Longitudinal Changes in Choroidal Thickness and Eye Growth in Childhood

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Abstract:

Purpose: To examine longitudinal changes in choroidal thickness and axial length in a population of children with a range of refractive errors.

Methods: One hundred and one children (41 myopes and 60 non-myopes), aged 10-15 years participated in this prospective, observational longitudinal study. For each child, 6-month measures of choroidal thickness (using enhanced depth imaging optical coherence tomography) and axial ocular biometry were collected 4 times over an 18 month period. Linear mixed models were used to examine the longitudinal changes in choroidal thickness and the relationship between changes in choroidal thickness and axial eye growth over the study period.

Results: A significant group mean increase in subfoveal choroidal thickness was observed over 18 months (mean increase 13 ± 22 µm, p<0.001). Myopic children exhibited significantly thinner choroids compared to non-myopic children (p<0.001), although there was no significant time by refractive group interaction (p=0.46), indicating similar changes in choroidal thickness over time in myopes and non-myopes. However, a significant association between the change in choroidal thickness and the change in axial length over time was found (p<0.001, β = -0.14). Children showing faster axial eye growth exhibited significantly less choroidal thickening over time compared to children showing slower axial eye growth.

Conclusions: A significant increase in choroidal thickness occurs over an 18 month period in normal 10 to 15 year old children. Children undergoing faster axial eye growth exhibited less thickening and in some cases, a thinning of the choroid. These findings support a potential role for the choroid in the mechanisms regulating eye growth in childhood.

Keywords: Choroid, Childhood, Myopia, Refractive Error, Optical Coherence Tomography
Introduction:

The development and application of new imaging methods, such as enhanced depth imaging (EDI) spectral domain optical coherence tomography (SD-OCT), and long wavelength OCT, has led to significant improvements in our understanding of the structural characteristics of the in vivo human choroid. Although high levels of between subject variability is a commonly reported feature of in vivo choroidal thickness measures, a number of recent cross-sectional studies of healthy eyes have established that two of the major factors that can impact upon in vivo choroidal thickness are age and refractive error. A reduction of choroidal thickness with increasing age in adults has been a consistent finding across a number of recent studies using a variety of OCT instruments, with estimates ranging from 14 µm to 54 µm decrease in subfoveal choroidal thickness per decade of increasing age. A number of recent studies have also examined the association between age and choroidal thickness in children. However, the nature of the changes occurring in choroidal thickness with increasing age in childhood has varied in these reports. Studies that have limited their investigations to children with refractive errors close to emmetropia have typically noted a positive association between choroidal thickness and age, suggesting that in normal pediatric eyes without significant refractive error, the choroid thickens with age. However, other studies including a wider range of refractive errors have noted a negative association between age and choroidal thickness in childhood. These apparently discordant results suggest an interaction between refractive error and age in childhood, particularly given that
myopia often develops in childhood and is known to be associated with a thinner choroid.\textsuperscript{20} This appears to be supported by the findings of Nagasawa et al\textsuperscript{8} who report that the negative association between age and choroidal thickness is no longer significant in multivariate analyses after adjusting for axial length.

Studies of \textit{in vivo} choroidal thickness in adults have also shown that the choroid exhibits significant variation with refractive error and axial length, with the thickest choroids typically noted in hyperopic eyes with shorter axial lengths and the thinnest choroids in myopic eyes with longer axial lengths.\textsuperscript{4,6,12,16-19} Marked choroidal thinning is also commonly found in subjects with high myopia.\textsuperscript{14,15} A thinner choroid has also been noted in the eyes of myopic children, consistent with choroidal changes occurring relatively early in the refractive error development process.\textsuperscript{20,21} The thinner choroid reported in cross-sectional studies of human myopic subjects is also consistent with previous research with a range of animal models demonstrating that a thinning of the choroid is one of the ocular changes that occurs at an early stage during the development of experimental myopia.\textsuperscript{22-24}

All of the recent studies examining the influence of age and refractive error upon the normal \textit{in vivo} choroidal thickness in adults and children have utilised cross-sectional study designs. This limits the insights that can be drawn from these data regarding the time-course of age-related and developmental changes in the thickness of the choroid and the causal nature of any links between choroidal thickness and refractive error. In order to provide a clearer understanding of the nature and time-course of the changes occurring in choroidal thickness in childhood, and the
relationship between these changes and eye growth, in this study we have examined
the longitudinal changes of macular choroidal thickness and axial length, over an 18
month period, in a population of healthy children with a range of refractive errors.

