nicotine in forty-three White-Leghorn chickens that had undergone monocular ID. Intraocular pressure (IOP) was measured and eyes were imaged with anterior segment OCT during accommodation.
Results: Iridectomy induced small but significant decreases in anterior and vitreous chamber depths and increase in lens thickness. IOP was similar in ID and control eyes from one week on. In vivo, nicotine induced similar accommodative changes in ID and control eyes. OCT images revealed a forward displacement of the iris during accommodation in control eyes. ID and control eyes showed similar increases in lens thickness.
Conclusions: Refraction and eye growth were minimally affected by ID in chickens, implying that emmetropization was unaffected, and supporting the use of ID as a tool in emmetropization and myopia studies. IOP was unaffected by the surgery, implying that the iris musculature is not essential to maintaining aqueous outflow pathways.

I.1 Introduction

Interest in manipulating optical aberrations and peripheral refractive errors as possible treatments for controlling myopia is driving on-going research involving specially designed lenses in animals models for myopia. The current study used the chick, the most commonly used model, to investigate the ocular effects of iridectomy, which has been used in such studies as a way of controlling and so avoiding the confounding effects of pupil size, which in turn is known to influence the magnitudes of higher order aberrations, peripheral refractive errors and thus retinal image quality[83-85] and ocular growth. Iridectomy provides a fixed, dilated pupil. However, its effects on ocular accommodation and intraocular pressure (IOP) in the chick remain unresolved yet changes in either or both of these parameters would introduce other confounding factors for such lens studies.

The role of accommodation in the development of myopia and emmetropization has not been fully resolved. While the apparent association between excessive near work and myopia opened the possibility that excessive accommodation might be responsible, it has not proven to be a factor in form deprivation myopia induced with translucent goggles in chicks, based on histological studies of ciliary body morphology,[86] and another study involving ciliary nerve section to eliminate accommodation.[80, 87] Results from studies using negative lenses to induce myopia lead to a similar conclusion; neither Edinger-Westphal lesioning[88] and ciliary nerve section,[80] both methods of eliminating accommodation, prevent these lens-induced eye growth changes. However, in a binocular lens experiment in which one eye also underwent ciliary nerve section, eyes showed similar rates of defocus induced eye growth, even though one eye was likely to have been accommodating to clear the imposed defocus. This observation and another in which ciliary nerve section was found to alter the response to competing defocus stimuli,[81] leave open the question of whether accommodation plays a role in emmetropization.

The possibility that raised intraocular pressure (IOP) may underlie the increased ocular enlargement seen in myopia remains unresolved. In three studies of young children, higher than normal IOP has been reported for myopic eyes. But in one of these studies, the differences were observed only after the children became myopic.[89] Results from the only two studies to address this question in chicks were also inconclusive. In chicks wearing either negative or positive lenses, IOP was observed to initially decrease with both types of lenses, although the faster growing eyes showed a later relative increase in IOP.[90] However, in a related study, topical timolol, an IOP lowering drug, failed to inhibit lens-induced myopic growth in young chicks.[91]

The mechanism of accommodation in the avian eye varies from that of mammals in a number of important ways and there are also differences between avian species. While mammals exhibit only lenticular accommodation mediated by smooth muscle bearing muscarinic receptors, birds have both corneal and lenticular accommodation, mediated by skeletal muscle bearing nicotinic receptors. In the former case, only the lens surfaces undergo steepening; in the latter case, the cornea also steepens, the net effect in both cases being an increase in the refracting power of the eye. Young chicks can accommodate over 25D through lenticular and corneal mechanisms.

Among avian species, the iris musculature plays a variable role in reshaping the lens during accommodation. Evidence for iris-mediated lenticular accommodation in birds was first offered in 1853, when Cramer observed that accommodative lens movements were absent after iridectomy in pigeons. In some diving birds, the anterior lens surface has been observed to protrude through the pupil of the iris, providing convincing evidence for a role of the iris in accommodation. In the chick, Glasser, et al. attributed accommodation-related changes in the curvature of the chick lens to the contraction of peripheral and oblique iris muscle fibers, based on anatomical observations and in vitro measurements of accommodation in response to electrical stimulation.[90, 91] In the latter studies, iridectomy was found to eliminate lenticular accommodation,[91] leading to their conclusion that during accommodation, the contracting muscle fibers at the peripheral edge (root) of the iris applied force through the ciliary processes to the equatorial surface of the lens, thereby deforming it.

