University of
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Circannual and Circadian Rhythms:

Implications for Physiological Ocular Growth

Dissertation for the degree of PhD
Person-centred Health Care

Faculty of Health and Social Sciences

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South-Eastern Norway

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Dedication

To Ellie and Tingting

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Nickolai G. Nilsen

Kongsberg, 21.05.2024

Sammendrag

Det er lav forekomst av nærsynthet i Norge og i andre skandinaviske land som skiller seg ut fra den verdensomfattende økende forekomsten, som er særlig høy i Sørøst-Asia. Økt utendørstid, som kan være knyttet til eksponering av dagslys, er rapportert til å ha en beskyttende effekt mot insidens og progresjon av nærsynthet. Basert på den lave forekomsten av nærsynthet i Norge, på tross av mørke vintre (6 timer med tilgjengelig dagslys sammenlignet med ≈19 timer om sommeren), kan man stille spørsmålet om sesongadaptasjon og forskjeller i tilgjengelighet av dagslys kan ha en rolle med å beskytte mot nærsynthet. Målet med denne avhandlingen var derfor å undersøke biologiske og miljøfaktorer knyttet til fysiologisk øyevekst — øyevekst som forekommer ved emmetropisering og ved opprettholdelse av emmetropi — for å øke forståelsen om utviklingen av brytningsfeil.

Avhandlingen består av tre artikler som utforsker biologiske og miljøfaktorer, spesifikt sesongvariasjoner (artikkel I og III) og døgnrytmer (artikkel I og II), og implikasjonene av disse for fysiologisk øyevekst. Prosjektet ble utført i Sørøst-Norge med et utvalg som bestod av deltakere i aldersgruppene 17–24 (artikkel 1), 19–25 (artikkel 2), og 7–11 år (artikkel III). Fysiologisk øyevekst (målt med AL) viste sesongendringer for 7–24-åringene med en raskere vekstrate om vinteren enn om sommeren (artikkel I og III), men med en saktere rate sammenlignet med sesongvariasjon under utvikling av nærsynthet. AL og SER for 7–8-åringer var sammenlignbare med kinesiske barn med samme alder, men forskjellene økte ved alderen 10–11 år, og enda mer ved 17–25 år. Choroidea var tykkere for alle aldersgruppene (artikler I–III) sammenlignet med kinesiske individer i tilsvarende aldre, dette gjaldt også ved alderen 7–8 år. Assosiasjonen mellom ΔAL og AL faseskifte fra vinter til sommer (artikkel I) indikerer en sesongadaptasjon, som støtter at okulære døgnrytmer er involvert i fysiologisk øyevekst. Dette støttes også av hvor forskjellig faseforholdet endres mellom AL og ChT i vinter når det er mer øyevekst (artikkel I), og når det er påvirkning av 1% atropin (artikkel II). Disse endringer i artikkel I og II har likheter med det som er rapportert i døgnrytmestudier på kyllinger ved akselerert og ved redusert øyevekstrate, henholdsvis. Det kreves mer forskning for å undersøke den mulige påvirkningen øyelinsen og dens døgnrytme kan ha for fysiologisk øyevekst.

Funnene i denne avhandlingen bidrar til forståelsen av de biologiske og miljøfaktorene som er involvert ved fysiologisk øyevekst. Dette kan ha implikasjoner for behandling av nærsynthet som bør å ha en person-orientert tilnærming.

Nøkkelord: Nærsynthet, brytningsfeil, fysiologisk øyevekst, aksiallengde, choroidal tykkelse, øyelinsen, døgnrytmer, sesongrytmer, person-orientert helsearbeid

Abstract

The low reported prevalence of myopia in Norway and other Scandinavian countries deviates from the increasing global prevalence, with a particularly high prevalence in South-East Asia. Increased outdoor time, potentially linked with daylight exposure, has been reported to have a protective effect against myopia incidence and progression. The low prevalence of myopia in Norway, despite the dark winters (6 hours of available daylight vs. ≈19 hours in the summer), raises the question of whether seasonal adaptation and the difference in daylight availability can have a role in protecting against myopia. The aim of this thesis was therefore to assess biological and environmental factors related to physiological ocular growth — ocular growth occurring during the stages of emmetropization and maintaining emmetropia — in order to comprehend refractive error development.

This thesis consists of three papers that explore the environmental and biological factors, specifically circannual (papers I and III) and circadian rhythms (papers I and II) and their implications for physiological ocular growth. The project was conducted in Norway and involved 17–24-year-olds (paper I), 19–25-year-olds (paper II), and 7–11-year-olds (paper III). Physiological ocular growth (by AL) exhibited a seasonal variation for 7–24-year-olds, with a faster rate during the winter compared to summer (papers I and III), but with an overall slower rate than seasonal changes during myopia development. AL and SER for the 7–8-year-olds were comparable to Chinese children of the same age, but the differences increased at ages 10–11-years, and more so at ages 17–25 years. The choroid for all age-groups (papers I–III) was thicker compared with Chinese individuals in each respective age-group, even at ages 7–8 years. The association between ΔAL and AL phase shift from winter to summer (paper I) indicates a seasonal adaptation, which provides support for the involvement of ocular diurnal rhythms in physiological ocular growth. Further support is shown in the differential alterations of the AL and ChT phase relationship during winter when there was more ocular growth than during summer (paper I), and when influenced by 1% topical atropine (paper II). These alterations in paper I and II held resemblance to those reported in chick studies during accelerated and slowed ocular growth, respectively. The potential involvement of the crystalline lens and its diurnal rhythm with physiological ocular growth warrants more research.

The findings of this thesis contribute to the understanding of environmental and biological factors involved with physiological ocular growth. This has potential implications for myopia control therapy that needs to have a person-centred health-care approach.

Keywords: Myopia, refractive errors, physiological ocular growth, axial length, choroidal thickness, crystalline lens, circadian rhythm, circannual rhythms, person-centred healthcare

List of papers

Paper I

Nilsen NG, Gilson SJ, Pedersen HR, Hagen LA, Knoblauch K, Baraas RC. Seasonal Variation in Diurnal Rhythms of the Human Eye: Implications for Continuing Ocular Growth in Adolescents and Young Adults. Investigative Ophthalmology & Visual Science. 2022;63(11):20-, doi:10.1167/iovs.63.11.20

Paper II

Nilsen NG, Gilson SJ, Pedersen HR, Hagen LA, Wildsoet CF, Baraas RC. The effect of topical 1 % atropine on ocular dimensions and diurnal rhythms of the human eye. Vision Res. 2024;214:108341, doi:10.1016/j.visres.2023.108341

Paper III

Nilsen NG, Gilson SJ, Lindgren H, Kjærland M, Pedersen HR, Baraas RC. Seasonal and Annual Change in Physiological Ocular Growth of 7- to 11-Year-Old Norwegian Children. Investigative Ophthalmology & Visual Science. 2023;64(15):10-, doi:10.1167/iovs.64.15.10

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Abbreviations

ACD	Anterior chamber depth
AL	Axial length
ANOVA	Analysis of variance
BrM	Bruch's Membrane
CCT	Central corneal thickness
ChT	Choroidal thickness
CI	Confidence interval
CR	Corneal radius
D	Dioptre
DLMO	Dim light melatonin onset
EDI	Enhanced depth imaging
ELISA	Enzyme-Linked Immunosorbent Assay
GAT	Goldman Applanation Tonometry
HST	Habitual sleep onset time
HWT	Habitual wake time
ICC	Intra-class correlation coefficient
ILM	Inner limiting membrane
IOP	Intraocular pressure
logMAR	logarithm of the minimum angle of resolution
LP	Crystalline lens power
LT	Crystalline lens thickness
MEL	Melatonin
MESOR	Midline estimating statistic of rhythm
NLME	Non-linear mixed effects models
NSAIDs	Non-steroidal anti-inflammatory drugs
OCT	Optical coherence tomography
RPE	Retinal pigment epithelium
RT	Retinal thickness
SAD	Seasonal affective disorder
SD	Standard deviation
SER	Spherical equivalent refractive errors
SFChT	Subfoveal choroidal thickness
USN	University of South-Eastern Norway
VCD	Vitreous chamber depth

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1 Introduction

1.1 Background

To understand the complexity of refractive error development, it is imperative to recognize what physiological ocular growth entails — that is, the ocular growth that is part of the stages of emmetropization and subsequently maintaining emmetropia. While there have been reported global increases in the prevalence of myopia over the past decades,[1] particularly in South-East Asia,[1-3] Scandinavian countries appear not to follow this trend with a low reported prevalence of myopia.[4, 5] Myopia aetiology is complex, having large between-individual variations. It involves genetic factors with a heritability component[6]; biological factors with ocular dimensions[7, 8] and diurnal rhythms;[9, 10] behavioural factors such as near-work[11] and outdoor time;[12, 13] and environmental factors with daylight exposure.[14, 15] Greater outdoor time has been found to particularly have a protective effect against myopia incidence and progression,[12, 13, 16] potentially linked to increased daylight exposure.[15] The low prevalence of myopia and the large differences in daylight availability between winter and summer in Scandinavia raise the question of whether being well-adapted to seasonal changes can have a protective effect against myopia, despite the dark winters.[17]

Understanding refractive error development and myopia susceptibility is of importance as severe myopia from excessive axial elongation has associated risks of sight-threatening ocular pathology later in life which can lead to visual impairment.[18, 19] Visual impairment can have severe consequences for an individual and their family in terms of becoming a social and economic burden. There are also high potential costs to governments for treatment, rehabilitation, and reduced productivity.[20-22] Uncorrected refractive errors (such as myopia) are also reported to be the leading cause of low vision worldwide.[18] It is therefore essential to increase our understanding of 1) what physiological ocular growth entails and how it might differ from myopic and hyperopic growth, and 2) the factors involved with maintaining emmetropia/mild hyperopia. This knowledge aims to facilitate a person-centred healthcare approach to clinical evaluation and follow-up of refractive errors and to contribute to personalized myopia control therapy.

This thesis explores the effects of seasonal variations and ocular diurnal rhythms on physiological ocular growth and the development of refractive errors. The sample population consisted of children, adolescents and young adults living in Norway.

1.2 Emmetropia and emmetropization

In this thesis, physiological ocular growth is defined as ocular growth (measured by axial length) during natural developmental stages, i.e., during emmetropization and the subsequent stage of maintaining emmetropia, and is differentiated from when failure of emmetropization occurs (as with hyperopia and myopia), or failure in maintaining emmetropia (as with myopia).[23] Emmetropia allows light rays that are parallel to the optic axis to enter the eye, refract through the cornea, the aqueous, the crystalline lens, and the vitreous and focuses onto the retina to create a clear image.[24] Refractive errors occur when the refractive state of the eye differs from emmetropia, and encompasses conditions such as myopia, hyperopia, astigmatism and presbyopia. Myopia and hyperopia can be described as the refractive state when the rays of light entering the eye focuses in front or behind the retina, respectively.[25] Myopia can be further classified into axial myopia and refractive myopia. Axial myopia is attributed to excessive axial elongation of the eye, while refractive myopia is attributed to changes in the corneal and/or crystalline lens power (LP).[26] This thesis will discuss different aspects about emmetropia, myopia and to a lesser extent hyperopia, while astigmatism or presbyopia will not be discussed any further.

Emmetropization has been defined as “the developmental process that matches the eye’s optical power to its axial length so that the unaccommodated eye is focused at distance”.[8] Emmetropization begins at birth and is characterized by changes in corneal and crystalline lens power, ocular elongation by the increase in axial length (AL), to achieve emmetropia.[27] At birth, children typically have hyperopia of +2.00–+4.00 D,[28] with the largest reduction the first 18 months of age.[27] At this age, there are substantial changes in corneal power, AL and the crystalline lens.[27] After this, corneal power appears to remain stable throughout childhood,[29-31] while ocular growth continues with a reduced rate until 5–6 years when emmetropization typically completes resulting in refractive errors of emmetropia/mild hyperopia.[28] Thereafter, there is a transition into the next developmental stage of maintaining emmetropia.[27, 32] This stage is characterized by the slower rate in ocular growth — compared to during emmetropization — where the changes in AL are compensating for the changes in the crystalline lens, and thus maintains emmetropia into adolescence.[27, 32] It was previously proposed that physiological ocular growth by AL was completed after the age of 13 years.[33] However, with the advent of instruments with higher resolution,[34] evidence emerged of a continued physiological ocular growth past the age of 13 and into late adolescence,[32, 34, 35] whereby these changes are compensated for by the crystalline lens power.[32] The crystalline lens has a role during the stages of emmetropization and maintaining emmetropia,[27, 32] by changing depending on refractive development and age.[27, 29, 32, 36, 37]

The choroid, apart from providing the outer retina with nutrients and oxygen,[38] has been suggested from animal[39] and human[8] studies to be a biomarker of ocular growth,[40] due to its location between the retina and the sclera.[41, 42] The choroid’s role involves release of growth

factors related to scleral extracellular matrix remodelling, and by modulating its thickness to ensure the retina is adjusted to the eye's focal plane in animals.[43, 44] In humans, thin choroids have been associated with longer AL and more negative spherical equivalent refractive errors (SER).[41, 42, 45] Furthermore, the choroid has been reported to thicken in a longitudinal study with long-term light exposure.[46] One study reported no associations between choroidal thickness (ChT) and AL in early childhood (2–5 years). In an older age-group (6–17 years), however, there was an association which suggests a clearer relationship when ocular growth slows down.[47] Thinner choroids have also been associated with lower birth length and weight.[48, 49] From previous observations, the choroid has been proposed to function as a buffer where thin choroids are associated with accelerated ocular growth and a thick choroid is protective against this growth.[7] However, the choroid has a malleable characteristic in which the thickness changes in response to a wide-variety of factors.[42] These include a physiological response with accommodation induced from hyperopic or myopic defocus,[50, 51] physical exercise,[52] water intake;[53] and light exposure.[54] In addition to large between-individual variations with refractive errors, axial length, sex and age,[55] as well as within-individual variation with the diurnal rhythm of ChT.[56-58]

The emmetropization process has been proposed to be visually guided based on the findings from animal studies where hyperopia and myopia is induced by positive and negative defocus, respectively,[59, 60] As ocular growth in animals still occurs when the optic nerve is sectioned, it has been inferred that ocular growth must be regulated locally at the retina.[8, 61] At the retina, there is a discrimination of the kind of blur imposed from the positive and negative lenses, respectively, which could be involved in stop and start signalling for the eye to grow.[8] The discrimination of blur has been discussed to be related to retinal contrast signalling[59, 62, 63] and/or longitudinal chromatic aberration.[64, 65] Despite this theory, there is still a considerable knowledge gap regarding several factors that are suggested to be involved with physiological ocular growth during emmetropization and when maintaining emmetropia, and the development of refractive errors. These factors will be discussed further in the following section.

1.3 Refractive errors

1.3.1 Prevalence of myopia

Generally, prevalence of myopia in children in Asia and Europe have been reported to be 60% and 40%, respectively, and below 10 % in South American and Africa.[66] There has been reported a trend in global increase in the prevalence of myopia the last decades,[1] with a particular increase in south-east Asia.[2, 3] The Scandinavian countries, however, appear not to follow the increasing trend, as myopia prevalence has been reported to be relatively stable,[4, 5] and considerably lower than that reported in south-east Asia. Myopia prevalence,

defined as <-0.50 dioptres (D), has been reported to be 10% in Swedish 8–16-year-olds,[67] 17.9% in Danish 14–18-year-olds,[68] and 13% in Norwegian 16–19-year-olds.[17] For comparison, studies in China reported that 6–15,[69] 15,[70] and 13–18-year-olds,[71] had a prevalence of 47%, 65%, and 83%, respectively. The reason for the lower prevalence of myopia in Scandinavia is not known, but could be related to delayed emmetropization,[72, 73] and/or possibly behavioural, biological and/or environmental factors — which will be introduced more closely in the following sections.

1.3.2 Ocular pathology and conditions associated with refractive errors

Uncorrected refractive errors, such as myopia, hyperopia and astigmatism, is the primary cause of preventable visual impairment in the world.[18] The risk of sight-threatening ocular pathology later in life, is associated with refractive errors and the elongation of the eye. For myopia and especially high myopia (<-6.00 D), these conditions include cataract, glaucoma, posterior vitreous detachment, myopia degeneration, posterior staphyloma, maculopathy, rhegmatogenous retinal detachment, and peripheral retinal degeneration.[22] The risk for myopic maculopathy increases considerably with every dioptre, suggesting the importance of myopia control therapy to reduce myopia development.[74] For hyperopia these conditions include an associated risk for early age-related macular degeneration[75, 76] and primary angle-closure glaucoma.[77] Visual impairment can have severe social and economic consequences for an individual and their families. Moreover, those with visual impairment have a reportedly lower quality of life, compared to those without visual impairment.[22, 78] Furthermore, rehabilitation, treatment and reduction of productive work hours[20] can potentially be costly for governments.[21] Hyperopia in children has also been associated risk factor for reading disability,[79] and poor academic performance.[80-82] Uncorrected refractive errors have also been associated with increased risk of amblyopia.[83] The risk for ocular pathology and conditions, and the severe consequences underline the importance in understanding the aetiology of myopia and hyperopia, in order to prevent or minimize progression.

