

**FACTORS OTHER THAN DEFOCUS THAT  
INFLUENCE EMMETROPIZATION AND EYE  
GROWTH IN CHICKS**

**By**

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**A Dissertation Submitted to the University of Newcastle for the  
Degree of Doctor of Philosophy**

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## Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository\*\*, subject to the provisions of the Copyright Act 1968.

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(Signed) \_\_\_\_\_

Xiaoying Zhu



## Statement of Collaboration

The work embodied in this thesis has been done in collaboration with other researchers. Here, I formally acknowledge the contribution of my collaborators:

**Chapter 1:** Introduction. Part of this chapter, Section 1.5, was adapted from a review titled *Temporal integration of visual signals in lens compensation (a review)*, published in *Experimental Eye Research* (2013). I wrote the manuscript. The late David Saffer gave me comments and proofread the manuscript.

**Chapter 3:** Evidence for a non-visual cue that guides recovery from abnormal eye sizes in the chick eye. I reviewed previous data that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, and performed statistical analyses under the guidance of McFadden. I wrote the manuscript.

**Chapter 4:** The effect of eye size on monocular lens compensation in chicks. This chapter was completed with the assistance of McFadden S. A., Wallman J., Sidhu A., and Cernota N. R. I designed the experiments under the guidance of McFadden and Wallman. I collected the data, assisted by Sidhu A. and Cernota N. R. I analyzed the data under the guidance of McFadden and Wallman and wrote the manuscript.

**Chapter 5:** Chick eyes can shorten to compensate for myopic defocus. A manuscript based on the work presented in Chapter 5 has been published in *Investigative Ophthalmology & Visual Science* (2013), titled *Eyes in various species can shorten to compensate for myopic defocus* by Zhu X., McBrien N. A., Smith E. L., Troilo D., and Wallman J. I designed the experiments and analyses under Wallman's guidance. I reviewed and analyzed the data on chicks that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, and wrote the manuscript.

**Chapter 6:** Interaction between paired eyes: Symmetrical growth, yoking, and anti-yoking. I reviewed previous data that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, collected new data, and performed statistical analyses under the guidance of McFadden. I wrote the manuscript.

**Chapter 7:** The effect of eye size on binocular lens compensation in chicks. I designed the experiments under the guidance of McFadden, collected the data assisted by

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Some of the work in this thesis or arising from the paradigms developed in this thesis has been published in either a paper or abstract form:

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2. Zhu X, McBrien NA, Smith EL, 3rd, Troilo D, Wallman J. Eyes in various species can shorten to compensate for myopic defocus. *Invest Ophthalm Vis Sci* 2013;54:2634-2644.
3. Zhu X. Temporal integration of visual signals in lens compensation (a review). *Exp Eye Res* 2013;114:69-76.
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# Table of Contents

<b>Declaration.....</b>	<b>i</b>
<b>Statement of Collaboration .....</b>	<b>iii</b>
<b>Acknowledgements .....</b>	<b>v</b>
<b>List of Figures.....</b>	<b>xii</b>
<b>List of Tables .....</b>	<b>xv</b>
<b>Abstract.....</b>	<b>1</b>
<b>1. Introduction.....</b>	<b>6</b>
<b>1.1 Myopia and its impact, globally and ocularly.....</b>	<b>7</b>
<b>1.2 Etiology of myopia .....</b>	<b>9</b>
1.2.1 Genetic risk factors for myopia.....	9
1.2.2 Environmental risk factors for myopia .....	10
<b>1.3 Animal research in myopia.....</b>	<b>12</b>
1.3.1 Lens compensation, recovery, and form deprivation .....	12
1.3.2 Animal models in myopia research.....	14
<b>1.4 Possible cues that guide ocular growth.....</b>	<b>15</b>
1.4.1 Accommodation .....	16
1.4.2 The magnitude of blur or sharp vision .....	17
1.4.3 Spatial frequency and image contrast .....	18
1.4.4 Image size .....	19
1.4.5 Chromatic aberration.....	19
1.4.6 Higher-order aberration.....	20
1.4.7 Conclusions .....	21
<b>1.5 Integration of myopic and hyperopic defocus.....</b>	<b>21</b>
<b>1.6 Myopia control.....</b>	<b>22</b>
1.6.1 Undercorrection .....	23
1.6.2 Bifocal and multifocal spectacle lenses .....	24
1.6.3 Soft bifocal contact lenses.....	24
1.6.4 Orthokeratology .....	25
<b>1.7 Non-visual mechanisms regulating eye growth.....</b>	<b>25</b>

