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PERIPHERAL REFRACTION, CENTRAL RETINAL FUNCTION AND THICKNESS IN CHILDREN WITH MYOPIA DEVELOPMENT

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Peripheral Refraction, Central Retinal Function and Thickness in Children with Myopia Development

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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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LI ZheChuang

To my families.

ABSTRACT

Myopia is reaching epidemic proportions with its increasing prevalence, especially in East and Southeast Asia. It is a multifactorial disorder. Findings in animal models with deprived non-foveal visual experience provided an insight about the role of peripheral image quality on central refraction development. Clinical human studies have demonstrated that refractive error varies with retinal eccentricity and different ametropic groups have distinct peripheral refractive error profiles. Longitudinal studies in children, however, were not able to establish a predictive role of peripheral refractive error on myopia onset or progression. It seems that the effect of peripheral refractive error alone cannot trigger or fully explain the myopia development mechanism in children.

Ample evidence from research using multifocal electroretinogram (mfERG) implies that myopia results in impaired retinal function. It was demonstrated that the severity of myopia in adults was negatively associated with mfERG responses, retinal adaptation responses and inner retinal function. Discrepancies of mfERG response characteristics between myopic adults and children were also observed. Findings of recent longitudinal studies have also indicated that central retinal function might be associated with myopia development in children. However, investigation of the relationship between retinal electrophysiology and myopia development is limited and further study on the role of retinal function would provide new insight in the understanding of myopia.

Ocular structural changes in myopia have been observed in animal models, and a direct relationship between retinal thickness and the severity of myopia was reported. With the application of optical coherence tomography *in vivo* imaging, clinical studies have revealed that children with

moderate myopia tended to have smaller total macular volume and thinner quadrant-specific macular thickness, indicating that early anatomical changes are present in the retina of myopic children. Further studies are required to understand how the relationship between retinal function and structure evolves along with myopia development.

This 3-year longitudinal study aims to demonstrate the changes of retinal electrophysiological function, peripheral refraction, and central retinal thickness associated with myopia development in children; to investigate the role of peripheral refractive error, retinal electrophysiological function, and central retinal thickness on juvenile myopia development; and to determine whether the retinal electrophysiological function, peripheral refraction, and central retinal thickness are possible precipitants of myopia development in young children aged 6 to 9 years.

106 subjects with emmetropic refractive errors were recruited at baseline visit and 88 subjects completed all four visits of the study. Cycloplegic objective central refractive errors changed from emmetropia at baseline visit to mild myopia at the last visit, with a concurrent axial elongation. Together with the central refraction change, peripheral refraction changed to become more myopic, while the relative peripheral refraction tended to more hyperopic. The electro-retinal activities showed significant changes over time, and the trends of changes were different in terms of retinal regions. Global Flash (MOFO) mfERG responses from the central retinal region displayed a significant decrease in amplitude and delay in implicit time. The response from other retinal regions also showed significant decreases in amplitude, but there were no changes in implicit time. However, with respect to retinal structure, central retinal thickness had no significant change over the study period. At the early stage of juvenile myopia development, the peripheral refraction (optics)

and electro-retinal activities (function) change over time, while the central retinal thickness (structure) is relatively preserved.

Subjects in our study were retrospectively divided into Emmetropic, Low Myopic and Moderate Myopic subgroups based on their central cycloplegic objective refraction at the last visit. The myopic groups displayed trends to have more peripheral hyperopic changes, delayed and decreased central retinal responses at baseline, and quadrant-specific thinning of outer macular thickness than those observed in the emmetrope group. Overall the results from our longitudinal study suggest that marked variance of retinal function and structure are noted earlier than that of retinal optical changes in myopia progression, which leads to the possible predictive role of central retinal function and structure in juvenile myopia development.

We demonstrated that the baseline amplitude of central induced component of mfERG response, which originates from the inner retina, was consistently correlated with subsequent myopic refraction changes and axial elongation in young children. This mfERG parameter was measured in emmetropic children with normal visual acuity, good ocular health, and within normative range of central retinal thickness, indicating that long-standing myopia effects, with respect to either pathological or structural changes in retina, could not account for the variance of the parameters obtained. Therefore, we hypothesize that the central inner retina is an essential determinant of the visual feedback process, where the peripheral retinal input could be decoded, and play a commanding role in the manipulation of juvenile myopia development. In summary, emmetropic children with subclinical decrease of inner retinal function, together with specific-quadrant thinning of the central retina, are more likely to develop myopia with faster progression.

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List of Abbreviations

AL	Axial length
ANOVA	Analysis of variance
ATR	Against-the-rule
CLEERE	The Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error
COMET	The American Study of the Correction of Myopia Evaluation Trial
DC	Direct component
DTL	Dawson-Trick-Litzkow
E	Emmetrope group
ERG	Electroretinogram
IC	Induced component
ISCEV	International Society for Clinical Electrophysiology of Vision
J0	Cylindrical component along the horizontal or vertical meridian
J45	Cylindrical component along the 45 ° or 135° meridian
LM	Low myope group
M	Spherical equivalent
mfERG	Multifocal electroretinogram
MM	Moderate myope group
MOFO mfERG	Global flash multifocal electroretinogram
N	Nasal
N1	First negative trough response in conventional mfERG
N2	Second negative trough response in conventional mfERG
N10	Nasal 10°

N20	Nasal 20°
N30	Nasal 30°
OCT	Optical Coherence Tomography
P1	First positive peak response in conventional mfERG
PR	Peripheral refraction
RPR	Relative peripheral refraction
S	Spherical power
SCORM	The Singapore Cohort Study of the Risk Factors for Myopia
SD-OCT	Spectral domain optical coherence tomography
T	Temporal
T10	Temporal 10°
T20	Temporal 20°
T30	Temporal 30°
V0	Baseline visit
V1	1st year follow up
V2	2nd year follow up
V3	3rd year follow up
VERIS	Visual Evoked Response Imaging System
WHO	World Health Organization

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Part I Literature Review

Chapter One

Myopia

1.1 Prevalence of Myopia

Worldwide myopia prevalence has been increasing rapidly in recent decades and is projected to affect approximately 50% of the global population by 2050 (Holden et al., 2016). It has become a worldwide public health issue, especially in East and Southeast Asian regions, including Japan, South Korea, Taiwan, Singapore, Hong Kong, and some areas of the mainland China. The above Asian regions have reported myopia prevalence of up to 80% to 90% in young adults (Matsumura and Hirai, 1999, He et al., 2004, Lin et al., 2004, Saw et al., 2006, Jung et al., 2012, Kim et al., 2013, Wu et al., 2013a).

The refractive error and visual impairment study carried out in Malaysia reported the myopia prevalence as 10% to 32% for children aged between 7 and 15 years (Goh et al., 2005). The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) noted 28% to 52% myopia prevalence in 7 to 9 years old children (Saw et al., 2006). Another Singapore epidemiological study found myopia prevalence in 14 to 19 years old students was as high as 74% (Quek et al., 2004). It was revealed that around 20% to 30% of 6 to 7 years old children in Taiwan were myopic, the average myopia progression was about 1D per year in schoolchildren, and the prevalence of myopia for high school students had reached 84% in 2000 (Lin et al., 2001). The rate of high myopia (over -6D) of young adults in Taiwan was as high as 24% (Lin et al., 2001). In mainland China, difference in prevalence of myopia between urban and rural regions in young adults has been observed. About 78% of 15-year-old teenagers were found to be myopic in Guangzhou, a well-developed south-eastern city in China (He et al., 2004), while only 27% of the same age group were myopic in the suburban area of Chongqing, which is located in the south-west China (Pi et al., 2010). It was found that higher population density of cities in China was associated with the higher prevalence of myopia (Zhang

et al., 2010). In Hong Kong, myopia prevalence of children of 2 to 6 years old was reported as 6% (Fan et al., 2011) while 18% and over 60% of children aged 6 and 12 years respectively were observed to be myopic (Lam et al., 2012). The prevalence of high myopia in young adults is now more than 10% and approaching 20% in Hong Kong (Jung et al., 2012, Wu et al., 2013a).

Myopia not only threatens the vision, but also affects the quality of life and imposes a societal burden. If myopia is left uncorrected or progresses in severity, the deprived vision and increased risks of developing permanently visually-impairing diseases, such as maculopathy, glaucoma and retinal detachment, will eventually adversely affect the quality of life (Jones and Luensmann, 2012). The World Health Organization (WHO) has recognized that myopia, if not fully corrected, is a major cause of visual impairment (Resnikoff et al., 2008). Therefore, the current situation of rapid increase in myopia prevalence calls for adequate diagnosis and correction of myopic refractive errors, effective treatment of myopic pathologies, and, above all, prediction of myopia onset and prevention of myopia (Morgan et al., 2012).

1.2 Pre-disposing Factors for Myopia

Myopia is a multifactorial disorder. Both genetic and environmental factors play a role in myopia development. Twin and parental myopia studies have demonstrated a genetic predisposition to myopia (Liang et al., 2004; Lam et al., 2008; Jones-Jordan et al., 2010; Zhang et al., 2011). However, epidemiological studies have revealed that myopia is more prevalent in younger than older adults within a population that shares the same gene pool. The increase in prevalence of myopia in recent generations is more rapid than which could be explained by genetic factors alone. It is now generally agreed that myopia development involves environmental factors as major risks with

contributions from a number of genes having a small effect. Environmental and lifestyle issues, especially reduced time spent outdoors, are strongly indicated as predisposing factors in recent epidemiological studies of myopia (Morgan et al., 2012).

1.2.1 Parental Myopia

There is evidence for a link between parental and child axial length, predisposing the child to myopia (Zadnik et al., 1994). Parental myopia has been proposed to be a risk factor for early myopia onset in children and the number of highly myopic parents increases the risk of high myopia development in their children (Liang et al., 2004). In an early epidemiological study of the heredity of myopia, 2888 children of Chinese ethnicity in China and Hong Kong were surveyed, and the prevalence of myopia was estimated to be three times greater in children who had one myopic parent compared to those with two non-myopic parents. If both parents are myopic, the risk for myopia is around six times higher than for children who have two non-myopic parents (Yap et al., 1993). The American Study of the Correction of Myopia Evaluation Trial (COMET) found that myopic progression and axial length changes in myopic children were associated with the number of myopic parents (Kurtz et al., 2007). Similar results were also observed in the Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) study, though the sensitivity of parental myopia for myopia prediction was low (Jones-Jordan et al., 2010). Lam et al (2008) reported that refractive errors of Hong Kong teenagers were negatively related to the number of myopic parents but axial length was positively associated with the number of myopic parents.

1.2.2 Time of Outdoor Activities

Increasing the hours of outdoor activity was reported to have positive protective effects on myopia, with a significant reduction in myopic shift in refraction and axial elongation and reduced odds ratios for incident myopia and fast myopia progression (Wu et al., 2018). This intervention is based on the successful Recess Outside Classroom intervention that increased time outdoors by locking children out of their classrooms during recesses (Wu et al., 2013b). The positive effects in this trial are consistent with the results of two other trials of increased time outdoors conducted in mainland China (He et al., 2015, Jin et al., 2015). This protective effect seems to be associated with total time outdoors, rather than with specific engagement in sport (Rose et al., 2008). Researchers have further suggested an target exposure of 11 hours outdoors every 7 days based on the epidemiological data (Jones et al., 2007, Rose et al., 2008).

Several mechanisms have been proposed for the protective effect of outdoor activity on myopia development. Some studies have negated the influence of physical exercise or simply reduced near work time (Mutti et al., 2002, Rose et al., 2008, Guggenheim et al., 2012, Read et al., 2014). Rose and colleagues postulated that increased levels of retinal dopamine triggered by exposure to sunlight might inhibit excessive eyeball elongation and thus reduce the incidence of myopia onset (Rose et al., 2008). Greater outdoor light intensity also causes pupil constriction and might inhibit myopic development through retinal image contrast improvement (Thibos et al., 2013). Given that being outdoors is also associated with distant fixation, relaxed accommodation, and increased myopic defocus, excessive eye growth may be inhibited (French et al., 2013). A role for vitamin D is also suggested, but has not obtained significant scientific evidence (Mutti and Marks, 2011).

1.3 Emmetropization

Emmetropization is a process regulating the growth of the eyeball to achieve clear vision and is an important mechanism in ocular growth. Peripheral defocus is found to be effective in manipulating central refractive changes in different animal models. Peripheral hyperopic defocus has been suggested as a possible trigger for myopia development and many clinical studies have been carried out to investigate this hypothesis.

It is believed that emmetropization is a visually guided active mechanism to modulate eye growth. Numerous animal studies in chick (Irving et al., 1992, Schmid and Wildsoet, 1996a, Schmid et al., 2006), mouse (Schaeffel et al., 2004), guinea pig (Howlett and McFadden, 2006, Howlett and McFadden, 2009), tree shrew (Norton et al., 2006), and monkey (Smith III et al., 1994, Smith III and Hung, 1999) have provided supportive evidence for this active feedback process. Positive lenses bring the focal plane to the front of the retina, inducing a positive or myopic defocus, while negative lenses take the focal plane further away behind the retina, producing a negative or hyperopic defocus. All the above studies showed that animal eyes can differentiate the sign of the defocus and respond to the magnitude of defocus by altering their ocular growth. Based on these findings, the development of myopia had been suggested to be regarded as a failure of the emmetropization process (Norton and Siegwart, 1995, Wildsoet, 1997). The myopia development with lens induced defocus was found to continue after optic nerve section (Wildsoet and Schmid, 2000), ciliary nerve section (Schmid and Wildsoet, 1996b), and foveal photo-ablation (Smith et al., 2007). Once the refractive errors of the animals' eyes reached the imposed power of defocus, the ocular growth ceased, with refractive errors similar to the imposed power (Wildsoet and Schmid, 2000,

Smith et al., 2007). Later studies further confirmed that the non-foveal visual experience was effective to regulate eye growth, which gave rise to the development of the peripheral defocus theory (Smith et al., 2007, Smith III et al., 2009, Huang et al., 2011).

1.4 Peripheral Refraction and Myopia

1.4.1 Effect of Peripheral Defocus on Myopia Development

Findings in animal models with deprived non-foveal visual experience have provided new insight into the role of peripheral field on central refraction development (Huang et al., 2009, Smith III et al., 2009, Huang et al., 2011). Hemi-field deprivation and defocus were shown to induce myopia in the corresponding visual field, and further studies in primates demonstrated that myopia could be produced when form deprivation and hyperopic defocus were restricted to the peripheral field with the central field spared (Smith et al., 2007, Smith III et al., 2009). These findings suggest that the peripheral retina can independently mediate ocular growth under defocus, and thus numerous clinical studies have been conducted to evaluate the peripheral refraction profile in the human eye (Chen et al., 2010, Ehsaei et al., 2011, Sng et al., 2011, Li et al., 2015).

There are a number of techniques to measure peripheral refraction of human eyes. The commonly used techniques include both subjective refraction and objective methods such as retinoscopy, autorefraction, photorefraction, scanning photoretinoscopy and aberrometry. All these measurements require subjects to fixate at different eccentric fixation points, either by head turn or eye turn. Among these techniques, open-view auto-refraction is the popular method used in most of the clinical studies.

Various clinical studies investigating peripheral refraction have demonstrated that refractive error varies with retinal eccentricity and different ametropic groups have distinct peripheral refractive error profiles. Emmetropes and hyperopes generally have relative peripheral myopia, while myopes tend to have relative peripheral hyperopia (Millodot, 1981, Mutti et al., 2000, Seidemann et al., 2002, Atchison et al., 2006, Chen et al., 2010, Ehsaei et al., 2011, Sng et al., 2011, Li et al., 2015). These refractive findings have also been confirmed by studies investigating ocular shape. The myopic eye has a prolate retinal shape, wherein the globe is more axially elongated compared to the equatorial diameter, and thus it is relatively hyperopic at the periphery (Atchison et al., 2004, Atchison et al., 2005, Singh et al., 2006). However, peripheral refraction along the vertical meridian does not vary among myopes, hyperopes, and emmetropes (Atchison et al., 2006, Chen et al., 2010). A large scale investigation in America found that relative peripheral refraction (RPR) varied with ethnicity: Asians, African Americans, and Hispanics had a less hyperopic central refraction and a less myopic RPR, while Native Americans and Whites had a more hyperopic central refraction and a more myopic RPR (Mutti et al., 2011). Kang and coworkers also observed similar discrepancies between East Asians and Whites (Kang et al., 2010). Myopic correction by single vision ophthalmic lenses or contact lenses impose hyperopic defocus on the peripheral retina along the horizontal meridian, and the magnitude of peripheral hyperopic refraction was reported to be correlated with the central myopic refractive error (Lin et al., 2010, Berntsen and Kramer, 2013, Berntsen et al., 2013, Kang et al., 2013). These observations, together with previous findings in animal models, support the theory that peripheral defocus plays an important role in myopia development.

1.4.2 Inadequacies of Peripheral Refraction Theory

Despite a number of longitudinal studies in children, a predictive role for peripheral hyperopia on myopia onset or progression has not been established (Mutti et al., 2011, Sng et al., 2011, Lee and Cho, 2013, Atchison et al., 2015). The CLEERE study, which followed up 2817 children in American, concluded that relative peripheral hyperopia had little consistent influence on the risk of myopia onset or the rate of myopia progression (Mutti et al., 2011). Another longitudinal study also reported that relative peripheral hyperopia and astigmatism in Singapore children measured at baseline could not predict the progression of myopia (Sng et al., 2011). These findings were further confirmed by a later study on Hong Kong children (Lee and Cho, 2013). A recent large-scale longitudinal study, which involved large sample groups of Chinese children, 2893 of 7 years old and 2,267 of 14 years old, found that hyperopic RPR did not predict the development or progression of myopia in either of the groups of children (Atchison et al., 2015). In addition, relative peripheral hyperopia was suggested to develop after the onset of myopia, rather than to trigger its onset (Sng et al., 2011, Hartwig et al., 2016).

Some researchers argue that peripheral retinal images are the result of the interactions of peripheral refractive errors and the visual environment. In normal daily life, the visual environment includes numerous focal distances and thus the peripheral retina experiences a wide range of magnitudes and types of defocus across different regions. With eyeball movement, the peripheral retinal exposures to different defocus stimuli changes rapidly. Evidence from animal models has indicated that interruptions of exposure to hyperopic defocus with brief periods of clear vision may suppress the myopigenic effect of imposed blur, which is a more realistic comparison to the typical human environment (Norton et al., 2006). Based on these findings, it has been suggested that defocus-dependent eye growth is integrated over time in a non-linear fashion (Zhu et al., 2013). Given the

complex interaction between the visual environment, the accommodation response, and the peripheral refraction of the eye, which determines the pattern of retinal defocus, the role of peripheral retinal defocus in myopia development cannot be fully understood based on current limited findings about peripheral refraction (Charman, 2011, Flitcroft, 2012). Hence, the concept of peripheral refraction theory in myopia development should be reconsidered. Referring to the findings of the animal studies using induced hyperopic defocus (Wildsoet and Schmid, 2000; Smith et al., 2007, 2009), the end-points of refractive errors in animal models with optic nerve section and foveal-ablation were found to fluctuate considerably, with large variations compared to the animals with intact ocular structures. It appears that the effects of peripheral refraction alone cannot fully drive ocular growth in emmetropization, suggesting that involvement of intact central vision (e.g. foveal function) may be necessary in the visually guided process of eye growth.

Chapter Two

Multifocal Electroretinogram and Myopia

Evidence from studies using multifocal electroretinogram (mfERG), which provides a topographical representation of retinal functions, has shown that myopia results in impaired retinal function. The severity of myopia in adults has been reported to be negatively associated with mfERG responses (Kawabata and Adachi-Usami, 1997), retinal adaptation response (Chen et al., 2006c), and inner retinal function (Ho et al., 2011). Longer axial length was also shown to be related to both reduced first-order kernel response and second-order kernel response of mfERG (Chan and Mohidin, 2003). The reduction in mfERG response in myopic adults is believed to be caused by the deterioration in retinal function associated with long-standing myopia. Discrepancies of the mfERG response characteristics between myopic adults and children were observed in terms of retinal regions and mfERG components (Luu et al., 2006, Ho et al., 2012b).

2.1 Multifocal Electroretinogram

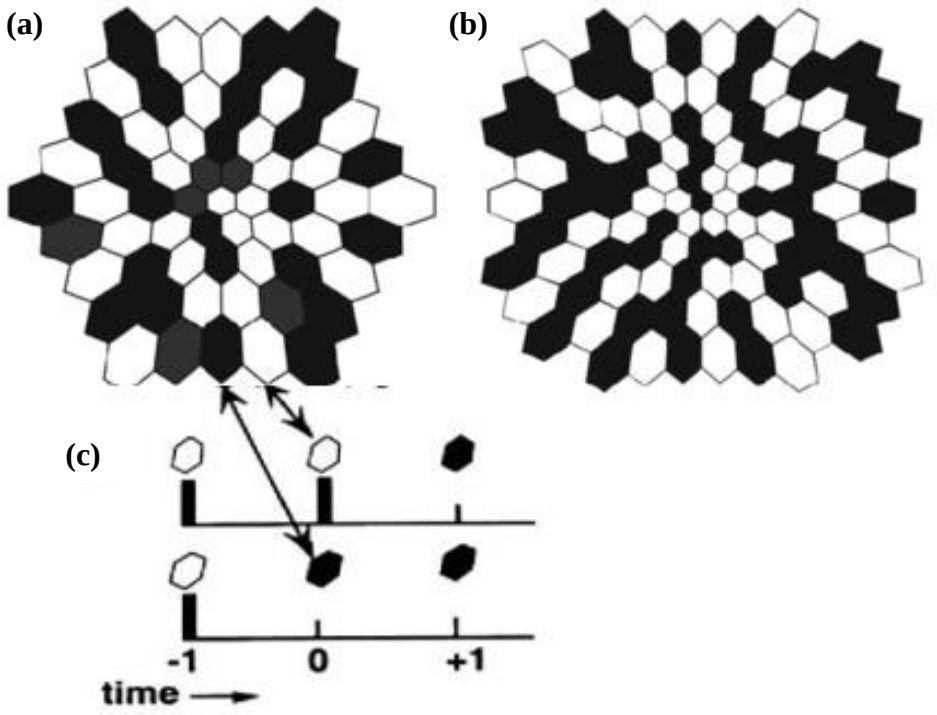
An electroretinogram (ERG) measures electrical activity produced by both neural and non-neural retinal cells in response to a light stimulation, mainly recording the function of the photoreceptors and the bipolar cells. The conventional multifocal electroretinogram (mfERG) was first introduced by Sutter and Tran in 1992. It permits ERG testing of multiple retinal loci simultaneously and makes it possible to quantify the spatial distribution of electro-retinal activities. As the mfERG provides a topographical representation of retinal functions, there is wide scope for application of mfERG in spatial, temporal, and contrast domains to improve the way in which they discriminate normal from abnormal visual functions.

2.1.1 Multifocal Electroretinogram Recording

The mfERG measurement guidelines have been published by the International Society for Clinical Electrophysiology of Vision (ISCEV) in 2011 (Hood et al., 2012). The stimulation display commonly consists of either 103- or 61-scaled hexagons (Figure 2.1). The stimulus hexagons are scaled by eccentricity according to the density of cone distribution (Sutter and Tran, 1992), whereby smaller hexagons are presented in the central region and larger hexagons are presented in the periphery. The stimulus pattern usually subtends a visual angle of 40 to 60º. Each hexagon of the stimulus is temporally flickered between dark and bright (flash) presentations according to a pseudo-random binary m-sequence stimulation. Different starting point of mfERG flickering presentation makes the sequence of stimulation of each region independent of each other, so as to facilitate the derivation of each local response through cross-correlation (Sutter and Tran, 1992). The frame rate of stimulation is normally at 60 or 75 Hz. The amplifier is set with a gain of 100,000 or 200,000. Use of a band-pass filter with a range of 3-300 or 10-300 Hz is advised. The subject's pupil is usually dilated for the measurement. It is suggested that the responses are averaged by a specifiable percentage with those from adjacent hexagons to smooth out responses to individual waveforms and to improve the signal-to-noise ratio (Sutter and Tran, 1992). However, the averaging process can affect the presentation of localized lesions and thus it should be used with caution. Reducing the contrast of the hexagon in the stimulation has also been suggested, which allows an enhanced detection of inner retinal activity in the human eye (Hood et al., 1999).

The results can be displayed as an array of traces from different retinal regions or averaged together with any number of traces for comparison of quadrants or successive eccentric rings from the centre to the periphery. Three-dimensional topographic response density plots can also be used to

show an overview of retinal responses across the field. The response density (i.e. response amplitude per unit of visual angle) decreases with eccentricity, which has been shown to be correlated with the cone density (Sutter and Tran, 1992). The first-order kernel is derived by averaging all the responses to dark stimulation, and then subtracting this from the average of all the responses to bright stimulation. It represents the averaged response of the retina to a light stimulus. The second-order kernel represents the interactive response between the preceding frame and current frame and thus reflects the adaptive response to consecutive stimulation. It is believed to be related to the activity of the inner retina and ganglion cells.



Adopted from: Hood, Donald C.; Bach, Michael; Brigell, Mitchell; Keating, Kondo, Mineo; Lyons, Jonathan S.; Marmor, Michael F.; McCulloch, Daphne L.; Palmowski-Wolfe, Anja M. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition) Documenta Ophthalmologica 2012; 124(1):1-13.
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Figure 2.1. The stimulation patterns of multifocal electroretinogram (mfERG). (a) Representative mfERG stimulus array with 61 hexagons scaled with eccentricity. (b) Same as in panel A for an array with 103 hexagons. (c) Each hexagon of the stimulation pattern is temporally flickered between dark and bright (flash) presentations.