1.3.3 Development and associated factors

At birth, refractive errors have been reported to follow a statistical normal distribution.[23] From the age of 2–5 years, the distribution becomes more and more leptokurtic, characterized by its high peak and heavy tails. When emmetropization completes, the high peak indicates that most individuals have reached emmetropia/low hyperopia, while the heavy tails indicate either failure of emmetropization for hyperopia and myopia, or a failure of maintaining emmetropia in myopia, respectively.[23] Myopia onset typically occurs during school-age at around 8–14 years and has been labelled *school myopia* or *juvenile-onset myopia*,[84] but has been reported to onset in young adults as well.[85] This thesis will address the development of school myopia rather than secondary myopia,[26] as the former is more common and appears to be more complex than the latter — secondary myopia is linked more directly to genetic

defects or rare childhood conditions.[26, 84] Myopia development is complex and is suggested to involve genetic, environmental, biological, and behavioural factors.[14, 15] The behavioural factors includes near-work,[11] and outdoor time.[12, 13] while biological factors include ocular dimensions,[7, 8] and diurnal rhythms.[9, 10] The genetic factors include heritability of refractive errors[6] — with parental myopia, there is a 2.83 odds ratio for myopia for individuals with two myopic parents compared to those with non-myopic parents,[26] albeit these genetic variations explain only 18.4% of heritability.[26] Another genetic factor is L:M cone ratio — in a low myopia prevalence country such as Norway, males had a higher L:M cone ratios compared to males in countries with high myopia, and higher L:M cone ratio in females were associated to less severe myopia.[63] While there are genetic factors in myopia development, it has been proposed that the environment plays a larger role,[66, 86, 87] and a particularly important environmental factor is daylight exposure.[12, 13] This thesis will thus focus on the environmental factors, the ocular dimensions and diurnal rhythms which best characterize growth.

1.3.4 Environmental factors — outdoor time

There is increasing evidence that time spent outdoors has protective effects against myopia incidence and progression in humans.[12, 13, 16] This is supported by the decrease in myopia incidence in children from cluster randomized trials where outdoor time was compulsory during recess at school.[13, 88, 89] One of the studies also reported a negative relationship between myopia incidence and both the intensity and duration of light, and interestingly, myopia incidence was not associated with time spent indoors nor the intensity of indoor lighting.[88] In young adults, emmetropes were reported to have higher amounts of daylight exposure than myopes, which was associated with slower ocular growth for the former group.[90] While outdoor time during recess at school appears to be optional in some countries, it is compulsory to be outdoors during recess in primary school in Scandinavia throughout the whole year.[5, 17, 67] A study reported that 6–17-year-olds in China spent on average 100 minutes (min) outdoors per day,[91] which is notably lower than the 170 minutes reported in 9–15-year-olds in Norway.[92] Norwegian 16–19-year-olds and Singaporean 15–20-year-olds, however, were found to spend a similar average amount of time outdoors every day (both ≈230 min).[17, 93] This suggests that factors such as environment, upbringing, and genetics (heritage) are involved, which could potentially be linked to the differences in myopia prevalence in Scandinavia, compared to countries in south-east Asia, such as China and Singapore. The mechanisms behind the protective effect of outdoor time against myopia are not fully understood, but proposed to be related to the intensity, spectral composition and duration of daylight exposure,[15] and possibly dim light exposure.[94] One suggested mechanism is that daylight exposure increases the release of dopamine in the retina, as dopamine inhibits ocular growth.[12] Another suggestion arises from the time-of-day effects on ocular parameters: in humans, the choroid thickened more from light stimulus in the morning compared to other periods of the day,[95] and in chicks myopic defocus[96] and light stimulus[97] more efficiently inhibited ocular growth in the evening. These observations allude to that

daylight exposure can contribute to an improvement of the diurnal rhythm[98] which also includes the ocular diurnal rhythm — possibly through the AL and ChT phase relationship, as implicated with ocular growth in animal studies.[9, 10]

1.3.5 Biological factors

1.3.5.1 Circadian rhythms

The circadian rhythm, which follows roughly a 24-hour period, is the collection of biological processes observed in humans and other organisms. These processes include regulation of the sleep and wake cycle, reproduction, thermoregulation, and metabolic control, among others.[99] The circadian rhythm is entrained by different zeitgebers where daylight is the strongest one, with the synchronization to the day and night cycle of light being defined as a diurnal rhythm.[99] Light entrainment in humans occurs via a non-image-forming mechanism in the retina, where melanopsin in the intrinsically photosensitive retinal ganglion cells absorbs light with a peak absorption around 480 nm,[100] thereafter signals are relayed to the suprachiasmatic nucleus where the master circadian clock is located.[99] Melatonin has been described to promote sleep by inducing sleepiness and reducing alertness.[101, 102] Melatonin secretion at dim-light is rhythm-regulated by signals from the master clock, and melatonin levels and particularly dim light melatonin onset (DLMO) can be used as a biomarker for the rhythm in the master clock.[103] There is an importance to have a well-regulated circadian rhythm to the day and night cycle,[98] as a circadian disruption increases the risk for cardiovascular disorders, metabolic syndromes, and cancer, and various other diseases.[98, 104]

1.3.5.2 Animal studies and species-differences

Findings from animal studies suggest that ocular diurnal rhythms have implications for ocular growth.[105] The strongest evidence has been found in chicks, where it has been suggested that specific optical and light conditions disrupt the ocular diurnal rhythm.[9, 10] This has been demonstrated in the alterations of the axial length and choroidal thickness phase relationship: during normal ocular growth, the AL and ChT relationship is near anti-phase, while during slowed and accelerated growth the relationship is in-phase and anti-phase, respectively (**Figure 1**).[9, 10] The association between accelerated growth and either constant light or darkness, also suggests that maintaining a balance of the light/dark cycle has importance for physiological ocular growth.[7, 105, 106] While the findings in chicks studies are promising and significant, there are however inter-species differences that need to be considered, particularly as findings in other species have been less clear.[8] As the strongest evidence of diurnal rhythms implicated with ocular growth has been found in chick studies,[8] the next section will focus on the differences between chick and human species.[60]

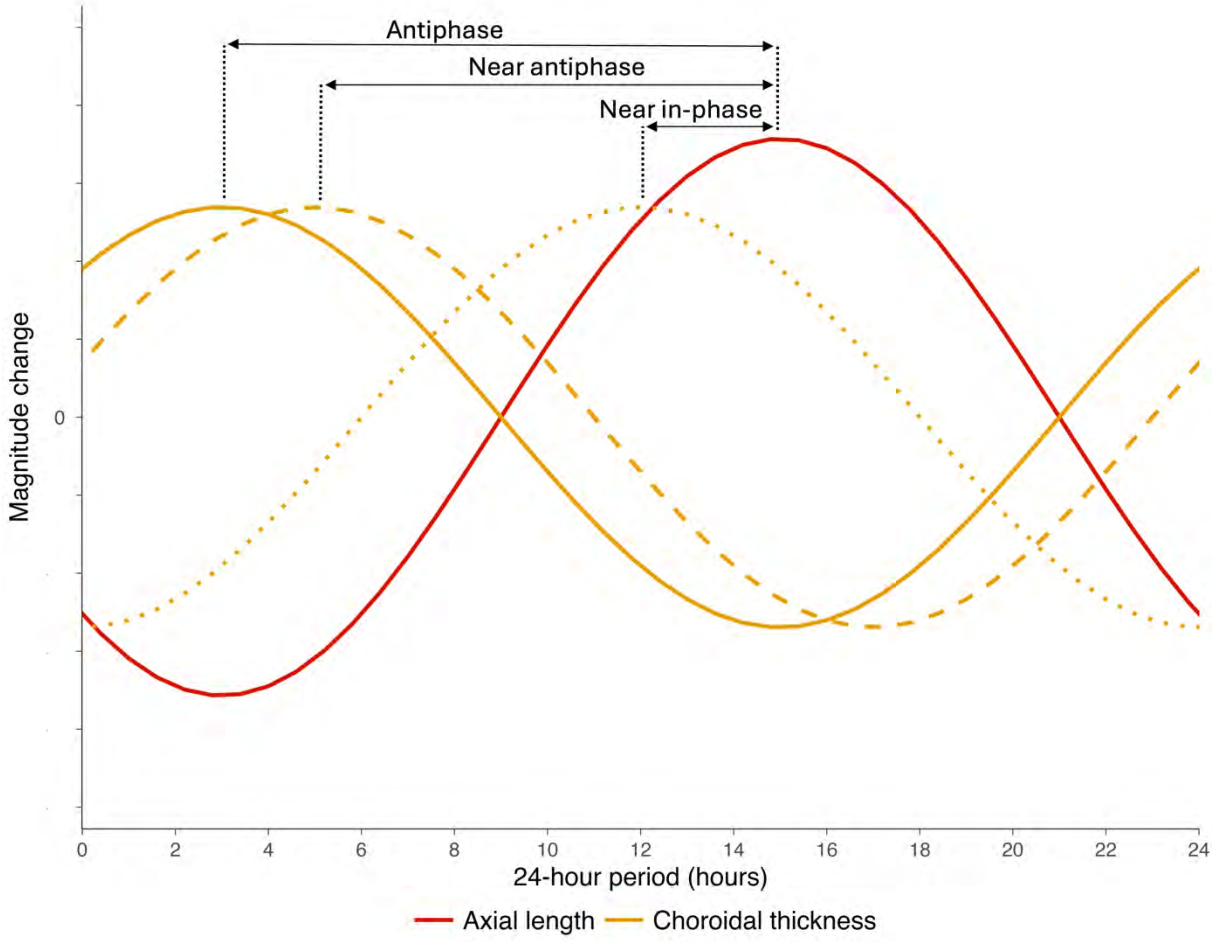

Figure 1. *An illustrative example of anti-phase, near anti-phase and near in-phase relationship between axial length and choroidal thickness. Each curve represents theoretical sinusoidal model fits plotted over a 24-hour period. The y-axis shows the magnitude change, while the x-axis shows the 24-hour period in hours. The illustration is loosely based on the findings reported in Ref. [10]. For simplification, it is only shown that the choroidal rhythm is shifted while the referenced study has shown that the axial length rhythm also shifts in response to stimulus.*

First of all, the accommodative system differs: during accommodation in humans it is suggested that the smooth muscle in the ciliary body contracts and moves forward, which releases the tension on the zonular fibres attached to the crystalline lens, and in turn the crystalline lens becomes more spherical and thus changes its power.[107] Chicks, on the other hand, have striated muscle in the ciliary body,[60] which applies force on the lens causing it to move and thus change its power.[108] Also, as the ciliary muscle in humans are muscarinic receptors and chicks have nicotinic receptors, topical atropine does not work as cycloplegia in chicks.[109]

Second, the magnitude of short-term change in ChT from hyperopic and myopic defocus differs:[42] Chicks can alter their choroids by over 100%,[38] while for humans it has been reported to be as small as 5–8%.[110] Similar differences have also been reported between chicks and other primates, such as marmosets.[39] These species-differences have been suggested to be due to that chicks use the choroid as a "local retinal focusing mechanism" as they do not have a fovea.[39]

Third, the circadian system between birds and humans differs considerably — unlike humans where the pineal gland produces melatonin in response to the signals from the suprachiasmatic nucleus based on light processed by the retina, birds have a pineal gland which is directly light sensitive and functions as an endogenous circadian clock.[8] The pineal gland in birds is also involved in the regulation of anterior chamber growth.[111] These functions could allow birds to adapt efficiently to changes in light — as experienced during migration in locations with large seasonal variations in daylight availability,[112, 113] or potentially from manipulating lighting conditions.[7, 10]

There are also other differences, such as chicks being tetrachromats while humans are trichromats, and a difference in amount of spherical aberration,[8] however, these differences are out of scope of this introduction. Overall, the findings from animal studies are important for myopia research, but the species-differences highlight the importance of careful considerations when translating findings from animals to humans.

1.3.5.3 Human studies

Several ocular parameters have been reported to exhibit diurnal variations in humans, including central corneal thickness (CCT), corneal radius (CR), anterior chamber depth (ACD), crystalline lens thickness (LT), vitreous chamber depth (VCD), axial length (AL), retinal thickness (RT), choroidal thickness (ChT), and intraocular pressure (IOP).[56-58, 114, 115] The AL and ChT phase relationship was reported to be between 10.5–12.1 hours (h) across studies in children and young adults.[56, 58, 116] For young adults, hyperopic defocus changed the amplitude of AL and ChT,[117] and myopic defocus changed the AL and ChT phase relationship from near anti-phase to more in-phase.[118] The latter finding is reminiscent of findings from chick studies with a more in-phase relationship during slowed growth.[9, 10] One study assessing diurnal rhythms and other factors, did not find statistical differences between young-adult non-myopes and myopes, which the authors discuss could be due to the age of participants (22–41 years) in that the myopes likely were stable in terms of their progression.[116] In young participants, there has also reported differences in diurnal rhythm and sleep characteristics between myopes and non-myopes. Melatonin (MEL) levels in the morning were found to be higher in myopes than non-myopes in a sample of 18–22-year-olds,[119] while a more recent study found no associations between MEL levels and SER, nor with AL in 12–15-year-olds.[120] Single measurements of MEL levels have been suggested to have questionable value,[121, 122] which could explain the conflicting findings. For other sleep-

related factors, myopes have been reported to have decreased sleep quality compared to non-myopes,[123-125] however also with conflicting findings[126, 127] — possibly attributed to methodological differences and limitations.[128] In a study with 8–15-year-olds, myopes had a later DLMO, delayed sleep and wake time, and worse sleep quality, than non-myopes, where the authors suggest these findings in myopes are related to circadian dysregulation.[129] Similar findings were also reported in young adults aged 18–25 years by the same authors.[130]

Another interesting finding from studies assessing ocular diurnal rhythm is that the anterior chamber deepens, and the crystalline lens thickens during the evening.[56, 57] These characteristics differ from accommodation, where the anterior chamber shallows as the lens thickens to increase its focus power,[107, 131] and has been suggested to be related to lens metabolism,[57] but remains unclear. These findings warrant more research, especially since the crystalline lens has a prominent role during emmetropization and maintaining emmetropia.[27, 32]

Considering the findings of ocular diurnal rhythms in humans,[56-58, 114, 115] it has yet to be established associations between ocular diurnal rhythms or circadian dysregulation[129] and ocular growth similar to that observed from chick studies.[9, 10] It has neither been proven that the observations are species-specific to chicks and other avian species. Interestingly, it has been indicated from a large meta-analysis that genetic factors which regulate circadian rhythm could be involved with refractive error development in humans.[87] So far, the majority of the human studies, assessing ocular diurnal rhythms, have been short-term, i.e., a duration of a 24-hour period,[56, 58, 115, 116] or in the span of a few days.[117, 118] One study in humans did not find a seasonal effect in daily variations in AL and ChT,[90] which could be due to the mere 3 h of daylight difference between winter and summer in north-eastern Australia (latitude 27°S).[132] This warrants a need for longitudinal studies in humans to assess how ocular diurnal rhythms over time could be associated with ocular growth.