1.7.1	Circadian rhythm.....	25
1.7.2	Eye size .....	26
1.7.3	Interactions between paired eyes .....	28
<b>1.8</b>	<b>Aims and hypotheses.....</b>	<b>34</b>
1.8.1	Chapter 3: Evidence for a non-visual cue that guides recovery from abnormal eye sizes in the chick .....	34
1.8.2	Chapter 4: The effect of eye size on monocular lens compensation in chicks .....	35
1.8.3	Chapter 5: Chick eyes can shorten to compensate for myopic defocus.....	35
1.8.4	Chapter 6: Interaction between paired eyes: Symmetrical growth, yoking, and anti-yoking.....	36
1.8.5	Chapter 7: The effect of eye size on binocular lens compensation in chicks .....	36
<b>2</b>	<b>General Methods.....</b>	<b>39</b>
<b>2.1</b>	<b>Animals .....</b>	<b>40</b>
<b>2.2</b>	<b>Spectacle lenses used.....</b>	<b>40</b>
<b>2.3</b>	<b>Measurements of refractive error and ocular dimensions.....</b>	<b>41</b>
<b>2.4</b>	<b>General data analyses .....</b>	<b>44</b>
<b>3.</b>	<b>Evidence for a Non-Visual Cue That Guides Recovery from Abnormal Eye Sizes in the Chick Eye .....</b>	<b>46</b>
<b>3.1</b>	<b>Forward .....</b>	<b>47</b>
<b>3.2</b>	<b>Abstract .....</b>	<b>48</b>
<b>3.3</b>	<b>Introduction.....</b>	<b>49</b>
<b>3.4</b>	<b>Methods .....</b>	<b>51</b>
3.4.1	Animals .....	51
3.4.2	Experimental procedures.....	51
3.4.3	Measurements .....	52
3.4.4	Analyses .....	52
<b>3.5</b>	<b>Results.....</b>	<b>53</b>
3.5.1	Recovery from prior positive lens wear .....	53
3.5.2	Recovery from prior negative lens wear .....	57
<b>3.6</b>	<b>Discussion .....</b>	<b>59</b>
3.6.1	The effect of dark rearing.....	59
3.6.2	Comparison between recovery rates in the light and dark .....	61
3.6.3	Comparison with previous studies .....	61

3.6.4	Possible mechanisms responsible for maintaining organ size or shape.....	62
3.6.5	The effect of starting lens treatment at different ages.....	63
3.6.6	Conclusions.....	63
<b>4.</b>	<b>The Effect of Eye Size on Monocular Lens Compensation in Chicks.....</b>	<b>64</b>
<b>4.1</b>	<b>Forward.....</b>	<b>65</b>
<b>4.2</b>	<b>Abstract.....</b>	<b>66</b>
<b>4.3</b>	<b>Introduction.....</b>	<b>68</b>
<b>4.4</b>	<b>Methods.....</b>	<b>70</b>
4.4.1	Animals.....	70
4.4.2	Experimental procedures.....	70
4.4.3	Analyses.....	75
<b>4.5</b>	<b>Results.....</b>	<b>76</b>
4.5.1	Exp. 4.1: Constant vs. stepped lens powers.....	76
4.5.2	Exp. 4.2: Stepped lens powers vs. recovery.....	86
<b>4.6</b>	<b>Discussion.....</b>	<b>91</b>
4.6.1	Summary of results.....	91
4.6.2	The effects of recovery.....	94
4.6.3	Possible reasons why chick eyes cannot compensate for the strong negative lens after the step-up.....	95
4.6.4	Contradictory results from early studies.....	96
4.6.5	Conclusions.....	97
<b>5.</b>	<b>Chick Eyes Can Shorten to Compensate for Myopic Defocus.....</b>	<b>98</b>
<b>5.1</b>	<b>Forward.....</b>	<b>99</b>
<b>5.2</b>	<b>Abstract.....</b>	<b>100</b>
<b>5.3</b>	<b>Introduction.....</b>	<b>101</b>
<b>5.4</b>	<b>Methods.....</b>	<b>102</b>
5.4.1	Animals.....	102
5.4.2	Experimental procedures and axial biometry measurements.....	102
5.4.3	Analyses.....	103
<b>5.5</b>	<b>Results.....</b>	<b>104</b>
<b>5.6</b>	<b>Discussion.....</b>	<b>107</b>
<b>5.7</b>	<b>Conclusions.....</b>	<b>109</b>