2.1.2 The Origin of Responses

For a conventional mfERG first-order kernel response, there is an initial negative trough (N1), a positive peak (P1), and a second negative trough (N2). The overall shape of the human mfERG responses is attributed mainly to bipolar cell contributions combined with smaller contributions from photoreceptors, which is based on the pharmacological dissection of the monkey's mfERG. (Hood et al., 2002). The human mfERG was significantly different from that recorded from the monkey (Frishman et al., 2000). However, once the inner retinal components were removed from the monkey's mfERG responses, the mfERG waveform of the monkey was found similar to that of humans (Hood et al., 1999b; Frishman et al., 2000). Based on these findings, a proposed model of human mfERG was deduced and this model suggests that the contribution to the human mfERG is mainly from the outer retina and partly from the inner retina (Hood et al., 2002). Figure 2.2 shows the model explaining how the bipolar cells contribute to the mfERG waveform (Hood et al., 2002). The N1, P1, and N2 components are influenced in different ways by the onset and offset of the bipolar cell responses. It is thought that N1 is the result of the hyperpolarization of the cone photoreceptors and OFF-bipolar cells, the following rising phase of the waveform (P1) is the response originated from ON-bipolar cells, and the OFF-bipolar cells are dominant the later response of N2 (Hood et al., 1997, Hood, 2000). The inner retina, however, exerts a subtle influence on the waveform. In short, the first-order kernel response involves major contribution from outer retinal activity and minor contribution from inter retinal activity.

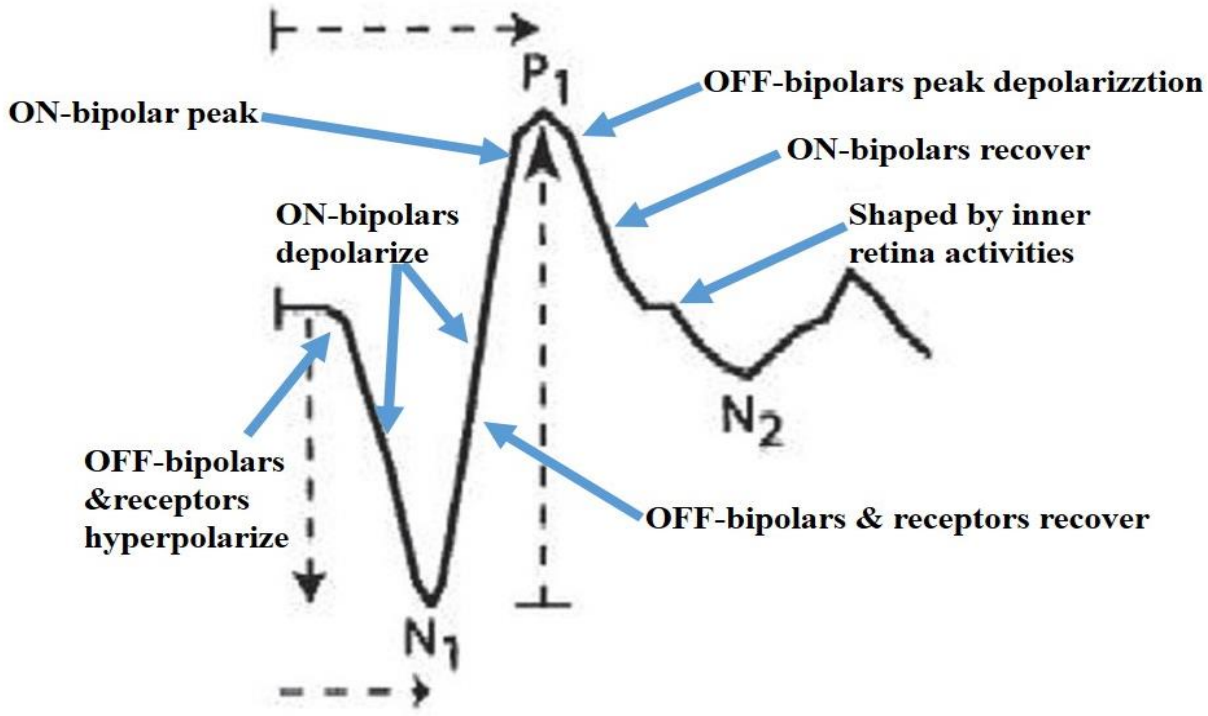


Figure 2.2. The retinal origin of the conventional multifocal electroretinogram response.

2.1.3 Effect of Age on Multifocal Electroretinogram

Age has been demonstrated to be a significant factor affecting mfERG responses. The central retinal response was reported to exhibit the greatest age-related decline (Fortune and Johnson, 2002, Gerth et al., 2002, Seiple et al., 2003, Tam et al., 2006). Seiple and co-workers reported the rate of loss was about 10.5% per decade for mfERG amplitude and the rate of amplitude reduction was the highest in the central retina region (Seiple et al., 2003). This reduction was suggested due to the age-related changes of the optical factors, such as pupil size and crystalline lens opacity (Fortune and Johnson, 2002). A later study, which recruited post-cataract surgery elderly subjects with insertion of intraocular lenses, in order to rule out optical effect on mfERG responses, found that age-related decline in mfERG responses was mainly due to optical factors before the age of 70 years, but neural factors significantly affected mfERG responses after the age of 70 (Tam et al., 2006).

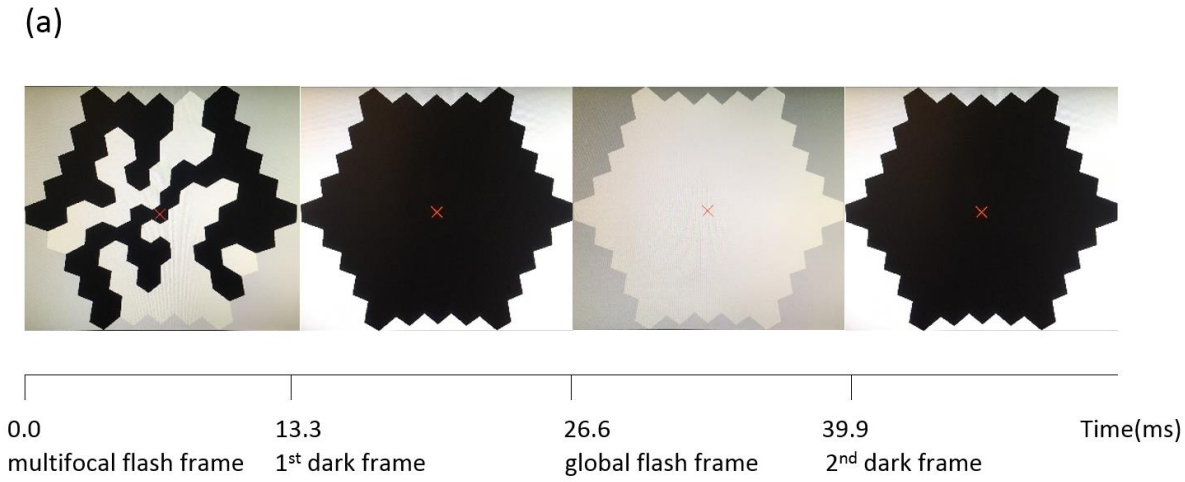
2.1.4 Global Flash Multifocal Electroretinogram

As conventional mfERG responses originate predominantly from the outer retinal layers, such as photoreceptors and bipolar cells, a new stimulation paradigm was developed to enhance measurement of inner retinal responses. The method is based on the rationale that retinal processing involves multiple stages of adaptation: starting at the photoreceptor level, followed by post-receptoral feedback and, finally lateral interaction in the inner retina. It is thus assumed that components due to these nonlinear mechanisms originate predominantly in the inner retina and are most pronounced in the retinal ganglion cells (Sutter et al., 1999). The global flash multifocal electroretinogram was designed to emphasize these components. This new paradigm of multifocal stimulation, which consists of the usual stimulus sequence of binary m-sequence frames, is slowed down by

inserting a given number of video frames between consecutive frames of the multifocal stimulation (Sutter et al., 1999). The inserted frames are either dark or flashes covering the entire stimulation array and the full-screen flash can be one or more periodic inserted. The global flash (MOFO) paradigm is one of several new mfERG stimulations suggested to be applied for measurement of the inner retinal response.

The MOFO mfERG paradigm, shown in Figure 2.3, involves four video frames in each stimulus interval. It commences with the first frame of multifocal stimulus (M), followed by a full-field dark frame (O), a global flash (F), and finishes with a second dark frame (O). The response due to the interaction between the focal flash and the global flash is a non-linear component, which is designed to measure the rapid adaptive retinal mechanism. It leads to the generation of two major components in the response waveform: a direct component (DC), which is the response to the focal flash, and an induced component (IC), which represents the interaction response of focal and global flash. The DC is believed to be analogous to a mfERG response using the conventional flickering protocol (Sutter et al., 1999). However, as the focal flashes are always preceded by the periodically occurring global flashes, the DC also reflects the resulting adapted or desensitized state of the retinal patch (Chu et al., 2011). The nonlinear IC has been suggested to predominantly reflect inner retinal function (Shimada et al., 2005). Cellular contributions to the MOFO mfERG responses were investigated in porcine eyes by using a pharmacologic dissection method (Chu et al., 2008). The measurement was performed on the eyes of Yorkshire pigs in control conditions and after suppression of inner retinal responses with inhalation of isoflurance (ISO), and injections of tetrodotoxin (TTX) and N-methyl-D-asparatic acid (NMDA). ON- and OFF-pathway responses

were isolated by injection of 2-animo-4-phosphonobutyric acid (APB) and cis-2,3-piperidinedi-carboylic acid (PDA) respectively. The porcine global flash mfERG consisted of an early DC and a late IC. ISO and TTX removed inner retinal contributions to the IC; NMDA application further abolished the oscillatory wavelets in the DC and removed the residual IC waveform. The inner retina contributed regular oscillating wavelets to the DC and shaped the IC. After removing the inner retinal contributions, the porcine global flash mfERG waveform left becomes comparable to that obtained by conventional mfERG stimulation. The remaining waveform was mainly formed by contributions of the ON- and OFF-bipolar cells, as revealed after APB and PDA injection re-spectively. Photoreceptors contributed a small signal to the leading edge of N1. Therefore, it has been summarized that the DC response is generated by photoreceptor, ON-, and OFF-bipolar cells, while the IC response mainly originates from inner retinal activity (Chu et al., 2008).



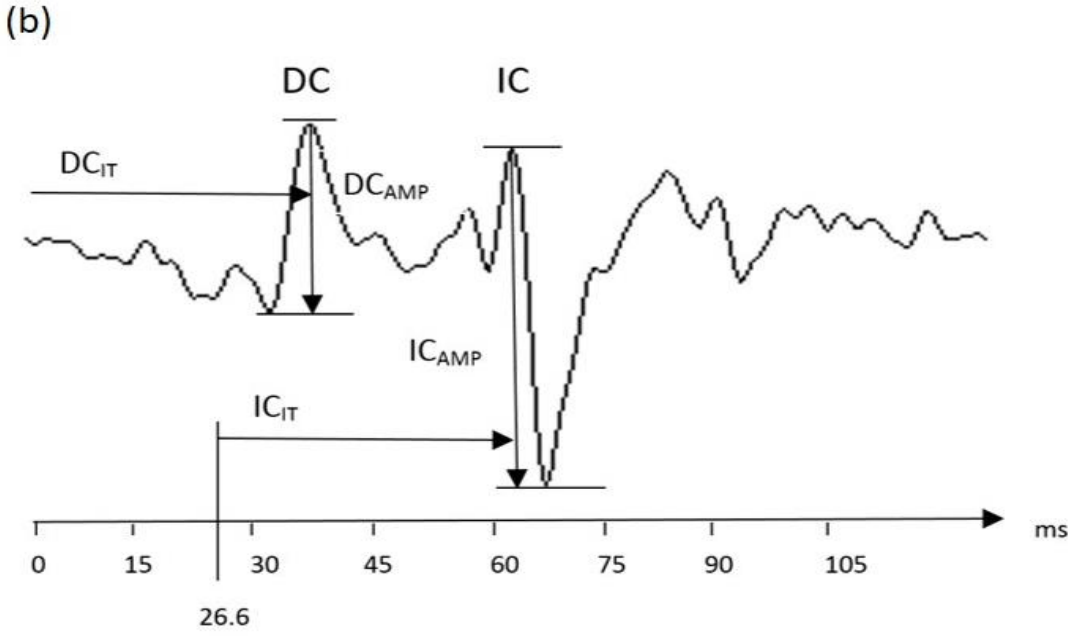


Figure 2.3. (a) The global flash (MOFO) paradigm is composed of four video frames: starting with a frame of multifocal flashes, followed by a dark frame, a full screen flash frame, and a second dark frame in each slice of the pseudorandom binary m-sequence. (b) A typical waveform of the MOFO mfERG response and its components (DC and IC).

In clinical studies, the DC has been shown to be sensitive to early changes in retinal function of subjects with diabetes (Shimada et al., 2001) and age-related maculopathy (Feigl et al., 2005). The IC was found to be reduced in glaucoma (Palmowski et al., 2002) and hydroxychloroquine retinopathy (Penrose et al., 2003). Fortune and co-workers investigated the characteristics of the MOFO mfERG response to discriminate glaucoma sufferers from normal subjects, reporting a sensitivity of 75%, with a specificity of 83% (Fortune et al., 2002). Chu and co-workers further developed a luminance modulation protocol for the global flash mfERG and derived an indicator, named adaptive index, to detect the early retinal function changes of glaucoma. This use of adaptive index was reported to have 93% sensitivity and 95% specificity for the detection of glaucomatous damage (Chu et al., 2006). Another similar modification of this protocol to detect glaucoma was the use of a "two global flash" paradigm, which was shown to have 85% sensitivity and 80% specificity (Palmowski-Wolfe et al., 2007).

2.2 Multifocal Electroretinogram Changes in Myopia

Several studies have investigated retinal function in myopia by mfERG measurement. It was found that the response amplitude decreased and implicit time increased in young adults with moderate or high myopia (Kawabata and Adachi-Usami, 1997, Westall et al., 2001, Chan and Mohidin, 2003, Chen et al., 2006a, Chen et al., 2006d). Response amplitudes from the retinal peripheral regions were decreased by a greater degree than those at central regions (Kawabata and Adachi-Usami, 1997, Chan and Mohidin, 2003). Implicit times were delayed more in the inferior region than the superior (Kawabata and Adachi-Usami, 1997). Westall and colleagues reported that there was a linear reduction in ERG amplitude with increasing axial length in healthy young adults (Westall et al., 2001). Chan and Mohidin demonstrated that the averaged mfERG amplitude decreased by

6-10% per millimeter elongation of axial length (Chan and Mohidin, 2003). All these changes in myopia are believed to originate from outer retinal function changes. The inner retinal function in myopia was studied using the modulation protocol of mfERG. In these studies with MOFO mfERG, there was a significant correlation between the DC or IC response amplitude and myopic refractive error, indicating that the retinal adaptation response varied according to the degree of myopia (Chen et al., 2006c). The implicit time of the oscillatory potentials from a modified mfERG stimulation in progressing myopes was also found to be significantly shorter than that of emmetropes or stable myopes (Chen et al., 2006b). These findings demonstrate that inner retinal change is involved in myopia progression.

The electro-retinal response changes in myopia had been suggested to be primarily due to the increased axial length that accompanies myopia development. Researchers proposed the rationale of effects of an increase in ocular resistance (Perlman et al., 1984) and the sub-retinal space (Huang and Karwoski, 1990), reduced image size, decreased retinal illumination, and the reduced retinal cell density in an elongated eyeball (Beresford et al., 1998, Chan and Mohidin, 2003). Perlman first suggested that axial elongation increased ocular resistance, which was the resistance to the electrical signal by ocular tissues and structures, which caused the reduction in electro-retinal responses. However, findings of later studies failed to confirm this hypothesis. Kawabata and chi-Usami believed that the reduction of electro-retinal activity in myopia measured by conventional mfERG protocol was due to the loss of cone cells, associated with ocular expansion (Kawabata and Adachi-Usami, 1997). Chan and Mohidin, examining the correlation between axial length and the mfERG, determined that the reduction in responses to morphological changes was associated with increased axial length (Chan and Mohidin, 2003). Chen and coworkers, investigating the

contribution of axial length to the mfERG responses, showed that axial length contributed to 15% of the implicit time total variance, while refractive error accounted for 27% (Chen et al., 2006a). Therefore, delayed mfERG responses observed in myopes were not completely attributable to the anatomical change in myopia, and the findings of the studies suggested that there were underlying differences in retinal function of myopic eyes. In addition, if the reduction in mfERG responses in myopic adults could be simply explained by the deterioration in retinal function with longstanding myopia, such an effect would not be expected to be observed in myopic children either. (Luu et al., 2006) and (Ho et al., 2012b) both observed differences in the characteristics of electro-retinal responses of myopic adults and children. Luu and his colleagues conducted a cross-sectional study of mfERG measurement in 104 children and 31 adults with a range of refractive errors. They found a significant correlation between refractive error and mfERG response in adults, but a similar correlation was not observed in the children. Ho and co-workers also illustrated different characteristics of retinal electrophysiological activities in adults and children in terms of retinal regions and mfERG components. Fifty-two children and 19 young adults with spherical equivalent refractive errors ranging from Plano to -5.50 D were examined using the MOFO mfERG at 49% and 96% contrast. It was reported that myopic children had central reduction in high contrast multifocal ERG response, while adults had paracentral reduction in low contrast response (Ho et al., 2012b).

In order to investigate the changes of retinal function with progressing myopia, longitudinal studies were performed on children with continuing myopia development. Luu and his co-workers followed 81 Singapore children for two years and divided these children into three subgroups according to their myopic progression rate (Luu et al., 2007). They reported that the fast myopic progression subgroup had decreased central mfERG amplitudes at the initial visit. A significant

correlation was also found between the baseline central mfERG amplitude and the change in vitreous chamber length, but not with the change in refractive errors. They suggested that central retinal function may be a predictor of myopia progression rate in children. The subjects recruited in their study were all myopic at the initial visit, with a large variation in refractive error ranging from -1D to -5.88D. As children with higher myopic refractive errors have been reported to have more changes in the electro-retinal activity (Ho et al., 2012b), this may have affected the findings. Another longitudinal study investigated changes in global flash mfERG responses in children with myopia progression. A total of 26 myopic children were followed for 1 year. With low contrast level stimulation global flash mfERG, central DC and IC amplitudes were significantly reduced and found to be correlated with the changes in myopic refractive error (Ho et al., 2012a). These findings further indicate that central retinal function may be associated with myopic development. Investigation of central retinal activity, including both outer and inner retinal functions, in young children may be useful in monitoring myopia progression, estimating final refractive error, or even predicting myopia development before it occurs. However, the electro-retinal changes preceding myopia have not been investigated previously.

In summary, myopia progression or axial elongation is associated with reduced amplitude and/or delayed implicit time. There are differences in the characteristics of retinal electrophysiological activities between adults and children in terms of retinal regions and mfERG components. The retinal functions are affected by myopia or axial elongation, and the investigation of retinal function could help to predict myopia progression or even future development.

Chapter Three

Retinal thickness and Myopia

As discussed in the previous chapter, changes of retinal function in myopic eyes have been widely reported. In terms of structural changes, retinal thickness was also reported to be altered in myopic eyes compared to emmetropic eyes (McBrien and Gentle, 2003, Rymer and Wildsoet, 2005, Rada et al., 2006). However, only limited studies have investigated how the changes of retinal structure are related to functional changes in myopia.

3.1 Retinal Thickness in Myopia

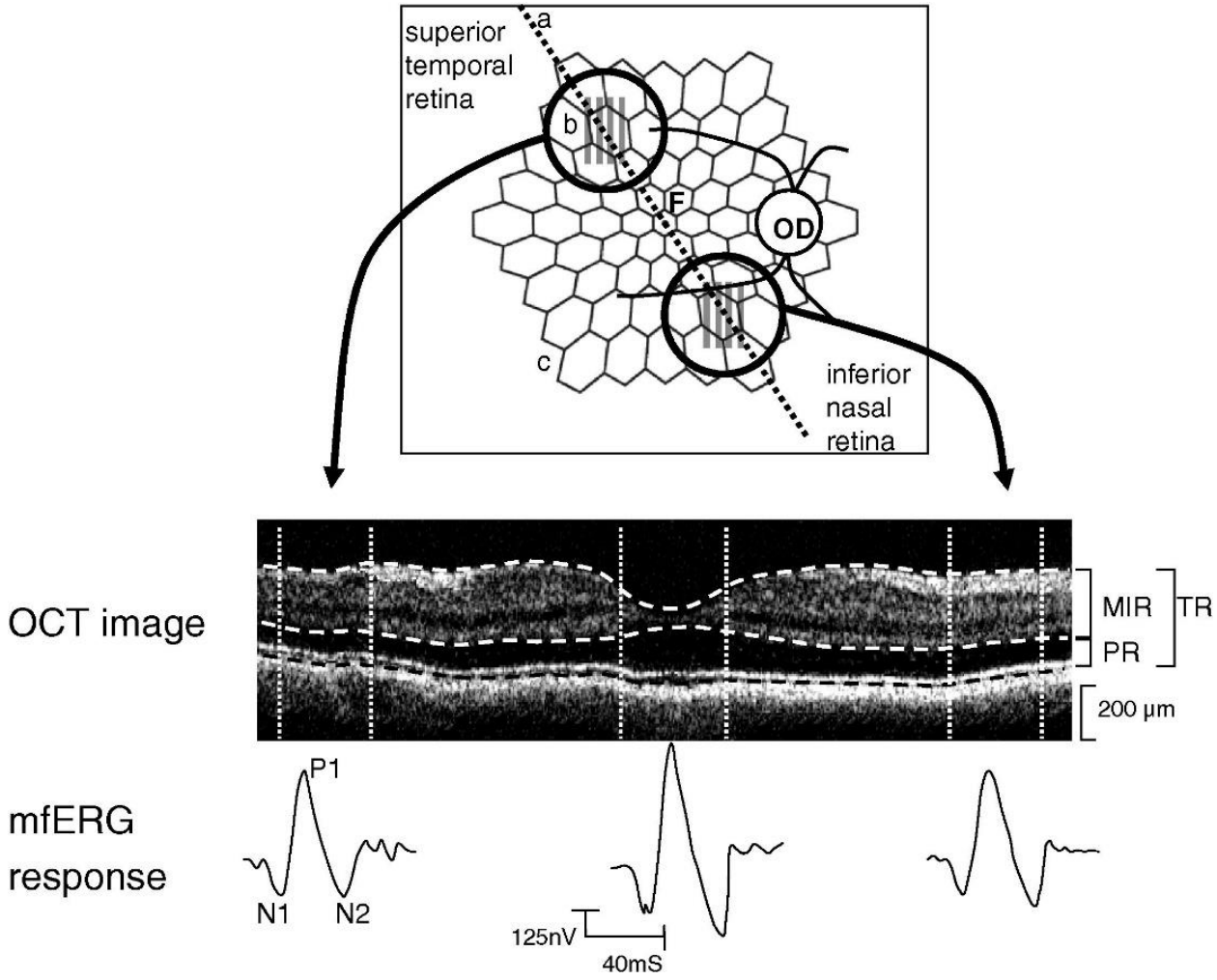
Ocular structural changes in myopia were observed in animal models with both lens-induced and form-deprived myopia, and a direct relationship between retinal thickness and the severity of myopia was reported (Hung et al., 2000, Beresford et al., 2001). In human myopic eyes, the mechanical forces that are involved in eyeball enlargement and retinal stretching make the fovea particularly vulnerable to change (Springer and Hendrickson, 2004). With the application of Optical Coherence Tomography (OCT) *in vivo* imaging, researchers have shown a significant correlation between macular total thickness/volume and axial length/refractive error (Sato et al., 2010, Song et al., 2014). Longer axial length was associated with a significantly thicker central retina (Wong et al., 2005). Thinning of the parafoveal retina, thickening of the foveal pit, and smaller macular volume were found in moderate and high myopic adults (Lim et al., 2005, Lam et al., 2007, Wu et al., 2008, Wolsley et al., 2008, Song et al., 2010). These changes were also observed in young myopic children. Studies in Singapore and China showed that children with moderate myopia usually had smaller total macular volume and thinner quadrant-specific macular thickness, indicating that early anatomical changes were present even in the retinas of myopic children (Luo et al., 2006b, Zhang et al., 2011). Another clinical study investigated retinal thickness in 276 Chinses

schoolchildren aged 7-13 years. It reported that myopic retinas were thinner than those with emmetrope or hyperope in the superior parafoveal and all 4 perifoveal subfields. Central foveal thickness was not shown any changes in children of different refractive status (Jin et al., 2016). These studies have provided detailed information on the normal range of retinal thickness and a subtle increase in total retinal thickness and change throughout childhood. It should be noted that all of these studies only had involved normal children in cross-sectional study designs. To date, only one study of Read and his team has investigated the changes in macular retinal layer thickness associated with myopia in childhood, and the normal changes occurring in retinal layer thickness over time in a healthy pediatric population (Read et al., 2017). Total retinal thickness and the changes in retinal morphology occurring over an 18-month period were examined in 101 children with a range of refractive errors. In childhood, the presence of myopia was associated with subtle but statistically significant changes in macular retinal thickness, with a thinning of the parafoveal retina. However, over 18 months, longitudinal changes in retinal thickness and individual layers were of small magnitude, indicative of a high degree of stability in retinal morphology in healthy adolescent eyes, despite significant eye growth over this same period of time (Read et al., 2017). In the current study, we aimed to further expand knowledge of the changes in macular thickness preceding the myopia onset in young children.

3.2 Correlation between Retinal Thickness and Function

Myopic changes to the retinal thickness have been observed in many studies and functional changes in myopic eyes are widely recognised. Therefore, further research to investigate the link between retinal structure and visual function has been conducted. Wolsley and co-workers found significant retinal disorders in moderate and high myopes, using both structural and functional

measures (Wolsley et al., 2008). They examined the eyes of 56 subjects aged between 19 and 45 years, with refractive errors of +0.50 to -15.00 D. For each subject's eyes, comparative retinal structure-function data were generated at the foveal region and 12°-16° in the inferior nasal and superior temporal retina (Figure 3.1). Reduced mid-inner retinal layer thickness was associated with myopia-related losses in neural activity derived from resolution acuity and mfERG timing in the peripheral retina.