1.3.5.4 Seasonal variations, adaptation, and circannual rhythms

Seasonal variations have previously been observed during myopia development in children.[133-137] The slower ocular growth rate during the summer months compared to the winter months, has been suggested to be related to more daylight hours during the summer.[134, 135] This is in line with findings from a 18-month follow-up study where daylight exposure was negatively associated with change in axial length in 10–15-year-olds.[138] In another paper from the same authors, there were indications of seasonal variations from the increase in AL and less thickening of ChT in the winter than in the summer, but did not reach significance.[139] Similar findings of no seasonal variation in AL and ChT was reported in young adults.[90] As previously mentioned, this lack of variation could be explained by the small differences in seasonal daylight availability in Australia.[132] However, as myopia development has been reported to follow seasonal variations,[133-137] it is plausible that

physiological ocular growth also would follow such trend — this might particularly be likely in a location with large seasonal variation. In Norway, located at 60° N, there are considerable differences in available daylight between seasons. In the south-east of Norway, there is as low as ≈6 h of available daylight at winter solstice, while it is as high as ≈18 h at summer solstice.[132] It has been argued that it may not necessarily be the outdoor time that Norwegian adolescents spend during the summer holiday that protects against myopia development, as Singaporeans have access to 12 h daylight all year while Norwegians only have this available during the summer months.[17, 132] Thus, the low amount of daylight availability during the winter and the low prevalence across Scandinavian countries,[17, 67, 68] raises the question whether circannual rhythms and being well-adapted to seasonal changes could be involved in promoting physiological ocular growth.[17]

Circannual rhythms are internal biological processes that undergo rhythmic changes in the period of approximately a year.[140] Resembling circadian rhythms, light — specifically the varying photoperiodicity between seasons— have been suggested to be the most consistent zeitgeber for the circannual rhythm.[99] All while circannual rhythms persists near equator where there are minimal differences between seasons in photoperiodicity also have been detected.[141, 142] Seasonal adaptation can be crucial for survival for various species.[140, 142] For example, plants adapt by being growth-oriented during the winter to protect their reproductive organs from the cold temperature and frost, and flower in the spring before the warm and dry weather in the summer.[143] Animals, such as sheep, time their reproductive cycles to occur in autumn in order to have an offspring in spring when food is plentiful, and the offspring can develop enough to survive the winter.[144] The adaptation can differ depending on species and is based on their biological needs and functions.[99, 142] While birds have a circadian system that adapts efficiently to migration and seasonal changes in daylight availability,[112, 113] it has been suggested that arctic reindeers adapt to periods of constant light or darkness in the summer and winter, respectively, by having a periodically weaker circadian rhythm, i.e., a less entrainable circadian system to environmental cues.[145-147] In order to time these events, it has been proposed that mammals detect the duration of light and dark in which for instance the period of melatonin secretion adjusts according to day length, by being longer in the winter and shorter in the summer.[99, 144] For humans, the modern society with electric lightning, controlled heating, and consistent food availability, largely minimizes the necessity to adapt to an environment that varies seasonally. Still, seasonal variations and the reduced daylight exposure in winter have been associated with seasonal affective disorder (SAD).[148] SAD has been hypothesized to be related to a mismatch between the circadian rhythm during the winter and the social/work schedule. Treatment consists on re-aligning the circadian phase with appropriately timed bright light exposure and melatonin supplements.[149] Although it is not understood why some individuals get SAD and others do not, it could be tied to individual adaptation to seasonal changes.[150] SAD and night-shift work are potential consequences of circadian disruption with behavioural factors related to the light/darkness balance, in which light treatment can re-align these conditions.

But how can seasonal variations or adaptation be assessed, specifically in relation to the eye? It can be inferred from previous studies that ocular structures are synchronized to the circadian master clock via melatonin,[103] as ocular structures have been reported to be near in-phase or near anti-phase with melatonin phase.[58, 116] This could entail that as melatonin phase shifts from winter to summer,[151, 152] there could be an observable phase shift in the same direction as ocular structures. Also, as daylight is suggested to promote a healthy phase relationship in the eye, it can be hypothesized that the daylight availability in winter and summer would have a differential effect on the phase relationships in the eye, with the potential implication on ocular growth observed from animal studies,[9, 10] when measured at a location with large differences in daylight availability between seasons.

1.3.6 Myopia control therapy and atropine

Myopia control therapies are interventions that reduces myopia progression. Treatment options are optical (with spectacle lenses, multifocal contact lenses or orthokeratology lenses); pharmacological (with anti-muscarinic agents, specifically topical atropine); or behavioural (increased outdoor time).[153] While the protective mechanism of optical treatments is suggested to be from imposing a peripheral myopic defocus on the retina which slows down myopia progression,[154] the protective mechanism of topical atropine and other anti-muscarinic agents, are far less understood.[155, 156] Topical atropine, an anti-muscarinic agent, induces temporary suspension of accommodation and pupil dilation by relaxing the ciliary muscles and iris sphincter, in which both of these contain muscarinic receptors.[157] Atropine's protective effect against myopia is proposed to be unrelated to accommodation, explained by that atropine reduces myopia in chicks but accommodation is unaffected as chicks have nicotinic receptors in the ciliary muscle,[109, 158, 159] as previously noted. Muscarinic receptors are also present in the retina, choroid and sclera,[160, 161] which are other possible sites of action where atropine has its protective effects. In line with this, short-term effects has been reported where axial length shortened and the choroid thickened after 60 minutes from 0.01% atropine,[162] and 2% homatropine.[163, 164] For long-term effects, the choroid was reported to thicken in a 2-year follow up study in children aged 4–12 years after daily use of topical atropine with the effect of thickening varying with concentration dosage,[165] which also varies the efficacy of myopia control.[155, 166] Atropine has also been reported to prevent thinning of the ChT induced by hyperopic defocus and to have an additive effect with choroidal thickening induced by myopic defocus in a 6-month study.[167]

When used as myopia control treatment, topical atropine is usually given at bedtime to reduce the side effects at daytime, such as glare, photophobia and blur.[168-170] From this practice, it can be suggested that atropine alters the ocular diurnal rhythm in humans and potentially influence ocular growth. There is support in this from the human studies reporting short-term effects of atropine on AL and ChT,[162-164] and defocus altering the AL and ChT phase relationship.[118] These alterations have resemblance to the phase relationship in AL

and ChT which have been suggested to influence ocular growth.[9, 171] Evaluating the potential protective mechanisms of topical atropine as myopia control therapy is not only important for increasing the understanding of myopia development, but also to observe the suggested characteristics of ocular diurnal rhythms potentially linked with physiological ocular growth. Furthermore, use of topical atropine would paralyze accommodation, which could give insight on the origin of crystalline lens diurnal rhythm.

2 Motivation and aim of research

2.1 Motivation

Considering the increasing prevalence of myopia and the potential consequences of ocular pathology and conditions associated with refractive errors, it is imperative to increase the understanding of the aetiology of refractive errors. Refractive error development appears to be complex where myopia susceptibility is linked to biological, environmental, genetic, and behavioural factors.[15] At the same time, it is equally essential to understand physiological ocular growth at its different stages and the associated factors for an eye to reach and maintain emmetropia/low hyperopia. In this regard, it is of great interest to investigate why there is a reported low prevalence in Scandinavia[17, 67, 68] — specifically in Norway.[17] This understanding could be used for future research to reduce myopia incidence and progression. It has previously been proposed that the low prevalence might be linked to the large seasonal variations in daylight availability in Scandinavia, and more specifically, with seasonal adaptation.[17] Daylight exposure has an important role in maintaining a well-regulated circadian rhythm,[98, 104] and could be involved with outdoor time protecting against myopia incidence and progression.[12, 13, 16] From animal studies, ocular growth has been associated with alterations of ocular diurnal rhythms from optical stimulus or light conditions — implying that regulation of ocular growth can be linked to ocular diurnal rhythms and the light-darkness balance.[9, 10] Corresponding with this, myopia development has been reported to undergo seasonal variations,[133-137] but it is currently not known if physiological ocular growth would also follow such trend. Overall, it is reasonable to assume that ocular diurnal rhythms and potentially physiological ocular growth during the different developmental stages would follow the seasonal variations of daylight. In this case, it warrants further investigation into whether being well-adapted to seasonal changes contributes to the promotion of physiological ocular growth, and whether circadian dysregulation can be linked to myopia development.

The protective mechanism of topical atropine as myopia control therapy is not entirely understood, but one suggestion is that its action takes place in the retina, choroid and/or sclera.[160, 161] Given the reported short-term and long-term effects on AL and ChT in humans,[163-165] it could be assumed that the ocular diurnal rhythms and the phase relationship also would be altered — with the potential implications observed with slowed, normal and accelerated growth in animal studies.[9, 10] If so, this information could contribute to the understanding of the characteristics of ocular diurnal rhythms during normal and/or slowed growth for maintaining physiological ocular growth.

The crystalline lens has been reported to increase in the evening,[56, 57] but it is not understood what these diurnal changes are, and whether they would persist when accommodation is minimized as with cycloplegia. As the crystalline lens has an important role during emmetropization and maintaining emmetropia/mild hyperopia,[27, 29, 32, 36, 37] this is an area of interest to investigate further. Atropine and ocular diurnal rhythms have not previously been investigated in human studies. In summary, this thesis assesses environmental, behavioural, and biological factors suggested to be involved with ocular development, which can increase the understanding of physiological ocular growth and refractive error development.

2.2 Aim and objectives

The aim of this thesis was to investigate circannual and circadian rhythms and their implications on physiological ocular growth and refractive error development in children, adolescents, and young adults. The project was conducted in the southeast of Norway where there are considerable seasonal variations in available daylight hours.[132] The study sample consisted of 17–24-year-old adolescents and young adults (paper I), 19–25-year-old young adults (paper II), and 7–11-year-old-children (paper III). The objectives in this thesis are covered in each paper with details mentioned below.

Paper I: To investigate the seasonal variations in diurnal rhythms in young adults, and examine the associations between diurnal rhythm parameters and physiological ocular growth

Paper II: To investigate the short-term effects of atropine 1% on the diurnal rhythms of ocular parameters in young adults.

Paper III: To investigate the seasonal variations of physiological eye growth in children and the potential implications for refractive error development.

3 Materials and methods

3.1 Study design and recruitment of participants

The first study (paper I) had a longitudinal, prospective, and repeated-measures design. Participants underwent several sets of ocular measurements and collection of saliva samples for melatonin analysis. The sets of measurements were scheduled throughout the day, to estimate the diurnal rhythm for a given parameter. To assess seasonal variations of diurnal rhythms, the measurement protocol was conducted first in winter and then repeated in summer, as close as possible to the winter and summer solstices. The second study (paper II) followed the same measurement protocol as paper I, but was only conducted in autumn/winter, and had an experimental, paired-eye and repeated-measures design. The paired-eye design involved monocular instillation of a cycloplegic agent (topical atropine 1%) to assess its influence on diurnal rhythms of ocular parameters while the fellow untreated eye was used as control. The third study (paper III) had a longitudinal and prospective design. Participants were measured at one time point at four separate occasions: November 2019, January 2020, June 2020, and November 2020, as close as practically possible to the winter and summer solstices. This was conducted to assess the seasonal variations of physiological eye growth as well as exploring refractive error related differences. An overview of study design, number of participants, and measurement sessions for the three papers is provided in **Figure 2**.

All individuals who participated in the three studies (**Figure 2**) were recruited in the town of Kongsberg (population count of ≈29 000),[172] located in the south-east of Norway. The participants from the first study (paper I) consisted of students from the University of South-Eastern Norway (USN) at campus Kongsberg, and pupils at one high school in Kongsberg, while the second study consisted solely of students from USN. The third study consisted of children attending one primary school in Kongsberg. Prior to participation, informed and written consent was obtained from all individuals. For the children, their parents/caregivers provided informed written consent.

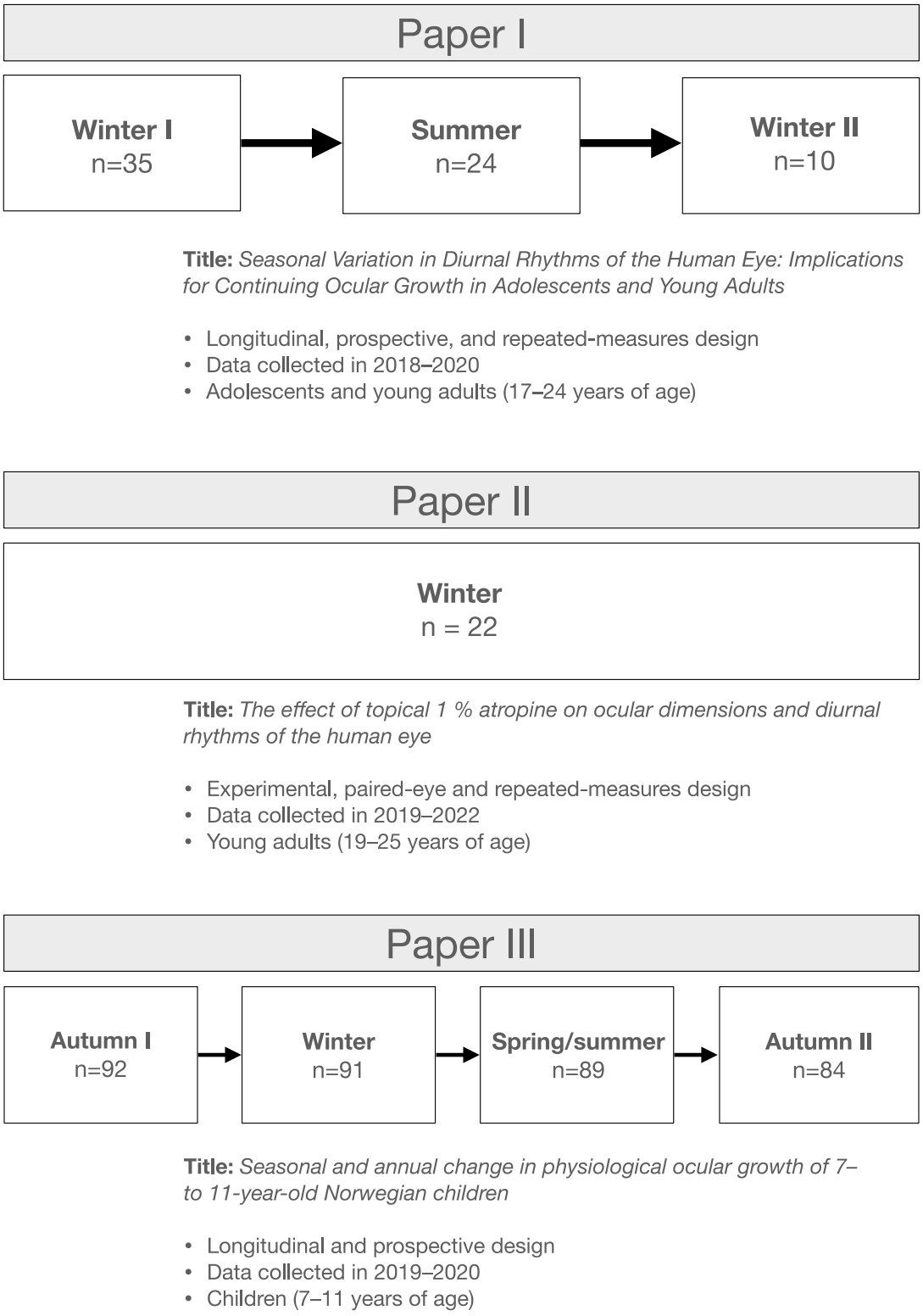

Figure 2. *An overview of title, study design, age of participants and when the data collections occurred for papers I–III. For papers I and III, each box represents the various data collection sessions in chronological order.*

3.2 Seasonal variations in Kongsberg, Norway

All data was collected in Kongsberg, Norway (60°N), where daylight availability varies from 5 hours and 59 minutes at the winter solstice to 18 hours and 44 minutes in the summer solstice (**Figure 3**). For papers I and III, participants were measured as close as possible to the solstices to capture the extreme of each season. The difference in available daylight at the measurement periods versus at the solstices varied with a maximum of ≈2 hours and a minimum of 1 minute for papers I and III (**Table 1**). The variability of up to 2 hours was due to consideration of participants having examination periods close to both solstices, and to minimize participant drop out (paper I). For paper III, the allocated time for measurements given by the rector at the primary school, to minimize disturbing the school day activity for a child, also varied with 2 hours from solstice for cycloplegic SER in January, and 45 minutes for ocular biometry and ChT measurements.