<b>6. Interaction between Paired Eyes: Symmetrical Growth, Yoking, and Anti-Yoking.....</b>	<b>110</b>
<b>6.1 Forward .....</b>	<b>111</b>
<b>6.2 Abstract .....</b>	<b>112</b>
<b>6.3 Introduction.....</b>	<b>114</b>
<b>6.4 Methods .....</b>	<b>115</b>
6.4.1 Animals .....	115
6.4.2 Experimental procedures and axial biometry measurements.....	115
6.4.3 Analyses .....	119
<b>6.5 Results.....</b>	<b>120</b>
6.5.1 Exp. 6.1. Binocular symmetrical size and growth in untreated chicks .....	120
6.5.2 Exp. 6.2. The effect of monocular-lens wear on binocular symmetrical growth, yoking and anti-yoking .....	122
<b>6.6 Discussion .....</b>	<b>128</b>
6.6.1 Possible mechanisms for interactions between paired eyes.....	128
6.6.2 The amount of yoking/anti-yoking depends on lens-wearing duration .....	130
6.6.3 Implications of these results for monocular experimental designs .....	131
6.6.4 Conclusions .....	132
<b>7. The Effect of Eye Size on Binocular Lens Compensation in Chicks.....</b>	<b>133</b>
<b>7.1 Forward .....</b>	<b>134</b>
<b>7.2 Abstract .....</b>	<b>135</b>
<b>7.3 Introduction.....</b>	<b>137</b>
<b>7.4 Methods .....</b>	<b>138</b>
7.4.1 Animals .....	138
7.4.2 Experimental Procedures .....	139
7.4.3 Measurements .....	142
7.4.4 Analyses .....	143
<b>7.5 Results.....</b>	<b>146</b>
7.5.1 Exp. 7.1: Stepped vs. constant lens powers .....	146
7.5.2 Exp. 7.2: Recovery vs. constant lens powers.....	150
<b>7.6 Discussion .....</b>	<b>154</b>
7.6.1 Equal binocular defocus dominates eye size signals .....	154
7.6.2 The effect of asymmetry between paired eyes.....	156

7.6.3	Conclusion .....	157
<b>8.</b>	<b>General Discussion.....</b>	<b>158</b>
<b>8.1</b>	<b>Summary of thesis findings.....</b>	<b>159</b>
8.1.1	Chapter 3 .....	159
8.1.2	Chapter 4 .....	161
8.1.3	Chapter 5 .....	165
8.1.4	Chapter 6 .....	165
8.1.5	Chapter 7 .....	167
<b>8.2</b>	<b>The proposed intrinsic factor is only observed when the refractive states of the two eyes are asymmetrical .....</b>	<b>169</b>
8.2.1	Monocular vs. binocular lens step-up .....	170
8.2.2	Monocular vs. binocular recovery .....	172
<b>8.3</b>	<b>Possible molecular pathway responsible for the non-visual cue(s) .....</b>	<b>174</b>
<b>8.4</b>	<b>Implications for human anisometropia .....</b>	<b>175</b>
<b>8.5</b>	<b>Implications for human myopia control .....</b>	<b>176</b>
	<b>References.....</b>	<b>178</b>
	<b>Appendix 1. Supplemental Table and Figures for Chapter 3.....</b>	<b>194</b>
	<b>Appendix 2. Supplemental Table and Figures for Chapter 4.....</b>	<b>198</b>
	<b>Appendix 3. Supplemental Figures for Chapter 7.....</b>	<b>208</b>
	<b>Appendix 4. Previous Publications.....</b>	<b>213</b>