Adopted from: Wolsley, Clive J.; Saunders, Kathryn J.; Silvestri, Giuliana; Anderson Roger S. Investigation of changes in the myopic retina using multifocal electroretinograms, optical coherence tomography and peripheral resolution acuity. Vision research 2008; 48(14):1554-1561.
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Figure 3.1. A schematic diagram shows the location the OCT scan lines and mfERG stimuli in relation to the fovea and optic disc (OD). Total retinal (TR), mid-inner retinal (MIR) and photoreceptor retinal (PR) thickness, measured from OCT were compared to mfERG responses in the foveal region, inferior nasal and superior temporal retina.

Another study confirmed that significant correlations existed between mid-inner retinal thickness and mfERG response amplitude and implicit time in the perifoveal retina of myopic eyes (Park et al., 2013). 90 myopic subjects underwent standard mfERG and OCT measurement. Retinal thickness was measured in horizontal scans at several regions: fovea, perifoveal 2.0 mm nasal and temporal from the foveola. Multifocal ERG responses from ring 1 and ring 4 (outer radii approximately 1.6° and 12° respectively), which correspond to the area measured by OCT, were obtained to evaluate structural and functional correlations. They reported that mid-inner retinal layer thickness in the peripheral retina was significantly correlated with implicit time as well as amplitude from mfERG. These findings were similar to those of Wolsley et al. (2008). A further two recent clinical studies have also illustrated correlation between mfERG parameters and OCT macular and retinal nerve fiber layer thickness (Koh et al., 2014, Song et al., 2016). Together these findings suggest that retinal thickness, especially the post-receptoral layer, and function are vulnerable to disruption in moderate and high myopia.

The origin of the structural/functional changes in myopia has mostly been attributed to axial elongation. The expansion of the eye length can lead to adverse effects on the retina as a result of retinal stretching and thinning. These changes may subsequently lead to impaired electro-retinal response. Although a strong association between reduced visual function/retinal thickness and presence of myopia has been reported, there are some discrepancies in the association when comparisons are made between the structural/functional changes observed in myopic adults and children. Hence, the relationship between retinal function and structure remains somewhat equivocal. Thus, further studies are required to determine this relationship in children and how this relationship evolves over time.

Chapter Four

Research Questions and Objectives

4.1 Research Questions

After consolidation of the findings from previous studies on myopia, there appears to be a lack of knowledge in terms of longitudinal changes in linking peripheral refraction (optics), electro-retinal activities (function), and retinal thickness (structure) to juvenile myopia development.

The hypothesis of peripheral defocus is inconclusive. Although it has been demonstrated in animal models that the peripheral retina could independently mediate ocular growth under defocus, longitudinal clinical studies in children were not able to establish a predictive role for peripheral defocus in either myopia onset or progression.

Electro-retinal responses were found to be altered in myopic eyes. However, different characteristics of the retinal electrophysiological activities were observed between myopic adults and children in terms of both the retinal regions involved and the ERG components. It is still unknown how the retinal function changes from childhood to adulthood with concurrent myopia development. Recently, the use of a modified mfERG protocol to derive different components of retinal function has shown increased potential to detect myopic retinal changes. Longitudinal studies could help to determine the predictive role of retinal function in myopia development.

Several studies suggested early anatomical changes presented in the central retina of myopic children. However, there is a lack of knowledge of the longitudinal changes in central retinal thickness in children with myopia development. Although a strong association between reduced visual function/retinal thickness and presence of myopia have been reported, the relationship between retinal

function and structure remains unclear, and further studies are required to determine this relationship in children.

4.2 Research Hypothesis

Most previous studies have used ophthalmic lenses to induce myopia in animal models. Once the refractive errors of the animal eyes reached the desired power of the induced lenses, the refractive errors would be stable and further changes limited. As the refractive error matched the induced power, the distance vision of the animal was assumed to be clear. These results indicated that the development of myopia in these studies is also an emmetropization process, which simply compensates for the induced defocus from the ophthalmic lenses in order to achieve clear distance vision. Therefore, the end points of refractive errors in these induced myopia models were likely controlled by a visual feedback mechanism to achieve clear central vision. In primate studies with foveal photo-ablation, myopia developed as a result of the presence of peripheral defocus (Smith et al., 2007). However, the end points of the refractive errors were found to fluctuate considerably, with large variations compared with use of the same model, but with intact foveal structure/function. The significant variation of the final refractive errors in the foveal photo-ablation model indicates that the feedback mechanism for emmetropization may not function properly if the central retina (ie. fovea) is not intact or not functioning well. The feedback system, hence, may be related to foveal activity. In addition, the central retina is the anatomical site with the highest spatial resolution, which may also be the optimal location to determine the end point of emmetropization.

Clinical observation has revealed that myopic children can still have obvious myopic progression even after they have been prescribed with spectacles providing full correction of refractive error.

This progression is not coherent with the findings in animal models of emmetropization. In addition, in various myopia intervention clinical trials involving application of peripheral myopic defocus, some subjects did not respond well to the treatment and still had myopic progression after they were fully corrected for both central and peripheral refraction. In the cases in which the treatment was not as effective as was expected, it is possible that the feedback system of the eye for the end-point determination did not function properly. It was noted that children with high myopia had weaker subclinical foveal responses, which were assessed with the mfERG, than those with low myopia (Ho et al., 2012a; Luu et al., 2007). Thus, the foveal function of children could be hypothesized to be a contributor to the feedback mechanism in juvenile myopia development. It is possible that peripheral defocus could trigger myopia development and that foveal function is involved in determining the end point of myopia development. Therefore, the foveal function in children could be important in this feedback mechanism facilitating ocular growth. As the foveal function can be altered by either neural or structural factors, the interaction between retinal electrophysiological function, peripheral refraction, and central retinal thickness in children may be the keys to understanding the onset of myopia and prediction of the rate of progression.

4.3 Research Objectives

1) To study the changes of retinal electrophysiological function, peripheral refraction, and central retinal thickness associated with myopia development in children.

2) To study the interaction between retinal electrophysiological function, peripheral refraction, and central retinal thickness on myopic progression in children over a 3-year study period.

3) To determine whether the retinal electrophysiological function, peripheral refraction, and central retinal thickness are possible precipitants of myopia development in children.

Part II General Methodology

Chapter Five

Study Design

5.1 Subject Recruitment and Schedule

The subjects were screened and recruited at the Optometry Research Clinic of The Hong Kong Polytechnic University. All subjects underwent a comprehensive eye examination, including objective cycloplegic refraction and ocular health assessment. The resolution of refraction was 0.25D. Visual acuity was determined with a Thomson computerized chart. Axial length measurement was conducted with an IOL master (V.4.08; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Five readings with a range of less than 0.10 mm were averaged. Color vision was evaluated with a 24-plate version of the Ishihara color vision test and the passing criterion was defined as correct recognition of all 24 plates. The inclusion criteria were best corrected logMAR visual acuity of 0.00 or better, normal color vision, cycloplegic refractive error between +1.00D and -1.00D, astigmatism less than 1.00D, and normal ocular health in both eyes. Subjects with a family history of inherited ocular diseases, clinically significant retinal degeneration, or any systemic disease were excluded from the study. A total of 106 subjects were recruited at the baseline visit. All subjects were aged 6 to 9 years with near-emmetropic refractive error.

All the subjects were scheduled to attend four visits for cycloplegic eye examination, initially and at 12-month intervals over the following 3 years. One drop of 0.4% oxybuprocaine (Agepha Pharmaceuticals, Wien, Austria) and two drops of 1% Tropicamide (Alcon Laboratories, Inc., Fort Worth, Tx, USA) were instilled at 5-minute intervals into both eyes 30 minutes before all tests. Two drops of 1% tropicamide have been proven to be equivalent to cyclopentolate in terms of the cycloplegic effect (Egashira et al., 1993). The MOFO mfERG was performed prior to peripheral refraction and OCT measurement to avoid any influence of previous tasks. During the 3-year study period, new pairs of spectacles with a fully corrected prescription would be provided to any of the

subjects who became myopic (defined as more than -1.00D) or had refractive error changes over -0.5D. All the subjects were prescribed with a same brand and design of single vision spectacles. Additional follow-up of refraction every 6 months was arranged to monitor their myopic progression.

The study was approved by the Human Ethics Committee of The Hong Kong Polytechnic University and adhered to the tenets of the Declaration of Helsinki. The parents of the subjects gave written informed consent for their children to participate in this study after fully understanding all of the details.

5.2 Instrumentation

The range of data collected included cycloplegic objective/subjective refraction, axial length with Zeiss IOL Master, peripheral refraction with Shin Nippon autorefractor (model NVision-K5001, Ryusyo Industrial CO., LTD, Kagawa, Japan), global flash mfERG recordings with VERIS system (VERIS Science 6.0.6d19, EDI, San Mateo, CA, USA), and macular thickness with spectral domain optical coherence tomography (SD-OCT, Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany).

5.2.1 Peripheral Refraction with Shin Nippon Autorefractor

The Shin Nippon autorefractor is a wide field binocular open-view autorefractor allowing objective measurement of refractive error. The repeatability and validity of the measurement has been established (Chat and Edwards, 2001, Mallen et al., 2001). It has been widely used in peripheral refraction studies and is considered to be the principle instrument of choice (Fedtke et al., 2009).

In order to measure peripheral refraction, modification of the instrument was required. A rod was attached to the autorefractor to present the fixation target of LED light at a viewing distance of 1 m (Figure 5.1). This swinging rod could be rotated to the desired angle of fixation. When the subject was fixating with the right eye to his/her temporal field, measurement was taken from the temporal retina (corresponding to the nasal visual field).

Peripheral refraction across the central 60° horizontal field was measured. During the measurements, subjects were asked to turn their eyes to follow the fixation target, and their heads were firmly pressed against the forehead and chin rests, such that they were not able to turn their head. Peripheral refraction was determined at 0° of center, and then at 10°, 20°, and 30° angles of the nasal (N10, N20, N30) and temporal retina (T10, T20, T30). Five repeated measurements were made at each angle and averaged. Refraction was measured at least 30 minutes after dilation. Enlarged pupil size enabled the acquisition of more peripheral refraction and its cycloplegic effect also minimized the accommodation effect on results. The measurement repeatability has been previously published (Lee and Cho, 2012) (Appendix A).

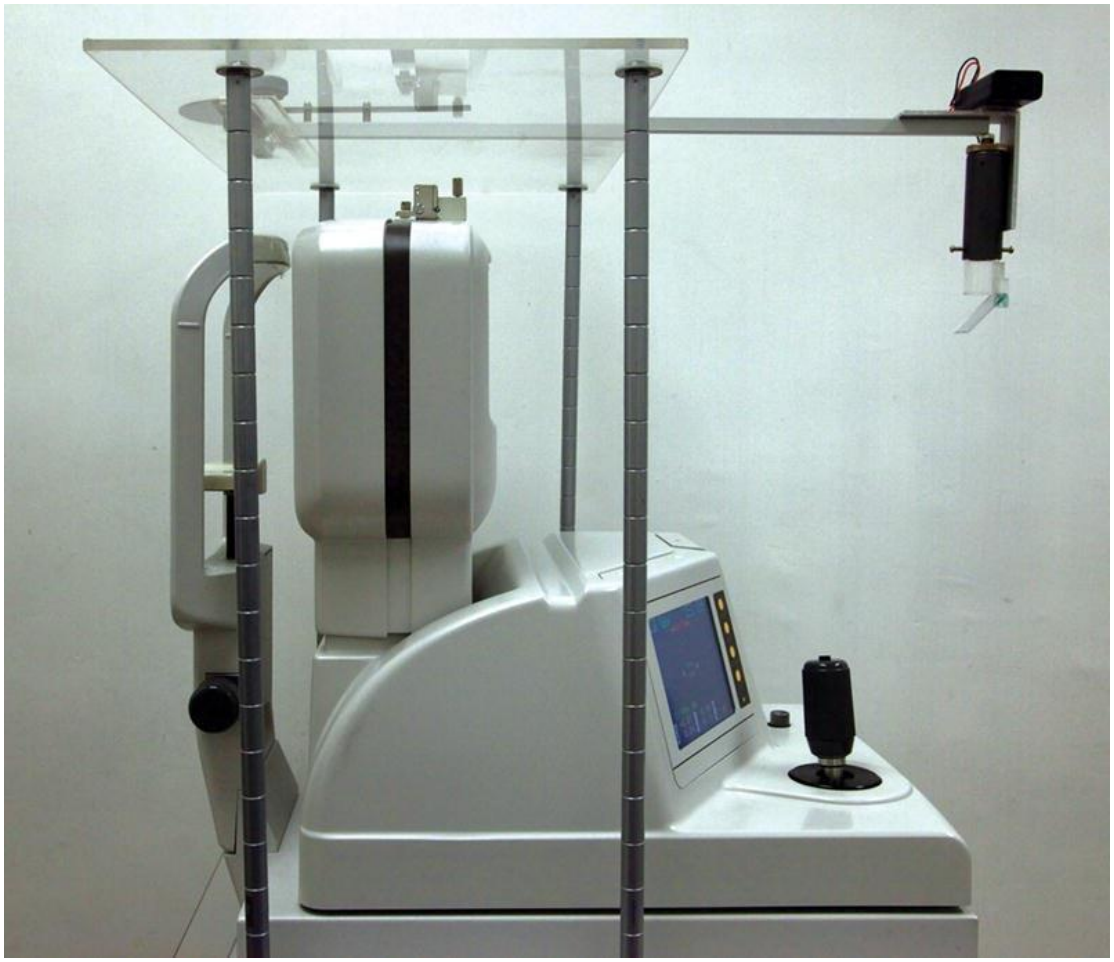


Figure 5.1. Shin Nippon open-view autorefractor (model NVision-K5001, Ryusyo Industrial CO., LTD, Kagawa, Japan) with an external fixation system.

5.2.2 Multifocal Electroretinogram

The mfERG setting is shown in Figure 5.2. The electro-retinal responses from the subject's right eye were recorded with Dawson-Trick-Litzkow (DTL) fiber acting as an active electrode (located on the cornea of the right eye) and gold-cup surface electrodes as reference (located at the outer canthus of the right eye) and ground (located at the forehead). The recordings were commenced after the pupil of the tested eye was dilated to at least 7 mm in diameter. The stimulus pattern was generated by the Visual Evoked Response Imaging System and displayed on a 22-inch LED monitor (model VG2239M-LED, ViewSonic, CA, USA.). The stimulus pattern consisted of 61 hexagons subtending 39º horizontally and 33º vertically at a working distance of 40 cm. Full correction was provided to compensate for subjects' sphero-cylindrical refractive error for the working distance.

The global flash (MOFO) mfERG paradigm was composed of four video frames as shown in Figure 2.3 (a): starting with a frame of multifocal flashes, followed by a dark frame, a full screen flash frame, and ending with a second dark frame in each slice of the pseudorandom binary m-sequence (2^{12}-1). Frame frequency of the monitor was set at 75 Hz. The luminance of the multifocal flash stimulus in light and dark states were 183 cd/m^2 and 4 cd/m^2, respectively, for 96% contrast level, and, 140 cd/m^2 and 48 cd/m^2, respectively, for 49% contrast level. The mean luminance of the multifocal flashes and the background was approximately 94 cd/m^2 for both contrast levels. The recording time of 4 min for each contrast level, was divided into 16 segments to allow the subject to rest between runs. A central cross in the stimulation pattern was used for fixation. The signal was monitored using the real-time responses provided by the VERIS program and any segment contaminated by blinks or fixation loss was re-recorded. An amplifier (model 15A54,

Physiodata Amplifier System; Grass Technologies, Astro-Med, Inc., West Warwick, RI) was used with a signal gain of 100,000 times and a band-pass of 10 – 300 Hz.

Groups of responses from the MOFO mfERG trace arrays were averaged into five successive rings from the center to the periphery. The peak-to-peak amplitudes of the DC and the IC responses were calculated (Figure 2.3 (b)). The implicit times of DC and IC response were counted from the onset of multifocal flash and global flash respectively, to the peak of the response. The validation and repeatability of the MOFO mfERG measurement in children has been reported previously (Li et al., 2017) (Appendix B).

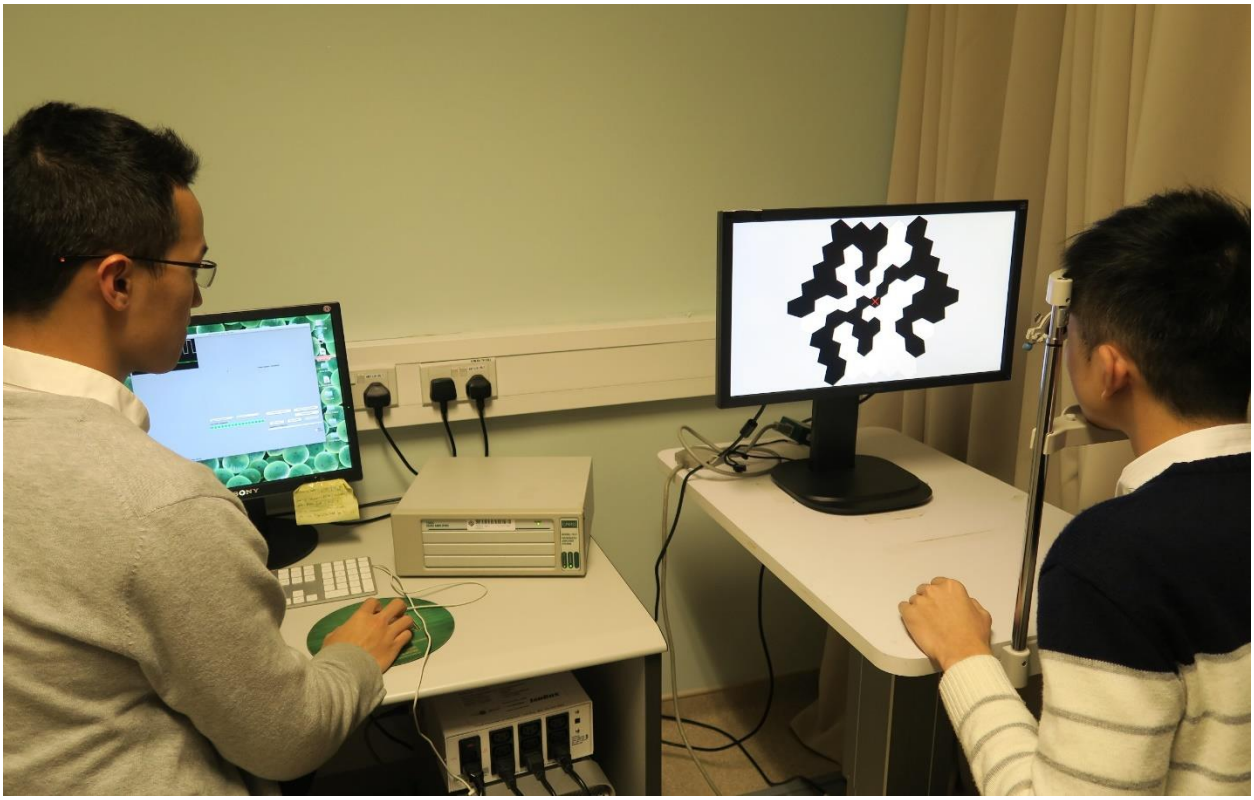


Figure 5.2. Experimental setting of the mfERG recording.

5.2.3 Spectral Domain Optical Coherence Tomography

Retinal structural imaging was captured by spectral domain optical coherence tomography. This device utilized a laser source with a peak wavelength of 870 nm and a scanning speed of 40,000 A-scans per second. The cross-sectional and volume scanned images were provided with axial resolution of 3.9 μm and transverse resolution of 14 μm. At each visit, 20°x20° fovea-centered volume scans (consisting of 19 line-scans) of subjects' right eyes were obtained. Automatic real-time tracking was employed with 35 frames, which enabled 35 averaged B-scans at each scanning position. Images with a scan quality index of greater than 20 dB were accepted.

Central retinal thickness, the distance from the Bruch's membrane to the inner limiting membrane, was segmented and measured automatically with built-in Heidelberg Eye Explorer software (Version 5.8.3.0, Heidelberg Engineering, Heidelberg, Germany). The average macular thickness was analyzed across three concentric annular rings around the fovea. As shown in Figure 5.3, macular thicknesses were averaged according to the ETDRS grouping: within a central foveal ring (central 1 mm diameter), an inner macular ring (from inner diameter of 1 mm to outer diameter of 2 mm), and an outer macular ring (from inner diameter of 2 mm to outer diameter of 3mm). The inner and outer macular rings were further divided into four segments: superior, temporal, inferior and nasal. Average thickness and volume of each segment was determined for analysis. The integrity of the segmentation was checked and segmentation errors were corrected by a professional optometrist. The validation and repeatability of the OCT measurement in children have been reported previously (Li et al., 2017) (Appendix C).

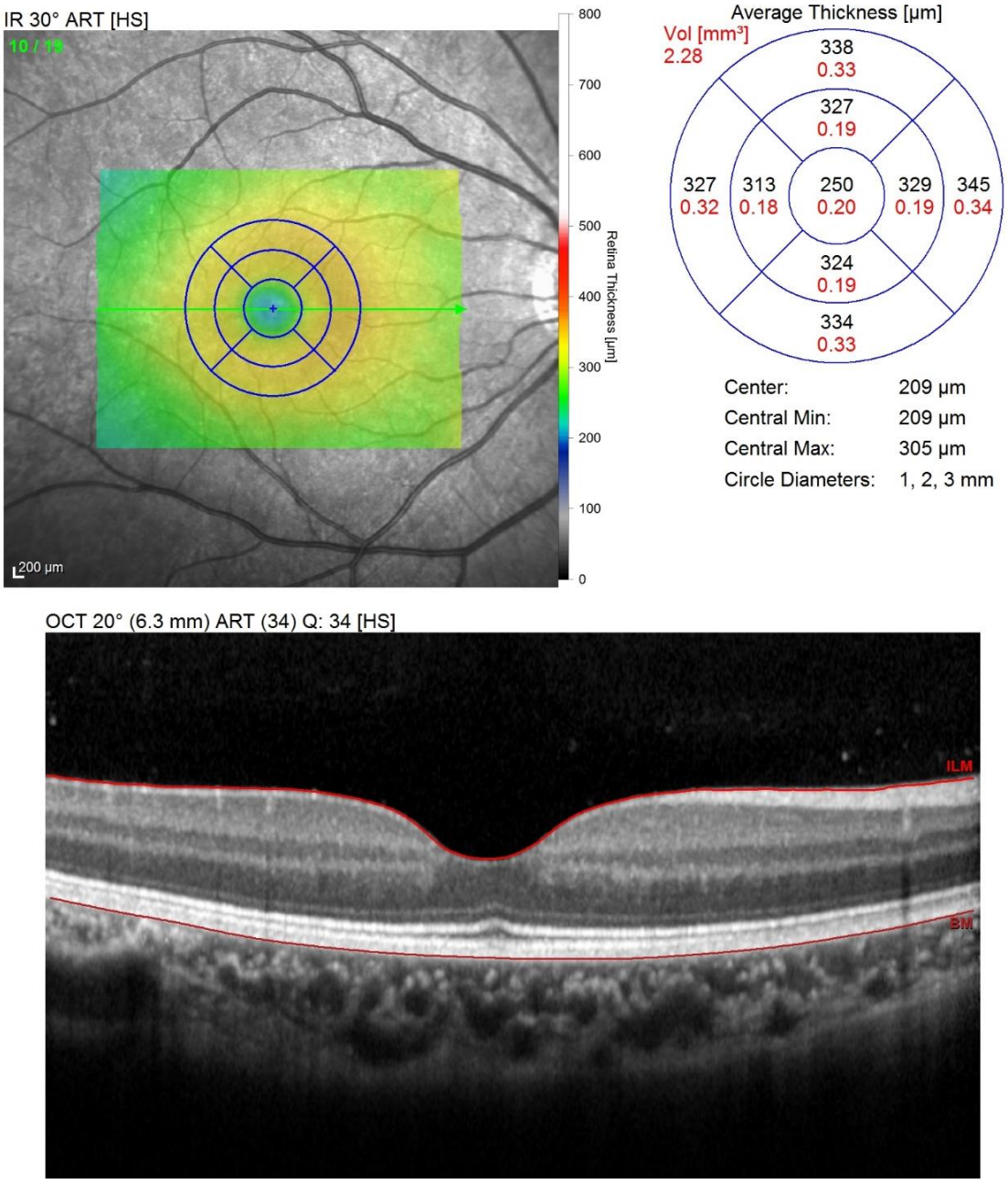


Figure 5.3. An example of a macular thickness report from a representative subject.

Part III Experimental Results and Discussion

Chapter Six

Peripheral Refraction and Myopia

6.1 Introduction

Variant studies have reported that emmetropes and hyperopes usually have relative peripheral myopia, while myopes tend to have relative peripheral hyperopia (Mutti et al., 2000, Seidemann et al., 2002, Atchison et al., 2006). However, it has not been fully elucidated whether eyeball elongation in the myopic eye results in peripheral refraction shifting or peripheral hyperopic defocus causes accelerated eye growth. The hypothesis that peripheral hyperopic defocus triggers myopia development in children, has not been substantiated (Mutti et al., 2011, Sng et al., 2011, Lee and Cho, 2013, Atchison et al., 2015). This chapter reports the peripheral refraction changes in a group of subjects followed up for three years, who had near emmetropic refraction at their baseline visit. The aim of this study was to determine the association between peripheral hyperopic refraction and development of juvenile myopia.