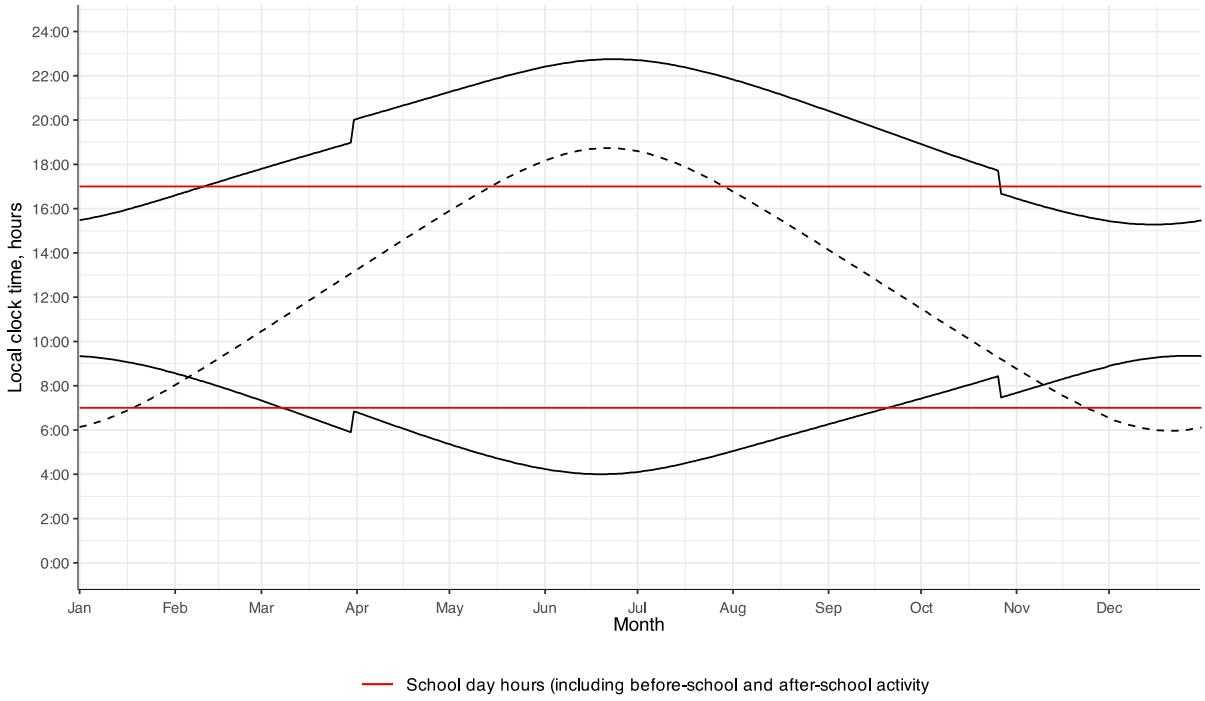

Figure 3. *Seasonal variation of available daylight, sunset and sunrise in Kongsberg, Norway (60°N). A 12-month period (January–December 2019) is shown on the x-axis. Sunrise and sunset (lower and upper solid lines, respectively) are in local time, while day length (dashed line) is in hours, both on the y-axis. The step changes at the end of March and October for sunrise and sunset are due to daylight saving with a 1-hour shift. Data was extracted from timeanddate.com.*[132] *The red lines illustrate the school day hours (07:00 to 17:00) which includes before- and after-school (BAS) program. The school hours, without the BAS program, were for the 7–8-year-olds and the 10–11-year-olds from 08:30 to 13:00, and 08:30 to 13:45, respectively.*

Table 1. *Estimations of available daylight at the measurement periods for papers I–III. The difference in number of daylight hours available at the measurement period versus at the winter (5 hours 59 minutes) or summer solstice (18 hours and 44 minutes) is shown in the rightmost column. Data was extracted from timeanddate.com.*[132]

	Measurement periods (dates)	Available daylight (hours)	Difference from daylight hours available at solstice
Paper I			
Autumn/winter	19 Nov–17 Dec	06:00–07:19	00:01–01:20
Spring/summer	13 May–25 Jun	16:54–18:43	00:01–01:50
Paper II			
Autumn/winter	25 Oct–13 Nov	07:48–09:23	01:49–03:24
Paper III			
Autumn 1	11 Nov–21 Nov	07:12–07:57	01:13–01:58
Winter	14 Jan–30 Jan	06:44–07:51	00:45–01:52
Spring/summer	08 Jun–16 Jun	18:31–18:43	00:01–00:13
Autumn 2	09 Nov–12 Nov	07:49–08:03	01:50–02:04

3.3 Data collection

Measurements of various ocular and biological parameters were taken in papers I–III. An overview of these measurements is provided in **Table 2**, with a more detailed description in sections 3.2.1–3.2.7.

Table 2. *Overview of the various measured parameters included in papers I–III.*

	Paper I	Paper II	Paper III
Cycloplegic SER	✓	✓	✓
Non-cycloplegic SER	✗	✓	✓
Ocular biometry	✓	✓	✓
Intraocular pressure	✓	✓	✗
Posterior segment	✓	✓	✓
Melatonin by saliva	✓	✓	✗
Sleep data via actigraphy	✓	✓	✗
Body height	✗	✗	✓

3.3.1 Collection of sleep data and measurement schedule (papers I–II)

An overview of which measurements that were taken and the measurement schedules for papers I and II, are shown in **Table 2** and **Figure 4**, respectively. For paper I, on Day 1, self-reported habitual wake time (HWT) for the preceding month was gathered from the participants. Actigraph GT3X (ActiGraph Corp., Pensacola, FL, USA), a wrist-mounted activity monitor (actigraph), was handed out for the participants to wear for 7 days and nights to collect sleep data (specifically habitual sleep onset times, HST). On Day 8, eight sets of measurements were conducted where each time point (epoch) was scheduled to an individual participants' wake and sleep times (HWT and HST). The first two epochs were scheduled 1 h and 4 h after the self-reported habitual wake time (HWT+1 and HWT+4), whereas the remaining 6 epochs were scheduled every hour from 4 h before HST (HST-4, HST-3, HST-2, HST-1), at HST (HST+0), and lastly 1 h after HST (HST+1), by using average weekday estimate of HST collected the week prior. This measurement schedule was used for the data collection in autumn/winter and then repeated in spring/summer (**Table 1**). Saliva samples for melatonin analysis were collected at HST-4–HST+1.

For paper II, a similar measurement schedule as paper I was used, with five major differences:

1. One additional saliva sample for melatonin analysis was self-collected at wake-up time on Day 8 (HWT+0),
2. A topical cycloplegic agent (atropine 1%) was monocularly instilled on Day 8 at HWT+2.
3. Participants were additionally measured at Day 7 and Day 9 at HWT+4.
4. There were no measurements taken after HST+0.
5. The experiment was not repeated at a later season.

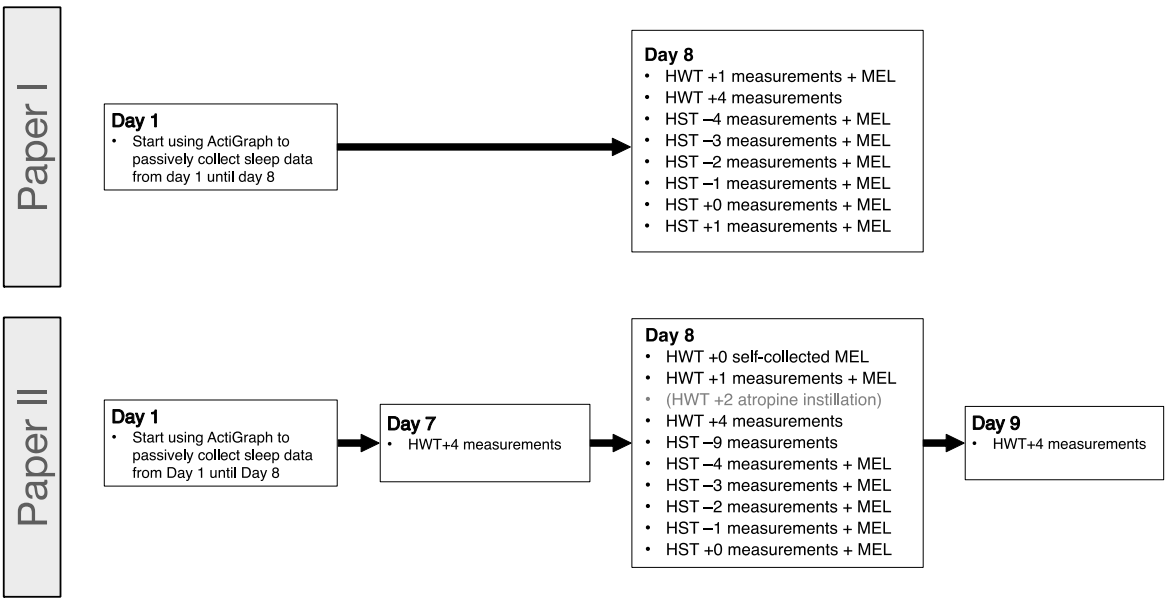

Figure 4. *Overview of the measurement schedules for papers I and II. For paper I, the measurement schedule was first used during autumn/winter and then repeated in spring/summer.*

3.3.2 Collection and analysis of melatonin saliva levels (papers I and II)

For papers I and II, saliva samples (Salivette saliva collection kit; Sarstedt, Nümbrecht, Germany) were collected from participants at specific epochs on Day 8 (**Figure 4**) for subsequent analysis for melatonin. After the collection, the samples were centrifuged immediately at -4°C, 4400 rpm, for 5 minutes (Centrifuge 5702R; Eppendorf SE, Hamburg, Germany) and then stored at −25°C in a freezer. The samples were transported in ice to VITAS Analytical Services (Oslo, Norway) where melatonin was extracted from the samples using an ELISA kit (Enzyme-Linked Immunosorbent Assay, Bühlmann Laboratories,

Schönenbuch, Switzerland). Saliva sampling has been described as a reliable method for melatonin extraction.[173]

Both papers followed previously described guidelines to minimize any effects from external factors on melatonin secretion.[173, 174] This entailed that the participants could not consume or drink certain substances (NSAIDs, nicotine, bananas, chocolate) 36 hours prior to or during Day 8, and no consumption of alcohol, or certain beverages (caffeine, artificial additives) 24 hours prior to and during Day 8. Also, participants could not travel across more than two time zones within the last month before the experiments. To avoid suppression of melatonin secretion from bright artificial light sources, light levels were kept below 20 lux during all measurements.

3.3.3 Measurements of non-cycloplegic and cycloplegic autorefraction, ocular biometry, posterior segment, and intraocular pressure

An overview of the various variables that were measured in papers I–III is provided in **Table 2**. Cycloplegic autorefraction is considered gold-standard when measuring refractive errors,[175] and was used throughout papers I–III, as non-cycloplegic autorefraction can underestimate hyperopia and overestimate myopia.[176, 177] Refractive error measurements were taken at least 20 minutes after administration of cycloplegia for papers I and II, and after 30 minutes for paper III. In papers I and III, topical 1% cyclopentolate (Minims single dose; Bausch + Lomb, Bridgewater, NJ, USA) was administered binocularly, while in paper II, topical 1% atropine (Minims single dose; Bausch + Lomb, Bridgewater, NJ, USA) was instilled monocularly in the dominant eye as a part of its paired-eye design. It is expected that cycloplegia in darker irides, compared to lighter irides, will be less effective due to melanin binding.[157, 178] Dosage was therefore dependent on iris colour; one drop if blue to green, and two drops if eyes were (darker) green to brown.[179]

For papers I and III, the HRK-8000A autorefractor (HRK-8000A; Huvitz Co., Ltd., Gyeonggi-do, Korea) was used to measure baseline cycloplegic refractive errors. The instrument uses an automatic three-axis eye tracker to compensate for the participant's movements, and a Hartmann-Shack wavefront sensor that measures lower and higher aberrations which improves accuracy.[180] The repeatability of HRK-8000A was estimated in paper I and is described in the section 5.3. For paper II, the Nvision-K 5001 Open-field autorefractor (Shin-Nippon, Tokyo, Japan) was used to measure refractive errors at far (6 m) and near (Maltese cross at 0.30 m) at every epoch from Day 7 to Day 9. The open-view design, as compared to a closed-view, has been reported to measure more positive values of non-cycloplegic refractive errors compared to its counterpart.[181, 182]

At every epoch after instillation of atropine 1% (Day 8 HWT+2), refractive error measurements were taken with cycloplegia (1 % atropine) in the dominant eye and non-cycloplegia in the non-dominant eye (fellow control eye). For paper III, in addition to using the HRK-8000A for cycloplegic autorefraction, the Nvision-K 5001 was used for non-cycloplegic autorefraction at far (6 m) in autumn, winter, spring, and the following autumn.

Ocular biometry was measured with IOLMaster 700 (IOLMaster 700; Carl Zeiss Meditec AG, Jena, Germany) for all three papers. The IOLMaster 700 uses swept-source optical coherence tomography (OCT) technology and has been reported to have a high repeatability.[183] The instrument measures CR, CCT, ACD, LT, and AL. AL is measured from the anterior corneal surface to the retinal pigment epithelium (RPE).

For papers I–III, the posterior segment was measured with spectral domain OCT imaging with the Spectralis (Heidelberg Engineering, Heidelberg, Germany). The Spectralis has the advantage of setting a participant's first image as a reference scan to ensure subsequent imaging are conducted at the same location at the retina. This is used in combination with eye tracking, which also helps to minimize noise from participant movement or unstable fixation. The instrument also uses enhanced depth imaging (OCT2 EDI) which optimizes imaging of the choroid. These qualities were important when conducting a wide variety of measurements of the retina and choroid throughout the day in order to capture the diurnal rhythm of retinal and choroidal thickness.

Intraocular pressure was measured for papers I and II with the hand-held rebound tonometer iCare TA01 (Tiolat Oy, Helsinki, Finland). The iCare TA01 has been reported to have a relatively high repeatability (ICC=0.93).[184] Although the Goldman Applanation Tonometry (GAT) is considered the gold-standard in IOP measurements with an even higher repeatability (ICC=0.98),[184] the iCare TA01 is far less invasive, more portable, and requires no anaesthetics, which was deemed more appropriate for the experiments in papers I–II. GAT also requires use of extensive lighting with a slit-lamp microscopy, which would likely affect melatonin levels[173] and choroidal thickness.[54]

For paper III, measurements of body height (Seca 217 stable stadiometer) were taken (without footwear) to assess its association with axial length.[73, 185, 186]

3.3.4 Posterior segment OCT imaging protocol and segmentation

For all three studies, a variation of the same measurement protocol was followed (**Figure 5**). The protocol consisted of a radial OCT-EDI scan with 6 orientations with 100 B-scans averaged for each orientation (**Figure 5A**). For paper III, where children as young as 7–11 years were measured, fewer orientations (horizontal + vertical scans or only horizontal scans) were used when fixation issues occurred, or the participant could not sit still. The first measurement on a participant was used as a reference image for all subsequent measurements, which used the instrument's retinal tracking system to ensure imaging at the same location on the retina during subsequent measurements.

The retinal and choroidal thickness layers were semi-automatically segmented with custom-made software (**Figure 5B**) using an active-contour method[187] with choroid-enhancing segmentation as described by ref.[188] The active-contour method used energy minimization to deform a contour to image features (bright/dark edges that represent retinal layers) while simultaneously satisfying rigidity constraints (preferring smooth shape changes to sharp corners) to segment retinal layers.[187] A set of default active contour parameters were derived that worked for the majority of OCT images. The default parameters were changed to improve the automatic contour fitting in images which had poor quality, uneven borders or artefacts in the images. The segmenter could also interactively manipulate the contour to improve the fitting process in cases needing manual adjustments.

Lateral scaling for all OCT-scans was corrected with a 4-surface schematic eye model,[189, 190] by using each individual's ocular biometry (IOLMaster 700) from the same epoch. The retina was defined as the distance between the inner limiting membrane to the retinal pigment epithelium, while the choroid was defined as the distance between the RPE and the choroid-scleral interface (**Figure 5C**). Mean values were extracted for the thicknesses at the retinal foveal point (RT), and central 1 mm retinal- and choroidal thickness (papers I and II), and the subfoveal choroidal thickness (SFChT), central 1 mm, and the nasal and temporal inner (1 mm) and outer (1.5 mm) macular areas (paper III).