## List of Figures

Figure 1.1. Schematics of ocular compensation for defocus of opposite signs.....	13
Figure 2.1. A photograph of the Hartinger refractometer use.....	42
Figure 2.2. Axial ocular dimensions measured using A-scan high-frequency ultrasound biometry.....	43
Figure 3.1. Comparison of recovery in the light and the dark after positive lens wear.....	56
Figure 3.2. Comparison of recovery in light and in the dark after negative lens wear.....	58
Figure 4.1. Schematics for the interactions between the hypothesized size- and defocus- factors.....	70
Figure 4.2. Schematics for lens-wearing paradigms and the proposed effects of the size- and defocus-factors at lens step-up.....	73
Figure 4.3. Time course of compensation for +15 D lenses and for first +7 D then +15 D lenses.....	78
Figure 4.4. Time course of compensation for -15 D lenses and for first -7 D then -15 D lenses.....	82
Figure 4.5. Time course of compensation for -10 D lenses and for -5 D then -10 D lenses. .....	84
Figure 4.6. Comparisons between positive lens step-up and recovery from negative lens wear.....	88
Figure 4.7. Comparisons between negative lens step-up and recovery from positive lens wear.....	90
Figure 4.8. A Scatter plot of change in refractive error after lens step-up or recovery.....	92
Figure 4.9. A Scatter plot of change in axial length after lens step-up.....	94
Figure 5.1. The frequency distributions of change in axial length.....	105
Figure 5.2. The frequency distributions of change in vitreous chamber depth.....	106
Figure 5.3. The frequency distributions of change in axial length in eyes that shrank after wearing positive lenses and their fellow eyes.....	107
Figure 6.1. Symmetry in axial length and axial growth in paired eyes in untreated chick eyes (Exp. 6.1). .....	121
Figure 6.2. Axial length in left eyes of normal, untreated chicks from 1 to 17 days of age	

(Exp. 6.1). .....	122
Figure 6.3. Correlation between the change in axial length in the fellow eye and that in the lens-wearing eyes.....	124
Figure 6.4. Adjusted change in axial length for both experimental and fellow eyes after either positive or negative lens wear for 1 to 7 days.....	125
Figure 6.5. The effects of lens-wearing duration on yoking and anti-yoking.....	127
Figure 7.1. Schematic for treatment paradigms and the potential effects of the proposed size- and defocus-factors at the time of step-up or lens removal.....	140
Figure 7.2. Time course of binocular positive lens treatment.....	147
Figure 7.3. Time course of binocular negative lens treatment.....	149
Figure 7.4. Comparison between positive lens recovery and negative lens wear.....	151
Figure 7.5. Comparison between negative lens recovery and positive lens wear.....	153
Figure 7.6. The correlation between change in refractive error and functional defocus after lens step-up or recovery. ....	155
Figure. 8.1. Comparison of change in experimental eyes between monocular and binocular lens step-up.....	171
Figure. 8.2. Comparison of change in experimental eyes between monocular and binocular recovery.....	173
Figure 8.3. Diagrams for the interactions between the size- and defocus-factors during positive (A) and negative (B) lens step up.....	174
Figure 8.4. A diagram showing future experiments to further study the effect of the Hippo pathway.....	175

**Figures in Appendices are listed below:**

Figure. A1.1. Comparison for recovery in either light or darkness after positive lens wear. ....	195
Figure. A1.2. Comparison for recovery in either light or darkness after negative lens wear. ....	196
Figure. A2.1. Time course of compensation for +15 D lenses and for first +7 D then +15 D lenses.....	202
Figure. A2.2. Time course of compensation for +5 D lenses and for first +5 D then +10 D	

lenses.....	203
Figure A2.3. Time course of compensation for –15 D lenses and for first –7 D then –15 D lenses.....	204
Figure A2.4. Time course of compensation for –10 D lenses and for first –5 D then –10 D lenses.....	205
Figure A2.5. Comparisons between positive lens step-up and recovery from negative lens wear.....	206
Figure A2.6. Comparisons between negative lens step-up and recovery from positive lens wear.....	207
Figure A3.1. Time course of binocular positive lens treatment.....	209
Figure A3.2. Time course of binocular negative lens treatment.....	210
Figure A3.3. Comparison between positive lens recovery and negative lens wear.....	211
Figure A3.4. Comparison between negative lens recovery and positive lens wear.....	212