Methods

Subjects and Procedures

This 18 month prospective, observational longitudinal examination of the changes in
macular choroidal thickness and axial length in childhood included the 102 children
aged between 10 and 15 years of age enrolled in the Role of Outdoor Activity in
Myopia (ROAM) study.25 Baseline measurements of choroidal thickness and axial
length were collected between May and November 2012. Subsequent measurement
sessions were then conducted every 6 months over 18 months (i.e. a total of 4 study
visits over 18 months). Approval from the Queensland University of Technology
human research ethics committee was obtained prior to commencement of the
study, and all parents provided written informed consent for their child to participate,
and all children provided written assent. All participants were treated in accordance
with the tenets of the declaration of Helsinki.

An ophthalmic examination was conducted for all children prior to enrollment in the
study in order to determine their refractive, visual, and ocular health status. All
children enrolled in the study exhibited best corrected visual acuity of logMAR 0.00
or better in each eye, no history or evidence of significant ocular disease, and no
hyperopic refractive errors greater than +1.25 DS. Eligible subjects were classified based upon the non-cycloplegic spherical equivalent subjective refractive error (SER) of their right eye, as being either myopic (SER of -0.75 D or more myopia) or non-myopic (SER less than +1.25 D and greater than -0.50 D). At the time of enrollment in the study, all of the myopic children wore conventional single vision spectacle corrections (although 4 children also sometimes wore spherical soft disposable contact lenses) and no children were under any optical or pharmacological treatments to slow myopia progression. Retinal imaging at the second 6-month study visit revealed signs that one of the non-myopic participants was developing a retinal dystrophy, and they were therefore excluded from all analyses, which meant that 101 children were included in the final analysis.

At the baseline study visit, these 101 children had a mean (± SD) age of 13.1 ± 1.4 years and consisted of 52% females. Forty-one children were classified as myopes (mean subjective SER -2.39 ± 1.51 D, mean cylinder -0.39 ± 0.49 D) and 60 as non-myopes (mean subjective SER +0.35 ± 0.31 D, mean cylinder -0.09 ± 0.21 D). The myopic and non-myopic children were well matched for both age (mean age 13.0 ± 1.5 years in the myopes and 13.1 ± 1.2 years in the non-myopes) and gender (51% of the myopes and 52% of the non-myopes were female). Over the 18 month study period, three children were lost to follow-up (two after their baseline visit, and one after their second ocular measurement visit) and four children were excluded after they began orthokeratology contact lens wear (after their second (n = 3) or third (n = 1) ocular measurement visit). Therefore, 94 (92% of enrolled participants) children (59 non-myopes and 35 myopes) completed all 4 study visits.
Choroidal thickness measurements

Choroidal thickness measurements were derived from OCT images collected with the Heidelberg Spectralis SD-OCT instrument (Heidelberg Engineering, Heidelberg, Germany). This device uses an 870 nm super luminescent diode for OCT imaging with a scanning speed of 40,000 A-scans per second, to provide chorio-retinal OCT images with an axial resolution of 3.9 µm and transverse resolution of 14 µm, and has been shown to provide highly repeatable measures of choroidal thickness. At each study visit, children had 2 series of OCT images of their right eye collected using a high resolution six line “star” scanning protocol, consisting of six 30° long, radial line scans centred on the fovea, each separated by 30°. All OCT images were captured using the instrument’s enhanced depth imaging (EDI) mode in order to optimise choroidal visibility. Automatic real time eye tracking was also employed, allowing each radial OCT image to be the average of 30 B-scans. Four children (2 non-myopes and 2 myopes) were unable to maintain stable fixation to allow all 6 radial OCT images to be captured, and a single horizontal scan image (the average of 30 B-scans) centred on the fovea was collected and analysed for these subjects. Following the baseline visit, all subsequent scans were collected using the instrument’s “Auto Rescan” feature which tracks features in the instrument’s scanning laser ophthalmoscope (SLO) retinal image in order to register follow-up OCT scans to the same retinal location as the baseline measurements. Only images with a scan quality index (QI) of >20dB were included in the analysis, with the mean QI from all scans at all visits being 32.8 ± 2.6 dB. All measurements were collected at a similar time of day between 2pm and 5pm to reduce the potential confounding influence of diurnal variations in choroidal thickness upon the results.
In addition to choroidal thickness measurements, measures of axial length were also collected at each study visit using an optical biometer which is based on the principles of optical low coherence reflectometry (Lenstar LS 900, Haag Streit AG, Koeniz, Switzerland). This instrument precisely measures a range of axial ocular dimensions,\textsuperscript{29} which were also used to correct the OCT transverse magnification. At each visit, 5 repeated measurements of ocular biometry were collected.