6.2 Methods

Subject recruitment and inclusion criteria have been described in Section 5.1. Subjects underwent cycloplegic refraction, axial length measurement, and peripheral refraction at 12-month intervals for three years. Only data from the right eye were included in the analysis. The instrument set-up and measurement procedure were reported in Section 5.2.1. Peripheral refraction (PR) was measured across the horizontal meridian at the eccentricities of 10, 20, and 30º of both the temporal and nasal retina. For a measurement of the right eye, when the fixation target was turned to the subject's temporal side, the results were taken from the temporal retina, corresponding to the refractive errors in the nasal visual field. Relative peripheral refraction (RPR) was computed by subtracting central refraction spherical equivalent from that of the peripheral refraction at each eccentric field

angle. Each sphero-cylindrical refractive error measurement was decomposed into vector components using the following equations:

$$J0 = -\frac{C \cos 2\theta}{2}$$

$$J45 = -\frac{C \sin 2\theta}{2}$$

$$M = S + \frac{C}{2}$$

Where J0 and J45 are the powers of Jackson cross-cylinder components; M is the spherical equivalent; S is the spherical power; C is the cylindrical power and θ is the axis of cylinder.

Subjects were retrospectively divided into three subgroups based on their central refraction at the last visit for analysis. At the end of the study, subjects with central refraction (spherical equivalent refraction) between -1.00D and +1.00D were placed in the emmetropic group (E), while the low myopic group (LM) was defined as between -1.00D and -3.00D, and the moderate myopic group (MM) was defined as equal to or higher than -3.00D of myopia.

The normality of the variables was determined by the Shapiro-Wilk Test (SPSS 23.0). Non-parametric tests were applied to those variables violating normal distribution. A Friedman test was conducted to determine whether there were differences in cycloplegic central refraction over the four visits. A one-way repeated measured analysis of variance (ANOVA) was conducted to determine whether there were differences in axial length, peripheral refractions, and relative peripheral refractions over the four visits. A Spearman's rank-order correlation was applied to assess the relationship between the 1-year, 2-year, and 3-year changes in central refraction and relative peripheral refraction in M component, while a Pearson's correlation was run for the relationship

among yearly changes in axial length. For the subgroup comparison, a one-way repeated ANOVA was conducted to determine if the 3-year changes in peripheral refraction and relative peripheral refraction were different among three subgroups. An adjusted significance level of $p < 0.01$ was applied in the statistical analysis to reduce the chance of Type 1 errors with multiple testing.

6.3 Results

6.3.1 Central Refraction and Axial Length at Each Visit

One hundred and six children who met with the inclusion criteria were recruited at the baseline visit. The age of the subjects was 7.53 ± 0.97 years (mean ± standard deviation). A total of 18 subjects dropped out: eight, four, and six subjects at the second, third, and last visits, respectively, leaving 88 (83% of enrolled subjects) children who finished the whole study. At the last visit, there were 54, 21 and 13 subjects in the emmetrope (E), low myope (LM), and moderate myope (MM) groups, respectively. The overall cycloplegic central refraction and axial length at each visit are summarized in Table 6.1.

Table 6.1 Cycloplegic objective central refraction and axial length at each visit

Visit/n	Cycloplegic Objective Refraction ± SD (D)			Axial Length ± SD (mm)
	M	J0	J45	
V0/106	+0.22±0.56*	+0.14±0.20	-0.05±0.11§	22.92±0.80*
V1/98	-0.12±1.11*	+0.15±0.20	-0.03±0.11	23.28±0.89*
V2/94	-0.47±1.40*	+0.18±0.23	-0.01±0.11§	23.55±0.98*
V3/88	-0.88±1.73*	+0.18±0.24	-0.02±0.15	23.79±1.05*

V0 – baseline visit, V1 – 1st year follow up, V2 – 2nd year follow up, V3 – 3rd year follow up

* Each pairwise difference was significant, $p < 0.0005$.

§ Difference between V0 and V2 was significant, $p = 0.008$.

All subjects displayed a myopic shift over the 3-year study period. A Friedman test was conducted to determine the differences in cycloplegic central refraction, in terms of M, J0, and J45 components, among the four visits. Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. M component differed significantly between different visits (χ^2 (3) = 89.299, $p < 0.0005$). The mean central refractive error changed from a mild hyperopic power (+0.22±0.56D) at baseline to a near myopic power (-0.88±1.73D) after three years. Post hoc analysis indicated that each pairwise difference was significant ($p < 0.0005$). The significant decrease in the M component of refraction over time suggested that the central refractive error of subjects continuously changed towards myopic. There were no significant differences in J0 component over the four visits, while J45 component was statistically significantly different between visits (χ^2 (3) = 8.363, $p = 0.039$). Post hoc analysis revealed a statistically significant difference in J45 component only between the baseline visit and the third visit ($p = 0.008$).

A one-way repeated measured analysis of variance (ANOVA) was conducted to determine the differences in axial length between the four visits. There were no outliers and the data were normally distributed, as assessed by boxplot and Shapiro-Wilk test ($p > 0.05$), respectively. The assumption of sphericity was violated, as assessed by Mauchly's test of sphericity (χ^2 (5) = 272.290, $p < 0.005$), and a Greenhouse-Geisser correction was therefore applied ($\varepsilon = 0.398$). There was statistically significant axial elongation over the 3-year period (F (1.193, 99.037) = 248.603, $p < 0.0005$, partial $\eta^2 = 0.750$), with a mean axial length of 22.92 mm at the baseline visit, 23.28 mm at the second visit, 23.55 mm at the third visit, and 23.79 mm after three years. Post hoc analysis with a Bonferroni adjustment revealed that each pairwise difference was significant ($p <$

0.0005). Thus, there was a significant increase in axial length over time, suggesting that subjects had a continuous eyeball growth.

6.3.2 Peripheral Refraction

6.3.2.1 Peripheral Refraction at Each Visit

The peripheral refraction data at each visit are listed in Table 6.2 to 6.5. A one-way repeated measures ANOVA was conducted to determine the changes in peripheral refraction, in terms of M, J0, and J45 components and spherical power over the 3-year study period.

The M component and spherical power of the peripheral refraction became more myopic and less hyperopic with time. M component and spherical power at all retinal angles were statistically significantly different over the four visits (all $p < 0.0005$). The J0 component at both the retinal temporal and nasal 30° angle differed significantly over the course of the study ($p < 0.0005$). However, there were no significant differences in the J45 component over the four visits at any retinal angle. These findings indicate that the spherical power and M component of peripheral refraction were changing, while peripheral oblique astigmatism (J45) had stabilized during the early childhood. The cardinal astigmatism (J0) at the far peripheral retina (30° eccentricity) showed a decrease in against-the-rule (ATR) astigmatism.

Table 6.2 The M component of peripheral refraction at each visit

Visit/n	Peripheral Refraction M ± SD (D)					
	Retinal Angles (°)					
	N30*	N20*	N10*	T10*	T20*	T30*
V0/100	+0.07±1.28	+0.02±0.93	-0.13±0.86	-0.18±0.81	-0.63±1.00	-1.25±1.69
V1/98	+0.19±1.09	-0.11±1.02	-0.22±1.00	-0.28±0.98	-0.64±1.08	-0.96±1.51
V2/94	+0.09±1.20	-0.34±1.20	-0.48±1.16	-0.62±1.19	-1.02±1.23	-1.42±1.61
V3/87	-0.18±1.32	-0.66±1.39	-0.83±1.30	-1.11±1.36	-1.37±1.34	-1.78±1.98

N - Nasal, T – Temporal

* M component differed significantly over the four visits, $p < 0.0005$.

Table 6.3 The J0 component of peripheral refraction at each visit

Visit/n	Peripheral Refraction J0 ± SD (D)					
	Retinal Angles (°)					
	N30*	N20	N10	T10	T20	T30*
V0/100	-0.66±0.76	-0.22±0.39	+0.06±0.21	-0.05±0.26	-0.58±0.48	-1.90±1.20
V1/98	-0.32±0.42	-0.21±0.36	+0.14±0.21	-0.04±0.27	-0.51±0.44	-1.40±1.06
V2/94	-0.19±0.43	-0.13±0.43	+0.11±0.27	+0.00±0.28	-0.45±0.44	-1.47±1.03
V3/87	-0.11±0.40	-0.11±0.43	+0.18±0.26	-0.04±0.34	-0.50±0.46	-1.74±1.25

N - Nasal, T – Temporal

*J0 component differed significantly over the four visits, $p < 0.0005$.

Table 6.4 The J45 component of peripheral refraction at each visit

Visit/n	Peripheral Refraction J45 ± SD (D)					
	Retinal Angles (°)					
	N30	N20	N10	T10	T20	T30
V0/100	+0.03±0.45	-0.04±0.31	+0.01±0.20	+0.04±0.23	+0.10±0.33	+0.15±0.50
V1/98	-0.03±0.34	-0.09±0.34	-0.02±0.14	+0.02±0.20	+0.07±0.30	+0.17±0.43
V2/94	+0.01±0.32	-0.05±0.34	-0.02±0.17	+0.02±0.19	+0.10±0.28	+0.18±0.46
V3/87	+0.04±0.26	+0.01±0.33	-0.02±0.16	+0.01±0.22	+0.09±0.34	+0.16±0.45

N - Nasal, T – Temporal

Table 6.5 The Spherical power of peripheral refraction at each visit

Visit/n	Peripheral Refraction Spherical Power ± SD (D)					
	Retinal Angles (°)					
	N30*	N20*	N10*	T10*	T20*	T30*
V0/100	+0.97±1.04	+0.52±0.92	+0.15±0.86	+0.13±0.83	+0.09±0.91	+0.71±1.05
V1/98	+0.76±1.03	+0.37±1.04	-0.01±1.03	-0.06±1.06	-0.04±1.07	+0.45±1.18
V2/94	+0.63±1.20	+0.18±1.25	-0.26±1.24	-0.41±1.30	-0.47±1.30	+0.19±1.31
V3/87	+0.25±1.31	-0.19±1.46	-0.65±1.39	-0.86±1.47	-0.76±1.53	+0.02±1.62

N - Nasal, T – Temporal

* Spherical power differed significantly over the four visits, $p < 0.0005$.

6.3.2.2 Subgroup comparison of peripheral refraction changes

The 3-year changes in peripheral refraction M component of the three subgroups are shown in Figure 6.1. A one-way ANOVA was conducted to determine the changes in M component for the subgroups of emmetropia, low myopia, and moderate myopia. It was found that the changes were statistically different among three subgroups at all retinal angles (all $p < 0.0005$). The largest changes were observed in the MM group (1.43D – 2.65D), while the smallest changes were observed in the E group (0.03D – 0.44D). The changes in peripheral refraction and central refraction were correlated, with the greater the central refraction changes, the greater the peripheral refraction changes.

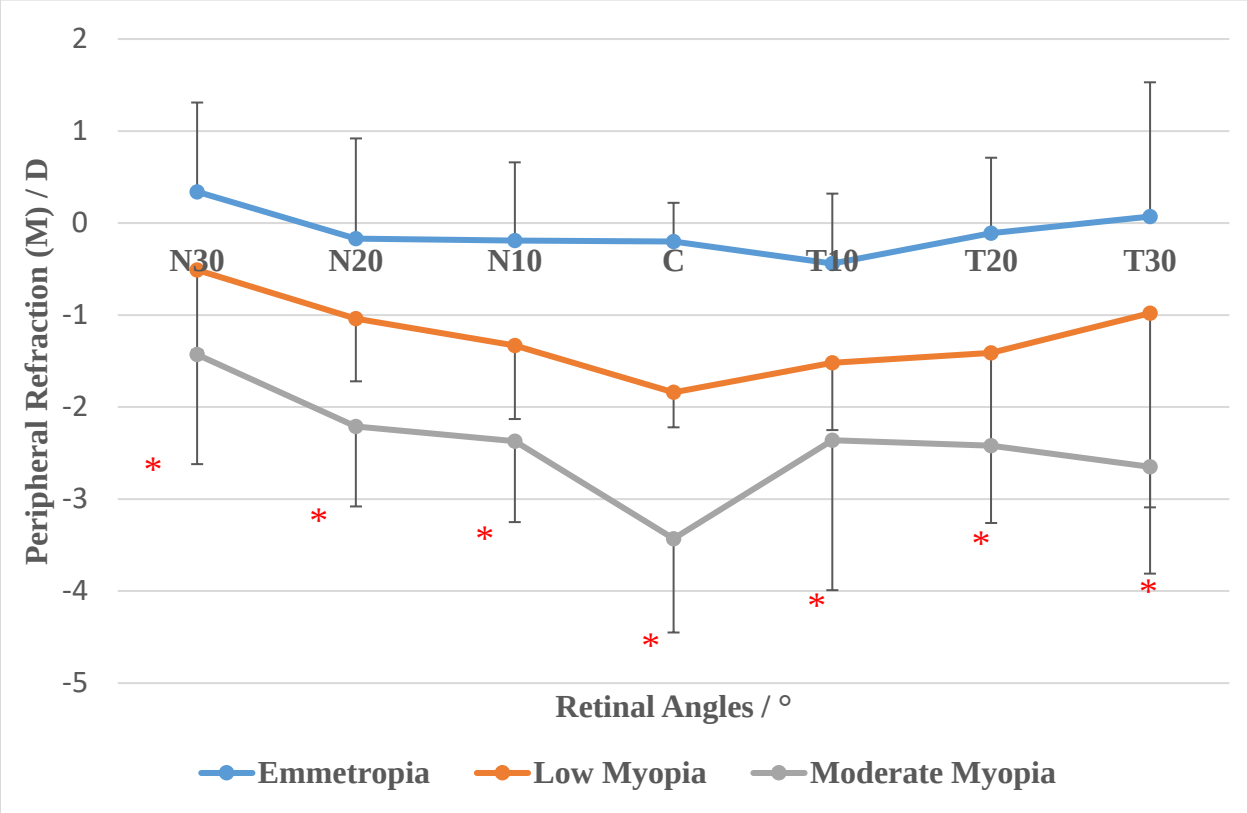


Figure 6.1. 3-year Changes in the M Component of Peripheral Refraction. Error bars denote standard deviations.

* 3-year changes in the M component differed significantly between the three groups, $p < 0.0005$.

6.3.2.3 Relative peripheral refraction at each visit

The findings of relative peripheral refraction at each visit are listed in Table 6.6 to 6.9. At baseline, the mean relative peripheral refraction was found to be myopic across the central 60º retina. It became less myopic or more hyperopic over time. After 3 years, relative hyperopia was observed in the nasal retina, while it remained relative myopic in the temporal retina. A one-way repeated measures ANOVA was conducted to determine the differences in relative peripheral refraction, in terms of M, J0, and J45 components and spherical power, over the 3-year study period.

The M component and spherical power of the peripheral refraction became more hyperopic or less myopic over time. M component and spherical power, at all retinal angles except T10, were significantly different between the four visits (all $p < 0.001$). J0 component at T30 and N30 also differed significantly over the course of the study (both $p < 0.0005$). There were no significant differences in J45 component over the four visits at any retinal angle. These findings indicate that spherical power and M component of relative peripheral refraction were changing towards being more hyperopic, with the nasal retina under hyperopic defocus initially. The far peripheral relative ATR astigmatism tended to be reduced, while the relative oblique astigmatism stabilized over the course of the study.

Table 6.6 The M component of relative peripheral refraction at each visit

Visit/n	Relative Peripheral Refraction M ± SD (D)					
	Retinal Angles (°)					
	N30*	N20*	N10*	T10	T20*	T30*
V0/100	-0.02±1.24	-0.07±0.73	-0.21±0.38	-0.26±0.43	-0.72±0.81	-1.40±1.75
V1/98	+0.39±1.01	+0.04±0.56	-0.05±0.31	-0.14±0.36	-0.48±0.70	-0.77±1.36
V2/94	+0.64±0.99	+0.24±0.71	+0.02±0.38	-0.12±0.34	-0.48±0.65	-0.79±1.28
V3/87	+0.86±1.07	+0.38±0.75	+0.11±0.34	-0.16±0.35	-0.38±0.70	-0.79±1.78

N - Nasal, T - Temporal

* M component differed significantly over the four visits, $p < 0.001$.

Table 6.7 J0 component of relative peripheral refraction at each visit

Visit/n	Relative Peripheral Refraction J0 ± SD (D)					
	Retinal Angles (°)					
	N30*	N20	N10	T10	T20	T30*
V0/100	-0.78±0.69	-0.37±0.36	-0.08±0.19	-0.20±0.19	-0.71±0.40	-2.05±1.16
V1/98	-0.48±0.41	-0.36±0.38	-0.01±0.18	-0.17±0.20	-0.66±0.41	-1.51±0.96
V2/94	-0.36±0.42	-0.28±0.44	-0.04±0.24	-0.16±0.21	-0.59±0.38	-1.59±1.00
V3/87	-0.29±0.35	-0.26±0.33	-0.00±0.14	-0.22±0.20	-0.68±0.37	-1.92±1.24

N - Nasal, T - Temporal

***J0 component differed significantly over the four visits, $p < 0.0005$.

Table 6.8 J45 component of relative peripheral refraction at each visit

Visit/n	Relative Peripheral Refraction J45 ± SD (D)					
	Retinal Angles (°)					
	N30	N20	N10	T10	T20	T30
V0/100	+0.02±0.48	-0.05±0.34	-0.00±0.22	+0.03±0.21	+0.09±0.31	+0.14±0.49
V1/98	-0.02±0.37	-0.07±0.34	-0.01±0.15	+0.04±0.18	+0.09±0.28	+0.17±0.43
V2/94	+0.00±0.36	-0.06±0.37	-0.04±0.20	+0.01±0.18	+0.09±0.27	+0.17±0.46
V3/87	+0.01±0.32	-0.02±0.37	-0.06±0.21	-0.02±0.20	+0.06±0.32	+0.13±0.45

N - Nasal, T - Temporal

Table 6.9 Spherical power of relative peripheral refraction at each visit

Visit/n	Relative Peripheral Refraction Retinal Angles (°)			Spherical Power ± SD (D)		
	N30*	**N20***	**N10***	**T10**	**T20***	**T30***
V0/100	+0.66±1.02	+0.20±0.71	-0.16±0.39	-0.19±0.41	-0.22±0.67	+0.38±0.94
V1/98	+0.73±0.93	+0.34±0.68	-0.04±0.32	-0.09±0.32	-0.06±0.58	+0.40±0.96
V2/94	+0.91±0.94	+0.49±0.75	+0.05±0.41	-0.11±0.33	-0.16±0.60	+0.48±0.88
V3/87	+1.00±1.02	+0.55±0.80	+0.10±0.35	-0.12±0.37	-0.01±0.74	+0.78±1.12

N - Nasal, T – Temporal

* Spherical power was statistically different over the four visits, $p < 0.001$.

6.3.2.4 Correlations between Central Myopic Shift/Axial Elongation, and Relative Peripheral Refraction Changes

The changes in central refraction, axial length, and relative peripheral refraction over 1-year, 2-year, and 3-year of the study period are summarized in Table 6.10 to 6.12. A Spearman's rank-order correlation was conducted to assess the relationship between the 1-year, 2-year and 3-year changes in central refraction and relative peripheral refraction in M component/spherical power.

There were statistically significant and moderate negative correlations between the 2-year and 3-year changes in central refraction and relative peripheral refraction M component at N30 (r = -0.334, $p = 0.001$ for 2-year change, r = -0.375, $p < 0.0005$ for 3-year change). There was a moderate negative correlation between the 2-year changes in central refraction and relative peripheral refraction M component at N20 (r = -0.280, $p = 0.008$ for 2-year change). There were also statistically significant and moderate negative correlations between the 2-year and 3-year changes in central refraction and spherical power at N30 (r = -0.273, $p = 0.009$ for 2-year change; and r = -0.362, $p < 0.001$ for 3-year change).

A Pearson's correlation was also performed to assess the relationship between the 1-year, 2-year and 3-year changes in axial length and relative peripheral refraction in M component/spherical power. For 1-year change, there was a trend between axial elongation and relative peripheral refraction change at N30, but it did not reach the adjusted significance of 0.01 (r = 0.238, $p = 0.022$). For the 2-year change, there was a statistically significant correlation between axial elongation and relative peripheral refraction changes at N30 (r = 0.351, $p = 0.001$ at N30). For 3-year change, axial elongation was a trend of correlation with relative peripheral refraction changes at all nasal

angles (r = 0.422, $p < 0.0005$ at N30; r = 0.228, $p = 0.038$ at N20, r = 0.231, $p = 0.035$ at N10), but only the correlation at N30 reached adjusted significance. There were statistically significant correlations between axial elongation and the spherical power of relative peripheral refraction change at N30 over the whole study period (r = 0.263, $p = 0.01$ for 1-year change; r = 0.331, $p = 0.001$ for 2-year change; and r = 0.454, $p < 0.0005$ for 3-year change). These results demonstrate that the relative peripheral refraction corresponding to the nasal retina is changed with myopia development and axial elongation, while these correlations were not found from the temporal retina.

Table 6.10 Changes in central refraction and axial length

Duration/n	Change in Cycloplegic Objective Refraction ± SD (D)			Change in Axial Length ± SD (mm)
	M	J0	J45	
1Yr/98	-0.33±0.68	-0.01±0.15	+0.02±0.09	0.34±0.22
2Yr/94	-0.67±0.98	+0.03±0.19	+0.03±0.12	0.61±0.36
3Yr/88	-1.07±1.31	+0.03±0.22	+0.03±0.16	0.83±0.46

Table 6.11 Changes in the M component of relative peripheral refraction

Duration /n	Change in Relative Peripheral Refraction M ± SD (D)					
	Retinal Angles (°)					
	N30	N20	N10	T10	T20	T30
1Yr/92	+0.36±0.76	+0.15±0.42	+0.17±0.41	+0.12±0.49	+0.25±0.62	+0.48±1.24
2Yr/90	+0.63±0.96	+0.33±0.74	+0.25±0.43	+0.16±0.46	+0.27±0.72	+0.54±1.27
3Yr/85	+0.79±0.96	+0.44±0.75	+0.33±0.47	+0.13±0.89	+0.34±0.62	+0.52±1.53

N - Nasal, T - Temporal

Table 6.12 Changes in spherical power of relative peripheral refraction

Duration /n	Change in Relative Peripheral Refraction Spherical Power ± SD (D)					
	Retinal Angles (°)					
	N30	N20	N10	T10	T20	T30
1Yr/92	+0.06±0.71	+0.13±0.70	+0.14±0.41	+0.09±0.45	+0.17±0.52	-0.03±0.76
2Yr/90	+0.22±0.85	+0.29±0.81	+0.22±0.52	+0.10±0.47	+0.07±0.68	+0.04±0.85
3Yr/85	+0.29±0.84	+0.34±0.87	+0.27±0.45	+0.05±0.46	+0.20±0.64	+0.32±0.96

N - Nasal, T - Temporal

6.3.2.5 Subgroup Comparison of Relative Peripheral Refraction Changes

The profiles of the changes of the subgroups over 3 years in M component of relative peripheral refraction are illustrated in Figure 6.2. A one-way ANOVA was conducted to determine the 3-year changes in relative peripheral refraction M component for the three groups. Although there was a trend for the myopic subgroups to have more hyperopic changes in relative peripheral refraction than the emmetropic subgroup, only the parameter change at N30 was statistically significant different between groups ($p < 0.0005$).

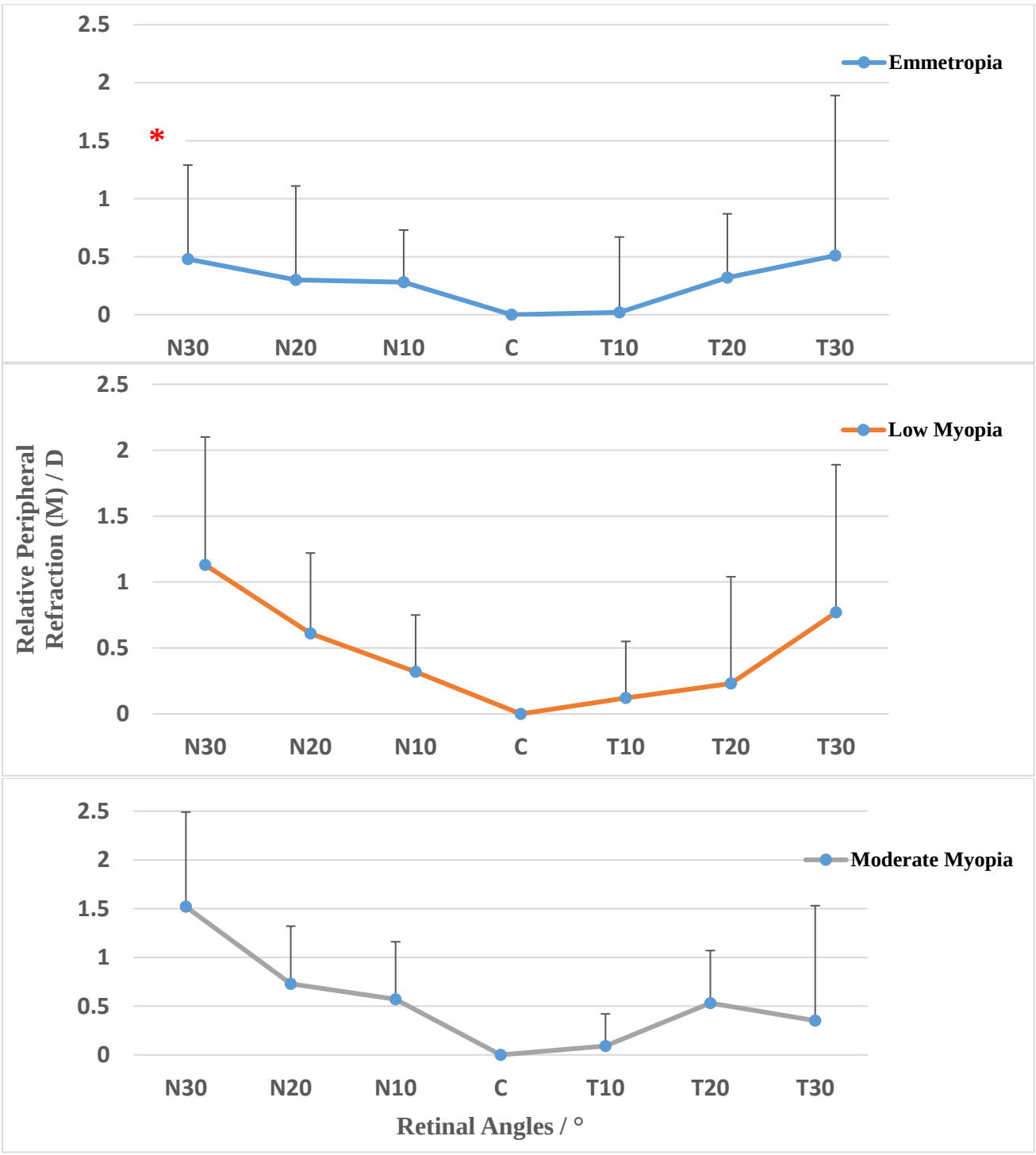


Figure 6.2. 3-Year changes in the M component of relative peripheral refraction.