In the study reported here, the effects of iridectomy on baseline biometric and refractive parameters, nicotine-induced accommodation, and IOP were evaluated in vivo.

I.2 Methods

This study into the ocular effects of iridectomy used forty-three White Leghorn chickens, which were housed in a 12 hour light/dark cycle with food and water available ad libitum. Details of the number and age of birds used in each experiment within this study are summarized in Table I-1. In brief, all chickens underwent monocular iridectomies. The effects of this surgery were subsequently studied in terms of refractive development, ocular growth, accommodation and intraocular pressure (IOP). Study protocols were approved by the UC Berkeley Animal Care and Use Committee and conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.
Table I-1: Number and age of birds at time of measurements used in each experiment within this study. Monocular iridectomies were performed at least 5 days before measurements.

Table I-1. Age and sample size distribution of chickens in each group

Group	Experiment	Sample Size	Age (days)
I	*In vivo* accommodation	4 15 5 5 7	16 22 33 45 54
II	*In vivo* anterior segment imaging	5	19-22
	In vivo IOP measurements	7	14-42

Unilateral iridectomies (ID) were performed under gaseous anesthesia (2% isofluorane in oxygen) on chicks aged between 8-32 days. A 1.5mm temporal limbal incision was made, and the iris grasped at its base with microfine, flat tipped forceps through the incision and180° from the incision; it was then gently detached from its root in one motion, taking care not to contact the anterior lens surface, and drawn out through the

incision. The anterior chamber was then irrigated with sterile PBS to remove any tissue debris and blood. Immediately following surgery and for the next four days, dexamethasone and vigamox were applied topically. The contralateral fellow eyes were not treated in any way and served as controls in all experiments.

In vivo measurements, comprised of streak retinoscopy, high frequency A-scan ultrasongraphy and corneal topography, were carried out five days after surgery. Retinoscopy and A-scan ultrasound were performed with the chicks on a custom made stand with beak bar. Gaseous anesthesia was delivered during ultrasound measurements. For corneal topography measurements, the birds were held in front of the instrument for alignment. The youngest group of four 17 day old birds did not undergo keratometry measurements. Both the iridectomized and fellow control eyes of each bird were measured before and at timed intervals after the topical instillation of two drops of 0.4% nicotine solution. The latter procedure reflected the composition of the intraocular muscles of the avian eye - skeletal rather than smooth muscle with nicotinic rather than muscarinic receptors.14 Baseline values provided indices of the effects of the ID surgery alone and the nicotine-induced changes provided indices of accommodation-related changes.

A TonoLab (iCare, Finland Oy) was used to measure IOP in both eyes of individual chickens, one day prior to the surgery, as well as 1 hour, 24 hours, 4 days post-operatively, and then weekly for four weeks. The TonoLab measures the pressure 6 times and gives an average of the most similar 4 readings. Each data point represents the average of 2 such measurements per eye. A topical anesthetic is not necessary for TonoLab IOP measurements. The chickens were gently restrained but not otherwise anesthetized to avoid the IOP lowering effects of general anesthesia.

In vivo imaging of the anterior ocular segments of some of the birds was undertaken using a non-contact OCT system (Visante, Zeiss) one week after their ID surgery. For imaging, birds were anesthetized with a combination of 15 mg/kg ketamine and 3 mg/kg xylazine, administered as an intramuscular injection. The birds were placed on a custom-built stand with beak holder that facilitated the alignment of each eye in turn with the instrument. Each eye was imaged both before and at one minute intervals over about five minutes after the topical instillation of two drops of 0.4% nicotine solution; the ID eye was measured first and then the bird was repositioned for measurement of the fellow eye. Custom software and calipers built into the instrument were used to measure anterior chamber depth and axial length thickness from captured images, and nicotine-induced changes were subsequently derived.