**Data analysis**

Following image acquisition at each study visit, all OCT images were exported from the instrument and analysed using custom written software. The image analysis procedures employed in this study have been previously described in detail.\textsuperscript{20} Briefly, an automated graph based method was initially used to segment the outer boundary of the retinal pigment epithelium (RPE) in all OCT images. An experienced masked observer then manually segmented the chorio-scleral interface (CSI), corrected any RPE segmentation errors and marked the centre of the fovea (defined as the position of the deepest portion of the foveal pit) in all OCT images. Following segmentation of the OCT images, the transverse scale of each subject’s OCT data was adjusted to account for ocular magnification factors using their individual ocular biometry data from that study visit.

Choroidal thickness (defined as the distance from the RPE to the CSI) across each OCT image was then calculated to determine the subfoveal choroidal thickness, and the average choroidal thickness across a series of concentric annular zones around
the fovea, including the central foveal zone (central 1 mm diameter), the inner
group (from an inner diameter of 1 mm to an outer diameter of 3 mm) and the
outer macula zone (inner diameter of 3 mm outer diameter of 6 mm). These data
were further analysed to determine the average thickness at 8 locations (temporal,
superior temporal, superior, superior nasal, nasal, inferior nasal, inferior and inferior
temporal) across each of the 3 zones (central fovea, inner macula and outer
macula).

All statistical analyses were carried out using IBM SPSS Statistics Version 21. The
longitudinal changes in subfoveal choroidal thickness (and axial length) over the 18
months of the study (and the influence of various predictor variables upon the growth
trajectory of the choroid) were examined using linear mixed model (LMM) analysis
with restricted maximum likelihood estimation. The LMM examined the effect of
study visit time (in years from baseline visit, as a time varying continuous variable)
upon subfoveal choroidal thickness, using a first order autoregressive covariance
structure (which assumes the correlation between measurements is higher for
measurements taken closer together in time). Individual subject’s slopes and
intercepts were included as random effects in the model (allowing for any pattern of
correlation between the random effects). In addition to classification according to
refractive error group (i.e. myope or non-myope), subjects were additionally
classified based on their axial eye growth over the course of the study (linear
regression analysis of each individual subject’s change in axial length over time was
used to derive an axial growth rate for each subject). This was based upon a tertile
split of the axial growth rate data into groups exhibiting either slow eye growth (<25
µm/year, n = 33), or medium rate of eye growth (between 25 and 67 µm/year, n =
33) or fast eye growth (>67 µm/year, n = 33). Categorical predictor variables (refractive error group, axial eye growth rate and gender) were included in the model as fixed factors, and continuous predictor variables (baseline axial length, change in axial length and age at baseline visit) were included as covariates. Separate analyses were carried out for refractive error group, axial growth rate and change in axial length, since these factors are typically related. A similar approach was used for the analysis of the longitudinal changes in the parafoveal choroidal thickness, with the additional fixed factors of parafoveal zone and location included in the LMM.