*The parameter change at N30 was statistically significant different between groups ($p < 0.0005$). Error bars denote standard deviations.

6.4 Discussion

Eighty-eight subjects completed the whole study. They had a mean near emmetropic central refractive error of +0.22±0.56D at the baseline visit and the refractive error continuously became more myopic, resulting in a mean central refraction of -0.88±1.73D at the last visit. There was also a concurrent axial elongation, with a mean axial length of 22.92±0.80 mm at baseline visit reaching 23.79±1.05 mm at the last visit, suggesting that subjects had continuous eyeball growth. Together with the central refraction change, peripheral refraction changed towards more myopic, while the relative peripheral refraction tended to be more hyperopic. The profile of relative peripheral refraction, which was not hyperopic at any eccentricity at baseline visit, in this study is consistent with the findings of previous studies. Sng and co-workers (Sng et al., 2011) studied peripheral refraction in young Chinese children in Singapore, of whom 84 with emmetropia (-0.49D to +1.00D) showed relative myopia at all peripheral eccentricities, and 81 with low myopia (-2.99D to -0.50D) displayed relative hyperopia only at peripheral 30º. The findings of the current study also agreed with other studies of both older children and Caucasian subjects (Mutti et al., 2000, Atchison et al., 2005, Atchison et al., 2006). However, these earlier studies were unable to establish the temporal relationship between relative peripheral refraction and myopia development using cross-sectional study designs. By employing a prospective longitudinal study of changes over 3 years, relative peripheral refraction was found to change from myopic towards hyperopic and the relative hyperopia emerged initially at the far peripheral eccentricity (30º) during early myopia development. Another consistent finding is the nasal-temporal difference, which has been observed in many studies (Millodot, 1981, Atchison et al., 2006, Berntsen et al., 2008). The relative peripheral hyperopia was shown to be greater in the nasal retina (corresponding to temporal visual field) compared to the temporal retina (Atchison et al., 2006, Pardhan and Rae, 2009,

Radhakrishnan et al., 2013). The asymmetry in the M component was suggested due to a combination of angle alpha and lack of rotational symmetry in the retinal surface (Charman and Atchison, 2009).

Off-axis astigmatism has been described in detail in previous reports (Seidemann et al., 2002, Atchison et al., 2006, Berntsen et al., 2008). In our study, it was noted consistently that as the amount of astigmatism increased progressively, cardinal astigmatism changed towards ATR astigmatism with the increasing eccentricity. Asymmetry in off-axis astigmatism was also observed, with the amounts of cardinal astigmatism being negatively increased in the temporal retina. This asymmetry has been attributed to asymmetries, rotation, or misalignment in the curvature of anterior optical surfaces, such as the asymmetrical corneal surface (Barnes et al., 1987, Dunne and Barnes, 1987, Charman, 2005). The longitudinal findings presented in the current study indicated that the in-axis oblique astigmatism showed a mild decrease, but the off-axis oblique astigmatism displayed no significant changes over time. For cardinal astigmatism, however, an opposite pattern of change was observed: the on-axis astigmatism had no change, while the off-axis ATR astigmatism decreased in early childhood.

The changes in M component of relative peripheral refraction measured from N20 and N30 were correlated with myopia development and axial elongation, suggesting that the visual input from the nasal retina (temporal visual field) was associated with myopic eye growth. This finding agrees with previous studies. A weak correlation had been revealed between the progression of myopia over two years and the change in relative peripheral refraction at the nasal retina, while other horizontal eccentricities did not show any significant correlation (Faria-Ribeiro et al., 2013,

Radhakrishnan et al., 2013). The weak correlation between the peripheral refraction profile and progression of myopia could be due to the result of analyses only considering spherical equivalent refractive errors. This approach assumed that the relative position of the circle of least confusion, rather than the tangential and sagittal image planes, was associated with the regulation of myopia development, but did not consider the possible effect of the astigmatic defocus. Therefore, both the spherical equivalent (M component) and the spherical power were analyzed in the current study. It was found that the spherical power change only at N30 was associated with myopia development, while spherical equivalent changes at both N20 and N30 were correlated. All these findings demonstrate that the magnitude of off-axis astigmatism is likely to be involved in the association between relative peripheral refraction and myopia development.

In subgroup analysis, there was a trend for the myope groups to have more peripheral hyperopic changes than the emmetrope group, but only the parameter change at N30 was significantly different between different groups. Several longitudinal studies in peripheral refraction, have failed to establish a strong correlation between relative peripheral hyperopia and myopia development (Mutti et al., 2011, Sng et al., 2011, Lee and Cho, 2013, Atchison et al., 2015). Initially, Mutti et al. (2002) showed that relative peripheral hyperopia preceded the onset of myopia by 2 years in children aged 6 to 14 years, but their later study with 2817 children reported that relative peripheral hyperopia had little consistent influence on the risk of myopia onset and on the rate of myopia progression (Mutti et al., 2011). The other teams studying peripheral refraction profile in Chinese children also showed that baseline peripheral refractive error was not predictive of myopia development (Sng et al., 2011, Lee and Cho, 2013). In our study, a group of young children with near emmetropia were investigated and those children that developed myopia after 3 years had more peripheral hyperopic defocus present at a certain nasal retinal eccentricity. In addition, the change

in relative peripheral refraction at this position was also associated with myopia development in terms of central refraction change and axial elongation. Our findings provide evidence that peripheral retinal profile undergoes asymmetrical changes during myopic eye growth and that visual input from the nasal retina may play a role in the myopia development to some extent. However, our peripheral refraction results were still not able to determine how the eye ignores the signal of clear foveal vision but recognizes the relative hyperopia signal only from the peripheral nasal retina to trigger myopia onset.

6.5 Conclusions

Peripheral refraction and relative peripheral refraction profile change over time during childhood. Children with myopic eye growth have more changes in peripheral refraction and relative peripheral refraction. These changes at the nasal retina eccentricity (temporal visual field) show a consistent correlation with axial elongation and central refraction changes, suggesting that visual input from nasal retina was involved in the process of myopia development to some extent.

Chapter Seven

Global Flash Multifocal Electroretinogram and Myopia

(Part of this chapter was published in Investigative Ophthalmology and Visual Science, 2017, 58, 4399-4406)

7.1 Introduction

Application of ERG techniques has provided ample evidence to confirm that myopia results in impaired retinal function. It has been reported that myopia in adults was associated with decreased nonlinear components of ERG responses (Yoshii et al., 2002), mfERG responses (Kawabata and Adachi-Usami, 1997), retinal adaptation response (Chen et al., 2006c), and inner retinal function (Chen et al., 2006b, Ho et al., 2011). Axial length was also shown to be linearly related to ERG amplitudes (Westall et al., 2001), the first-order kernel, and the first slice of second-order kernel of mfERG responses (Chan and Mohidin, 2003). The reduction in mfERG responses in myopic adults is believed to be due to the deterioration in retinal function associated with long-standing myopia. However, this explanation cannot be applied to the situation in myopic children, and discrepancies of ERG characteristics have been noted between myopic adults and children (Luu et al., 2006, Ho et al., 2012b). Luu and his colleagues conducted a cross-sectional study of mfERG measurement in 104 children and 31 adults with a range of refractive errors (Luu et al., 2006). They found a significant correlation between refractive error and mfERG response in adults, but this correlation was not observed in children. Ho and his team also demonstrated different characteristics of retinal electrophysiological activities in adults and children in terms of retinal regions and mfERG components (Ho et al., 2012b). These studies suggest that electro-retinal changes in myopes are not simply related to the myopia status but may happen earlier than the onset of myopia.

To the best of our knowledge, no studies have previously investigated electro-retinal function in young children with emmetropia, nor the correlation between electro-retinal function and subsequent myopia development. This longitudinal study sought to use the MOFO mfERG program to

investigate myopic induced retinal electrophysiological change in children, and the correlation between electro-retinal function and juvenile myopia development.

7.2 Methods

Subject recruitment and inclusion criteria have been described in Chapter 5, Section 5.1, and the retrospective grouping division (E, LM and MM subgroups) was the same as defined in Chapter 6. Subjects underwent cycloplegic refraction, axial length measurement, and the MOFO mfERG measurement at 12-month intervals for three years. Only the right eye data were included for analysis. The instrumental set-up and measurement procedure have been reported in Chapter 5, Section 5.2.2. As shown in Figure 7.2, mfERG measurement comprised 61-hexagonal stimulation at contrast levels of 96% and 49%. Groups of responses were averaged to five successive rings (Ring 1 to Ring 5). Amplitudes and implicit times of DC and IC responses were adopted and extracted for analysis.

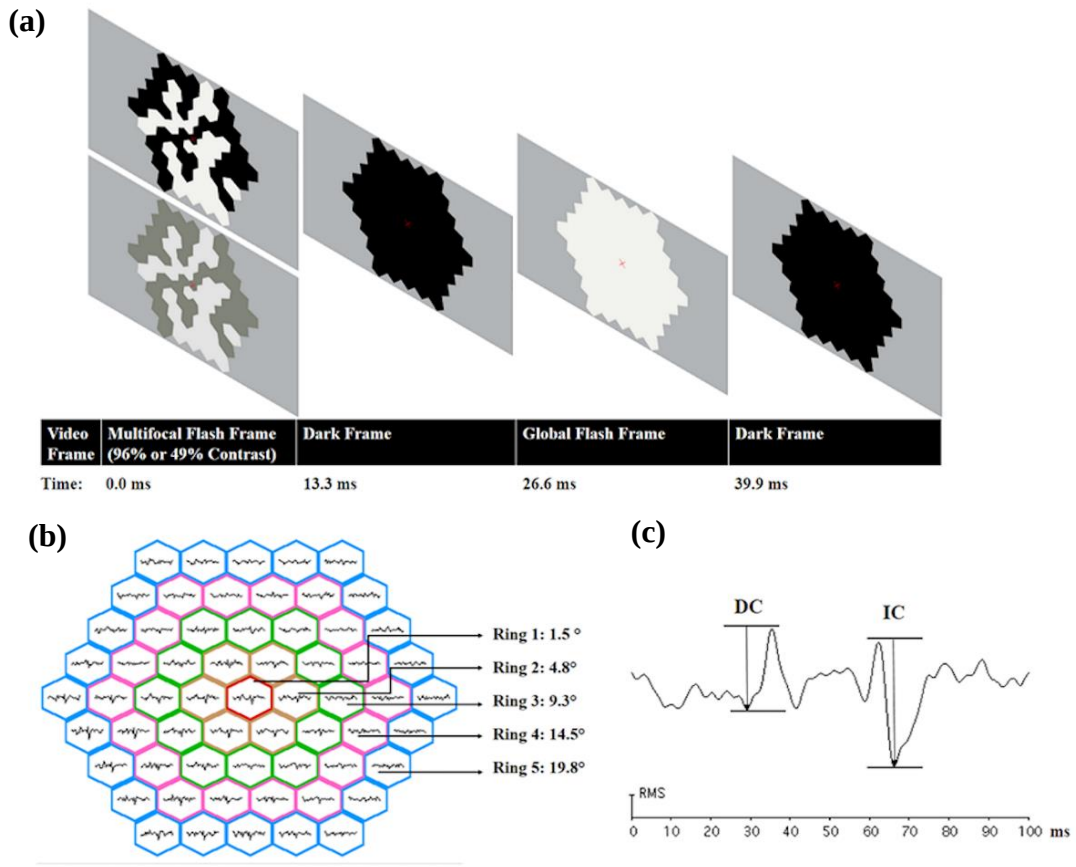


Figure 7.1. (a) The MOFO mfERG measurement was set with 61-hexagonal stimulation at contrast levels of 96% and 49%. (b) The trace arrays of 61 responses were averaged to five successive rings from center to periphery. (c) A representative waveform and its components of the MOFO mfERG response.

The normality of the variables was determined by the Shapiro-Wilk Test (SPSS 23.0). Non-parametric tests were applied to those variables violating normal distribution. A one-way repeated measured analysis of variance (ANOVA) was conducted to determine whether there were statistically significant differences in mfERG parameters among four visits (i.e. baseline (V0), 1-year (V1), 2-year (V2) and 3-year (V3)). As the data of central refraction changes were not normally distributed, a Spearman's rank-order correlation was run to assess the relationship between the 1-year, 2-year and 3-year changes in central refraction and the changes in mfERG parameters, while a Pearson's correlation was used for the changes in axial length. For the subgroup comparison, a one-way repeated ANOVA was conducted to determine if the baseline mfERG parameters were different between the three subgroups. The correlation between baseline IC response and central refraction change was further tested by a Spearman's rank-order correlation, and the correlation between baseline IC response and axial elongation was tested with the Pearson's correlation. An adjusted significance level of $p < 0.01$ was applied in the statistical analysis to reduce the chance of Type 1 errors with multiple testing.

7.3 Results

7.3.1 Global Flash Multifocal Electroretinogram Parameters at Each Visit

Parameters of the MOFO mfERG responses at each visit are shown in Figure 7.1 - 7.4. The response amplitude of both DC and IC decreased dramatically with increasing retinal eccentricity, while the response implicit time of both DC and IC slightly shortened with increasing retinal eccentricity.

A one-way repeated measures ANOVA was conducted to determine whether there were statistically significant differences in each ERG parameter over the 3-year study period. There were no significant changes in the DC implicit time measured at high contrast (96%) stimulation (Figure 7.2). The DC implicit time of Ring 1 at relative low contrast (49%) level was significantly increased from 36.59±0.19 ms at the baseline visit to 37.60±0.22 ms after three years ($p = 0.003$) (Figure 7.2). In addition, the implicit time at Ring 2 was also significantly delayed from 36.40±0.17 ms to 37.01±0.20 ms ($p < 0.0005$) (Figure 7.2). Post hoc analysis indicated that the pairwise differences between V3 and V0 at Ring 1, V3 toV0, V1 and V2 to V0 at Ring 2 were significant ($p < 0.05$). In summary, the DC implicit time under high contrast level showed no changes over time, while central DC implicit time (Ring 1 and 2) under low contrast condition had a delayed response.

For the IC implicit time, the response time showed significant increment under both high and low contrast stimulation for all retinal regions except at Ring 5 (Ring 1 - 4, $p < 0.0005$; Ring 5, $p > 0.05$) (Figure 7.3). Post hoc analysis indicated that the pairwise differences between baseline and all the other visits at Ring 1 were significant ($p < 0.05$), while the pairwise differences between V3 and all the other visits at Ring 2 to Ring 4 were also significant ($p < 0.05$). These results indicate that central IC implicit time (Ring 1) increased significantly after one year, while the implicit time at para-central (Ring 2 - 4) regions had more significant increase at the last visit after three years.

DC and IC amplitude of both contrast conditions at all retinal regions decreased significantly over time (all $p < 0.0005$) (Figure 7.4 and Figure 7.5). Post hoc analysis revealed that the pairwise differences between baseline and last visit at all retinal regions were significant ($p < 0.05$). These

findings showed that the electro-retinal activities of normal young children changed significantly in both response time and amplitude over their childhood.

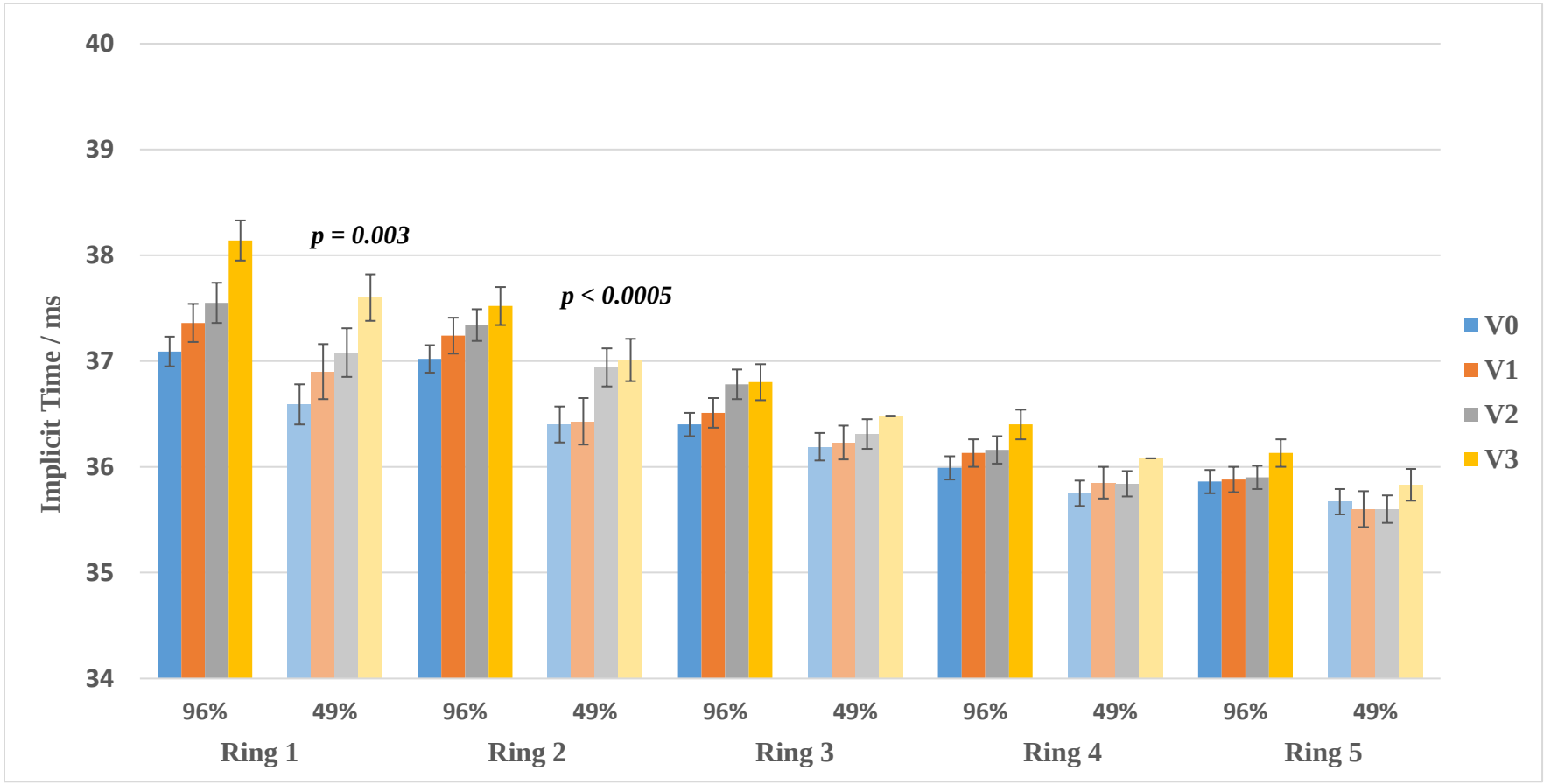


Figure 7.2. DC implicit time at different retinal regions at each visit. Error bars denote standard error means.

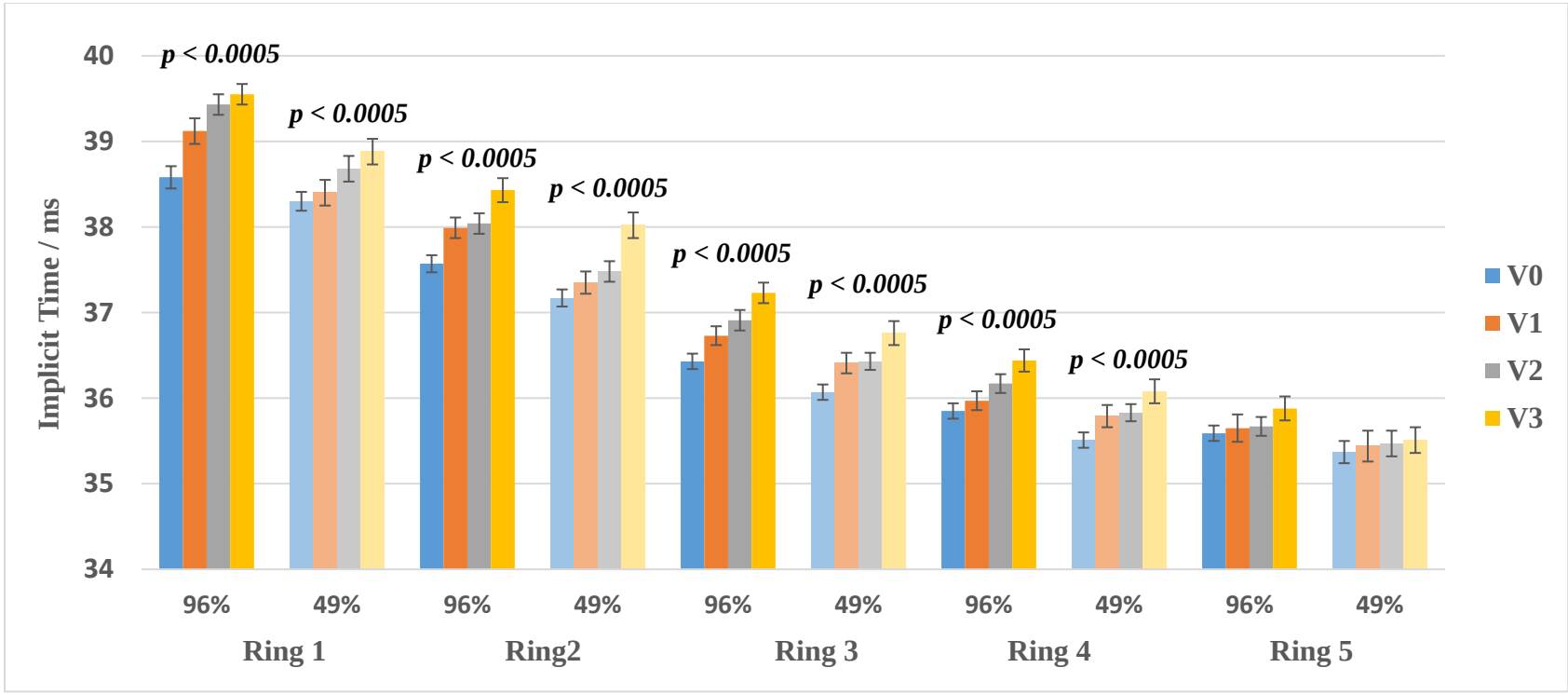


Figure 7.3. IC Implicit time at different retinal regions at each visit. Error bars denote standard error means.

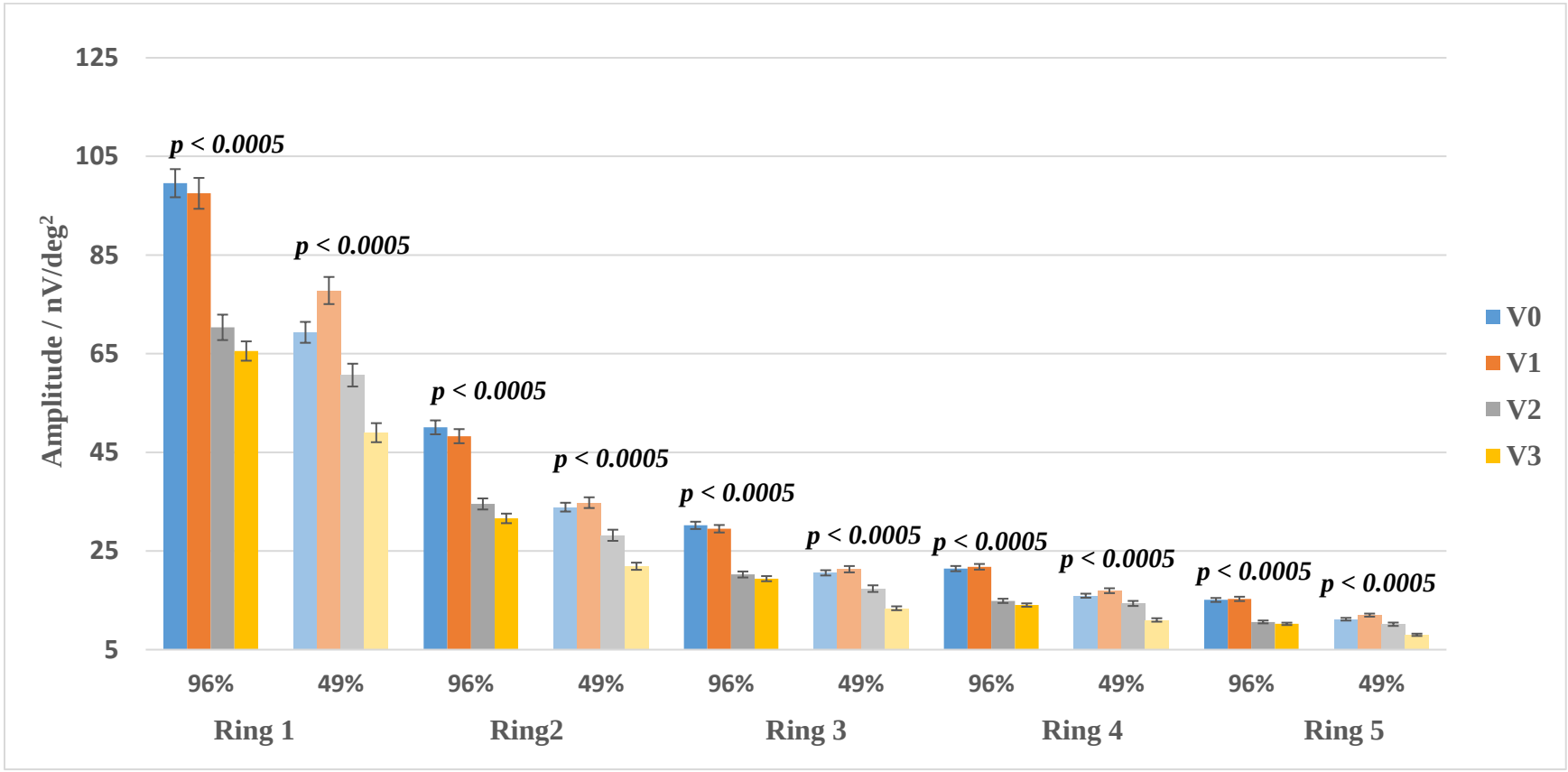


Figure 7.4. DC amplitude at different retinal regions at each visit. Error bars denote standard error means.