## List of Tables

Table 1.1. Summary of recent reviews (from 2005 to 2016) on various aspects of myopia .....	11
Table 1.2. Summary of studies reporting effects of monocular lens wear on the untreated eye and ocular interactions.....	30
Table 1.3. Summary of literature reporting opposite changes between the two eyes .....	33
Table 1.4. Summary of hypotheses for Chapters 3 to 7.....	38
Table 3.1. Summary of treatment details, the predicted effects of the size- and defocus- factors, and sample size (n).....	52
Table 3.2. Summary of inter-ocular difference ( $X - N$ , Mean $\pm$ SEM) for ocular dimensions and refractive error .....	53
Table 4.1. Summary of treatment details, the effects of the proposed size- and defocus- factors, and sample size (n) in Exp. 4.1 and 4.2 .....	71
Table 4.2. Summary of inter-ocular difference ( $X - N$ , Mean $\pm$ SEM) for ocular dimensions, refractive error, and $p$ values for Exp. 4.1 .....	77
Table 4.3. Summary of inter-ocular difference ( $X - N$ , Mean $\pm$ SEM) for ocular dimensions, refractive error and $p$ values for Exp. 4.2 .....	86
Table 5.1. Summary of treatment details and sample size (n).....	103
Table 6.1. Summary of the measurement age, ocular dimensions (Mean $\pm$ SEM) of the left eyes, the $r^2$ and $p$ values for axial length between paired eyes, and sample size normal chicks (Exp. 6.1).....	116
Table 6.2. Summary of the treatment details, the change in ocular dimensions over the course of each experiment (Mean $\pm$ SEM), $p$ values, and sample size (n) for Exp. 6.2 .....	118
Table 7.1. Summary of the treatment details, the effects of the proposed size- and defocus- factors, and sample size (n).....	139
Table 7.2. Actual values in ocular dimensions and refractive error (Mean $\pm$ SEM) .....	144
Table 7.3. Summary of inter-ocular difference ( $X - N$ , Mean $\pm$ SEM) for ocular dimensions and refractive error and $p$ values .....	145

**Tables in Appendices are listed below:**

Table A1.1. Summary of actual values for ocular dimensions, refractive error, and sample size (n) for Chapter 3 (Mean $\pm$ SEM) .....	197
Table A2.1. Summary of actual values for ocular dimensions, refractive error, and sample size (n) for Chapter 4 (Mean $\pm$ SEM) .....	199

## Abstract

**Purpose:** While it is well known that growing human and animal eyes respond to imposed defocus by changing their growth to compensate for and eliminate the defocus (referred to as the “defocus-factor” in this dissertation), non-visual factors may also be involved. For example, it is common knowledge that body parts are under an intrinsic homeostatic control to firstly obtain the “right” length or size during development and secondly maintain this size after development. Previous experiments have shown evidence supporting non-visual factors playing a role in eye growth, e.g., chick eyes can restore their normal shape during recovery from form deprivation even though the retina has been damaged by tunicamycin. Therefore, it is possible that an intrinsic, homeostatic, non-visual mechanism also exists to control eye growth and to prevent the eye from deviating from the age-appropriate eye length or size (referred to as the “size-factor” in this thesis). In addition, it has been discovered that there are interactions between the paired eyes in the same animal, another factor that might be involved in eye growth regulation. Specifically, previous studies have shown that the fellow eyes might change in either the same direction as the lens-wearing eyes (the “yoking” effect), or the opposite direction compared with the lens-wearing eyes (the “anti-yoking” effect), in terms of both refractive error and axial length. The aim of this thesis is to investigate the existence and role of factors other than local defocus that may influence eye growth control. This is undertaken using the well-known chick lens-compensation model as it provides the gold standard providing the largest effect sizes available within animal models.