Repeatability of the imaging and analysis procedures were assessed through analysis of the two repeated OCT measurements collected at each study visit. The mean difference and 95% limits of agreement between the differences were determined for these data at each visit using the methods of Bland and Altman.\textsuperscript{30}

**Results**

**Choroidal thickness measurement repeatability**

The mean ± 95% limits of agreement of the difference in subfoveal choroidal thickness between the two repeated series of OCT measures at each visit was 0.2 ± 3.7 µm (Visit 1: 0.2 ± 3.7 µm, Visit 2: 0.4 ± 3.4 µm, Visit 3: 0.3 ± 3.8 µm, Visit 4: 0.0 ± 3.9 µm), indicating excellent repeatability for the subfoveal analysis of the two repeated scans, that appeared consistent across all 4 study visits (Figure 1a). The parafoveal analysis also revealed good repeatability (although the limits of
agreement were observed to be greater in the inner and outer macula zones compared to the central foveal zone), with the mean ± 95% limits of agreement of the difference between the two repeated scans at all visits being 0.1 ± 4.6 µm (Visit 1: 0.1 ± 4.1 µm, Visit 2: 0.4 ± 4.8 µm, Visit 3: 0.1 ± 4.2 µm, Visit 4: -0.2 ± 5.2 µm) for the mean thickness in the central foveal zone, 0.4 ± 8.4 µm (Visit 1: 0.5 ± 7.4 µm, Visit 2: 0.6 ± 10.0 µm, Visit 3: 0.5 ± 7.8 µm, Visit 4: 0.1 ± 8.0 µm) for the inner macula zone and 0.7 ± 8.7 µm (Visit 1: 1.0 ± 9.3 µm, Visit 2: 0.6 ± 9.8 µm, Visit 3: 0.9 ± 7.6 µm, Visit 4: 0.4 ± 8.0 µm) for the outer macula zone (Figure 1).

**Longitudinal changes in subfoveal choroidal thickness and axial length**

Figure 2 illustrates the longitudinal changes in subfoveal choroidal thickness and axial length observed in this population of children over the 18 months of the study. The subfoveal choroid was found to increase significantly in thickness over time (p<0.001; β = 8 µm/year; 95% CI: 2 to 14 µm/year). For all subjects considered together, a mean increase of 13 ± 22 µm in subfoveal choroidal thickness was observed over the 18 month study period. A significant main effect of refractive error group was also observed, indicative of a significantly thinner subfoveal choroid in the myopic children (mean choroidal thickness was 303 ± 79 µm at baseline) compared to the non-myopic children (mean 360 ± 77 µm) (p<0.001). However, there was no significant time by refractive error interaction (p=0.40), suggesting that although on average the non-myopic children (mean change of 15 ± 19 µm in 18 months) showed slightly greater choroidal thickening than the myopic children (mean change 11 ± 28 µm), the change in subfoveal choroidal thickness over time was not significantly different between them. There were no significant main effects of
gender or age at baseline, and no significant gender by time or age at baseline by
time interactions (all p>0.05). Figure 2 illustrates a trend for a potential seasonal
variation in the changes in choroidal thickness, with smaller changes in thickness
observed at visit 3 (which coincided with the winter months in the majority of
participants) compared to visits 2 and 4. However, pairwise comparisons of the
average 6-monthly changes in choroidal thickness observed at visits 2, 3 and 4,
were not significantly different (all p>0.05).

Over the 18 months of the study, axial length also increased significantly over time
(mean increase in axial length for all children over 18 months was 105 ± 155 µm,
p<0.0001) (Figure 2b). Significant main effects of gender and refractive group were
also seen (both p<0.001), indicative of a significantly longer axial length in boys
(baseline axial length was 70 µm longer in boys) and myopic children (baseline axial
length was 119 µm longer in myopic children). Significant interactions were also
observed between refractive group and time (p<0.001; indicating a greater axial
growth rate for myopic children (β = 119 µm/year) compared to non-myopic children
(β = 42 µm/year)) and age at baseline and time (p<0.001, β = -20; indicating a
greater increase in axial length over time in younger children).

For all subjects considered together, a significant association between the change in
subfoveal choroidal thickness and the change in axial length (p<0.001, β = -0.14;
95% CI: -0.20 to -0.09) was found. This association remained significant for
analyses considering only the myopic children (p<0.001, β = -0.11; 95% CI: -0.16 to -
0.06 ), as well as for analyses considering only the non-myopic children (p<0.001, β
= -0.27; 95% CI: -0.37 to -0.17). This negative association indicates that those children exhibiting larger amounts of axial eye growth over the 18 months of the study exhibited less subfoveal choroidal thickening (Figure 3a). It is evident from Figure 3a that those children exhibiting the greatest rate of axial eye growth over the 18 month study also typically exhibited a thinning of the subfoveal choroid. There was no significant association between the baseline axial length and the change in subfoveal choroidal thickness over time (p = 0.7).