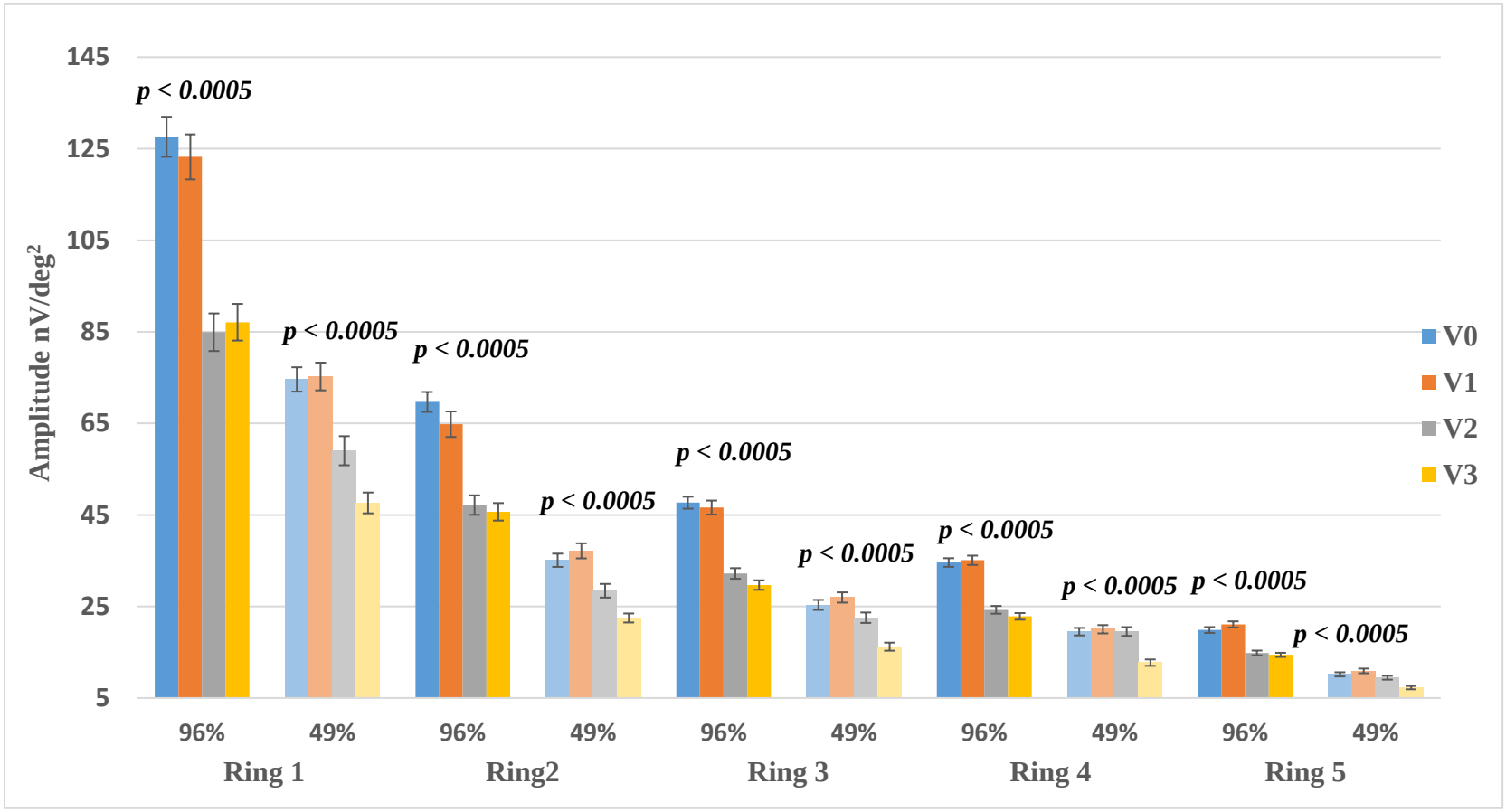


Figure 7.5. IC amplitude at different retinal regions at each visit. Error bars denote standard error means.

7.3.2 Correlations between the Changes in Central Refraction and the Changes in mfERG Responses

Table 7.1 to 7.4 show the percentage changes of mfERG response at 49% and 96% contrasts for different retinal regions after 1-year, 2-year, and 3-year periods of the study, and the correlations between those changes and central refraction change/axial elongation. After three years, the mean implicit time of responses increased by 0.5% to 2.9% and the mean amplitude of responses decreased by 11.7% to 31.9%. A Spearman's rank-order correlation was run to assess the relationship between the changes in central refraction and mfERG parameters. Overall, there was no statistically significant correlation, which reached an adjusted significance of 0.01, between the changes in refraction and mfERG parameters, indicating the changes in mfERG response, were not associated with the central refraction changes.

7.3.3 Correlations between Axial Elongation and the Changes in mfERG Responses

A Spearman's rank-order correlation was also run to assess the relationship between the axial elongation and percentage changes in mfERG parameters (Table 7.1 - 7.4). There was a weak correlation between the 2-year and 3-year axial elongation and the changes in Ring 1 implicit time and amplitude under 96% contrast (rho = 0.249, $p = 0.038$ for 2-year changes of IC implicit time; rho = 0.260, $p = 0.044$ for 3-year changes of IC implicit time; rho = 0.284, $p = 0.022$ for 2-year changes of the DC amplitude; and rho = 0.254, $p = 0.05$ for 3-year changes of IC amplitude). However, these observed correlations did not reach the adjusted significance. This finding demonstrates that electro-retinal response changes were not related to the axial elongation over early childhood.

Table 7.1 One-year, two-year and three-year percentage changes in the DC implicit time and their correlations with central refraction change/axial elongation

	Retinal Region	R1		R2		R3		R4		R5	
	Contrast Level	96%	49%	96%	49%	96%	49%	96%	49%	96%	49%
% Change (Mean ± SEM)	1Yr	0.63±0.54	1.35±0.85	0.37±0.44	0.56±0.68	0.53±0.38	0.47±0.47	0.27±0.30	0.47±0.33	-0.03±0.30	-0.16±0.45
	2Yr	1.18±0.66	1.75±0.74	0.92±0.48	1.82±0.57	1.25±0.31	0.71±0.39	0.65±0.31	0.49±0.34	0.38±0.30	-0.08±0.32
	3Yr	2.36±0.69	2.94±0.75	0.90±0.54	1.93±0.66	1.09±0.36	0.80±0.46	1.17±0.35	0.89±0.33	0.79±0.29	0.50±0.39
Correlation with Refraction Change (rho Sig.)	1Yr	-0.045	0.040	-0.054	0.099	-0.096	0.037	0.004	0.017	-0.017	0.181
		0.674	0.715	0.616	0.361	0.372	0.731	0.972	0.877	0.875	0.092
	2Yr	0.141	-0.201	-0.075	-0.143	-0.063	-0.026	-0.053	-0.074	0.068	0.135
		0.244	0.060	0.536	0.185	0.605	0.809	0.666	0.488	0.575	0.207
	3Yr	0.144	-0.110	-0.005	-0.092	-0.095	-0.055	-0.095	-0.107	-0.135	-0.130
		0.267	0.336	0.972	0.422	0.465	0.628	0.469	0.348	0.301	0.257
Correlation with Axial Elongation (rho Sig.)	1Yr	0.009	-0.084	0.070	-0.115	0.153	-0.096	0.030	0.006	-0.095	-0.108
		0.934	0.445	0.515	0.288	0.151	0.372	0.783	0.955	0.374	0.319
	2Yr	-0.106	0.101	0.204	0.011	0.110	-0.023	-0.028	0.043	-0.141	-0.121
		0.384	0.348	0.090	0.918	0.366	0.834	0.820	0.689	0.245	0.258
	3Yr	-0.153	0.022	0.038	0.000	0.095	-0.001	0.102	0.057	0.045	0.135
		0.239	0.846	0.770	1.000	0.468	0.995	0.436	0.615	0.733	0.240

Table 7.2 One-year, two-year and three-year percentage changes in IC implicit time and their correlations with central refraction change/axial elongation

	Retinal Region	R1		R2		R3		R4		R5	
	Contrast Level	96%	49%	96%	49%	96%	49%	96%	49%	96%	49%
% Change (Mean ± SEM)	1Yr	1.56±0.51	0.31±0.49	1.21±0.39	0.68±0.35	1.01±0.31	0.82±0.24	0.51±0.29	0.89±0.34	0.13±0.29	0.15±0.45
	2Yr	2.11±0.50	0.95±0.48	1.30±0.36	1.05±0.36	1.33±0.30	1.14±0.29	0.98±0.25	1.21±0.37	0.32±0.25	0.34±0.36
	3Yr	2.70±0.45	1.82±0.49	2.05±0.35	2.37±0.40	2.00±0.31	1.72±0.36	1.51±0.29	1.33±0.37	0.74±0.27	0.48±0.43
Correlation with Refrac-tion Change (rho Sig.)	1Yr	0.023	-0.061	-0.100	0.080	-0.087	-0.046	0.073	-0.003	0.033	0.133
		0.833	0.575	0.352	0.459	0.415	0.671	0.496	0.979	0.758	0.218
	2Yr	-0.123	0.044	0.079	0.066	-0.250	0.036	-0.060	-0.065	-0.058	0.082
		0.309	0.686	0.516	0.539	0.037	0.737	0.622	0.543	0.633	0.444
	3Yr	-0.161	-0.124	0.043	-0.142	-0.193	-0.124	-0.126	-0.199	-0.063	-0.060
		0.219	0.278	0.747	0.216	0.137	0.277	0.333	0.081	0.628	0.607
Correlation with Axial Elongation (rho Sig.)	1Yr	-0.018	-0.011	0.096	-0.035	0.096	0.067	-0.100	0.024	-0.138	-0.111
		0.869	0.923	0.371	0.745	0.372	0.537	0.351	0.826	0.196	0.302
	2Yr	0.249	-0.084	-0.187	-0.048	0.136	-0.024	-0.076	0.051	-0.130	-0.064
		0.038	0.436	0.121	0.655	0.262	0.821	0.533	0.635	0.283	0.553
	3Yr	0.260	0.116	0.034	0.124	0.228	0.164	0.114	0.200	0.022	0.037
		0.044	0.311	0.800	0.278	0.077	0.148	0.382	0.078	0.865	0.750

Table 7.3 One-year, two-year and three-year percentage changes in the DC amplitude and their correlations with central refraction change/axial elongation

	Retinal Region	R1		R2		R3		R4		R5	
	Contrast Level	96%	49%	96%	49%	96%	49%	96%	49%	96%	49%
% Change (Mean ± SEM)	1Yr	7.15±4.69	31.63±8.84	3.56±4.14	14.56±6.28	1.73±3.22	11.10±5.29	4.98±3.46	10.77±4.20	6.14±3.89	17.90±5.10
	2Yr	-22.85±3.43	-3.96±6.14	-25.64±3.52	-12.71±4.36	-29.44±3.40	-12.45±4.28	-26.35±3.16	-8.31±3.49	-23.98±3.18	-3.68±4.17
	3Yr	-26.58±2.99	-22.57±4.50	-29.86±2.88	-29.70±3.19	-31.85±3.17	-29.54±3.17	-30.26±2.58	-28.46±2.43	-26.56±3.54	-24.17±2.76
Correlation with Re-fraction Change (rho Sig.)	1Yr	0.077	-0.078	-0.021	-0.094	-0.100	-0.027	0.039	0.022	0.204	0.036
		0.475	0.476	0.847	0.386	0.351	0.802	0.714	0.836	0.056	0.742
	2Yr	-0.194	0.056	-0.021	0.033	-0.025	-0.002	0.031	0.069	0.087	0.030
		0.107	0.604	0.864	0.760	0.835	0.988	0.800	0.518	0.475	0.779
	3Yr	-0.025	0.062	0.009	-0.003	-0.085	0.045	0.050	0.128	0.080	0.084
		0.850	0.588	0.948	0.978	0.516	0.692	0.700	0.259	0.538	0.462
Correlation with Axial Elongation (rho Sig.)	1Yr	0.014	0.059	0.168	0.078	0.228	-0.013	0.038	0.066	-0.145	0.045
		0.898	0.591	0.116	0.474	0.032	0.904	0.720	0.541	0.174	0.679
	2Yr	0.284	0.116	0.210	0.105	0.164	0.023	0.195	-0.007	0.072	0.016
		0.022	0.280	0.083	0.330	0.176	0.833	0.106	0.949	0.554	0.878
	3Yr	0.113	-0.048	0.150	0.045	0.247	0.015	0.079	-0.066	-0.065	-0.113
		0.385	0.679	0.249	0.691	0.055	0.893	0.547	0.564	0.621	0.326

Table 7.4 One-year, two-year and three-year percentage changes in IC amplitude and their correlations with central refraction change/axial elongation

	Retinal Region	R1		R2		R3		R4		R5	
	Contrast Level	96%	49%	96%	49%	96%	49%	96%	49%	96%	49%
% Change (Mean ± SEM)	1Yr	6.04±5.72	10.47±5.73	-2.27±4.76	16.69±7.59	0.69±3.72	24.81±11.11	5.01±3.75	17.70±9.29	14.17±5.12	35.13±13.62
	2Yr	-21.24±5.98	-7.18±7.69	-27.30±4.02	0.66±8.47	-29.06±3.17	8.05±9.73	-26.14±3.19	16.72±8.86	-18.39±4.01	16.51±12.13
	3Yr	-23.10±4.76	-28.97±4.60	-30.69±3.31	-22.35±6.35	-35.28±2.39	-19.92±7.71	-31.87±2.68	-22.71±6.82	-22.52±3.89	-11.69±8.13
Correlation with Refraction Change (rho Sig.)	1Yr	0.083	-0.049	0.060	0.076	0.138	-0.058	0.221	-0.048	0.261	-0.097
		0.438	0.652	0.576	0.486	0.198	0.594	0.037	0.657	0.013	0.367
	2Yr	0.028	0.066	0.041	0.056	-0.041	-0.081	0.007	-0.094	0.140	0.073
		0.817	0.543	0.737	0.602	0.737	0.451	0.952	0.380	0.247	0.498
	3Yr	-0.206	-0.147	-0.056	-0.010	0.016	-0.085	0.132	0.025	0.149	0.104
		0.115	0.199	0.675	0.931	0.905	0.455	0.311	0.828	0.250	0.368
Correlation with Axial Elongation (rho Sig.)	1Yr	0.042	-0.007	0.035	-0.109	-0.058	0.035	-0.098	0.023	-0.121	0.076
		0.698	0.951	0.746	0.313	0.591	0.748	0.360	0.835	0.258	0.482
	2Yr	0.060	-0.057	0.090	-0.012	0.165	0.101	0.140	0.024	0.050	-0.031
		0.622	0.595	0.459	0.912	0.173	0.347	0.249	0.823	0.681	0.772
	3Yr	0.254	0.121	0.113	-0.003	0.140	0.143	0.005	0.023	-0.044	-0.055
		0.050	0.292	0.395	0.977	0.281	0.210	0.967	0.840	0.739	0.632

7.3.4 Subgroup Comparison of the Baseline MOFO mfERG Parameters

A one-way ANOVA was conducted to determine if the baseline MOFO mfERG parameters were different among the three subgroups with different refraction changes. The results are shown in Table 7.5 -7.6.

At the central retinal region (i.e. Ring 1), the baseline IC implicit time and amplitude under 49% contrast were statistically different among the three refractive groups. The implicit times of the LM and MM groups were longer than those of E group. The amplitude decreased from that of the E group's 84.02 nV/deg^2, to LM group of 63.34 nV/deg^2, and to MM group of 58.93 nV/deg^2. Tukey post hoc analysis revealed that the difference of implicit time between E group and LM group was statistically significant *($p = 0.013$*). The decreases of amplitude from E group to LM group (*$p = 0.001$*), as well as to the MM group (*$p = 0.001$*) were statistically significant. These results indicate that different refractive error groups have significantly different characteristics in baseline central IC responses at the 49% contrast level.

At the peripheral retinal region of Ring 4, baseline IC amplitude under 49% contrast was statistically different among the three subgroups. The E group had a mean response amplitude of 21.05 nV/deg^2, LM group a response amplitude of 15.50 nV/deg^2, and MM group a response amplitude of 18.33 nV/deg^2. Tukey post hoc analysis revealed that the difference between E group and LM group was statistically significant (*$p = 0.028$*). There were no differences among the three subgroups at the other retinal regions.

Table 7.5 Responses of three subgroups at 96% contrast level

		Ring 1		Ring 2		Ring 3		Ring 4		Ring 5	
Parameters	**Group**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**
DC	E	36.82±0.21	1.545	37.00±0.17	0.914	36.36±0.12	1.806	36.05±0.13	1.168	35.95±0.14	2.218
Implicit	LM	37.33±0.37	(0.219)	37.44±0.34	(0.405)	36.7±0.37	(0.171)	35.93±0.28	(0.316)	36.12±0.31	(0.115)
Time (ms)	MM	37.50±0.28		37.02±0.30		35.92±0.29		35.56±0.27		35.34±0.25	
DC	E	97.31±4.06	0.803	49.82±1.98	1.290	30.98±1.04	0.166	21.56±0.74	0.114	15.14±0.59	0.766
Amplitude (nV/deg^2)	LM	100.76±5.64	(0.451)	47.27±2.71	(0.281)	29.88±1.40	(0.848)	22.10±1.18	(0.892)	15.11±0.86	(0.468)
	MM	109.32±11.5		55.41±4.68		30.87±2.34		22.21±1.76		16.70±1.15	
IC	E	38.66±0.20	0.038	37.64±0.11	0.194	36.55±0.11	1.304	35.93±0.12	0.979	35.63±0.12	1.000
Implicit	LM	38.59±0.29	(0.962)	37.55±0.32	(0.824)	36.56±0.30	(0.277)	35.88±0.28	(0.380)	35.78±0.30	(0.372)
Time (ms)	MM	38.56±0.34		37.46±0.28		36.06±0.26		35.51±0.26		35.29±0.21	
IC	E	127.98±5.69	0.026	70.16±2.81	1.038	49.17±2.03	0.811	35.68±1.39	1.311	20.50±0.97	0.843
Amplitude	LM	128.43±7.29	(0.975)	66.02±4.03	(0.359)	45.02±2.63	(0.448)	32.01±2.09	(0.275)	18.45±1.39	(0.434)
(nV/deg^2)	MM	131.10±8.77		76.56±6.73		50.16±3.24		36.73±2.29		21.05±1.72	

Table 7.6 Responses of three subgroups at 49% contrast level

		Ring 1		Ring 2		Ring 3		Ring 4		Ring 5	
Parameters	**Group**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**	**Mean ± SEM**	**F (Significance)**
DC	E	36.45±0.24	1.230	36.33±0.23	1.200	36.07±0.17	2.459	35.67±0.14	1.268	35.52±0.14	3.381
Implicit	LM	36.97±0.46	(0.297)	25.57±0.44	(0.306)	36.75±0.38	(0.092)	35.95±0.32	(0.287)	36.19±0.35	(0.059)
Time (ms)	MM	35.95±0.51		35.72±0.42		35.85±0.25		35.30±0.32		35.57±0.34	
DC	E	72.85±3.23	1.681	34.22±1.24	0.266	20.53±0.68	1.066	15.72±0.47	1.296	11.12±0.34	0.080
Amplitude	LM	73.07±5.02	(0.192)	34.58±2.05	(0.767)	22.06±1.26	(0.349)	16.98±1.11	(0.279)	11.13±0.46	(0.924)
(nV/deg^2)	MM	60.35±4.84		32.36±2.48		19.51±1.52		14.78±1.27		10.82±0.92	
IC	E	38.00±0.16	4.849	37.14±0.12	0.194	36.11±0.12	0.604	35.59±0.11	1.276	35.31±0.12	0.165
Implicit	LM	38.85±0.27	**(0.010*)**	37.30±0.28	(0.824)	36.30±0.28	(0.549)	35.66±0.27	(0.284)	35.46±0.34	(0.848)
Time (ms)	MM	38.62±0.27		37.13±0.33		35.93±0.23		35.18±0.23		35.24±0.40	
IC	E	84.02±2.99	10.881	36.21±1.70	0.808	27.35±1.44	2.641	21.05±1.11	3.525	10.68±0.67	2.110
Amplitude	LM	63.34±5.50	**(< 0.001*)**	31.83±3.69	(0.449)	20.94±2.80	(0.077)	15.50±1.99	(0.034)	8.43±1.04	(0.128)
(nV/deg^2)	MM	58.93±4.46		33.55±3.97		24.56±2.45		18.60±1.98		10.26±1.21	

* The baseline IC implicit time and amplitude under 49% contrast were statistically different among the three refractive groups.

7.3.5 Correlations between Baseline IC Response at 49% Contrast and Central Refraction Changes

As the baseline Ring 1 and Ring 4 responses were found to differ among different refractive error groups, a Spearman's rank-order correlation was run to assess the relationship between the baseline IC responses of Ring 1 and Ring 4 at 49% contrast and the central myopia shift over the study period (Table 7.7).

There were statistically significant, moderate, and positive correlations between the 1-year, 2-year, and 3-year refraction changes and the IC amplitude of Ring 1 (r = 0.317 to 0.366, all $p < 0.001$). There was also a weak and negative correlation between the 3-year change in central refraction and the IC implicit time of Ring 1 (r = -0.213, $p = 0.047$). However, no significant correlation was found between refractive changes and the baseline Ring 4 responses. These results demonstrated that baseline central IC amplitude was closely correlated with subsequent myopic refraction changes in young emmetropic children, and baseline IC implicit time was also weakly associated with longer time change in refractive errors.

7.3.6 Correlations between Baseline IC Response at 49% Contrast and Axial Elongation

As observed in the above section, a Spearman's rank-order correlation was applied to determine the relationship between the baseline IC responses of Ring 1 and Ring 4 at 49% contrast condition and the axial elongation over the study period (Table 7.8). There were statistically significant, moderate negative correlations between the 1-year, 2-year, and 3-year axial elongation and the IC amplitude of Ring 1 (r = -0.335 to -0.361, all $p < 0.001$). There was also a significant, weak positive correlation between the 3-year changes in axial length and the IC implicit time of Ring 1 (r = 0.261,

p = 0.0014). These findings indicate that the baseline IC response at Ring 1, either amplitude or implicit time, was consistently associated with the subsequent longitudinal eyeball growth in young children.

For the retinal response at Ring 4, there were statistically significant, weak negative correlations between the 1-year and 3-year axial elongation and the IC amplitude (r = -0.290, *p = 0.004* for 1-year of AL change; r = -0.274, *p = 0.010* for 3-year of AL change). There were also weak positive correlations between the 1-year and 2-year changes in axial length and the IC implicit time of Ring 4 (r = -0.234, *p = 0.022* for 1-year of AL change; r = -0.232, *p = 0.026* for 2-year of AL change). These findings indicate that the baseline IC response at Ring 4 was consistently associated with the subsequent longitudinal eyeball growth in young children.

Table 7.7 Spearman's rank-order correlation between the changes in refraction and baseline IC response at Ring 1 and Ring 4 under 49% contrast level

Time of Rx Change	Retinal Region	ERG Parameters	Spearman's rho	Significance
1 Year	Ring 1	IC Implicit Time	-0.088	0.391
		IC Amplitude	0.342	**0.001***
	Ring 4	IC Implicit Time	0.189	0.066
		IC Amplitude	0.194	0.057
2 Year	Ring 1	IC Implicit Time	-0.137	0.189
		IC Amplitude	0.317	**0.002***
	Ring 4	IC Implicit Time	0.196	0.061
		IC Amplitude	0.122	0.240
3 Year	Ring 1	IC Implicit Time	-0.213	0.047
		IC Amplitude	0.366	**<0.001***
	Ring 4	IC Implicit Time	0.204	0.060
		IC Amplitude	0.209	0.051

* There were statistically significant correlations between the 1-year, 2-year, and 3-year refraction changes and the IC amplitude of Ring 1 (r = 0.317 to 0.366, all $p < 0.001$).

Table 7.8 Spearman's rank-order correlation between the changes in axial length and baseline IC response at Ring 1 and Ring 4 under 49% contrast level

Time of AL Change	Retinal Region	ERG Parameters	Spearman's rho	Significance
1 Year	Ring 1	IC Implicit Time	0.135	0.188
		IC Amplitude	-0.335	**0.001***
	Ring 4	IC Implicit Time	-0.234	0.022
		IC Amplitude	-0.290	**0.004***
2 Year	Ring 1	IC Implicit Time	0.148	0.154
		IC Amplitude	-0.354	**< 0.001***
	Ring 4	IC Implicit Time	-0.232	0.026
		IC Amplitude	-0.194	0.060
3 Year	Ring 1	IC Implicit Time	0.261	0.014
		IC Amplitude	-0.361	**0.001***
	Ring 4	IC Implicit Time	-0.172	0.112
		IC Amplitude	-0.274	**0.010***

***For the retinal response at Ring 1, there were statistically significant correlations between the 1-year, 2-year, and 3-year axial elongation and the IC amplitude. For the retinal response at Ring 4, there were statistically significant correlations between the 1-year and 3-year axial elongation and the IC amplitude.