I.3 Results

I.3.1 Effects of iridectomy on refractive development, ocular growth & IOP

Ocular measurements for the iridectomized (ID) eyes for all ages were averaged and compared to control eyes (CE) using the 2-tailed paired t-test. ID eyes had only slightly shorter albeit statistically significant anterior chambers (-0.09 mm, $p<0.001$) and vitreous chambers (-0.12 mm, $p<0.001$), and thicker lenses (+0.06 mm, $p<0.001$) compared to

contralateral control (fellow) eyes (Table I-2). However, these interocular differences in axial dimensions were not reflected in significant differences in refractive error (-0.10±0.98D, *p*=0.57). Interocular differences in corneal power also did not reach statistical significance at any age (+0.31±1.25D, *p*=0.16; Figure I-2).

Table I-2. Baseline and accommodated biometric and refractive measurements, measured 5-7 days post-operatively with high frequency A-scan ultrasonography, corneal topography and retinoscopy respectively.

Parameter	Eye	Baseline (no nicotine)		After topical nicotine	
			Δ (ID-C)		Δ (ID-C)
Anterior chamber depth (mm)	ID	1.63 ± 0.30	-0.09*	1.50 ± 0.33	-0.03
	CE	1.72 ± 0.27	(p<0.001)	1.53 ± 0.29	(p<0.05)
Lens thickness (mm)	ID	2.62 ± 0.26	+0.06*	2.85 ± 0.23	+0.02
	CE	2.56 ± 0.26	(p<0.001)	2.83 ± 0.26	(p=0.09)
Vitreous chamber depth (mm)	ID	5.81 ±0.69	-0.12*	5.75 ± 0.70	-0.13*
	CE	5.93 ± 0.62	(p<0.001)	5.88 ± 0.60	(p<0.001)
Retinal thickness (mm)	ID	0.24 ± 0.02	0	0.24 ± 0.02	-0.01*
	CE	0.24 ± 0.01	(p=0.53)	0.25 ± 0.02	(p<0.001)
Choroidal thickness (mm)	ID	0.32 ± 0.17	0	0.31 ± 0.16	+0.03*
	CE	0.32 ± 0.16	(p=0.71)	0.28 ± 0.16	(p<0.01)
Corneal power (D)	ID	93.64 ± 11.33	+0.31	100.10 ± 15.53	+0.09
	CE	93.33 ± 11.38	(p=0.16)	100.01 ± 14.67	(p=0.84)
Refractive error (D)	ID	+1.77 ± 0.77	-0.10	-15.22 ± 8.40	-0.09
	CE	+1.87 ± 0.64	(p=0.57)	-15.13 ± 7.33	(p=0.78)