Further analysis was performed, stratifying the population according to their rate of axial eye growth. Each of the three axial eye growth rate groups included both myopic and non-myopic children, with the slow eye growth group (mean SER -0.1 ± 1 D) consisting of 24% myopes within the group, the medium eye growth group (mean SER -0.6 ± 2.1 D) consisting of 24% myopes and the fast eye growth group (mean SER -1.6 ± 1.5) consisting of 73% myopes. Figure 3b illustrates the mean changes in subfoveal choroidal thickness for the slow, medium and fast axial eye growth groups. A significant interaction between eye growth and time was observed (p = 0.03) indicating that the amount of choroidal thickening observed in the children exhibiting faster axial eye growth (mean change of 6 ± 27 µm in 18 months) was significantly less than the choroidal thickening in the children exhibiting slow (18 ± 21 µm) and medium (16 ± 18 µm) axial eye growth.

These results demonstrate that the changes in choroidal thickness in childhood appear to be closely linked to the axial growth of the eye. The individual changes observed in two of the subjects in our study provide examples that illustrate this
relationship (Figure 4, Supplementary Movie S1). In one of these examples, a child (S_079) who was non-myopic at baseline, began to develop myopia between the 6 and 12 month study visits. After showing less than 20 µm of axial length change over the first six months of the study, an increase in axial length of ~200 µm was observed in the next six months of the study and this was accompanied by a substantial choroidal thinning (51 µm thinner subfoveally compared to baseline). In the second example, a myopic child (S_090) who exhibited rapid axial elongation (412 µm increase in axial length) over the first 12 months of the study (accompanied by a 12 µm choroidal thinning), shows a thickening of the choroid (33 µm thicker subfoveally compared to baseline) and a substantial slowing of axial eye growth in the final 6 months of the study (only 2 µm difference in axial length between the 12 and 18 month visits).

Longitudinal changes in parafoveal choroidal thickness

Figure 5 illustrates the average parafoveal choroidal thickness at the baseline visit, and the mean changes in parafoveal choroidal thickness over the 18 months of the study for the 90 children with complete parafoveal data from all 4 study visits. A significant increase in parafoveal choroidal thickness was found over time (p<0.001; $\beta = 8$ µm/year; 95% CI: 5 to 11 µm/year). Significant variations in the mean choroidal thickness were found across the parafoveal zones (with the mean thickness being greatest in the central foveal zone compared to the inner and outer macula zones) and locations (with the choroid being thickest in the superior and temporal locations and thinnest in the nasal and inferior locations) (both p<0.001). Although there was a trend for the central foveal (13 ± 22 µm mean change in 18
(13 ± 21 µm) zones to exhibit larger increases over time than the outer macula zone (mean increase 11 ± 18 µm), and for the superior location (mean increase of 14 ± 21 µm) to exhibit the largest and the temporal location (11 ± 22 µm) the smallest change over time, there was no parafoveal zone by time, or location by time interactions (p>0.05), indicating that the changes in choroidal thickness across the various parafoveal regions were similar over time.

A significant main effect of refractive group (p<0.001) was also found for the parafoveal choroid, with the myopic children (mean parafoveal choroidal thickness across all zones and locations at baseline 294 ± 72 µm) having significantly thinner parafoveal choroids compared to the non-myopic children (341 ± 71 µm) (Figure 5a). However, there was no time by refractive group interaction (p = 0.4) indicating that the changes in the parafoveal choroidal thickness over time were not significantly different between the myopic children (mean increase of 10 ± 25 µm in 18 months across all parafoveal regions) and the non-myopic children (mean increase of 14 ± 16 µm) (Figure 5b).