**Methods:** The refractive error and axial dimensions of chick eyes were measured with a Hartinger refractometer and A-scan Ultrasound biometry, respectively. (1) The existence of a non-visual-factor was studied in Chapter 3: To investigate whether a non-visual factor exists in chick eyes to guide eye growth independent of the defocus-factor, recovery after wearing +7 D (n = 8) or -7 D (n = 11) lenses while the chicks were kept in darkness was compared to chicks that recovered in light after wearing +7 D (n = 8) and -7 D (n = 5) lenses. (2) After demonstrating the existence of a non-visual factor that can guide eye growth, the

effect of manipulating eye length or size on subsequent monocular lens-compensation was studied in Chapter 4: Chicks first wore a weak positive or negative lens (+7 D, n = 4; -7 D, n = 25) over one eye for a few days then the lens power was stepped up to a strong positive or negative lens (+7 D to +15 D; -7 D to -15 D), respectively. The size- and defocus-factors would be working in opposite directions at the time when lens power was increased, so studying lens compensation after the step-up can reveal which of these factors predominates in guiding eye growth. Furthermore, recovery from prior lens treatment vs. lens compensation after the step-up in lens power was compared when experimental eyes in both groups experienced the same amount of defocus (chicks recovering from -7 D lens wear vs. chicks that wore +15 D lenses after compensating for +7 D lenses; and chicks recovering from +7 D lens wear vs. chicks that wore -15 D lenses after compensating for -7 D lenses. The major difference between the two groups was their asymmetric eye sizes, which could act to facilitate recovery and reduce further lens compensation after the step-up. (3) The previous Chapter found that local defocus dominated in the case of positive lens wear (myopic defocus caused the eye to further compensate for the strong positive lenses, against the size-factor), so analyses were performed in Chapter 5 to investigate the ability of chick eyes to shorten axially, against the size-factor, to compensate for myopic defocus. Previous data from chicks from the Wallman database that wore a positive lens over one eye (n = 219) was compared to that from a group of normal, untreated chicks (n = 48). (4) To study another non-visual factor, the inter-ocular interactions between the paired eyes in the same chick, axial length from both eyes from a large group of untreated chicks from the Wallman database (n = 2960) were obtained to study the correlation in axial length between paired eyes and changes with age (1-17 days) in Chapter 6. Another group of untreated chicks (n = 48) were measured on days 7 and 10 to study the axial length growth in paired eyes. In addition, another group of chicks (n = 169) wore spectacle lenses of various powers (+/- 5, 7, 10, and 15 D) over one eye for various durations (1 to 7 days) and were measured before and after the treatment. The change in axial length in the fellow eyes was compared to that estimated from eyes of age-matched untreated animals. (5) Taking into account the discoveries related to the effects of asymmetric eye sizes and interactions between the two eyes (yoking) in previous chapters, the effect of eye size versus defocus was re-examined

under binocular conditions in Chapter 7: Chicks first wore a weak positive or negative lens ( $\pm 5$  D,  $n = 6$  and  $14$  for positive- and negative lens-wearing eyes, respectively) over one eye for a few days then the lens power was stepped up to a strong positive or negative lens ( $\pm 10$  D) on the same eye, respectively. At the same time, the fellow eyes started to wear a weak positive or negative lens, so both eyes would experience defocus of the same sign and magnitude after the step-up. Chapter 7 addressed whether the size-factor can still prevent the eyes from further elongating to compensate for the strong negative lenses if the defocus signal was similar in both eyes.

**Results:** (1) Chapter 3: Compared with chick eyes that recovered from prior lens treatment in the light (i.e. with visual input), chick eyes recovered more slowly in darkness. However, all chick eyes partially recovered from prior positive or negative lens treatment despite being kept in the darkness for 3 days, suggesting that a factor independent of visual input does exist and that it alone can guide eye growth. For convenience, we refer to this as a “size-factor”. (2) Chapter 4: Chick eyes fully compensated for  $+15$  D lenses after they had compensated for  $+7$  D lenses, despite having reduced axial length at the time of lens step-up, suggesting that myopic defocus dominated the eyes growth response, despite inter-ocular differences in eye size. In contrast, while chick eyes could fully compensate for  $-15$  D lenses if they wore them from the beginning, chick eyes did not fully compensate for  $-15$  D lenses after having compensated for  $-7$  D lenses, suggesting that some intrinsic factor interfered with the ability of the eye to respond to hyperopic defocus. Similar findings were discovered with weaker-powered lenses. It was also discovered that chick eyes that wore  $+15$  D lenses after the step-up reduced their rate of ocular elongation more than those recovering from prior  $-7$  D lens wear, confirming the dominance of the defocus-factor in positive lens treatment. On the other hand, eyes recovering from prior  $+7$  D lens wear developed a greater myopic shift compared with  $-15$  D lens-wearing eyes after stepping up from  $-7$  D lenses, confirming the involvement of a non-defocus related factor in the eyes response to negative lens treatment. Similar findings were discovered with lower-powered negative lenses. (3) Chapter 5: Chick eyes wearing positive lenses reduced their rate of ocular elongation by two-thirds, including 38.5% of eyes in which the axial length became shorter than before (mean change in axial length over the course of the experiment, experimental vs. fellow eyes, 40