7.4 Discussion

Over the three-year follow-up in young children, the MOFO mfERG responses from the central retinal region (Ring 1) had a significant decrease in amplitude and delay in implicit time. The response from other retinal regions also showed significant decreases in amplitude, but there were no obvious changes in implicit time. These findings demonstrate that the electro-retinal activities of normal young children changed over time, and the trends of changes differ in terms of retinal regions. Another study followed up 26 children with mild to high myopia for 1 year to investigate the changes of the MOFO mfERG with myopia progression (Ho et al., 2012a). Central response amplitude under low contrast stimulation significantly reduced and was associated with refractive error changes after one year, hence suggesting myopia progression was associated with functional changes in the inner retina. Our study further demonstrates that this reduced inner retinal function appears before myopia development. In addition, several studies have shown that the response amplitude decreased and implicit time increased in adults with moderate or high myopia, and the response amplitudes from the peripheral retina were decreased more than that from the central retina (Kawabata and Adachi-Usami, 1997, Chan and Mohidin, 2003, Ho et al., 2011). Collectively, we speculate that electro-retinal activities change with respect to different response components at different retinal regions from childhood to adulthood. In the central retinal region, the response amplitude and implicit time may change in early childhood but are relatively preserved over later adulthood; while in the peripheral retinal region, response amplitude continues to decrease while the response implicit time is reserved from childhood to adulthood with myopia development. However, a longer term longitudinal study, following up subjects from childhood to adulthood with various degrees of myopic progression, is necessary to provide more conclusive evidence.

In this study, correlation between changes in electro-retinal activities and myopia development was only found between the increased central IC response time and the axial elongation over 3 years. IC response represents the effect of the focal flash on the responses evoked by the global flash and reflects predominantly inner retinal function (Sutter et al., 1999, Shimada et al., 2005, Chu et al., 2008). Therefore, our results suggest that axial elongation in children was associated with the delay in central inner retinal response only. A stronger correlation between mfERG responses, in both amplitudes and implicit times, and the severity of myopia have been reported in myopic adults (Westall et al., 2001, Chan and Mohidin, 2003, Chen et al., 2006a, Chen et al., 2006c). It was suggested that the electro-retinal response changes were primarily due to the increased axial length, resulting in an increase in ocular resistance (Perlman et al., 1984), the subretinal space (Huang and Karwoski, 1990), reduced image size, decreased retinal illumination and the reduced retinal cell density (Beresford et al., 1998, Chan and Mohidin, 2003). Thus, the reduction in mfERG responses in myopic adults could be explained by the deterioration in retinal function with longstanding myopia. Our study further demonstrated a correlation between the delay in central inner retinal implicit time and axial elongation in young children with early myopia development. The discrepancy of the electro-retinal characteristics between myopic adults and children has also been reported in a previous cross-sectional study (Luu et al., 2006). Response amplitudes and implicit times were found to be significantly correlated with the severity of myopia in adults, while only the implicit time was correlated with the refractive error in children. Therefore, the difference between adults and children implies that the effect of myopia-induced change alone is not the cause of the characteristic changes in retinal electrophysiology in children, and thus other mechanisms are likely to be involved.

By means of retrospective subgroup comparison, we found different refractive error groups had different baseline characteristics in IC responses at the 49% contrast level. The subjects who had myopia development, were observed to have a delayed and decreased central IC response (Ring 1) and a decrease in amplitude at the peripheral retina (Ring 4) at baseline comparing to the subjects who remained emmetropia over the study period. Therefore, we further examined the correlation between baseline responses derived from Ring 1 and Ring 4 and the subsequent myopic changes. The baseline central IC amplitude was found to be consistently correlated with subsequent myopic refractive changes and axial elongation in young children. This mfERG parameter was measured in young emmetropic children with normal visual acuity and good ocular health, which indicates that the variance of this mfERG parameter could not be attributed to longstanding myopia or any other myopia-induced pathological effects. As IC represents predominantly inner retinal function, and a low contrast stimulus is demonstrated superior to a high contrast stimulus in revealing inner retinal activity (Bearse and Sutter, 1998, Hood et al., 1999), it could be concluded that emmetropic children with subclinical decreased inner retinal function in the central retina are more likely to develop myopia. This finding concurs with previous studies. Luu and co-workers recruited 81 myopic children, with refractive errors ranging from -1.00 D to -5.88 D at their initial visit, and followed up their refractive changes for 2 years, before dividing them into three subgroups according to their myopic progression rate (Luu et al., 2007). They reported that children in the fast myopic progression subgroup had decreased central response amplitudes of conventional mfERG at the initial visit. A significant correlation was also identified between the central mfERG amplitude and the change in vitreous chamber length, but not with the change of refractive errors. They suggested that the central retinal function may be a predictor of children's myopia progression rate.

Collectively, we hypothesize that the central inner retina plays a significant role in the manipulation of myopia development.

Our results from the near-emmetropic children demonstrated that reduced central IC response of the MOFO mfERG appeared before myopia development, and, thus it is more likely to be an inducement to myopia rather than a secondary effect. We suggest that children with a decreased central inner retinal function are more prone to eyeball elongation and myopia development. The decrement in retinal function seems to be inversely proportional to the rate of myopia development. Over years of myopic progression as children grow into adults, the reduced mfERG amplitude becomes significantly associated with the severity of myopia. We believe this decreased retinal function may interact with other myopigenic mechanisms and that the level of the reduction is mutable.

7.5 Conclusions

The central IC amplitude obtained using the MOFO mfERG under 49% contrast stimulation was significantly correlated with later changes of refractive error in young children. This finding indicates that subclinical reduction of the central inner retinal function in emmetropic children could be a myopigenic factor and this retinal response could be a potential reference for juvenile myopia development.

Chapter Eight

Central Retinal Thickness and Myopia

8.1 Introduction

Retinal thickness has been reported to be altered in myopic eyes compared to emmetropic eyes (Lim et al., 2005, Luo et al., 2006a, Wu et al., 2008, Cheng et al., 2010). The eyeball elongation in myopic eyes causes retinal stretching, making the fovea particularly vulnerable to change (Springer and Hendrickson, 2004). With the application of OCT *in vivo* imaging, researchers have shown a significant correlation between macular thickness and axial length/refractive error in moderate or high myopic adults (Wong et al., 2005, Sato et al., 2010, Song et al., 2014). A smaller total macular volume and thinner quadrant-specific macular thickness were also reported in myopic children (Luo et al., 2006a, Zhang et al., 2011). These studies suggest that early anatomical changes may be present in the central retina of myopic children. Only one study of Read and his team has investigated the changes in macular retinal layer thickness associated with myopia in childhood, and the normal changes occurring in retinal layer thickness over time in a healthy adolescent population (Read et al., 2017). However, there is a lack of knowledge of the longitudinal changes in macular thickness preceding the myopia onset in young children.

Myopic changes to either the retinal thickness or function have been widely reported in the literature. However, only four studies have investigated the link between retinal structure and function (Wolsley et al., 2008, Park et al., 2013, Koh et al., 2014, Song et al., 2016). Two studies reported that reduced mid-inner retinal layer thickness was associated with myopia-related losses in neural activity derived from mfERG timing in the peripheral retina (Wolsley et al., 2008, Park et al., 2013). The other two studies illustrated correlation between mfERG first order responses and macular thickness (Koh et al., 2014, Song et al., 2016). All these studies involved myopic adults but the relationships between myopia and function in children has not been reported. The current study

aimed to examine the central retinal thickness changes in children with myopia development, and to determine the relationship between retinal structure and function. The comparative retinal structure-function data were also determined at specific retinal locations within the same eyes.

8.2 Methods

Subject recruitment and inclusion criterion have been described in Chapter 5, Section 5.1, and the retrospective grouping division (E, LM and MM subgroups) is as defined in Chapter 6, Section 6.2. Subjects underwent SD-OCT measurement, which started at the first follow-up visit of the study and was repeated at 12-month intervals for two years. Hence, there were only 3 visits of OCT data in this experiment. Only the data of right eye were collected for analysis. The procedure of the SD-OCT and the MOFO mfERG measurement have been reported in Chapter 5, Section 5.2.2 and 5.2.3. The MOFO mfERG data were discussed in the last chapter. The mean central retinal thicknesses were determined across three concentric annular rings with diameters of 1 mm, 2 mm, and 3 mm around the fovea, and referred to as the central foveal ring, inner macular ring, and outer macular ring, respectively. The inner and outer macular rings were further divided into four segments: superior, temporal, inferior, and nasal. As shown in Figure 8.1, the OCT and mfERG data were compared at two retinal regions: between foveal ring and mfERG Ring 1 (outer radii =1.5º); and between outer macular ring and mfERG Ring 2 (outer radii = 5º).

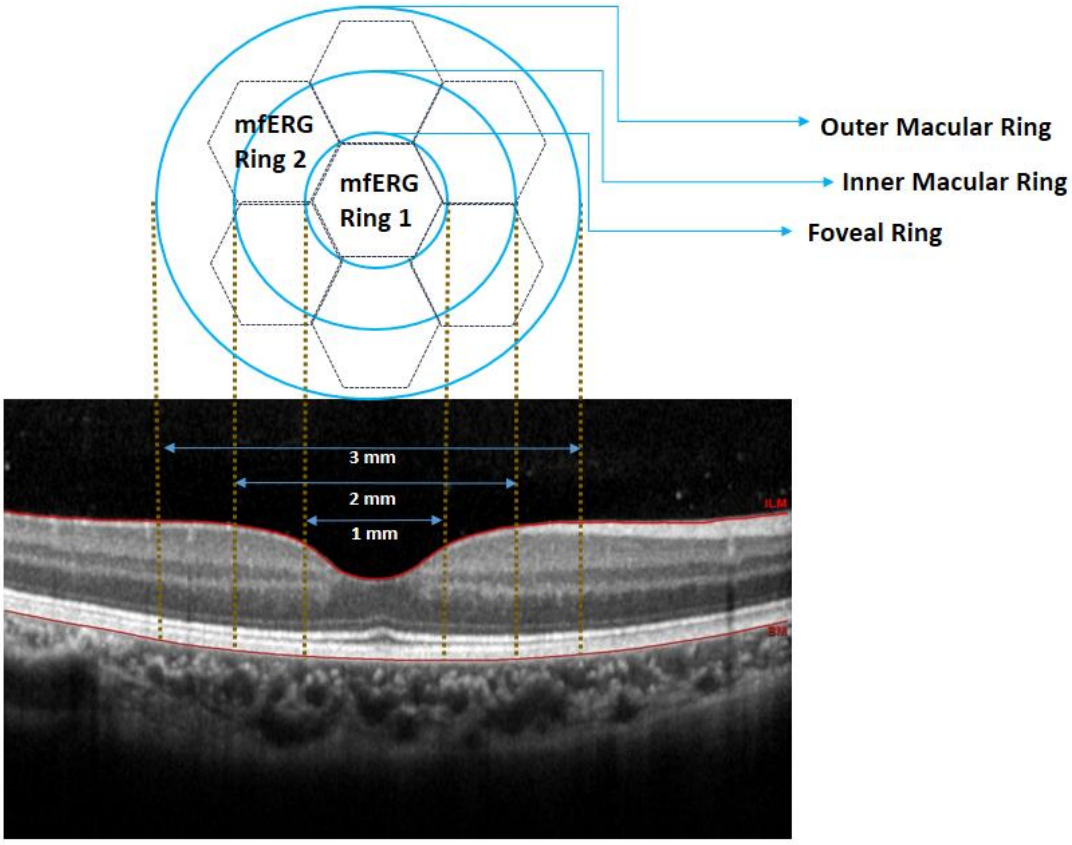


Figure 8.1. A schematic diagram shows that the OCT and mfERG data were compared at two retinal regions: between foveal ring and mfERG Ring 1; and between outer macular ring and mfERG Ring 2.

The normality of the variables was determined by the Shapiro-Wilk Test (SPSS 23.0). Non-parametric tests were applied to those variables violating normal distribution. A one-way repeated measures ANOVA was conducted to determine whether there was a significant difference in each segment of central retinal thickness among the three visits. A Spearman's rank-order correlation was run to assess the relationship between the retinal thickness measured at the last visit and the 3-year change in central refraction/axial length. The structure-function relationship was also determined using Pearson's correlation at two retinal locations of the foveal ring and outer macular ring, where mfERG and OCT results could be matched. For the subgroup comparison, a one-way repeated measure ANOVA was conducted to determine if the central retinal thickness measured at the last visit differed between various refractive groups. An adjusted significance level of $p < 0.01$ was applied in the statistical analysis to reduce the chance of Type 1 errors with multiple testing.

8.3 Results

8.3.1 Central Retinal Thickness at Each Visit

The results of averaged retinal thickness in terms of three annular rings and four segments are illustrated in Table 8.1. A one-way repeated measured ANOVA was conducted to determine whether there was difference of thickness in each retinal segment among the three visits. The results show that retinal thickness was consistent not only childhood but also during myopia development.

Table 8.1 Segment thickness across the central 3 mm of the posterior pole

Visit / Retinal Region	Retinal Thickness ± SD (µm)								
	Foveal Ring	Inner Macular Ring				Outer Macular Ring			
		Superior	Nasal	Inferior	Temporal	Superior	Nasal	Inferior	Temporal
V1	255.66±15.57	332.27±14.15	327.30±16.02	330.55±13.96	321.98±14.60	348.20±13.39	350.36±14.71	343.19±14.77	334.98±14.41
V2	255.70±15.41	332.24±14.68	327.01±16.11	329.88±14.57	320.93±13.79	347.68±13.95	349.56±14.04	343.08±14.32	334.22±13.07
V3	257.33±15.88	333.55±14.68	327.68±16.74	331.08±13.97	322.16±13.10	348.56±13.38	350.55±14.30	344.05±13.93	334.92±12.66

8.3.2 Correlation between Central Retinal Thickness and Central Refraction Change/Axial Elongation

A Spearman's rank-order correlation was run to assess the relationship between the retinal thickness measured at the last visit and the 3-year change in central refraction/axial length. The results are shown in Table 8.2. There were statistically significant positive correlations between the 3-year central refraction change and the thickness of the outer macular ring (r = 0.281 to 0.420, all $p < 0.01$). There were also weak and positive correlations between the central refraction change and the average thicknesses of the superior and nasal inner retinal segments (r = 0.250 and r = 0.255, both $p < 0.05$). However, the significance of the correlation did not reach the adjusted level of $p < 0.01$.

Statistically significant, negative and moderate correlations between the 3-year axial elongation and the thickness of the outer macular ring were observed (r = 0.315 to 0.432, all $p < 0.01$). There were also weak and negative correlations between the axial elongation and the average thicknesses of the superior and nasal inner retinal segments (r = 0.227 and r = 0.244, all $p < 0.05$), however, the significance of the correlation did not reach the adjusted level of $p < 0.01$. These findings show that in healthy pediatric eyes, thinning of outer macular thickness is associated with axial elongation and central refractive changes. In addition, myopia-induced ocular changes have different effects on various retinal regions, with a relative reserved foveal thickness and reduced outer macular thickness.

Table 8.2 Spearman's rank-order correlation between central retinal thickness and change in central refraction/axial Length

Retinal Region	Change of parameter	Spearman's rho	Significance
Foveal Ring	Central refraction	0.014	0.901
	Axial length	0.018	0.870
Superior Inner Macular Ring	Central refraction	0.250	0.021
	Axial length	-0.227	0.037
Nasal Inner Macular Ring	Central refraction	0.255	0.018
	Axial length	-0.244	0.024
Inferior Inner Macular Ring	Central refraction	0.185	0.092
	Axial length	-0.168	0.126
Temporal Inner Macular Ring	Central refraction	0.213	0.050
	Axial length	-0.175	0.109
Superior Outer Macular Ring	Central refraction	0.374	**<0.001***
	Axial length	-0.415	**<0.001***
Nasal Outer Macular Ring	Central refraction	0.420	**<0.001***
	Axial length	-0.432	**<0.001***
Inferior Outer Macular Ring	Central refraction	0.375	**<0.001***
	Axial length	-0.400	**<0.001***
Temporal Outer Macular Ring	Central refraction	0.281	**0.009***
	Axial length	-0.315	**0.003***

*There were statistically significant correlations between the 3-year central refraction change and the thickness of the outer macular ring.

8.3.3 Structure-Function Relationship

The structure-function relationship was determined using Pearson's correlation at two retinal locations. The correlation between the foveal ring thickness and Ring 1 response from the MOFO mfERG, and the correlation between outer macular ring thicknesses and Ring 2 responses measured at the V1, V2 and V3, were assessed. However, there was no significant structure-function correlation found at these two retinal regions. Therefore, the variance of electrophysiological responses at the central retina is not affected by the corresponding retinal thickness.

8.3.4 Subgroup Comparison of Central Retinal Thickness

One-way ANOVA was conducted to determine if the retinal thickness measured at the last visit differed between the various refractive groups. As shown in Figure 8.2, quadrant thicknesses, except for the temporal, within the outer macular ring were significantly different among different refractive groups ($F = 7.22$, $p = 0.001$ at superior retina; $F = 7.964$, $p = 0.001$ at nasal retina; and $F = 6.716$, $p = 0.002$ at inferior retina). Tukey post hoc analysis revealed that the retinal thickness of E group was significantly more thickened than LM and MM groups at the superior, nasal, and inferior segments of the outer macular ring (all $p < 0.05$). The subgroup comparison revealed a quadrant-specific thinning of outer macular thickness in myopic children with normal ocular health.

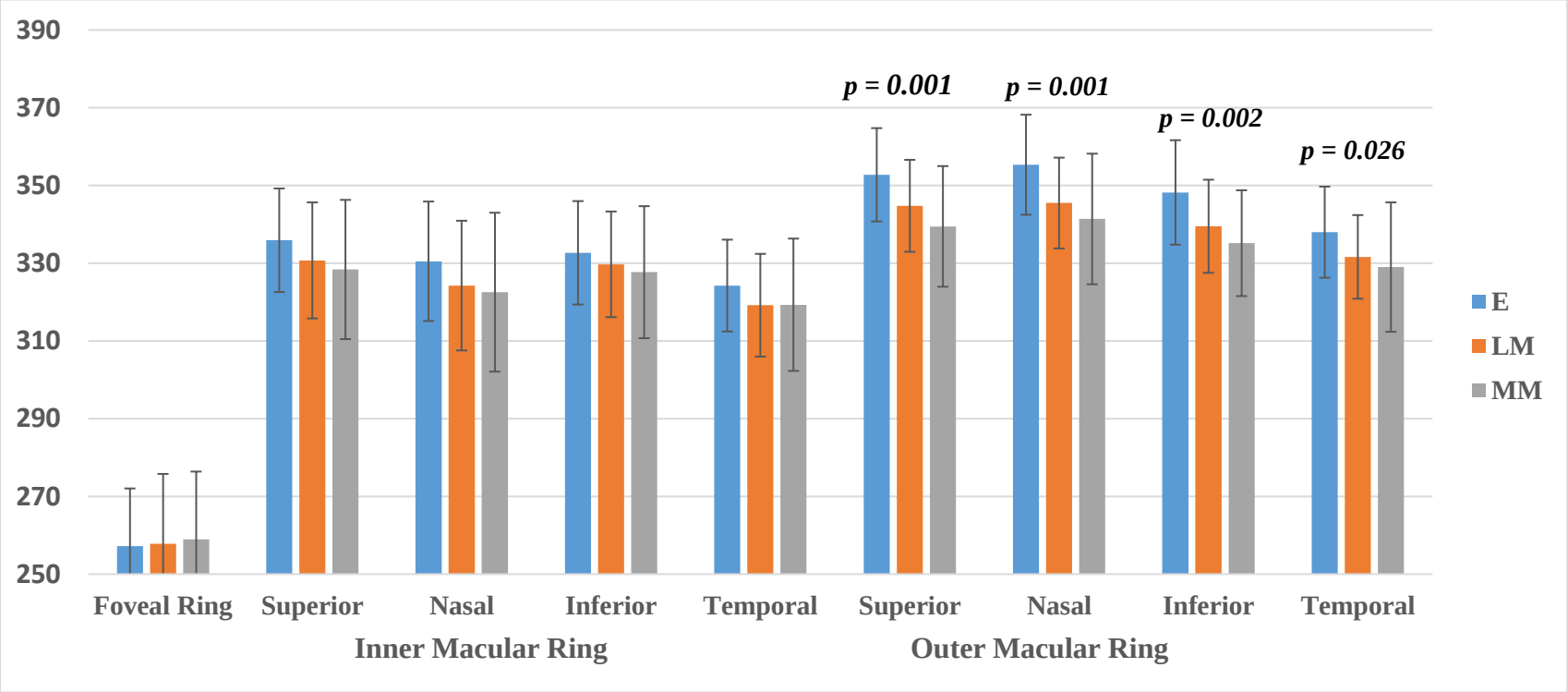


Figure 8.2. Segment thicknesses of three refractive subgroups. Error bars denote standard deviation.

8.4 Discussion

Central retinal thickness was found to undergo no significant change over early childhood. To our knowledge, this study is the first longitudinal study investigating central retinal thickness preceding the myopia onset in a group of Chinese children with normal ocular health. The mean retinal thickness in the central 1-mm foveal zone in this study was 255.66 μm, 255.70 μm, and 257.33 μm at the first, the second, and the third visits respectively, which are within the established normative range from 253 μm to 271 μm in the pediatric population (Turk et al., 2012, Barrio-Barrio et al., 2013, Yanni et al., 2013, Read et al., 2015b). Several studies have also reported a mild increase in the foveal thickness with age (Barrio-Barrio et al., 2013, Yanni et al., 2013, Read et al., 2015b). Read's cross-sectional study recruited 196 children aged 4 years to 12 years and documented a mean increase of 1.8 μm per year in the central 1-mm foveal zone (Read et al., 2015b). In their later longitudinal study, they showed the presence of myopia was associated with subtle but statistically significant changes in macular retinal thickness, with a thinning of the parafoveal retina. However, over 18 months, longitudinal changes in retinal thickness and individual layers were of small magnitude, indicative of a high degree of stability in retinal morphology in healthy adolescent eyes, despite significant eye growth over this same period of time (Read et al., 2017). In the current study, we also observed a small increase of mean thickness over two years. However, the increase magnitude did not reach statistical significance. This discrepancy may be due to the age range of the children being limited to 6 years to 9 years at the baseline visit and follow-up time in the study did not extend into adolescence. Despite the limitation of time-course, this study demonstrates that central retina development in terms of thickness stabilizes during early childhood.

We observed that myopia-induced ocular changes have different effects on different central retinal regions, with a relatively preserved foveal thickness and reduced outer macular thickness. In addition, subgroup comparison further demonstrated evidence of a quadrant-specific thinning of outer macular thickness in myopic children. Quadrant thicknesses of outer macular ring, expect the temporal segment, showed significant differences in thinning among E, LM, and MM subgroups and were correlated with the longitudinal axial elongation and refractive changes. Topographic variations in central retinal thickness (Turk et al., 2012, Barrio‐Barrio et al., 2013, Yanni et al., 2013, Read et al., 2015b), and the variation of retinal thickness in myopic and non-myopic eyes (Luo et al., 2006a, Cheng et al., 2010, Zhang et al., 2011), were also reported in other studies. Zhang's study (2011) investigated primary school aged Chinese children and found macular thickness varied with refractive errors. The Singapore Cohort Study of the Risk Factors for Myopia (SCORM) reported a quadrant-specific thinning of macular thickness in children with moderate myopia (SE at least -3.0D). Cheng and colleagues' study (2010) of young adults measured 30 myopic eyes with SE between -6.00D and -13.63D, and 30 non-myopic eyes with SE between +2.75D and -0.50D. At all macular zones within central 10º, retinal thickness was significantly thinner in myopic eyes compared to non-myopic eyes. Together with our findings, we speculate that macular thickness decreases with the severity of refractive error, and changes from childhood to adulthood with changes differing between quadrants.

We did not observe any significant structure-function correlation within the central retinal region in children with myopic progression. Some previous studies, using mfERG and OCT measurement, have compared retinal structure-function data in moderate and high myopic adults (Wolsley et al., 2008, Park et al., 2013) and reported a significant correlation between mid-inner retinal thickness

and mfERG responses at peripheral retina (12°-16°), while no correlation was found between the central mfERG responses and foveal thickness (0-1.5°). Therefore, the variance of electrophysiological responses at the central retina seems not to be associated with the corresponding retinal thickness in either myopic children or adults.

8.5 Conclusions

This study demonstrates that central retina thickness in children has topographical variations, with quadrant-specific thinning in myopic subgroups and is correlated with refractive errors. There was no structure-function correlation within the central retina region, which suggests that the variance of the central electro-retinal responses does not originate from the variance of corresponding retinal thickness.

Chapter Nine

Overall Analysis, Discussion, Conclusions, and Future Studies

9.1 Overall Analysis

The data presented in Chapters 6-8 show longitudinal changes in various ocular parameters from a group of reasonably large number of children. A multiple regression analysis was further applied to include all of the potential predictors for myopia development measured at baseline visit, and to exam which predictors or combination of factors provide the best prediction for future myopia development.

9.1.1 Prediction of Central Refractive Error Change

A multiple linear regression was calculated to predict 3-years central refractive error changes (Dependent Variable, DV_{rx}) based on the baseline parameters, including relative peripheral refraction, mfERG responses, and central retinal thickness. A significant regression equation (Equation 1) was found as $F(3,64) = 11.188$, $p < 0.0001$, with an R^2 of 0.344. The prediction of 3-years refractive error change in children is equal to $-3.907 + 0.035*IV_1 + 0.013*IV_2 - 0.266*IV_3$, where IV_1, IV_2, IV_3 are the baseline values of nasal outer segment thickness, IC amplitude at 49% contrast level, and IC implicit time at 49% contrast level, respectively.