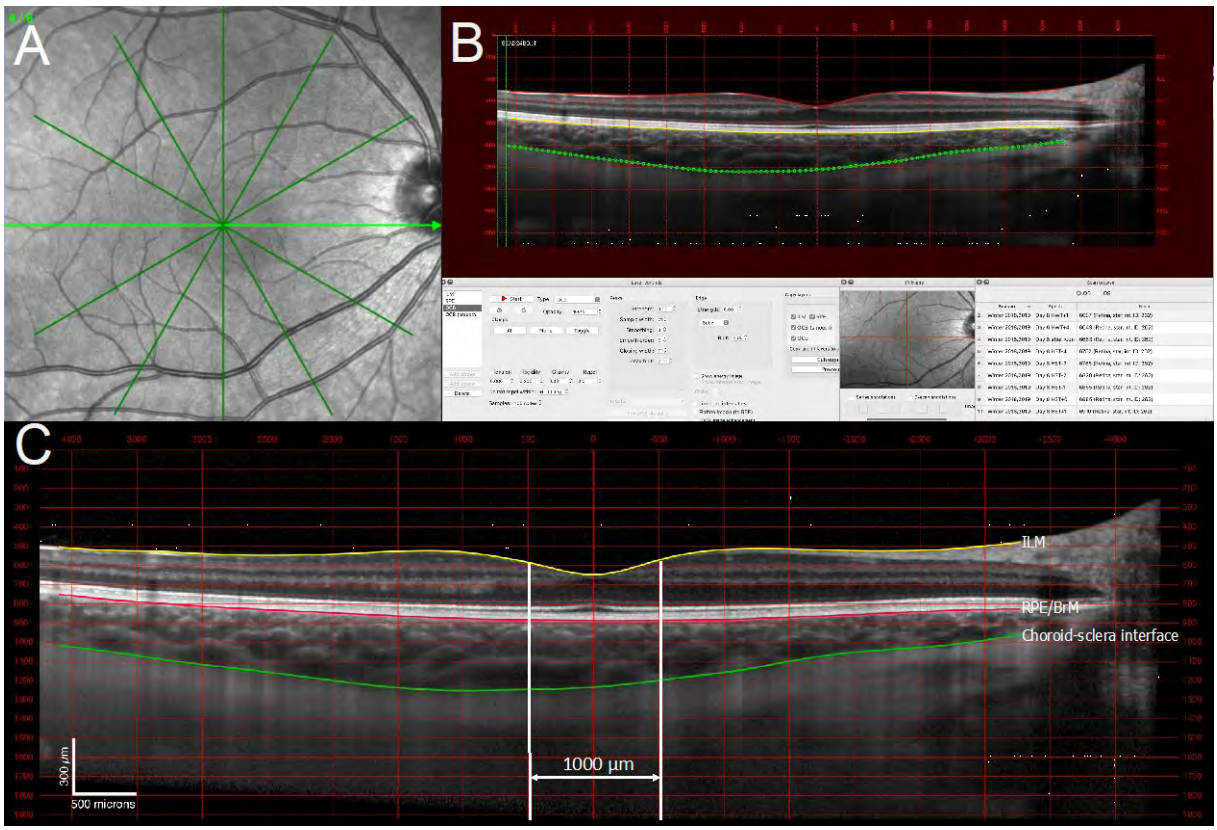

Figure 5. (A) *An infrared image from Spectralis OCT showing the radial scan with 6 (papers I and II) or 2 (paper III) orientations aligned to the fovea used in papers I and II.* ***(B)*** *An illustration of the custom-made software that was used for semi-automatically segmenting the retina and the choroid. The software implemented active-contour method to segment the layers of interest,[187] and featured an additional enhancement to improve segmentation for the choroid.[188]* ***(C)*** *Example of segmentation of the retinal and choroidal layers used for papers I and II. The retina was defined as the area between the inner limiting membrane (ILM) and the retinal pigment epithelium (RPE), and the choroid was defined as the area between RPE/Bruch's Membrane (BrM) and the choroid-sclera interface.*

3.3.5 Order of measurements and control of factors

For papers I and II, the order of measurements is shown in **Figure 6**. The participants first sat down on a chair with wheels and looked at a television at 5 m for 15 minutes to relax their accommodation (accommodation washout) for resetting any prior effect accommodation might have had on ChT before the session started.[117] Saliva samples for melatonin were collected at the last 5 minutes of the 15 minutes accommodation washout at specific epochs (**Figure 4**). Then, the participants were rolled on the chair to the OCT-imaging station, and thereafter, rolled to the IOLMaster 700 for ocular biometry measurements. For paper I, the participant would then be rolled further for IOP measurements. For paper II, the participants were first rolled to the autorefractor for measurements, then lastly for the IOP measurements. For paper III, the order of measurements was 1) body height, 2) Nvision-K 5001

autorefractor, 3) IOLMaster 700 for ocular biometry measurements, 4) accommodation washout for 15 minutes 5) OCT-imaging.

Having measurements of ChT right after the accommodation washout should minimize any effects from accommodation on ChT that might occur if any other measurements were taken before OCT-imaging.[117] IOP measurements were obtained last (paper I and II), since the probe is in contact with the cornea during measurements, and the extent on how measurements involving the cornea (ocular biometry and autorefraction) are potentially affected is not known.

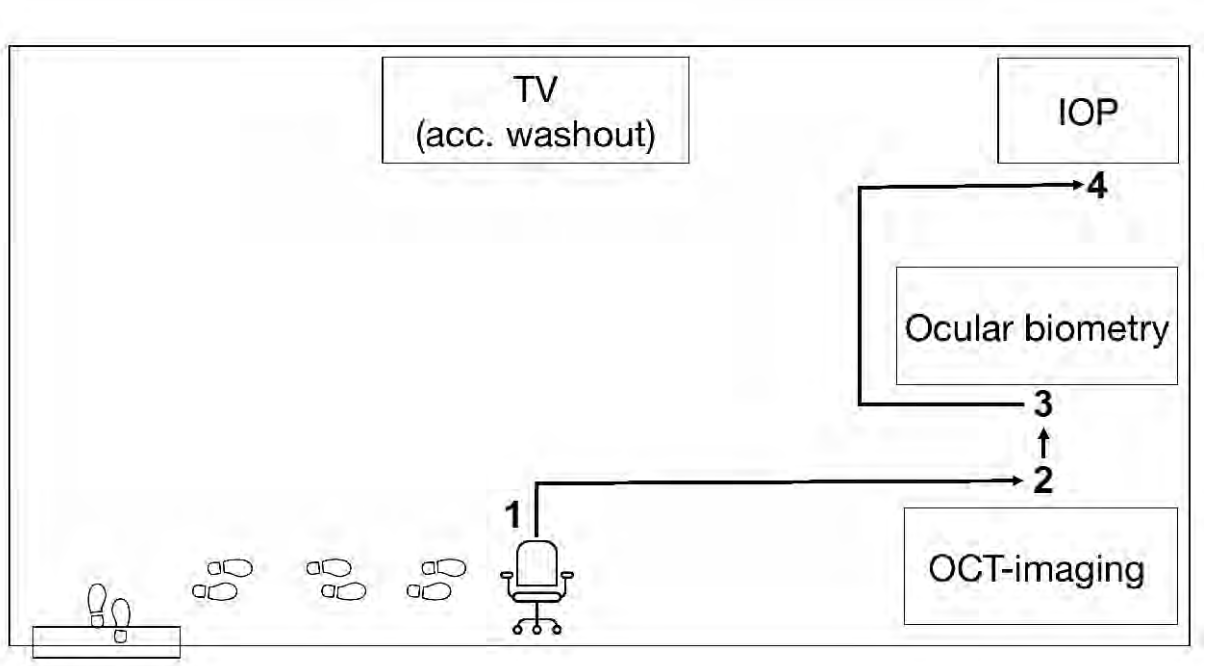

Figure 6. *Order of measurements for papers I and II: (1) The participant entered the room and sat down on a chair with wheels to undergo accommodation washout by watching a TV for 15 minutes at 5 meters, (2) then, the participant would be rolled on the chair to the Spectralis for OCT imaging, (3) thereafter, IOLMaster 700 for the ocular biometry measurements, (4) and lastly to a table for the intraocular pressure (IOP) measurements with ICare. For paper II, measurements of refractive errors with the Open-field autorefractor at 6 and 0.30 meters, were taken between step 4 and 5 (after ocular biometry and before intraocular pressure measurements). Saliva samples for melatonin extraction were collected the last 5 minutes of the accommodation washout, at HWT+0 for paper II, HWT+1 and HST-4 to 0 (papers I–II), and HST+1 (paper I). Participants were rolled on the chair between all measurements and did not stand up until finishing all measurements within an epoch.*

Participants were rolled on wheeled-chairs through the measurements during an epoch, to avoid suppressing melatonin secretion[121] and affecting IOP measurements.[191, 192] Light levels were also controlled to avoid suppressing secretion of melatonin (papers I–II),[173] and to minimize the effect of different light intensities on ChT (papers I–III).[54] In papers I and II light levels were measured to be below 20 lux. In paper III, light levels were kept similar throughout the study, curtains were closed, and global- and local-illuminances were measured as 170–190 lux and 20–50 lux at the headrest of the autorefractor and OCT, respectively.

3.3.6 Inclusion and exclusion criteria

In papers I and II, all participants had a best-corrected visual acuity of ≤0.00 logMAR (TestChart 2000; Thomson Software Solutions, London, UK) at distance, anisometropia of <1.00 D, stereo acuity of ≤120 seconds of arc (TNO Stereotest; Laméris Ootech, Ede, Netherlands), and no errors on colour vision tests on either Ishihara (24-plate edition, Kanehara Trading Inc., Tokyo, Japan) or Hardy–Rand–Rittler Pseudoisochromatic Plate Test (4th edition, Richmond Products, Albuquerque, NM, USA). Exclusion criteria included systemic or ocular disease, sleep or mental disorders, use of melatonin supplements, or any current or previous myopia control therapy. In paper III, the participants had a habitual distance high contrast visual acuity in the range of –0.18 to 0.70 logMAR. All participants were healthy with no ocular disease, as reported by their parents.

3.4 Statistical analysis

Statistical analysis was performed with R statistical software (R Foundation for Statistical Computing, Vienna, Austria),[193] with the packages nlme,[194] lme4,[195] lmerTest,[196] and irr.[197] Statistical significance level was set to $\alpha = 0.05$. Parametric tests were used when data were normally distributed, tested with Shapiro Wilks test,[198] otherwise a non-parametric alternative was used. For papers I and III, the right eye was arbitrarily chosen for analysis as there were no differences in ocular biometry or SER between right and left eyes.

For papers I–III, SER was calculated as spherical power + ½ cylindrical power. The ratio between AL and CR was calculated as AL/CR. CR was calculated as the mean value of the two corneal meridians and was expressed in millimetres (mm). VCD was calculated as AL-(ACD+LT+RT) and included in the analysis in papers I–II. For paper II, accommodation response was calculated as the difference between the far and near measurements of SER with the Nvision-K 5001 autorefractor. For IOP, one measurement series consisted of 6 single measures. The average of the three measurement series was used in the analysis in papers I–II.

3.4.1 Classification of refractive errors (papers I–III)

In papers I–II, the adolescent and young adults (17–25 years of age) were characterized by the cycloplegic SER with the following definitions: Myopia as SER ≤-0.50 D,[26] emmetropia as -0.50 D < SER <+0.50 D, and hyperopia as SER ≥+0.50 D.

In paper III, the 7–11-year-old children were also characterized by the cycloplegic SER, but with age-group specific thresholds: myopia/risk-of-myopia at <+0.50 D for the 7–8-year-olds, and ≤+0.25 D for the 10–11-years-olds. These thresholds were chosen as it has been shown that less hyperopia for a given age in children can indicate a risk for developing myopia.[199] Mild hyperopia has been described as a more natural endpoint for refractive development than emmetropia.[200] Given the age of the children this was defined as ≤+2.00 D, as it was expected the children would experience physiological ocular growth. Significant hyperopia was defined as >+2.00 D.

3.4.2 Analysis of sleep data (papers I and II)

The Actigraph GT3X collected sleep and activity data from each participant for a week prior the data collection of ocular and biological data. This data was processed in the Actigraph ActiLife software where the Choi algorithm was used to check wear time validation,[201] and the Sadeh algorithm was used to score the sleep periods as this algorithm was recommended for the age-group in papers I and II.[202] The ActiLife software estimated the habitual sleep time for each night for the individual, which was averaged for weekdays, to get the individual's habitual sleep time (HST) which was used in the scheduling of the epochs.

3.4.3 Diurnal rhythms and fitting sinusoids to the data (papers I and II)

The measured values, at the various epochs on Day 8 for a given ocular parameter or melatonin level, were modelled with a sinusoid with a fixed 24-hour period with the following equation:[203]

$$y(t) = M + A\sin(2\pi(t + \varphi)/24) \qquad \text{(Equation 1)}$$

Equation 1 was used in a nonlinear mixed effects (NLME) model analysed in R, where *M* was the midline estimating statistic of rhythm (MESOR), *A* was the amplitude, *y*(*t*) the measured value at the epoch hour, *t*, where t=0 was the participant's HST, and φ was the phase of the sine wave. The estimation of φ was transformed into a logistic distribution which constrained φ to be between 0 and 1. This ensured that the model estimations of φ were in

the same cycle (e.g., -9 hours instead of +15 hours, twenty-four hours later) for all participants.

Saliva melatonin levels throughout the day are known to have a *bathtub curve*,[204] i.e., a sharp decrease in the morning, plateau during daytime, and have a rapid increase in the evening under dim-light when secretion starts, or dim-light melatonin onset (DLMO). In order to capture the profile of melatonin secretion better throughout the day, sinusoids were fitted to the logarithm of the melatonin values log(y(t)).[205] For each participant, DLMO was derived by solving equation 1 and substituting log(y(t)) in equation 2 with log(3) as 3 pg/ml was used as the threshold to detect DLMO.[151, 173, 206]

$$\mathrm{DLMO} = \frac{24\sin^{-1}\left(\frac{\log(3)-M}{A}\right)}{2\pi} - \varphi \qquad \text{(Equation 2)}$$

A linear mixed-effects model (LMM) (Paper I) and a one-way repeated-measures ANOVA (paper II) were used to assess if a given ocular parameter or melatonin underwent significant diurnal variation[57, 58, 90, 115], with participant as within-subject factor and SER group as a between-subjects factor.

3.4.4 *Adjusted* SER (paper III)

As cycloplegic SER was only obtained in January 2020, and non-cycloplegic SER was obtained at all four time points, *adjusted* SER was estimated from cycloplegic SER as the dependent variable, and AL/CR, non-cycloplegic SER and age as independent variables. The model estimations and choice of model are shown in the supplementary material for paper III.

3.5 Ethical considerations

All three studies (papers I–III) was carried out in accordance with the tenets of the Declaration of Helsinki, and were approved by the Regional Committee for Medical and Health Research Ethics (REK, Southern Norway Regional Health Authority). Application number(s) for these approval(s) were 2018/1322 (papers I and II) and 2019/578 (paper III). The projects were also registered at the Norwegian Centre for Research Data (SIKT) with project numbers 513699 (papers I–II) and 618 194 (paper III).

The individuals who were invited to participate were given thorough oral and written information about the projects. Informed consent was obtained from the ones who chose to participate. For the participants in paper III, both parents and caregivers provided written

consent for their child to participate in the study. Participants could withdraw from the study at any time, without having to provide an explanation.

The study aimed to acknowledge and implement person-centeredness at all stages of the research process.[207] Person-centredness has been described as "*promoting care of the person, for the person and by the person*",[208] which should be considered when involving participants in a research process. This can also include the proposed treatment for a patient — such as myopia control which can be tailored for a person's needs and interests, whereas choice and decision of treatment options are discussed with the person (and parents) to encourage active involvement and autonomy.

Person-centredness aims to understand and respects the complexity of what an individual consists of. Similarly, the human eye is complex system with large between-individual variation.[209] Understanding the development of refractive errors, why certain individuals remain with hyperopia and some individuals develop myopia, appears to be a complicated developmental process which involves environmental,[12, 13] genetic,[6] behavioral,[11] and biological[7, 8] factors. This complexity emphasizes the need to acknowledge and understand between-individual variation, which requires measurements of a wide variety of variables in an individual.[210]

Person-centredness was implemented in the research process with the following points:

- Speaking to every individual during the recruitment phase for the projects (Papers I–II). This was carried out in a one-to-one session to explain about the project, its objectives, and implications of participation. The goal was to engage the participants with the project's background, and also motivate the individual for participation.
- A subgroup of the participants was invited for a discussion about the data collection and the practicalities of them staying in the laboratory an entire evening for measurements without leaving the laboratory and with minimal smart-phone usage (papers I–II). This was vital in understanding what the participants would experience as acceptable and was in consideration for their participation.
- Acknowledging the individual normal variation in sleep behaviour by collecting habitual wake time and habitual sleep time for an individual, and thereafter making an individualized measurement schedule for the participants (papers I–II).
- Acknowledging the complexity of the eye by measuring a wide variety of ocular and biological parameters such as ocular biometry, retinal and choroidal thicknesses, intraocular pressure, and melatonin, respectively.

- Linear mixed effects modelling (papers I–III), which allows for analysis on a population level (fixed effects), and on an individual level (random effects).[194]
- Analysis which also considers biological differences between females and males[211] as there are known differences in ocular biometry and refractive errors[17, 30, 212-217]
- Preliminary results were presented to a subset of participants (paper I), where also participants were shown their own data which were explained to them accordingly to their knowledge level. A larger session is planned after the PhD dissertation, which will be made in respect for the participants and for their great contribution.

4 Main results

This chapter contains a short summary of the main results for the three papers. Detailed results are provided in each of the attached papers.