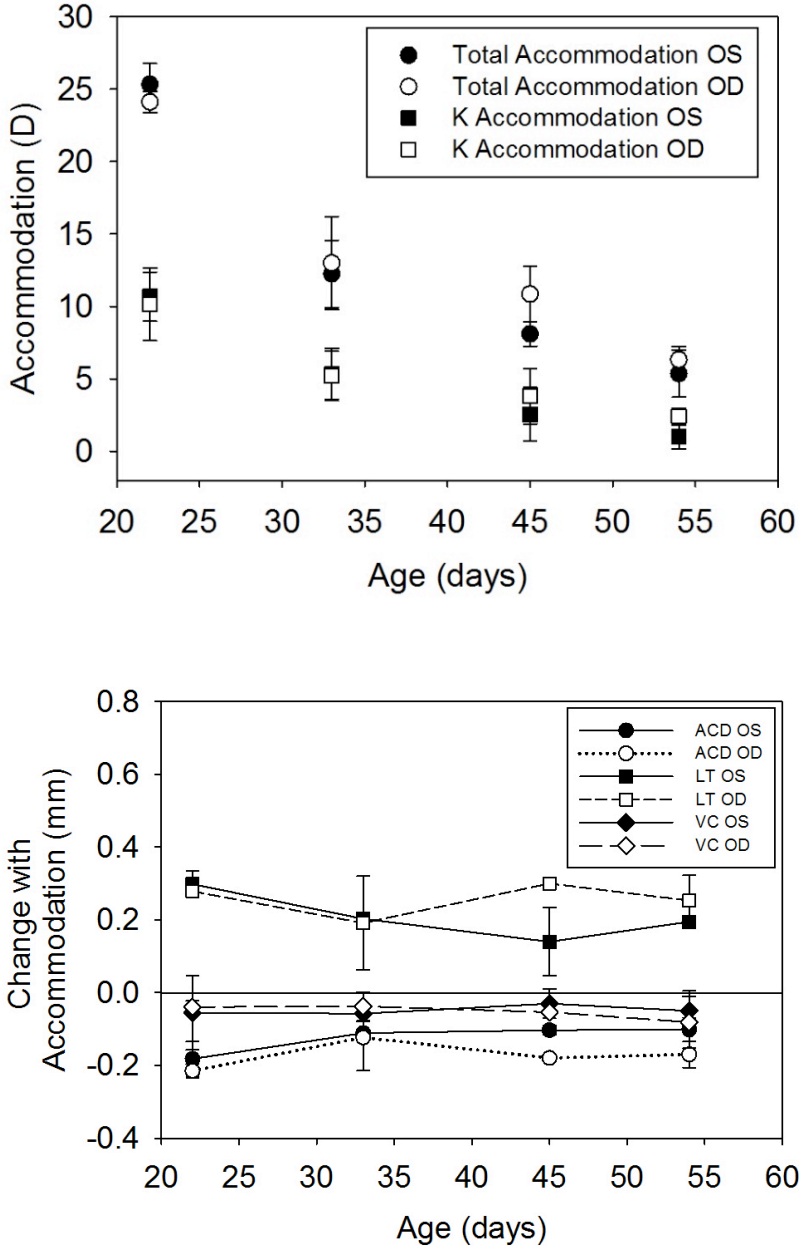


Figure I-1: A) Total accommodation and corneal accommodation were similar for iridectomized and control eyes, both showing an age related decrease. B) For all ages, there was a decrease in anterior chamber depth (ACD), and vitreous change depth (VC) and an increase in lens thickness (LT) in both eyes. These changes also showed age related decreases in magnitude.

For the seven birds in which IOP was measured following monocular iridectomies, there was no significant difference in IOP measured four weeks post-operatively between the ID and control eyes (Figure I-2). ID eyes exhibited an acute lowering in IOP, which returned to normal within the week following the surgery. Mean IOPs for ID eyes were 19.9±2.70 mmHg prior to surgery. At one and 24 hours after the surgery, IOPs for the ID

eyes were 15.1±2.6 and 16.1±2.62 mmHg respectively. The equivalent values for control eyes were 19.9±3.62 prior to surgery (p=093 compared to ID eyes) and 20.4±1.4 (p<0.0001) and 24.4±2.0 (p<0.0001) mmHg at one and 24 hours. From one week and further after the surgery, the IOPs of ID and control eyes were not significantly different.

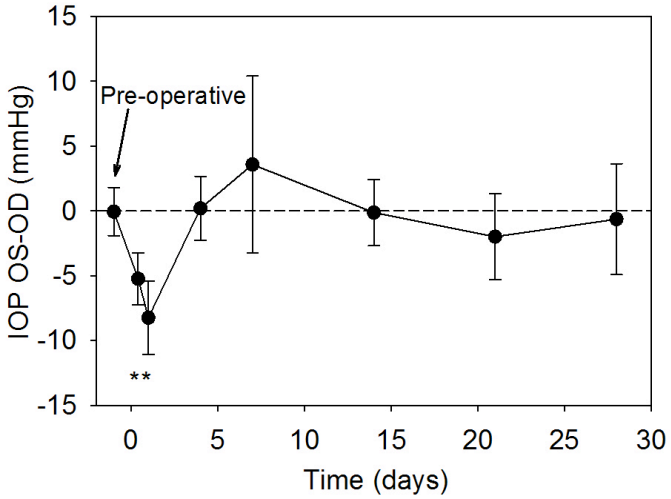


Figure I-2: Intraocular pressure (IOP) was measured in seven birds before monocular iridectomy, at one and 24 hours post-operatively, and 1 week intervals for four weeks. IOP was significantly decreased in the ID eye at the one and 24 hour measurement periods.