Similar to the subfoveal analysis, a significant interaction between axial eye growth and time was also observed for the parafoveal choroidal thickness (p = 0.04), with a greater choroidal thickening over time observed in the children with slow (mean increase of 16 ± 17 µm in 18 months) and medium (mean increase of 14 ± 15 µm) axial eye growth compared to the children exhibiting fast axial eye growth (mean increase 6 ± 25 µm) (Figure 6). There were no significant parafoveal zone (or location) by eye growth by time interactions, indicating that the change in choroidal
thickness associated with axial eye growth did not vary significantly in the different regions examined (all p>0.05).

Discussion

This study provides the first prospective longitudinal evaluation of the changes in choroidal thickness occurring in vivo in childhood. The major finding in this study is that in normal children, a significant increase in both subfoveal and parafoveal choroidal thickness occurs over an 18 month period. Over this same period of time, significant increases in axial length were also observed, which suggests that the changes in choroidal thickness cannot be explained by a simple mechanical mechanism related to alterations in eye size during childhood. However, the increases in choroidal thickness during childhood do appear to be influenced by the rate of axial eye growth, with those children exhibiting slower axial eye growth showing greater choroidal thickening compared to children exhibiting faster axial eye growth. Children demonstrating rapid axial eye growth over the 18 months of the study tended to exhibit less choroidal thickening and often showed evidence of choroidal thinning. The choroidal changes that we have observed therefore may represent the balance of two separate phenomena: a thickening of the choroid related to normal ocular growth and development in childhood, and a thinning of the choroid related to the rapid axial eye growth that is typically associated with myopia development and progression.
Previous animal studies examining developmental choroidal thickness changes in primates have also reported evidence of choroidal thickening occurring from birth to adolescence in normally developing animals with unrestricted vision.\textsuperscript{23,24} Our findings are broadly consistent with these choroidal changes in animals. Troilo and colleagues\textsuperscript{24} speculated that the increase in choroidal thickness may function to slow juvenile eye growth. Our finding of slower eye growth in childhood being associated with greater choroidal thickening tends to support this notion. Animal studies have also documented that the normal developmental changes in choroidal thickness are altered when deviations in the normal rate of eye growth are induced experimentally.\textsuperscript{22-24} Choroidal thinning has consistently been found to be associated with the increases in eye growth occurring with experimentally induced myopia and choroidal thickening is known to accompany the slowing of eye growth during the development of hyperopia.\textsuperscript{22-24} Although our study did not include hyperopic children, the association between axial eye growth and choroidal thickness observed in the myopic and non-myopic children in our study is consistent with these previously documented associations between changes in choroidal thickness and altered eye growth in animals.

Some previous cross-sectional studies examining primarily emmetropic children have reported a positive association between choroidal thickness and age in childhood\textsuperscript{10,13} with these studies demonstrating age-related increases in choroidal thickness of around 9 µm per year. This agrees closely with our longitudinal findings of an increase in choroidal thickness of 8 µm per year. Conversely, other cross-sectional studies of children\textsuperscript{8,9,11} have reported a negative association between age and choroidal thickness, which is inconsistent with our current longitudinal results.
However, these studies included children with a relatively wide range of refractive errors, which leaves open the possibility that this negative association is being driven by an increased proportion of myopia (and associated thinner choroid) in the older children of the populations examined in these studies, rather than an independent negative association between age and choroidal thickness in childhood.

Recently Li and colleagues\textsuperscript{21} reported on the subfoveal choroidal thickness of a large population of 11 and 12 year old Danish children. In their cross-sectional study, a significant relationship between choroidal thickness and stage of pubertal development was found in girls, with more advanced pubertal development being associated with thicker subfoveal choroids. Although an assessment of pubertal development was not included in our current study, the age of the children examined (10 to 15 years) suggests the possibility that hormonal related changes associated with puberty may have contributed towards some of the choroidal changes we observed over time.