4.1 Paper I

***Title**: Seasonal Variation in Diurnal Rhythms of the Human Eye: Implications for Continuing Ocular Growth in Adolescents and Young Adults*

This paper investigated seasonal variations (winter and summer) in diurnal rhythms of melatonin and ocular parameters in adolescents and young adults aged 17–24 years. The aim of this study was to examine seasonal variations in ocular growth and rhythm parameters in southeast Norway where there are 12 h difference in daylight availability between winter and summer.

In both winter and summer, all ocular parameters (IOP, ACD, LT, VCD, AL, RT and ChT) and melatonin underwent significant diurnal variation. When comparing summer to winter, there was a significant ≈1-hour advance of DLMO, and the acrophase of melatonin and axial length. Acrophase for VCD and retinal thickness was advanced with half an hour and ≈2 hours, respectively. The phase relationship between AL and choroidal thickness shifted from being anti-phase in winter (12-hour difference) to near anti-phase in summer (11-hour difference) which was due to the AL phase shifted across seasons while ChT phase remained similar. There was a significant negative association between changes in MESOR of AL and ChT from winter to summer, and a significant positive association between changes in AL MESOR and AL phase shift from winter to summer (all $p<0.05$). The crystalline lens thickened in the evening and had an acrophase at 03:00, and was near in-phase with ACD in both seasons.

4.2 Paper II

***Title**: The effect of topical 1% atropine on ocular dimensions and diurnal rhythms of the human eye*

This paper investigated the potential short-term effects of atropine 1% on the diurnal rhythms of ocular parameters in young adults aged 19–25 years. The main aim was to examine the diurnal phase relationships between AL and ChT rhythms under the effects of atropine, while a secondary aim was to assess whether the crystalline lens thickness would still exhibit diurnal variation when accommodation was suspended by atropine. Topical 1% atropine was instilled in the dominant eye while the fellow eye served as control. The paired-eye design allowed us to make comparisons with the untreated eye. Also, additional comparisons could be made with the untreated eyes from paper I because the same methodology was implemented.

Pre- versus post-treated eyes (Day 7 HWT+4 vs. day 8 HWT+4) showed lower IOP, deeper ACD, decreased LT and shorter AL. Similar trends were found when comparing treated eyes against fellow control eyes at Day 8 HWT+4, with the exception for IOP and AL where there were no differences. Although the ocular parameters still showed a significant diurnal variation throughout Day 8 in treated eyes, certain rhythm parameters were significantly affected by atropine when comparing the two eyes: MESOR was increased for ACD, while decreased for LT and VCD; amplitudes increased for AL and RT, while decreased for VCD; acrophase was advanced for ACD and VCD. When comparing treated eyes in this paper to untreated eyes in paper I, atropine affected ChT acrophase to be advanced ~6 hours earlier which changed the AL and ChT phase relationship from 11 h 54 mins (paper I, November–December) to 6 h 22 mins. Additionally, ChT in the fellow untreated eyes had an earlier acrophase compared to untreated eyes in paper I (20:25 versus 03:14, respectively) (all $p<0.05$).

4.3 Paper III

***Title**: Seasonal and annual change in physiological ocular growth of 7–11-year-old Norwegian children*

This paper investigated the seasonal variations of physiological eye growth in children in 2nd grade (7–8-years-old) and 5th grade (10–11-year-old). The aim was to assess seasonal and annual changes in physiological ocular growth and choroidal thickness in southeast Norway where there are 12 hours difference in daylight availability between winter and summer. A secondary aim was to assess if annual physiological growth for the 7–11-year-old participants were in the state of emmetropization, or whether it was coordinated (maintaining mild hyperopia/emmetropia).

Participants were measured on four occasions: autumn (November 2019, baseline), winter (January 2020), summer (June 2020) and autumn (Nov 2020). ChT was not obtained in the second autumn (Nov 2020). For participants grouped to *mild hyperopia* by cycloplegic autorefraction (cycloplegic SER +0.50–+2.00 D 7–8 years and +0.25–+2.00 D 9–10 years), it was assumed that they would experience physiological ocular growth. Each seasonal transition showed a significant increase in physiological ocular growth (measured by axial length), however with age- and sex-group differences: 7–8-year-olds had significantly larger changes over winter-spring (+0.080 mm) and annually (+0.099 mm) than the 10–11-year-olds; males had overall larger changes annual than females (+0.034 mm). There was a significant inverse relationship between ΔAL and ΔSFChT, with a significant interaction between age-group and season (n=79, adj R^2=0.65) from autumn–winter and winter-spring (all $p<0.05$).

5 Discussion

5.1 Summary of main results

This thesis explored the seasonal variations in physiological ocular growth in children, adolescents, and young adults (papers I and III), seasonal variations of diurnal ocular rhythms in adolescents and young adults (paper I), and the influence of topical 1% atropine on diurnal ocular rhythms in young adults (paper II).

The study revealed significant seasonal variations in physiological ocular growth with a higher rate during winter–spring than summer–autumn. This was independent of age, which included children (7–8-years), early adolescents (10–11-years), and late adolescents/young adults (17–25-year-olds). The rate of change was the highest in childhood during emmetropization, with a slower rate during early adolescence — a stage we argue as the maintenance of emmetropia — and slowed down even more during late adolescence/young adulthood. The choroid was the thickest for the late adolescents/young adults, followed by the early adolescents, and thinnest in the children. There was a decrease in choroidal thickness from winter to summer in the children and the late adolescents/young adults, while it increased in the early adolescents.

Another finding was the diurnal rhythm of the crystalline lens, whereby its thickness decreased in the mid-day and increased in the evening. There was still an intact diurnal rhythm in LT, even when the ciliary muscle and accommodation were suspended by topical 1% atropine. Physiological ocular growth, circannual and circadian rhythms

Ocular diurnal rhythms also showed seasonal variations in late adolescents and young adults, with indications of being synchronized to the master clock: Axial length followed suit with the ≈1 h melatonin phase advance from winter to summer. Less phase shift towards summer in AL was strongly associated with more change in AL, indicating that being well-adapted to seasonal variations may have a role in maintaining emmetropia. The phase relationship between AL and ChT was in anti-phase during winter and less anti-phase during summer, which was reminiscent of the alteration in the AL and ChT phase relationship observed in chicks stimulated for accelerated growth and normal growth, respectively. The phase relationship was also altered with topical 1% atropine: ChT phase was advanced, leading the phase relationship to be more in-phase which was reminiscent of the AL and ChT phase relationship observed in chick studies during slowed growth, and from myopic defocus in humans.

5.2 Refractive and ocular status in children, adolescents, and young adults in Norway

The study samples consisted of children, adolescents, and young adults in Kongsberg, Norway (Papers I–III). It is acknowledged that papers I–III have a relatively low sample size. This may have introduced some variation or inaccuracy in the prevalence of myopia — 5% and 2% for 7–8 and 10–11-year-olds, respectively (paper III) — considering prevalence of myopia is reported to be low in Norway.[17] The low sample size is further addressed and discussed in section 5.3 regarding strengths and limitations.

Table 3 provides an overview of SER, AL, CR and AL/CR from papers I–III and various population studies for each sex at different ages in different countries. The 7–8-year-olds in Norway (paper III), compared to children in China,[212, 214, 215, 218] Netherlands,[30] USA,[216] and Australia,[213] had an AL which was close to the median of the range, and a comparable SER (**Table 3**: compare rows 7 with 9 for AL, and row 7 with corresponding rows for age-groups 5.5–8.4 years for SER). The 10–11-year-olds (paper III) had an AL that was shorter than that reported for Dutch,[30] Chinese,[212, 215, 218] and American[216] children, which corresponded with SER in paper III being more hyperopic (**Table 3**: compare rows 15 with corresponding rows for age-groups 9.8–10.5 years for AL and SER). AL and SER for the 10–11-year-olds in paper III were the most similar to Dutch children.[30] At 17–25 years, both Norwegian females and males had a considerable more positive SER and shorter AL than Chinese adolescents aged 15–18 years (**Table 3**: compare row 23 with rows 17, 19 and 21).[215, 218]

Table 3. *Average refractive errors, axial length, corneal radius and AL/CR. These are presented by sex and age, and are independent of SER grouping. Refractive errors were measured with cyclopentolate 1% unless indicated otherwise. Females and males are indicated with "F" and "M", respectively. Papers I–III are marked in green colour. Not available data is indicated with "-". Latitudes are approximated.*[132]

Study	Country, latitude	Age	n		SER [D]		AL [mm]		CR [mm]		AL/CR	
			F	M	F	M	F	M	F	M	F	M
Guo et al 2017[214]	China, 22°N	≈6	89	99	+1.20	+1.26	22.40	22.84	7.73	7.81	2.90	2.92
Tideman et al 2018[30]	Netherlands, 52°N	6.2	3051	3033	-	-	22.09	22.63	7.70	7.84	2.87	2.89
Twelker et al[216]	USA, 30–39°N	≈7	381	443	+0.75[a]	+0.71[a]	22.55	23.00	7.69	7.81	2.93	2.95
He et al[218]	China, 31°N	≈7	1783	2091	+0.93	+0.79	22.66	23.22	7.78	7.9	2.91	2.94
Diez et al 2019[215]	China, 30°N	≈7	1062	1228	+0.29[b]	+0.27[b]	22.84	23.31	7.73	7.85	2.95	2.97
Li et al 2022[212]	China, 36°N	7.2	1194	1641	+0.98	+0.91	22.39	22.95	7.71	7.86	2.90	2.92
Paper III[219]	Norway, 60°N	7.4	19	24	+1.46	+1.27	22.59	22.77	7.84	7.79	2.88	2.92
Ojaimi et al 2005[213]	Australia, 34°S	5.5–8.4	849	875	+1.34	+1.20	22.32	22.89	7.72	7.85	2.89	2.92
Range	34°S–60°N	5.5–8.4	19–3051		+0.3–+1.5	+0.3–+1.3	22.09–22.84	22.63–23.31	7.69–7.84	7.79–7.90	2.87–2.95	2.89–2.97
Tideman et al 2018[30]	Netherlands, 52°N	9.8	2679	2617	+0.73	+0.74	22.84	23.36	7.72	7.85	2.96	2.98
He et al[218]	China, 31°N	≈10	319	384	-0.82	-0.62	23.61	24.06	7.77	7.88	3.04	3.05
Diez et al 2019[215]	China, 30°N	≈10	839	899	-1.37[b]	-1.29[b]	23.92	24.44	7.75	7.88	3.09	3.10
Li et al 2022[212]	China, 36°N	≈10	793	1091	-0.53	-0.35	-	-	-	-	-	-
Twelker et al[216]	USA, 30–39°N	≈10	193	177	+0.09[a]	+0.40[a]	23.18	23.53	7.74	7.85	2.99	3.00
Paper III[219]	Norway, 60°N	10.5	25	24	+1.18	+1.15	22.46	23.43	7.63	7.89	2.94	2.97
Range	30–60°N	9.8–10.5	24–2679		-1.4–+1.2	-1.3–+1.2	22.46–23.92	23.36–24.44	7.63–7.77	7.85–7.89	2.94–3.09	2.97–3.10
He et al[218]	China, 31°N	≈15	122	93	-3.12	-2.88	24.72	25.25	7.91	7.81	3.13	3.23
Tideman et al 2018[30]	England, 51°N	15.5	1328	1167	-	-	23.18	23.68	7.77	7.88	2.98	3.01
Diez et al 2019[215]	China, 30°N	≈16	58	61	-2.72	-2.68	24.73	25.19	7.86	7.88	3.15	3.19
Hagen et al 2018[17]	Norway, 60°N	≈16	129	95	+0.56	+0.74	23.21	23.62	7.78	7.85	2.98	3.01
He et al[218]	China, 31°N	≈18	350	589	-3.75	-3.39	24.99	25.23	7.92	7.83	3.16	3.22
Hagen et al 2018[17]	Norway, 60°N	17–19	102	67	+0.31	+0.65	23.36	23.73	7.79	7.89	3.00	3.01
Paper I[220] and II[221]	Norway, 60°N	17–25	32	19	-0.83[c]	+0.49[c]	23.77	23.65	7.75	7.86	3.07	3.01
Range	30–60°N	15–25	19–1328		-3.8–+0.6	-3.4–+0.7	23.18–24.99	23.62–25.25	7.74–7.92	7.81–7.89	2.98–3.16	3.01–3.23

[a] 1 drop 1% cyclopentolate was used when iris was graded dark in addition to 2 drops 1% tropicamide, otherwise and in the Orinda study the latter was used.
[b] 2 drops 0.5% cyclopentolate were used.
[c] 1% atropine was used.

The differences in AL and SER increased with increasing age between European and Chinese children (**Table 3**, including only studies with 1% cyclopentolate[215, 216]). At ages 5.5–8.4 years, the differences between studies were small both for AL (F/M: differences of 0.57 and 0.59 mm) and SER (F/M: difference of 0.53 and 0.48 D). At ages 9.8–10.5 years, however, the differences increased considerably (F/M: AL differences 1.15 and 0.70 mm, and SER differences 2.00 and 1.77 D), and even more so at ages 15–25 years (F/M: AL differences of 1.81 and 1.63 mm, and SER differences of 4.31 and 4.13 D). In line with previous observations,[215] the differences in AL between Chinese and European (Dutch and British)[30] increased from ages 6 (≈0.50 mm) to 9 (≈1.00 mm), and to 15 (≈1.30 mm) years. The smaller variation at early childhood (5.5–8.4 years) is consistent with a study reporting no differences at ages 6–7 years between different ethnicities in Britain,[222] and with the lower prevalence of myopia (≤-0.50 D) in 5–8.4-year-olds across countries; 5% in Norway (paper III), 1.43% in Australia,[213] and 3.7%,[214] 14.3%,[223] and 6%[212] in China. The larger differences for ≈10–year-olds in AL and SER (**Table 3**) are in line with the higher prevalence of myopia of 36–42% in China,[212] compared with the lower prevalence of 2% in Norway, 11.4% in the Netherlands, and 11.9% in Australia (11–14-year-olds).[224] It has been suggested that the higher prevalence of myopia in Asians cannot be explained by genetics alone,[15, 224] but also from behavioural and environmental aspects.[15] Given the larger differences between Chinese and Norwegian children aged 10 years than at 7 years, it is an interesting observation that environmental risk factors for myopia, such as less outdoor time, is less associated with change in refraction in 3–5-year-old children.[225] The lower prevalence of Asians living at other countries,[224] further elucidates that that environment plays a role, which is complicated by urban/rural differences,[226, 227] and daylight availability differences given that spending time outdoors protects against myopia.[15] The increasing differences in AL, SER and myopia prevalence with age between children of European and Asian descent, suggest a variability based on ethnicity, location and/or latitude, and possibly behaviour. This variability appears to start around at the age of 9–10 years. This is consistent with findings in multi-ethnic studies in 10–11[228] and 12–13-year-old Britons,[222] 10-year-olds in USA,[216] 11–15 year-old Australians.[224]

Emmetropization tends to be completed by the age of 6 years,[72] and thereafter transitions into a state where emmetropia/mild hyperopia is maintained by the crystalline lens power compensating for changes in AL.[27, 32] That there is an apparent delayed emmetropization in the 7–8-year-olds, and that the 7–11-year-olds (paper III) are mild hyperopic with a low prevalence of myopia (5%), are indeed supported by the previous observation of less myopia incidence when emmetropization completes after the age of 6 years.[72] At 10–11-years, the Norwegians had a SER of +1.15 D, and by 17–19 around +0.50 D, with indications of minimal changes after this into young adulthood. This in line with mild hyperopia being a more natural endpoint, and obtaining emmetropia at too early age could be a risk for myopia.[200] It was observed that mild hyperopia was maintained in 15-year-olds in locations with poorly developed educational systems.[200] Contrary to this, Norwegian children and adolescents are in a high level education system,[229] which are further indications that there

are other factors involved to protect against myopia onset. The data from paper III are also in line with past findings[17, 230] further supporting Norwegian children and adolescents do not follow the myopia epidemic trend reported particularly in South-east Asia.[2, 3]

Typically, females have shorter AL and steeper CR (paper III and Refs. [17, 30, 212-216]), smaller AL/CR ratio,[30, 212, 214, 215, 217] stronger crystalline lens power,[231] and higher prevalence of myopia at ages 6–12[217] and 16–19 years.[17] The sex differences at 7–8-years in ΔSER was discussed in paper III to be due to females finishing emmetropization earlier and/or have an earlier onset of puberty,[232] as males had larger changes in SER, but not in AL (paper III). Although there are established sex differences for SER and AL, these tend to be smaller than age and location-specific differences (**Table 3**).