I.3.2 Nicotine-induced changes recorded in vivo

Topical nicotine induced accommodative changes in both ID and control eyes, with changes reaching a peak around 2min after instillation and mostly of similar dimensions in the two eyes. Thus both eyes showed significant reductions in anterior and vitreous chamber depths (ACD, VCD), and increases in lens thickness (LT, Figure I-1B). Peak values for ACD and LT for the two eyes were not significantly different (Table I-2). However, ID eyes recorded a significantly shorter accommodated VCD than control eyes (-0.13 mm, p<0.001). Control eyes showed a slight increase in retinal thickness (RT) and decrease in choroidal thickness (CT) with accommodation, while only a change in the latter was evident in ID eyes. While these changes were small, they introduced significant interocular differences at peak accommodation for both RT (-0.01 mm, p<0.001) and CT (+0.03 mm, p<0.01).

Nicotine-induced corneal steepening and myopic shifts in refractive error were observed in both ID and control eyes at all ages, and while these changes for the two eyes were not significantly different (Table I-2, p=0.65 and p=0.64, respectively), the magnitude of the changes decreased with age (Table I-3). On average, corneal accommodation accounted for 37.4±6.4% of the total accommodation-related change in refraction, although its contribution decreased with age in ID eyes ($F_{3,28}$=8.54, p=0.0003).

Table I-3: Total and corneal accommodation induced by topical nicotine instilled in both iridectomized (ID) and fellow control eyes (CE) of chickens aged between 22 and 54 days; accommodation decreased with age, as did the corneal contribution for ID eyes.

Age (days)	Eye	Total Accommodation (D)	Corneal Accommodation (D)	Corneal Contribution (%)
22	ID	25.3±1.4	10.7±1.7	42±7
	CE	>24	10.1±2.5	< 42
33	ID	12.3±2.3	5.4±1.8	43±9
	CE	13.0±3.2	5.2±1.7	41±12
45	ID	8.1±0.8	2.6±1.9	31±23
	CE	10.9±1.9	3.8±1.9	33±13
54	ID	5.6±2.6	1.4±1.0	16±12
	CE	6.7±2.5	2.6±0.8	38±7

Similar to the trends evident in the A-scan ultrasonography data, for OCT anterior segment imaging ID eyes recorded significantly shorter baseline ACDs compared to control eyes, by 0.10±0.06 mm ($p<0.05$) and thicker lenses, by 0.022±0.04 mm, although the latter interocular difference did not reach statistical significance ($p=0.33$, Figure I-3). Likewise, the instillation of nicotine resulted in a reduction in ACD and increase in LT in all eyes (Table I-4). The magnitude of LT change was significantly smaller in ID eyes by 0.11mm ($p<0.005$).

Table I-4: Nicotine-induced changes in anterior chamber depth (ACD) and lens thickness (LT) of iridectomized (ID) and control (CE) eyes, measured by OCT anterior segment imaging (n=5) and high frequency A-scan ultrasongraphy (US) in chicks age 17 days (n=4).

Eye	Δ ACD (mm)		Δ LT (mm)	
	OCT	US	OCT	US
ID	-0.14±0.08	-0.12±0.06	+0.18±0.03	+0.19±0.06
CE	-0.19±0.05	-0.22±0.07	+0.29±0.05	+0.27±0.04
ID-CE	+0.05 (p=0.33)	+0.1* (p<0.05)	-0.11* (p<0.005)	-0.08* (p<0.05)