The myopic children in our current study were found to have significantly thinner choroids than the non-myopic children, which agrees with previous cross-sectional reports of choroidal thickness and refractive error in children\textsuperscript{20,21} and adults.\textsuperscript{4,6,12,16-19} However, the changes in choroidal thickness over time in myopes (7 µm/year) and non-myopes (9 µm/year) were not significantly different, with both groups exhibiting on average an increase in choroidal thickness over the 18 months of the study. Although the myopic children exhibited significantly greater axial eye growth than the non-myopic children, the mean changes in axial length in our myopic children were
slightly smaller in magnitude than a number of previous studies of axial eye growth in myopic children. A number of the myopic participants did exhibit relatively slow axial eye growth, and also demonstrated thickening of the choroid over time, which is the likely factor underlying the lack of statistically significant difference between the longitudinal changes in choroidal thickness between the myopic and non-myopic children. Although our study population included children exhibiting a wide range of axial eye growth rates and provides new insights into the relationship between changes in choroidal thickness and eye growth, future studies examining greater numbers of myopic children exhibiting more rapid myopia progression and axial elongation (particularly younger children in the early stages of myopia development) are likely to provide additional insights and may reveal more substantial differences between the longitudinal choroidal changes of myopic and non-myopic children.

Our findings demonstrate that faster axial elongation is associated with less choroidal thickening over time, with evidence of a choroidal thinning exhibited by a number of children showing rapid axial elongation. It has been shown previously that the fastest axial elongation associated with myopia in childhood typically occurs in the 12 months before and after the initial development of myopia. Since our myopic children all had established myopia when they were enrolled in the study, it is likely that the more rapid period of axial elongation (and potentially associated choroidal thinning) for some of our myopic children had already occurred before they were enrolled in the study. The association between axial eye growth and choroidal thickness change does provide a mechanism through which myopic children could develop a thinner choroid than non-myopic children over time. The rapid axial elongation occurring during the early period of myopia development, and the likely
associated choroidal thinning, would be expected to eventually result in myopic children developing thinner choroids compared to non-myopic children (who over the same period of time would be expected to show a thickening of the choroid).

Although there is evidence, particularly from animal studies, that changes in choroidal thickness accompany the development and progression of refractive errors, the exact role that the choroid plays in the control of eye growth is still a matter of speculation.\(^{36}\) The choroid may have a direct influence on eye growth through the secretion of growth factors that act on the sclera,\(^{37}\) or may have an indirect role by acting as a barrier to diffusion of retina-derived growth factors or signalling molecules,\(^{36}\) or act as a mechanical buffer to expansion of the globe.\(^{38}\) While our findings demonstrate an association between choroidal thickness changes and the axial growth of the eye in childhood, further research is required to determine whether this relationship between axial eye growth and choroidal thickness change is due to an active or passive choroidal mechanism or an association between choroidal thickness and other eye growth signals.

Our analysis of the parafoveal choroid revealed a similar magnitude of increase in choroidal thickness over time across the parafoveal region as was observed in the subfoveal region. Although examination of Figures 5 and 6 indicate some apparent subtle regional differences in choroidal thickness change (with trends towards greater choroidal thickness changes in central and superior regions), there were no statistically significant differences in the changes in choroidal thickness observed in the different parafoveal regions, suggesting a relatively uniform pattern of change in
choroidal thickness across the parafovea. Future work exploring wider field imaging

techniques, denser scanning protocols and examining larger populations of children

may help to provide a clearer picture of any regional differences in longitudinal

choroidal thickness change across the posterior pole.

Since our study provides the first report of longitudinal changes in choroidal

thickness in childhood, our results provide a reference for pediatric clinical imaging

and for future longitudinal studies examining factors influencing choroidal thickness

in childhood. The mean magnitude of subfoveal choroidal thickening over the course

of our study (13 µm) is larger than both the axial resolution of the OCT instrument

used and the repeatability (95% limits of agreement) observed in the results (Figure

1a), and this suggests that measurable changes in choroidal thickness are likely to

be present in normal children who are assessed clinically with OCT imaging over

time. Furthermore, knowledge of the magnitude and time course of the

developmental increases in choroidal thickness in normal children will aid in the

interpretation of the findings of future studies examining the potential influence of

ocular diseases and various pharmacological and optical ocular treatments upon

choroidal thickness in childhood. Our results also emphasise the importance of

knowledge of the magnitude of axial eye growth in the interpretation of choroidal

thickness changes.