CR is reported to stabilize at early childhood, [27, 30, 31] so differences between females and males could be assumed to be established within the first 2 years of living.[27] In line with previous findings,[17, 30, 212-216] CR was steeper in the females than in the males (paper III). There were however some discrepancy when comparing females' CR to other studies — the 7–8 and 10–11-year-old females had a flatter (7.84 mm) and steeper corneal radius (7.63 mm), respectively, than females in other studies (**Table 3**, range at ≈7 and ≈10 years: 7.69–7.74 and 7.72–7.75 mm, respectively). For both cases, this variation may be explained by the low sample size, and for the youngest children the low number of myopes in the sample. At 17–25 years, the Norwegian late adolescent and young adult females had a more negative SER (-0.83 D) and longer AL (23.77 mm) than Norwegian males (SER: +0.49, AL: 23.65 mm). Although a difference between females and males is expected, the difference in SER of 1.32 D was higher than what seen in 17–19-year-old Norwegians (F/M difference in SER of 0.34 D).[17] This could be due to selection bias (paper I) as more myopes were invited to the first study to have more equal SER group comparisons. This is also evident from the atypical longer AL in the females than the males at this age-group. Nonetheless, the females aged 17–25 years in papers I–II were considerably less myopic than Chinese children aged 15 (-3.12 D),[218] 16 (-2.72 D),[215] and 18 years (-3.75 D).[218] The males aged 17–25 years (papers I–II) had similar SER and AL as 17–19-year-old males in Norway.[17]

For the 7–8-year-olds (paper III), SER was more hyperopic (F/M: +1.46 and +1.27 D) when compared to same aged children in China (F/M: +0.29 and +0.27 D)[215] and USA (F/M: +0.75 and +0.71 D).[216] The former study in China used a lower dosage of cyclopentolate (0.5%, and not 1%) than other studies, while the latter study limited use of 1 drop 1% cyclopentolate to dark irides (in addition to 2 drops 1% tropicamide), which could have underestimated SER.[233] Non-cycloplegic autorefraction can underestimate SER[176, 177] up to -2.00 D[234], particularly with hyperopia[235] which is commonly found during early childhood.[27] Estimations of SER are not only dependent on the appropriate cycloplegic agent,[233] but also dosage, and ensuring adequate relaxation of accommodation.[214] These considerations need to be taken in order to obtain meaningful estimates of SER in children, adolescents and young adults.

5.2.1 Annual changes in physiological ocular growth

The annual rate during physiological ocular growth is faster during emmetropization, and when emmetropization is over, the rate slows down during the state of maintaining emmetropia[236] where the crystalline lens power compensates for the axial length changes leaving SER mainly unchanged.[27, 32] This was also evident from the rate of annual changes in AL for the 7–8-year-old and 10–11-year-old mild hyperopes with AL change of 0.21 mm and 0.11 mm, respectively, and the 19–22-year-olds non-myopes with 0.03 mm, whereas SER only changed significantly in the youngest age-group, as expected. This variable rate of AL at different growth stages are consistent with past findings in non-myopic children and adolescents: Children aged 3, 5, and 6 years in China had an AL change of 0.30, 0.24 and 0.27 mm/year, respectively.[236] Children aged 6.4 years in Denmark had an AL change of 0.23 mm/year;[34] children aged ≈9 years in the Netherlands had an AL change of 0.19 mm/year;[30, 34] at 12.8 years in Denmark with 0.09 mm/year.[34] Emmetropization is usually completed by 6 years,[72] but continues past this age in some countries,[72, 73] coinciding with a low incidence of myopia in the same countries.[72] That the 7–8-year-olds still were undergoing emmetropization as noted by the significant annual changes in SER (paper III), along with the low prevalence in Norway,[17] are in support of those observations. It is not known when emmetropization is completed in Norwegian children, but from the data in paper III it appears to be between the age of 8–9 years as the 10–11-year-olds did have a significant annual change in AL, but not in SER, indicating emmetropization was complete and the AL changes were compensated by the crystalline lens power.[27, 32] There is support in that there is a continued physiological ocular growth into late adolescence,[32, 34] and even into young adulthood.[90] Although it is a small sample, the ocular growth with no SER change in the 19–22-year-olds (Paper I) is another indication for this continued growth, which underlines that a healthy eye should grow,[35] but in a coordinated fashion in which the crystalline lens power decreases to maintain emmetropia/mild hyperopia.[72, 200]

The annual rate in physiological ocular growth by AL is generally slower than during myopic growth, where the former can vary by age, developmental stage (emmetropization or maintaining emmetropia/mild hyperopia) and the latter can vary by age at myopia onset. This has been shown in persistent myopes where the rate was 0.44 mm/year at 8 years, and 0.35 mm/year at 10 years. With a myopia onset at age 10 years, the rate was 0.35 at 8 years and 0.37 mm/year at 10 years.[37] While the rate can appear similar during physiological ocular growth and during myopic growth, the rate for the former will slow down after emmetropization while the latter can continue at a faster rate.

For mild myopes (-3.00 < SER ≤-0.50 D), 1 dioptre change was equal in 6–7 and 12–13-year-olds to 0.28 mm and 0.32 mm change in AL, respectively.[237] In the age-range 6–25 years for moderate myopes (≤ -3.00 D), 1 dioptre change was equal to 0.58 mm.[237] For the myopes aged 7–8 and 10–11 years (paper III), 1 dioptre change was equal to 0.70 and 0.61

mm, respectively. There are some variations here which may be complicated by myopia onset, but also by crystalline lens development.[37] In a clinical setting, these distinctions between physiological ocular growth and myopic growth are important to understand in order to assess whether myopia control treatment is necessary or not, and whether it is working or not. There are several factors that need to be taken into account when assessing myopia risk in a child; first and foremost, the refractive error relative to the child's age — for example at 7–8 years cycloplegic SER of <0.50 D can be considered a risk factor.[199] Second, the rate in AL is dependent on age and refractive development — larger changes are expected during emmetropization. Third, changes in AL relative to changes in SER — there are changes in SER during emmetropization, but would be subject to minimal change during maintenance of emmetropia/mild hyperopia, with the crystalline lens compensating for the continual AL change during this stage. Fourth, seasonal changes in AL and SER need to be considered as there appear to be larger changes during the winter than in the summer.

5.2.2 Circannual rhythms in physiological ocular growth

This thesis' findings show seasonal variation in physiological ocular growth rate (as measured by ocular axial length) during childhood and early adolescence (paper III), and young adulthood (paper I) in Norway where there are ≈12 hours of difference in daylight availability between seasons. There was a faster AL growth rate during winter compared to summer for all three age-groups. Both winter and summer growth rates appeared to slow down after emmetropization. After this, in the state of maintaining emmetropia, the winter rate stayed relatively stable from early adolescence into young adulthood. This is also in line with data from Czech Republic (50° N) and Australia (27° S).[90, 238]

As discussed in paper III, there is a resemblance in the seasonal pattern in myopic growth[90, 133, 134, 137, 238] to physiological ocular growth, with more growth occurring during the winter than in the summer. Overall, the ratio of growth between winter and summer during myopic growth was similar to physiological ocular growth (**Figure 7).** A notable distinction, however, is that the growth is overall faster in each season (particularly during winter) with myopia development than physiological ocular growth during childhood and adolescence (c.f. ΔAL for mild hyperopes and non-myopes vs. myopes, **Figure 7**). The myopes in the Czech Republic have slower progression during the summer compared to the other studies with myopes, cycloplegia was however not used which may have underestimated hyperopia and overestimated myopia when classifying the refractive error groups — ocular growth in hyperopia have been reported to be slower.[32, 35] Danish myopic children aged 8–11 years eyes grew by 0.19 mm during winter, and 0.12 mm in summer.[136] The few myopes in paper III (Table 4 and Figure 3 in paper III) had overall more growth from autumn to winter, with a similar rate to the other refractive error groups the other seasonal periods.

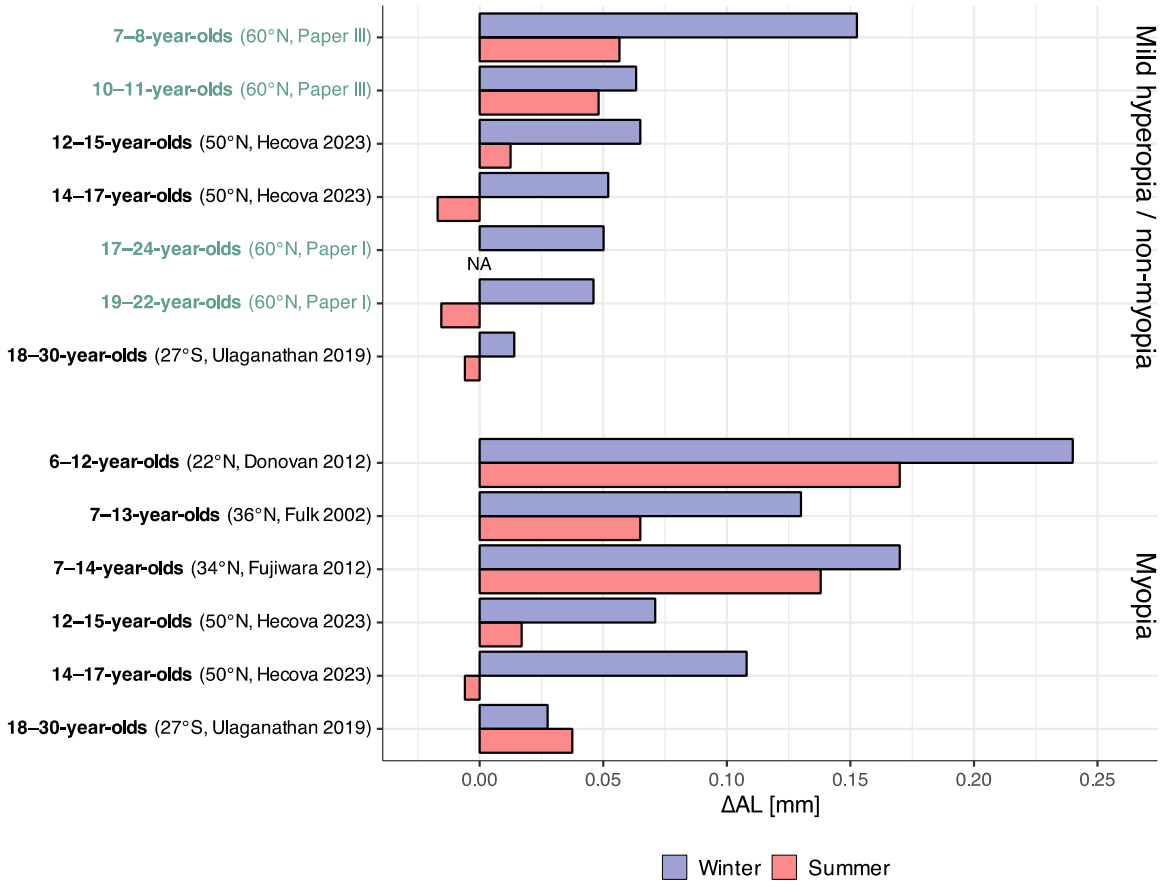

Figure 7. *Comparison between studies investigating seasonal variation in axial length. All references had an estimated measurement interval of 6 months. The horizontal bars represent the axial length change in winter (blue colour) and summer (red colour). "NA" indicates that no data were available. Data from Papers I and III are highlighted in green colour.*

There was a reversal in AL change in paper I, as also shown in **Figure 7**. This is an interesting observation, but it was beyond the scope of this thesis to be discussed any further. More details are enclosed in the discussion in paper I. For the majority of the studies in **Figure 7**, myopic growth during the summer varied by 0.2–1.4 times the rate during winter. Possible explanations for this are the differences in duration and distribution of school holidays, ethnicity, precipitation and iris pigmentation.[133, 137] This could also be due to the wide age-ranges, as seasonal variation in physiological ocular growth appears to vary by age and/or stage of growth (Papers I and III & Ref. [238]). It can be assumed that this could be generalized to myopia development as well, which progresses faster in younger children (7–10 years) than older children.[239]

The seasonal variations in ocular growth, by axial length during myopia development, have been discussed to mainly be due to variations in daylight exposure (day length, intensity and duration) with more time spent outdoors during the summer holiday than during winter, and variations in amount of near-work.[133-135, 137] One study conducted in Denmark challenged the

latter argument as school holidays in Denmark are evenly distributed across the year and still found a seasonal variation in myopic development.[136] The school holidays are similarly distributed in Norway, and shown in Table 1 in paper III. That Singaporean myopic children had a faster rate in AL in January (after examination periods) even when day length is similar all year (9 min variation),[240] appears to be more linked to behavioural variation as near-work is typically spent indoors (less outdoor time),[15] rather than attributed to a combination of behavioural variation and a seasonal variation of daylight availability. It has been hypothesized that seasonal variations could have a protective effect in relation to the relative low prevalence of myopia in Scandinavia[1, 5, 17] — this has been linked to seasonal adaptation and diurnal rhythms is discussed further in section 5.2.5.

5.2.3 Choroidal thickness

While the choroid has been implicated to have a role in regulation of ocular growth,[41, 42] it is however a challenging structure for inter-study comparisons as the thickness can vary from a wide variety of factors (section 1.2), due to its malleable characteristic. Also, the measurement of ChT can vary from the methodological differences between studies,[42] such as different instrumentation, the width of area on the choroid, and the use of cycloplegic agents.[162, 164] Additionally, there are many studies that do not report using participant-specific lateral scaling[189, 190] and retinal magnification,[241] which can affect the thickness when specific eccentricities or widths on the choroid are assessed. As all these factors make inter-study comparisons challenging, precise guidelines or standardized protocols are warranted to minimize external and internal factors in research studies involving ChT,[42] particularly in the context of refractive error development. There can still be value in what can be inferred from making comparisons across studies, but with the understanding that smaller differences may be due to other factors. The studies chosen for comparisons in **Table 4** were mainly limited to subfoveal choroidal thickness as there were few studies that had accounted for lateral scaling when needed.

When comparing ChT at age-groups 7–8, 10–11 and 17–25 years (papers I–III) with other studies, the Norwegian children had a comparable ChT to Danish[242] and Turkish children,[243] but overall thicker ChT when compared with Chinese children (**Table 4**).[244, 245] The latter observation was consistent even at the age of ≈7 years when AL and SER were similar between Norwegian and Chinese children (**Table 3**: compare rows 6 with 7). Similarly to AL and SER, the differences in ChT between European and Chinese children increased with increasing age (**Table 4**).

From the cross-sectional data in **Table 4**, it can be implied that there occurs a thinning of the choroid during emmetropization. At birth (term babies) ChT has been measured to be 456 µm,[246] with an ongoing decrease during emmetropization in childhood, followed by a thickening from childhood into adolescence for those who maintain emmetropia (or mild

hyperopia),[247] and thereafter stays relatively stable into early adulthood.[243, 248] These trends were also observed with the 7–8-year-olds having thinner choroids than the 10–11-year-olds in paper III, and the latter age-group had a slightly thicker choroid than 17–25-year-olds (papers I–II). As described in paper III, the thickening of the choroid during adolescence is a signature of continued ocular growth where the refractive errors are maintained.