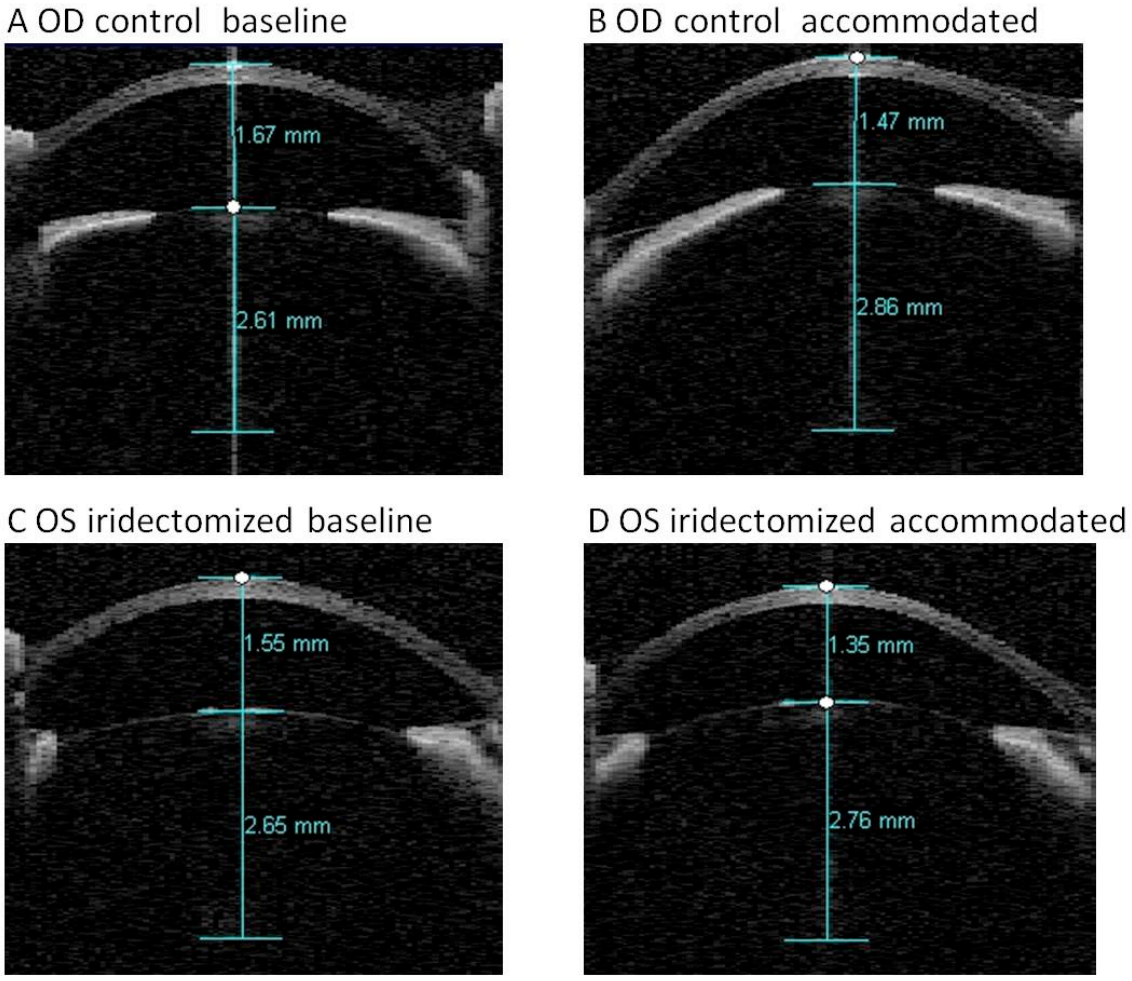


Figure I-3: Anterior segment OCT images captured from the control eye (A, B), and ID eye (C, D) of one chick. Panels A and C show the eyes in their unaccommodated state, while B and D shows accommodating eyes, after the topical instillation of 0.4% nicotine. For both control and ID eyes, ACD decreased and lens thickness increased with accommodation. The iris appears to move forward with the anterior lens surface during accommodation in the control eye (B).

I.4 Discussion

The near normal ocular dimensions and lack of difference in refractive errors of iridectomized eyes imply that emmetropization was not disrupted by surgical iridectomy. The iridectomy -related decrease of anterior chamber depth and increase of lens thickness suggest that the iris exerts a small, albeit significant restraining influence on the unaccommodated lens. The decrease in vitreous chamber depth may reflect expansion of the lens into the vitreous chamber. These iridectomy related changes in axial dimensions in the chicks are similar to those that have been reported with total iridectomy in rhesus monkeys.[92]

In vivo, nicotine stimulated accommodation was similar in iridectomized and contralateral control eyes as measured with streak retinoscopy, A-scan ultrasonography and corneal topography. However, the iridectomized eye required a longer time and more

drops of nicotine in order to reach the peak accommodative response. This is likely due to the fact that the nicotine quickly drains out of the anterior chamber of the iridectomized eye, while in the control eye, the pigment of the iris serves as a depot for sustained release of the drug. Measurements of ACD and LT taken with the Visante showed decreased accommodative change in the iriedectomized eye. For these measurements, only a single drop of 0.4% nicotine was instilled into the iridectomized eye to avoid systemic interactions sometimes seen with ketamine and xylazine anesthsia, and therefore the accommodative change was likely not at its peak of its potential response.
Glasser, et al, attributed lenticular accommodation in the chick and pigeon to contraction of the iris muscle.[90] As we have shown in chicks, in vivo accommodation is not affected by iridectomy, thus arguing against a role for the iris in accommodation. Glasser suggests that the peripheral muscle fibers of the iris squeeze the peripheral margins of the lens to increase central lens curvature.[90] However, OCT imaging of the anterior segment performed here illustrate that the iris appeared to passively move anteriorly with the lens (figure I-3B).