The strengths of our current study include the longitudinal data analysis with good

subject retention (less than 10% attrition over the 18 months), which allows greater

insight into the changes in choroidal thickness with age and eye growth compared to
cross-sectional studies. Our analysis approach accounted for the influence of ocular magnification, and eye tracking and image registration were employed by the instrument for the OCT imaging during the follow-up visits in the study. Furthermore, the time of day of the data collection at each study visit was also controlled to limit the potential confounding influence of diurnal variations of choroidal thickness. The main limitations of the current study include the relatively small sample size with unequal numbers of myopic and non-myopic children and short follow-up time. Future work examining longitudinal changes of choroidal thickness in larger numbers of both myopic and non-myopic children, over a longer period of time are likely to provide further insights into these changes. The lack of cycloplegic refraction measures may also reduce the reliability of refractive error determination in children, however there is evidence that anticholinergic cycloplegic agents can influence choroidal thickness in adults, and their effect on choroidal thickness in children is currently unclear. Since most of the analyses concentrated upon the relationship between choroidal thickness and axial eye growth changes over time, this is unlikely to be systematically influenced by the lack of cycloplegia.

**Conclusions:**

This study provides the first report of longitudinal changes of *in vivo* pediatric choroidal thickness, and demonstrates a significant increase in choroidal thickness occurs over 18 months in childhood. The changes in choroidal thickness were also found to be associated with the rate of axial eye growth, with children exhibiting
faster axial eye growth exhibiting less choroidal thickening over time, supporting a potential role for the choroid in the control of eye growth in childhood.

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References


Figures:

**Figure 1:** Bland-Altman plots illustrating the repeatability of the choroidal thickness measures from the two repeated OCT scans collected at each of the 4 visits in the study, for the subfoveal choroidal thickness (a), the central foveal zone (b), the inner macula zone (c), and the outer macula zone (d) parafoveal choroidal thickness.
**Figure 2:** Mean ± SEM change in subfoveal choroidal thickness (ChT) (a) and axial length (b) from baseline for all children (black line), myopic children (red line) and non-myopic children (blue line) over the 18 months of the study. Horizontal error bars represent the SEM of the study visit time.
Figure 3: Relationship between the rate of change (derived from regression analysis of each individual subject’s change over time) in subfoveal choroidal thickness (ChT) and axial length for the myopic (red circles) and non-myopic (blue circles) children over the 18 months of the study (dashed lines show the best fit regression line for the myopic (red) and non-myopic (blue) children) (a). Shading in (a) illustrates the tertiles of the axial eye growth data used to stratify children as exhibiting either fast (red shading), medium (blue shading) or slow eye growth (green shading). The mean ± SEM changes in subfoveal choroidal thickness in children exhibiting fast (red line), medium (blue line) and slow (green line) axial eye growth are also illustrated (b). Horizontal error bars in (b) represent the SEM of the study visit time.
**Figure 4:** Examples of longitudinal changes in choroidal thickness (ChT) and axial length for two subjects, illustrating the close relationship between the changes in choroidal thickness (red line) and axial length (blue line) in the study. Subject 079 (top) was a non-myopic male at baseline and exhibited minimal axial elongation and small magnitude choroidal thickening in the first 6 months of the study, and then exhibited marked axial elongation and the development of myopia along with choroidal thinning in the second half of the study. Subject 090 (bottom) is a myopic female who exhibited substantial axial elongation and a small degree of choroidal thinning over the first 12 months of the study, and then showed a slowing of axial elongation and a choroidal thickening in the final 6 months of the study. (Supplementary Movie S1 file illustrates the changes in the horizontal OCT scan choroidal images at each of the study visits).
Figure 5: Average choroidal thickness (ChT) at the baseline visit across the central 6 mm parafoveal region for all children (left), myopic children (middle) and non-myopic children (right) with complete parafoveal choroidal thickness data at all visits (a). The average change in choroidal thickness from baseline at each 6-month study visit for each group is also illustrated (positive values indicate a choroidal thickening and negative values indicate a choroidal thinning over time) (b). T indicates temporal, and N indicates nasal.
**Figure 6:** Average choroidal thickness (ChT) at the baseline visit across the central 6 mm parafoveal region for children exhibiting fast (left), medium (middle) and slow (right axial eye growth with complete parafoveal choroidal thickness data at all visits (a). The average change in choroidal thickness from baseline at each 6-month study visit for each group is also illustrated (positive values indicate a choroidal thickening and negative values indicate a choroidal thinning over time) (b). T indicates temporal, and N indicates nasal.