Equation 1. $\mathbf{DV_{rx} = -3.907 + 0.035*IV_1 + 0.013*IV_2 - 0.266*IV_3}$

DV_{rx} is the dependent variable of 3-years refractive error change.

IV_1 is the nasal outer segment thickness measured at baseline visit.

IV_2 is IC amplitude at 49% contrast level measured at baseline visit.

IV_3 is IC implicit time at 49% contrast level measured at baseline visit.

This equation indicates that there is less 0.035 diopter of central refractive error change for each micrometer increase of the nasal retinal thickness; there is less 0.013 diopter of central refractive error change for each unit of nV/deg^2 increase of IC amplitude; and there is more 0.266 diopter of central refractive error change for each micro-second increase of IC implicit time. All these three independent variables are significant predictors of the 3-years central refractive error changes, and 34.4% variance of 3-years central refractive error changes could be explained by these three predictors.

9.1.2 Prediction of Axial Elongation

A multiple linear regression was also calculated to predict 3-years axial elongation (Dependent Variable, DV_{al}) based on the baseline parameters, including relative peripheral refraction, mfERG responses, and central retinal thickness. A significant regression equation (Equation 2) was found as $F(4,63) = 9.796$, $p < 0.0001$, with an R^2 of 0.383. The prediction of 3-years change in axial length in children is equal to $1.635 - 0.010*IV_1 - 0.005*IV_2 + 0.101*IV_3 - 0.094*IV_4$, where IV_1, IV_2, IV_3, IV_4 are the baseline values of nasal outer segment thickness, IC amplitude at 49% contrast level, IC implicit time at 49% contrast level, and the subject age, respectively.

Equation 2. $\mathbf{DV_{al} = 1.635 - 0.010*IV_1 - 0.005*IV_2 + 0.101*IV_3 - 0.094*IV_4}$

DV_{al} is the dependent variable of 3-years axial elongation.

IV_1 is the nasal outer segment of retinal thickness measured at baseline visit.

IV_2 is IC amplitude at 49% contrast level measured at baseline visit.

IV_3 is IC implicit time at 49% contrast level measured at baseline visit.

IV_4 is the subject age at baseline visit.

This equation indicates that axial length changes 0.01 mm less for each micro-meter increase of the nasal retinal thickness; axial length changes 0.005 mm less for each unit of nV/deg^2 increase of IC amplitude; axial length changes 0.101 mm more for each micro-second increase of IC implicit time; and axial length changes 0.094 mm more for each year increase of age. All these four independent variables are significant predictors of the 3-years axial elongation, and 38.3% variance of 3-years central refractive error changes could be explained by these four predictors.

9.2 Overall Discussion

9.2.1 Ocular Parameters' Changes in Children

In this three-year longitudinal study, 88 subjects completed all four study visits. Cycloplegic objective central refractive errors changed from emmetropia (+0.22±0.56D) at baseline visit to mild myopia (-0.88±1.73D) at the last visit, with a concurrent axial elongation of 0.87 mm. Together with the central refraction change, peripheral refraction changed towards more myopic, while the relative peripheral refraction tended to become more hyperopic. The electro-retinal activities showed significant changes over time, and the trends of changes were different in terms of retinal regions. Global Flash (MOFO) mfERG responses from the central retinal region displayed a significant decrease in amplitude and delay in implicit time. The response from more peripheral retinal regions also showed significant decreases in amplitude, but there were no changes in implicit time. However, with respect to retinal structure, central retinal thickness had no significant change over the study period. To our best knowledge, this study is the first longitudinal study following up the ocular dynamics of optic, functional, and structural changes in a group of young normal emmetropic children. Based on these findings, we summarize that at the early stage of juvenile

myopia development, the peripheral refraction (optics) and electro-retinal activities (function) change overtime, while the central retinal thickness (structure) is relatively preserved.

In contrast to the early ocular changes in myopic children, moderate or high myopic adults were reported to be associated with a more hyperopic profile of relative peripheral refraction (Millodot, 1981, Mutti et al., 2000, Seidemann et al., 2002, Atchison et al., 2006, Chen et al., 2010, Ehsaei et al., 2011, Sng et al., 2011, Li et al., 2015), functional deterioration from paracentral to mid-peripheral retinal regions (Ho et al., 2011, Ho et al., 2012b), and thinning of the parafoveal retinal thickness (Lim et al., 2005, Lam et al., 2007, Wolsley et al., 2008, Wu et al., 2008, Song et al., 2010). Together with the findings of the current study, it may be hypothesized that ocular parameters change with a dynamic pattern in terms of retinal regions from childhood to adulthood. In the central retinal region, the electro-retinal activities first change in early childhood, but then are relatively preserved over later adulthood; while the non-central retinal region, relative peripheral refraction, electro-retinal activities, and retinal thickness continue to change with myopia development from childhood to adulthood. These regional variations during myopia development appear to indicate different underlying causes at different development stages.

9.2.2 Subgroup Comparisons

Subjects in our study were retrospectively divided into E, LM and MM subgroups based on their central cycloplegic objective refraction at the last visit. The myopic groups displayed trends to have more peripheral hyperopic changes over the study period, delayed and decreased central retinal responses at baseline, and quadrant-specific thinning of outer macular thickness at the last visit than those observed in the emmetrope group. These findings have been reported separately in

previous studies. For example, children with myopia development within a study period of 1 year were found to develop relative peripheral hyperopia at the nasal and temporal 30º, but baseline peripheral refraction did not predict the subsequent onset of myopia (Sng et al., 2011, Lee and Cho, 2013); decreased foveal function was associated with a high rate of myopia progression (Luu et al., 2007); studies in Singapore and China showed that children with moderate myopia usually had smaller total macular volume and thinner quadrant-specific macular thickness (Luo et al., 2006a, Zhang et al., 2011). These results together with our findings, provide evidence that changes in central retinal function appear to precede myopia development, but the peripheral retinal profile undergoes changes during myopic eye growth, and the outer macular thickness thins with the severity of myopia. Overall the results from our longitudinal study suggest that marked variance of retinal function is noted earlier than that of optical and structural changes in myopia progression, which leads to the possible predictive role of central retinal function in myopia development.

9.2.3 Prediction of Myopia Development in Children

In our study, relative peripheral refractive error at certain nasal retinal eccentricities was found to be associated with the progression of myopia, which implies visual input from the nasal retina (temporal field) is associated with myopic eye growth. This finding agrees with previous studies. A weak correlation had been revealed between the progression of myopia over two years and the change in relative peripheral refraction at the nasal retina, while other horizontal eccentricities did not show any significant correlation (Faria-Ribeiro et al., 2013, Radhakrishnan et al., 2013). The asymmetry of off-axis astigmatism may account for this finding. In this study, consistently larger against-the-rule astigmatism was observed in the temporal retina, and the same trend of asymmetry in off-axis astigmatism was also described in previous studies (Seidemann et al., 2002, Atchison

et al., 2006, Berntsen et al., 2008). This asymmetry of peripheral retinal refractive profile may indicate that particular peripheral retinal regions, such as at nasal 20º to 30º, is more prone to myopic changes. In addition, with the electronic revolution of cellphones and computers, working and living styles nowadays are changing towards longer time of near work. While temporal retina receives more overlapping visual inputs due to binocular summation than nasal retina during near work, the nasal retina receives wider field of view and more surrounding stimulations. Hence, in normal daily life, the nasal retina is likely more exposure to a wider range of defoci than temporal retina and may evoke more visually guided feedbacks. It may be the possible reason to explain why the nasal retina was found to correlate with myopia development.

To date, clinical intervention trials in myopia progression using optical means have been designed on the basis of a hypothesis regarding the amount of peripheral defocus at the retina, and are reported to have a ceiling efficacy of about 60% (Huang et al., 2016). In animal studies, myopic defocus limits eye growth and promotes the development of hyperopia in growing eyes. In humans, myopic defocus can be created by under-correcting pre-existing myopia. Deliberate under-correction of myopic children on that basis should slow myopic progression and conversely optically correcting myopes may promote myopic progression by eliminating myopic blur. However, the opposite effect was observed on both refraction and axial length in clinical studies, which showed that under correction of myopia enhanced rather than inhibited myopia progression (Chung et al., 2002, Adler and Millodot, 2006). This paradoxical finding indicates that an in-focus foveal image is critical in human eye growth. In addition, in the animal model of lens-induced-myopia, animal eye growth tended to match with the induced power of the lens and then the ocular growth would halt with little further progression, while the refractive end point of the animal eye with optic nerve

section or foveal photo ablation fluctuated more dramatically than those with an intact retinal system (Wildsoet and Schmid, 2000, Smith et al., 2007). This observed variation of refractive end points also provides insight into the significance of intact central retinal structure and function. In summary, the peripheral defocus theory alone, cannot fully explain how an eye ignores a clear foveal image, but recognizes the relative defocused input from the peripheral retina to trigger myopia development. The variability of previous studies' results and existence of contradictory evidence clearly indicate that other factors must also play a role and therefore peripheral refraction may not be the primary or dominant determinant of final refraction. It seems that the retina must have a certain mechanism to determine the end point of refractive error even under peripheral defocus.

Some of the missing essential determinants could be the central retinal structure and function. We found central retinal thickness did not change over study period, but with specific-quadrant thinning preceding myopia development. The baseline central IC response was consistently correlated with subsequent myopic refraction changes and axial elongation in young children. This mfERG parameter was measured in young emmetropic children with normal visual acuity, good ocular health, and within normative range of central retinal thickness, which indicates that longstanding myopia effects, with respect to either retinal pathological or structural changes, could not account for the variance of the parameters obtained. Therefore, we hypothesize that the central inner retina is an essential determinant of the visual feedback process, where the peripheral retinal input could be decoded, to play a commanding role in the manipulation of juvenile myopia development. In summary, emmetropic children with subclinical decreased central inner retinal function, together with thinning macular thickness, are more likely to develop myopia with faster progression.

Myopia is reaching epidemic proportion worldwide. A recent systematic review and meta-analysis predicted that the prevalence of myopia and high myopia in the global population by 2050 will be 49.8% and 9.8%, respectively (Holden et al., 2016). With the known ocular complications of high myopia, such as retinal detachment, glaucoma (Shen et al., 2016), maculopathy (Wakazono et al., 2016), and cataracts (Ripandelli et al., 2003), it is particularly important to identify children who are at high risk of myopia development and to provide them with early intervention for myopia control. The common myopia control methods, especially in Asia, include defocus incorporated optical corrections, orthokeratology, and atropine. Clinical trials of these interventions in slowing myopia progression have shown an efficacy from 20% to 60% (Cho and Cheung, 2012, Lam et al., 2014, Chia et al., 2016, Chamberlain et al., 2018). Although the current clinical prediction of future myopia progression is based on the age of children and current refractive status, it is difficult for clinicians to identify emmetropic children who are prone to myopia development and need early myopia intervention. The present study recognized the retinal electrophysiological and structural characteristics of young emmetropic children with subsequent myopia development. Our findings indicate that myopia development in children could be predicted through assessing central retinal function and structure by the measurement of the Global Flash (MOFO) mfERG and OCT, respectively. Children with subclinical decreased IC responses under the low-contrast measurement and retinal thickness thinning at specific-quadrant, should be considered for early myopia control interventions. It would be especially beneficial for those children to prevent future myopia-related morbidity, such as high myopia, ocular complications, and degenerations.

9.3 Conclusions

The human retina undergoes a regulatory emmetropic process that guides an eye to grow, and conversely, failure of such a mechanism can lead to development of refractive errors. In order to examine the role of peripheral refraction (optics), electro-retinal activities (function), and retinal thickness (structure) in this retinal-related regulatory process, we strategically recruited a group of young emmetropic children and followed up their myopia status from baseline over a three-year period. We found that central induced component (IC) amplitude obtained using Global Flash (MOFO) mfERG under low (49%) contrast stimulation, and thinning of central retinal thickness measured by OCT, were significantly correlated with later progression of myopia in young children. Peripheral refraction and relative peripheral refraction profile changed over time during childhood. Children with myopic eye growth had more changes in peripheral refraction and relative peripheral refraction. These changes at the nasal retinal eccentricity (temporal visual field) showed a consistent correlation with axial elongation and central refraction changes. In addition, central retinal thickness had topographical variations, with specific-quadrant thinning associated with the severity of myopia. However, no structure-function correlation within the central retina region was observed. We suggest that central inner retinal function, together with thinning of central retinal thickness, could be myopigenic factors and potential references for juvenile myopia development**.**

9.4 Further Studies

We have suggested functional and structural integrity of the central retina likely influences myopia development. To further validate this myopia development prediction method, studies with larger sample size and longer follow-up, from childhood to adulthood with various degrees of myopia

progression, are needed. In addition, the current Global Flash mfERG recording paradigm is laborious and, as only central IC response among variant mfERG parameters was found to be significantly related to myopia development, simplifying the protocol could allow a more efficient measurement of central retinal function in children.

We did not observe any structure-function correlation regarding only a limited coverage of central retinal thickness. Future studies should examine more peripheral retinal regions that encompass the entire field assessed by the mfERG, as well as isolate the individual retinal layer in the OCT scans. In addition, it has been reported that there was a significant increase in choroidal thickness over childhood, and children undergoing faster axial eyeball growth exhibited less thickening (Read et al., 2013, Read et al., 2015a). The potential role for the choroid in the mechanisms regulating eye growth, and the correlation between subfoveal choroidal thickness and central retinal function is not fully understood. Future work examining longitudinal changes of ocular parameters with myopia development should include *in vivo* changes of choroidal thickness, and to investigate the interactive effect of this parameter with peripheral refraction and retinal electroretinal functions.

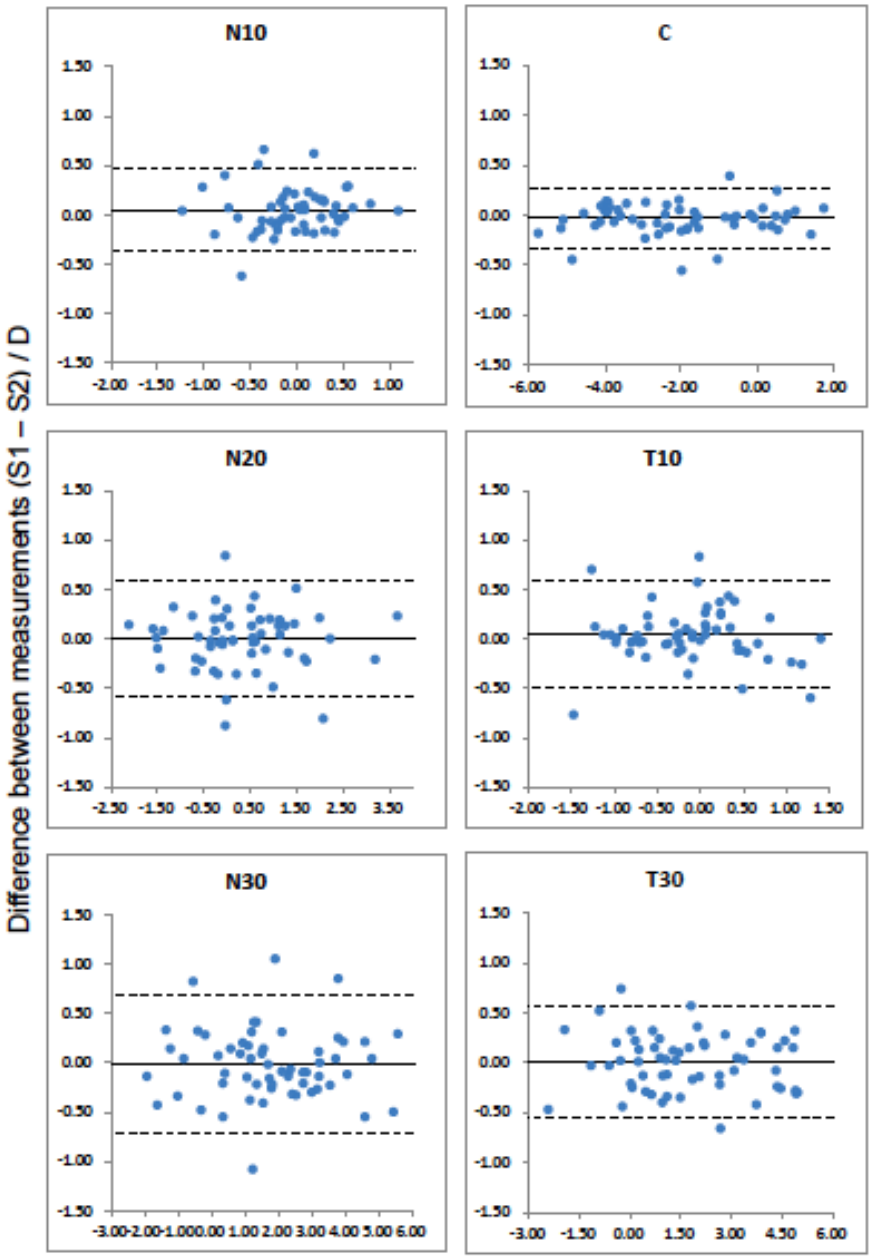
In this study, environmental factors have not been considered. Our visual environment (indoors and outdoors) has objects located at varying distances, resulting in a range of defocus across different regions of the retina (Flitcroft, 2012). Consequently, other than the focused foveal image, the magnitude and location of retinal defocus on the peripheral retina are constantly changing as the eye scans the environment. Therefore, information regarding both the viewing habits and off-axis refraction of an individual are required to understand the pattern of peripheral retinal defocus

in real life. In addition, the fact that the visual environment also contains a wide variety of bright ness, which would result in a variety of levels of retinal illuminance in different environments. Understanding of the nature of the interactions between the environment, the optical defocus and structure of the eye, and the retinal electrophysiology is essential to further enrich our existing knowledge in myopia development and for the design of more effective myopia control treatment.

Appendices

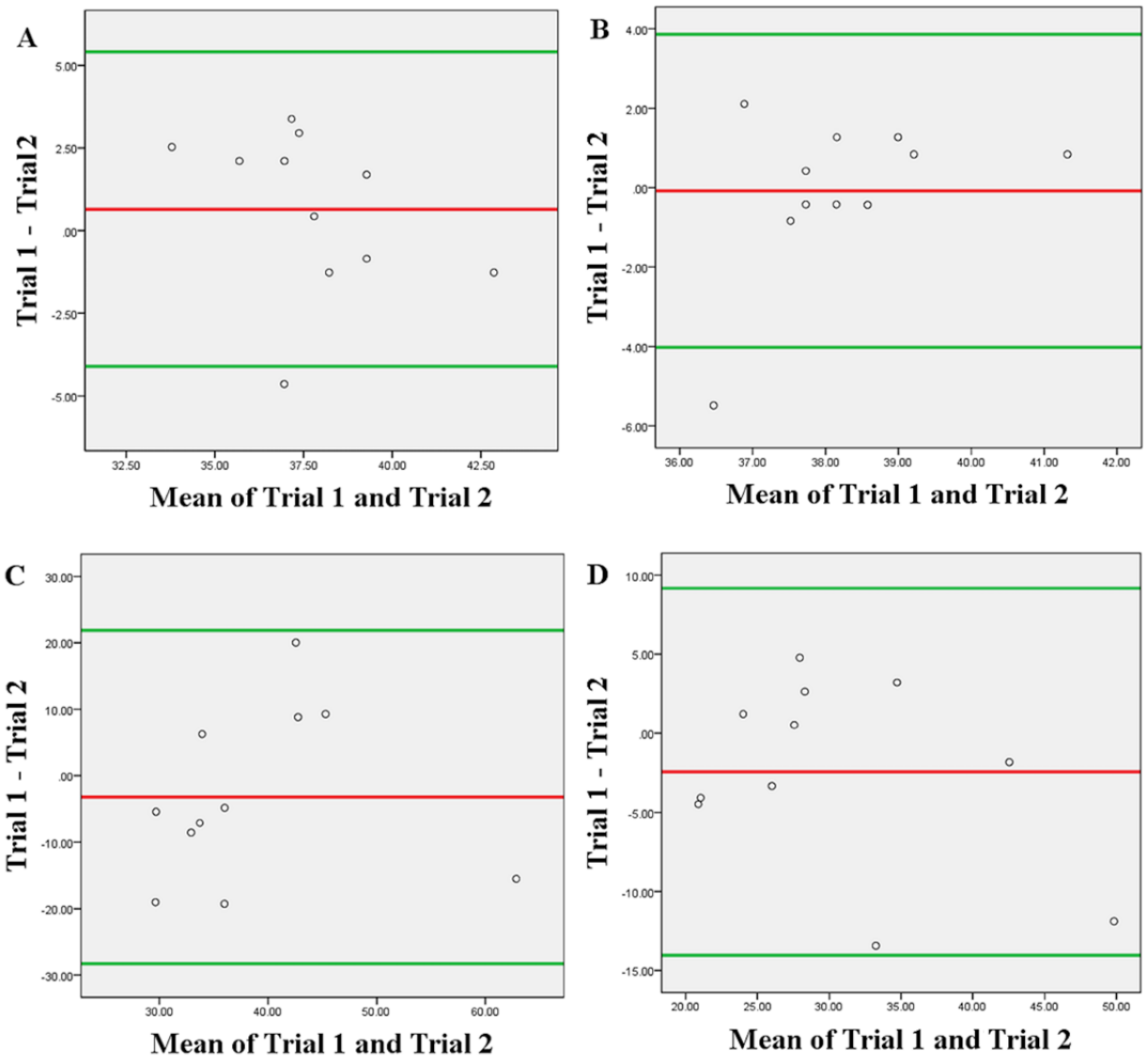
Appendix A-Repeatability of Peripheral Refraction Measurements

Bland-Altman plot of the repeated measurements in central refraction and relative peripheral refraction (M vector only) on spectacle-wearing eyes. Result of T20 was excluded. S1: 1st set of measurement; S2: 2nd set of measurement; C: central; N: nasal; T: temporal; 10: eccentricity of 10 degree; 20: eccentricity of 20 degree; 30: eccentricity of 30 degree

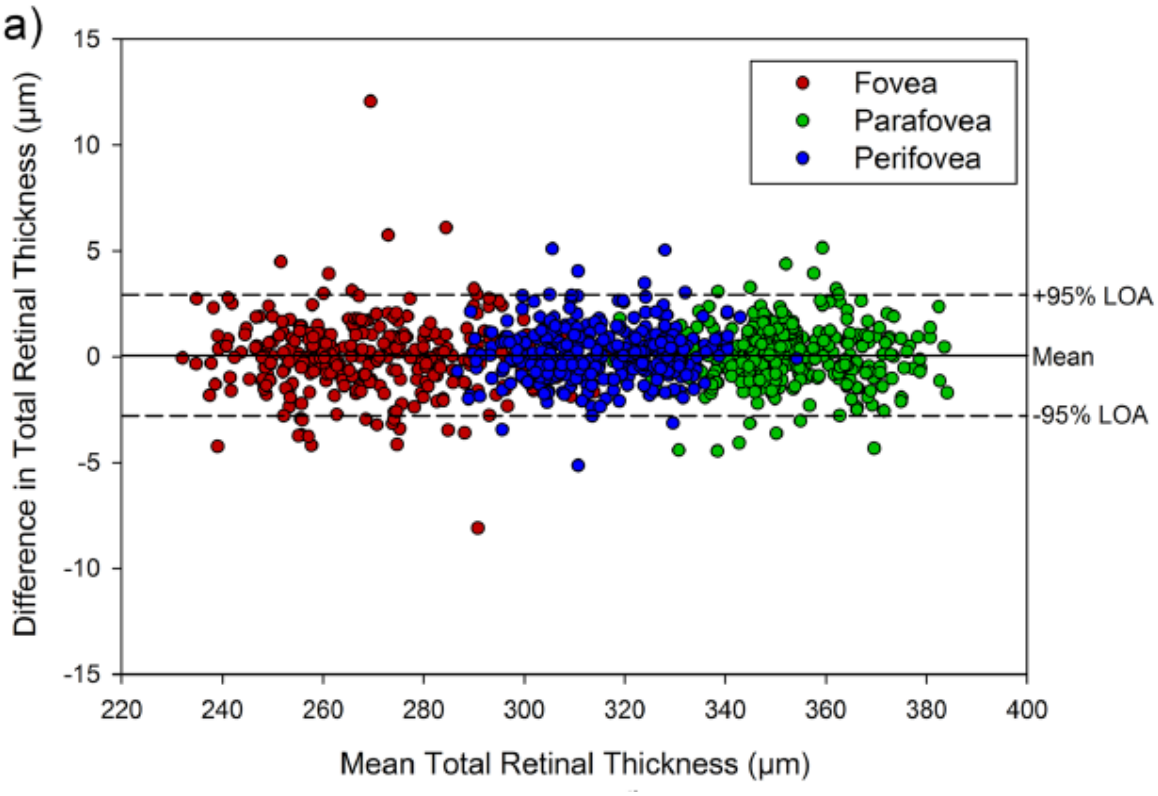


Adopted from: Lee, Tsui-Tsui; Cho, Pauline. Repeatability of relative peripheral refraction in untreated and orthokeratology-treated eyes. Optometry and Vision Science October 2012; 89(10):1477-1486.

Appendix B-Repeatability of Global Flash Multifocal Electroretinogram Measurements Bland-Altman analysis of repeatability for (A) DC implicit time; (B) IC implicit time; (C) DC amplitude; and (D) IC amplitude.



Appendix C-Repeatability of Optical Coherence Tomography Measurements
Bland-Altman plots demonstrating the repeatability of the total retinal thickness



Adopted from: Read, SA.; Alonso-Caneiro, D.; Vincent, SJ. Longitudinal changes in macular retinal layer thickness in pediatric populations: myopic vs non-myopic eyes. PLos One 2017; 12(6): e0180462.

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