While our results suggest that the iris is not responsible for in vivo lenticular accommodation in chicks, there may be a role for the iris in accommodation in aquatic birds. When the birds are underwater, the corneal accommodation is neutralized. It has been shown that some aquatic birds have enlarged iris musculature, which may support larger lenticular changes during accommodation.[93] Two specific types of diving birds were shown to have large anterior lenticonus during accommodation,[94] as well as in penguins[95] and cormorants.[96] This is attributed to the iris sphincter squeezing the lens and inducing extensive anterior lens surface changes. However, in the chicks measured here, accommodation measured in vivo was not affected by iridectomy, and anterior lenticonus was not observed during anterior segment OCT imaging.

West, et al, attributed lenticular accommodation in the chick to a contraction of the ciliary muscle, forcing the ciliary processes against the anterior surface of the lens.[97] Another report suggested that contraction of the ciliary muscle stretches the choroidal coat and increases the vitreous pressure behind the lens.[98] Based on our results, it is most likely that the former case is occurring, as the vitrious pressure behind the lens is absent in our in vitro preparations.

Early literature is inconsistent on the existence of corneal accommodation in various avian species. Corneal accommodation was not present in pigeons or ducks during nicotine induced accommodation,[94] electrical stimulation in chicks,[99] or in behavioral studies in owls.[100] More recently, Schaeffel and Howland demonstrated the presence of up to 15D of corneal accommodation in freely accommodating chicks and pigeons[101] and Troilo and Wallman showed that 40% of nicotine stimulated accommodation in chicks can be accounted for by changes in corneal curvature.[78] Similar to those reports, we found that corneal steepening contributes significantly to accommodation in chickens. Corneal accommodation accounts for 37.4±6.4% of the total accommodative change in refraction in both our control and iridectomized chick eyes, comparable to the roughly 40% reported by others.[78, 92] The magnitude of the corneal steepening decreased with age for both iridectomized and normal eyes, implying age-related changes in either corneal

stiffness or ciliary muscle contractility in chicken eyes. It has been shown that corneal accommodation in chicks is a direct ciliary muscle-mediated mechanism.[92] Although the ciliary muscle is normally assumed to contribute minimally to presbyopia, the avian ciliary muscle is comprised of striated rather than smooth muscle, and so may be more susceptible to aging.

Our finding of iridectomized chicken eyes having no significant decrease in nicotine stimulated accommodation contrasts with in vivo findings in primate models.[92] Total iridectomy is a commonly used tool in rhesus monkeys for the study of accommodation, allowing the entire lens diameter, zonules and ciliary processes to be viewed.[102] Studies have shown that accommodation induced by carbachol iontophoresis is reduced by 40% in iridectomized eyes compared to contralateral control eyes.[92] However, accommodation induced by Edinger-Westphal stimulation and intramuscular pilocarpine infusion is not decreased. The authors ruled out surgical damage to the accommodative apparatus as a possible explanation for the decrease in carbachol stimulated accommodation. Similar to results found here in chicks, the resting corneal curvatures were not significantly different in iridecomized monkey eyes, while the anterior chamber depth decreased. The investigators suggested that the intact iris exerts some pressure on the lens to account for the deep anterior chamber depth compared to iridectomized eyes. They attributed the decreased carbachol induced accommodative response to an iridal component to primate accommodation during which supramaximal pharmacological stimulation constricts the iris sphincter, pulling the ciliary muscle forward and inducing additional accommodation. The authors did not observe iridogenic anterior lenitconus, and their results also supported the use of iridectomy as a tool to study other ocular functions.

Overall, our results support the use of iridectomy as a tool for enlarging the pupil in emmetropization and myopia studies using the chicken model.

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