vs. 171  $\mu\text{m}$ ). The axial shortening was caused mostly by the reduction in vitreous chamber depth. (4) Chapter 6: Paired eyes in untreated chicks were well correlated in their axial lengths 24 hours after hatching (mean axial length 8.55 and 8.53 mm for the right and left eyes, respectively;  $r^2 = 0.77$ ,  $p < 0.0001$ ) and thereafter, demonstrating symmetrical length or size and symmetrical growth. While monocular lens treatment caused significant compensation in the treated eyes, there was still a significant correlation in axial length in paired eyes after 3 to 7 days of treatment. Furthermore, yoking and anti-yoking, as defined by significant differences compared to growth predicted from untreated animals, were observed in approximately half of the experiments. In general, monocular lens treatment tended to reduce eye growth in the fellow eyes after shorter lens wearing durations (1-2 days, anti-yoking for positive lens treatment and yoking for negative lens treatment) and to increase eye growth after longer lens wearing durations (longer than 4 days, yoking for positive lens treatment and anti-yoking for negative lens treatment), and had minimal effect on the fellow eyes if the treatment duration was around 3-4 days. (5) Chapter 7: When chicks experienced defocus of the same sign over both eyes, chick eyes fully compensated for the strong positive lenses and especially, the strong negative lenses after the step-up, suggesting that the defocus-factor dominated in binocular lens compensation and that there is yoking between paired eyes.

**Conclusions:** Other than the defocus-factor that plays a crucial role in regulating eye growth, there are other intrinsic, non-visual, homeostatic mechanisms that are also involved in eye growth regulation: One of the non-visual mechanisms, which we refer to as a “size-factor”, can guide the eyes to grow towards the direction to regain the normal, age-appropriate eye size, in the absence of visual cues. Additionally, some unknown intrinsic mechanism, possibly non-visual, refrains the eye from becoming longer than normal in the case of monocular hyperopic defocus. However, defocus still has a huge impact in eye growth regulation, as shown by the results that chick eyes fully compensated for the strong positive lenses after the step-up (at the step-up, the size-factor could act to reduce further compensation for the strong positive lenses since the lens-wearing eyes were already shorter than normal after compensating for the weak positive lenses) and that chick eyes can shorten axially to facilitate compensation for the myopic defocus, both against that predicted by any

intrinsic size-factor. Another non-visual mechanism, the inter-ocular interactions between paired eyes (symmetrical growth, yoking and anti-yoking), also influences eye growth: Growth in paired eyes was well correlated despite monocular lens treatment. Yoking and anti-yoking seemed to be lens-wearing duration dependent. Importantly, experiments which use the fellow eye as a control under conditions which may induce yoking and anti-yoking can still be used but are conservative and may underestimate the actual effect sizes by up to 27% if the lens treatment duration is around 3-4 days. Shorter and longer treatment durations, on the other hand, seem to have a larger effect on the fellow eyes, and caution should be taken when interpreting results of longer term monocular treatments. Finally, it might be prudent to have a group of untreated animals as a control. These non-defocus factors have significant implications for human myopia control, and may partially explain why the current mainstream optical treatments for myopia control attempting to project myopic defocus to reduce axial elongation have only proven to be moderately effective at best. Therefore, it is worthwhile further investigating the molecular pathways underlying the possible non-visual mechanisms and developing potential pharmaceutical treatments that enhance this intrinsic growth limiting system. It might be possible to maximize the effect of myopia treatment if the optical and pharmaceutical treatments can be combined.