Table 4. *Comparisons of choroidal thickness with findings from past studies. Results from papers I and III are highlighted in green colour, and shows data for each age-group regardless of refractive errors.*

				Mean ± SD	
Study	**Country, latitude**	**Age**	**n**	**ChT [µm]**	**AL [mm]**
Huang 2021[246]	USA, 48°N	At birth	39	456 ± 94	-
Biyik 2020[232]	Netherlands, 52°N	≈9	1018	290[a]	-
Hansen 2020[242]	Denmark, 55°N	11.5	714	361 ± 77	23.20 ± 0.80
Paper III[219]	Norway, 60°N	7.4	43	317 ± 78	22.67 ± 0.67
Paper III[219]	Norway, 60°N	10.5	49	332 ± 95	22.94 ± 0.86
Paper I[220] and II[221]	Norway, 60°N	17–25	51	339 ± 97	23.73 ± 1.16
Read 2013[247]	Australia, 27°S	4–6	57	312 ± 62	22.40 ± 0.68
Read 2013[247]	Australia, 27°S	7–9	99	337 ± 65	22.84 ± 0.61
Read 2013[247]	Australia, 27°S	10–12	38	341 ± 61	23.13 ± 0.78
Xiong 2017[244]	China, 31°N	6–9	276	283 ± 63[b]	-
Xiong 2017[244]	China, 31°N	10–13	326	244 ± 59[b]	-
Xiong 2017[244]	China, 31°N	14–18	145	218 ± 69[b]	-
Zengin 2014[243]	Turkey, 40°N	4–7	32	307 ± 42	22.33 ± 0.70
Zengin 2014[243]	Turkey, 40°N	8–11	32	298 ± 48	23.02 ± 0.91
Zengin 2014[243]	Turkey, 40°N	16–19	32	327 ± 57	24.12 ± 1.05
Zengin 2014[243]	Turkey, 40°N	20–23	32	326 ± 36	23.79 ± 1.12
Zhu et al 2017[245]	China, 42°N	≈7	108	288 ± 38	-
Zhu et al 2017[245]	China, 42°N	≈10	148	296 ± 45	-
Zhu et al 2017[245]	China, 42°N	≈17	78	273 ± 47	-
Zhu et al 2017[245]	China, 42°N	18–21	146	258 ± 46	-

[a] Combined average for females and male

[b] Central 1 mm foveal circle

From winter to summer, the choroid thinned in the 7–8-year-olds (paper III) and the 17–25-year-olds (paper I), while it thickened in the 10–11-year-olds (paper III). The thinning of the choroid during emmetropization for the youngest children can be linked to the larger changes of AL, with a higher association between ΔAL and ΔChT for the 7–8-year-olds than the 10–11-year-olds (Figure 6 in paper III). Indeed, choroidal thickening and thinning has been linked with annual AL changes up to 0.20 mm, and from 0.20 mm and higher, respectively, even in non-myopic children aged 3–14 years.[249] These findings coincide with findings in paper III, where the 7–8 and 10–11-year-olds had annual ΔAL of 0.21 mm and 0.12 mm, and a decrease in ChT from winter to summer of -12 µm and +4 µm, respectively). The 17–25-

year-olds had a ∆AL from winter to summer of 0.04 mm and decrease in ChT of –11 µm. Data from summer to winter was not obtained for ChT for any of the age-groups, and it is therefore not known to which degree the choroid would change from summer to winter. For Norwegian children, adolescents and young adults, there was a strong association between ∆AL and ∆ChT between winter and summer (papers I and III) in line with previous findings.[139] But, some participants had the same change in AL but differed in ∆ChT, indicative of other factors affecting ChT other than AL (Figure 4 in paper I and Figure 6 in paper III). This was also evident in a younger cohort (10–15 years), where different participants had the same change in AL, but varied between having a thickening or thinning of ChT (Figure 3a in Ref. [139]). One study reported the choroid to thicken after long-term light exposure, shown in a 18-month follow-up study in 10–15 year-olds.[46] It is reasonable to assume that this could be the case for the children, adolescents, and young adults (papers I and III) from the increased daylight exposure during the summer, as it has been reported that Norwegians (n=8278) aged 9–79 years, spend 4 hours a day outdoors during summer (2 h during winter).[92] It is possible the thickening would be accompanied by the reduced AL rate from summer to winter — that was about 0.40 times the change from winter to summer (**Figure 7**). A subgroup of the main sample in paper I aged 19–22 years (n=10), had no change in AL from summer to winter (0.002 mm) which could indicate that there would be minimal changes or continued thickening in the choroid annually at this age.

There are reported sex differences in terms of choroidal thickness, however with contradictory findings.[42] At birth ChT has been reported to be thicker in females, however, the study used a hand-held prototype OCT and no repeatability data was included.[246] Paper III showed an overall thicker choroid in females than males. With a shorter AL for the females at 10–11-years than males (paper III), it could be assumed that the faster rate in AL for the males between the ages of 7–8 to 10–11 would have contributed to the differences in ChT. It has also been proposed that differences between sexes can be related to differences in growth hormones, possibly linked with different puberty onset, which can affect both axial elongation and choroidal thickness from animal studies.[232] On the other hand, no differences between female and male Chinese children (6–19 years) were found when adjusting for age, SER and AL.[244] Females and males aged 16–19 years have been reported to spend an equal amount of time outdoors,[17] suggesting there are no differences related to daylight exposure.

There are certain differences in ChT between physiological ocular growth and myopic growth. Typically longer AL and more negative SER are associated with thinner choroids,[41, 42] non-myopes typically have a thicker choroid than myopes,[45] and the former group typically have a thickening of the choroid while the latter have a thinning during childhood and adolescences.[41] A decrease of ChT during myopia development needs to be distinguished from the decrease during emmetropization. A thin choroid has been suggested to be biomarker for physiological ocular and myopic growth in humans, but with no clear consensus.[42] This can also be seen in the context of countries with higher myopia

prevalence also have thinner choroids, and a thinning of the choroid throughout adolescence (**Table 4**) — well-designed longitudinal studies are warranted to verify whether a thin choroid is a predictor for axial elongation.[42] Although there were distinctions between the *Mild hyperopia*, *Myopia/risk of myopia* and *Significant hyperopia* groups in terms of AL change across seasons (Table 4 in paper III), it was also observed between-individual variation for AL, ChT, and *adjusted SER*, and change from winter to summer with no clear pattern. For example, the most myopic participant (-1.48 D) had the largest AL change (0.34 mm) from winter to summer, but had an increase of 4 µm in ChT; the least myopic participant in the myopia group (+0.54 D) had a modest change in AL (0.131 mm) from winter to summer, but had the largest decrease in ChT of -26 µm. It was also an interesting observation that participants in the *significant hyperopes* group had changes larger than physiological ocular growth (*mild hyperopia*) in AL and change in SER.

In humans, the suggested role of the choroid is to act as a biomarker for ocular growth,[42] a role less prominent than in chicks[43, 44] possibly due to species differences (see section 1.3.5.2).[8] While short-term effects on ChT in humans have been shown to be inconsistent in relation to predicting ocular growth, long-term effects lasting at least a month have been shown to predict ocular growth rate.[42] In line with this, the longitudinal design in papers I and III demonstrated an association between axial length and changes in ChT, which support the role as a biomarker. Considering this, the thinner ChT in the Chinese children compared to Norwegian children in **Table 4** could then be related to the increasing myopia prevalence and axial elongation with age. It is widely accepted that the choroid has a role in refractive development, although the exact mechanisms are unclear. Another important aspect of how the choroid may be implicated in these processes is through the diurnal rhythm of its thickness on ocular growth, which will be discussed in section 5.2.5.

5.2.4 The crystalline lens

Changes in the crystalline lens depend on age and refractive development.[27, 29, 32, 36, 37] In emmetropes, the crystalline lens reportedly thins until the age of 11 years and then start to thicken, in which its power decreases to compensate for the changes in AL to maintain emmetropia/mild hyperopia.[29] For myopes, lens changes depend on myopia onset; increase start at 11 years for early onset, and at 12–13 years for late onset (>12 years).[29] A thin crystalline lens and low lens power have also been reported to increase the risk of developing myopia.[199] Norwegian 16-year-olds maintaining emmetropia/mild hyperopia in a 2-year follow-up study, had an annual change in AL of +0.03 mm and SER of -0.03 D. The changes in AL were maintained by the 0.03 D reduction in LP.[32] The 10–11-year-old mild hyperopes (paper III) had annual ΔAL that were more than three-fold greater (+0.11 mm) than 16–year-olds,[32] with similar low changes in SER (-0.07 D). LP or annual changes in LP were not assessed in paper III as cyclopentolate was not used in the follow-up measurements. It is fair to assume that LP for the 10–11-year-olds would decrease to maintain the refractive errors, but with a larger reduction than the 16-year-olds, given the larger AL changes in the former group. Supporting this, 10–year-old persistent emmetropes in Singapore had annual changes in AL of +0.17 mm, SER of -0.14 D, and LP of -0.43 D.[37]

The crystalline lens development has been considered a passive process during refractive error development.[36, 250] Conversely, a study argues for both a passive process, where lens changes are passive as a result of axial elongation, and an active process, where the lens is actively reshaping itself to compensate for the axial elongation.[251] Furthermore, findings from studies on fish[252] and chick[253, 254] suggest that properties in the lens can be altered by modulating the light environment (monochromatic light and/or light deprivation).[36] These are interesting suggestions particularly when considering crystalline lens thickness (paper I and Refs. [57, 58]) and power[57] appear to follow a diurnal rhythm in humans. As light is the strongest zeitgeber for entraining diurnal rhythms,[99] it could be speculated that it is the diurnal rhythm of the lens that is altered in the mentioned animal studies.[252-254] It was demonstrated in paper II that the LT rhythm persists in humans even when accommodation is suspended with topical 1% atropine, but it is not known whether altering the light/dark cycle or active use of accommodation, could alter the lens' rhythm, and its development in thickness and power over time.[36] Given that ocular parameters have been shown to vary based on the time of day from optical[96, 255] and light stimuli,[95, 97] it would be of interest to assess whether light exposure or near-work at certain hours in the daytime or evening, could have implications for the development of the lens, which in turn could influence refractive error development. The crystalline lens thickness did not exhibit seasonal variation in its rhythmic parameters (MESOR, amplitude or acrophase) from winter to summer in 17–24-year-olds (paper I). This could be explained by the age of the participants and possibly non-cycloplegic measurement. It is reasonable to assume that changes in the lens, rhythmic or not, would be more likely to be observed in younger participants as the lens thins or thickens, dependent on age.[29] Further work is warranted to elucidate this hypothesis.

5.2.5 Seasonal adaptation of diurnal rhythms and implications for ocular growth

There is supporting evidence in that increased outdoor time has a protective effect against myopia,[12, 15] suggested to be related to the duration of exposure, intensity and spectral composition of light.[15] While the daylight's protective mechanisms are not understood, it has been suggested that light exposure increases dopamine levels in the retina, which in turn have a protective effect against myopia-inducing stimuli.[12] Another suggestion, which is the main focus in this section, is daylight exposure and its influence on bodily diurnal rhythms,[15, 98] which also includes ocular diurnal rhythms and how it potentially affects ocular growth. This has support in findings from animal studies — particularly with the AL and ChT phase relationship (section 1.3.5.2).[10, 105] While these are notable findings, there is a lack of strong evidence of a causal relationship between diurnal rhythms and ocular growth in humans.[15] This could be due to the differences between birds and humans as described in the introduction (section 1.3.5.2), with particularly importance with the difference in the circadian system, where birds have a light sensitive pineal gland that functions as a endogenous circadian clock,[8] which is also suggested to be involved in regulation of anterior chamber growth.[111] These differences between birds and humans underscore that certain insights from animal studies may not translate directly to humans.[8]

With this in regard, the low prevalence reported in Norway[17] compared to the high prevalence of myopia reported in South-East Asia,[2, 3] has been hypothesized to be linked to adaptation to environmental changes. Being well-adapted to seasonal variations could potentially play a protective role against myopia.[17] In Norway, children grow up with large seasonal variations in daylight exposure,[132] which may suggest that their physiological mechanisms are particularly attuned to the darker periods of the year. One study reported that less than 1 h of exposure per day to natural daylight of above >1000 lux is sufficient for the entrainment of the diurnal rhythm.[256] It is likely that children and adolescents in Norway spend this amount of time outdoors, with the minimum of 45–55 minutes outdoors even in winter (paper III) during compulsory recess at school.[17]

Regarding the eye, the hypothesis of seasonal adaptation has support from the findings in paper I, suggesting that in late adolescents and young adults in Norway, the ocular diurnal rhythm (by axial length) synchronizes with changes in the master circadian clock as observed in the transition from winter to summer. Also, findings from paper I showed similar characteristics in the AL and ChT phase relationships during normal and accelerated ocular growth in animal studies[10, 105]: in late adolescents and young adults, the AL and ChT relationship was anti-phase during the winter when there was less daylight available and near anti-phase during the summer (paper I). These characteristics of the phase relationship coincide with the faster physiological ocular growth during the winter than during the summer in children and adolescents (paper III). An interesting, but anecdotal observation from paper I

is that the three participants who had the largest annual change in AL (Table 6 in paper I), also had the smallest phase advance from winter to summer (**Supplementary Table 1**), it must however be acknowledged that a larger sample is needed to expand upon these observations. Furthermore, paper II demonstrated that topical 1% atropine alters the AL and ChT phase relationship to be in-phase, which resembles the phase relationship in chicks during slowed growth.[10, 105] Even though the findings in paper II are promising, the experiment was carried out over only a few days. A longitudinal study would be warranted to assess whether the in-phase relationship between AL and ChT would be one of the mechanisms in which atropine slows down ocular growth in humans. If this is the case and considering that myopic growth has been reported to vary seasonally,[90, 133, 134, 137, 238] one could infer that myopia control treatment with atropine may only need to be applied during the winter, and not the whole year around. Or, alternatively the concentration of atropine could be adjusted dependent on season,[257] as the summer rate during myopia development is higher than during physiological ocular growth (**Figure 7**).

Adaptation for seasonal variations have been shown to be crucial for survival and reproduction for plants and animals (see introduction).[99, 142] For humans in modern society, an inability to adapt to seasonal variations is suggested to be associated with seasonal affective disorder.[148] It is also suggested that circadian disruption, such as the case for night-workers, can have adverse health outcomes.[98, 104] There is a notable between-individual variation in melatonin suppression light sensitivity,[258] how circadian phase is affected by night-shift work,[259] and there are larger differences between individuals in the phase-angle difference of DLMO and sleep onset during winter than in the summer.[151] There are also suggestions that females are more resilient to circadian disruption than men.[260] Taken together, these are indications that individuals exhibit a varying degree in their ability for circadian adaptation to their environment. A seasonal adaptation in the eye implies an inherent ability to align the ocular rhythm by AL (alone or in combination with ChT, as suggested from animal studies) to environmental changes, as shown in paper I. When timed correctly, bright light exposure has been suggested to re-align the phase in the circadian system, and aids in recovering from jetlag more efficiently.[261, 262] The re-alignment of the circadian system has potential to be one mechanism in which the minimum 45 minutes of outdoor light exposure may protect against myopia incidence.[88, 89] It must be noted that, as estimated from the cumulated recess time during a school day, the Norwegian children and adolescents exceeded the 45-minutes threshold spent outside even in winter when there were larger changes in physiological ocular growth than in the summer (paper III). In addition, another study reported 9–15-year-old Norwegians to spend 3 hours in average outdoors every day.[92]

On the other hand, can disruptive patterns — such as irregular wake and sleep times relative to an individual's diurnal rhythm — lead to circadian disruption and thereby affects ocular growth? Indeed, late sleeping has been reported to increase the risk of incidence myopia and progression in 6–9-year-olds,[263] and similarly in 8–15-year-olds, myopes had a later DLMO

and sleep onset, poorer sleep quality, and later wake-time than emmetropes.[129] Conversely, there are conflicting findings assessing sleep-related factors between refractive errors groups, which can be tied to differences in methodology and lack of objective methods.[128] There are also possible confounders; late sleeping could be tied to reduced outdoor time and intense schooling.[264] Paper I also showed a strong association between ΔAL phase and ΔAL from winter to summer, but no associations between ΔMEL phase and neither ΔAL phase nor ΔAL over the same period, which could suggest independent regulation of the phase of both. Interestingly, it is common that children and adolescents in China aged 6–17 years have daytime naps,[265] which is less common for similar-aged Norwegians. While daytime naps can have learning benefits,[266] they have also been reported to phase shift the circadian rhythm depending on time-of-day in 20–30-year-olds.[267] It is not known whether the phase shift could have a negative effect on ocular growth in children and adolescents. Conversely, one study reported that not taking daytime naps increased the risk for myopia.[268]

As physiological ocular growth is most active during childhood and adolescence, and myopia usually onsets at school-age, it is of interest to assess the relationship between diurnal rhythms and seasonal adaptation in these age-groups. The acrophase in axial length for children[58] coincides with that observed in late adolescents and adults (paper I and Refs. [56, 57]). However, it remains unclear whether the axial length acrophase in children also adapts and shifts seasonally as shown in late adolescents and young adults (paper I), and would also have a phase-shift associated with ocular growth similar to findings in paper I. There are no longitudinal studies on ocular diurnal rhythms in children, which represents an important area for further research. In a broader context, it can be implied that the inherent ability in adapting the ocular diurnal rhythm to seasonal variations, also would have to adjust to disruptive behavioural patterns affecting the circadian rhythm.

5.3 Strengths and limitations

There are three major strengths of the methodology for papers I–III. First, papers I and II had the same rigorous methodology which included controlling for several confounding variables (see section 3.2.6 for details), and estimating measurements after individual wake and sleep times, and choosing hourly consecutive epochs in the evening to capture the sharp increase or decrease of a given ocular parameter or melatonin level by saliva samples. This methodology seem to have given consistent results as axial length acrophase had a range of 2 h (unpublished winter NLME data from paper I), in contrast to other studies that reported a range of 19 h (estimated from Figure 3 in Ref. [116]) and 10 h and 47 min.[115]

Second, reporting of the repeatability for the measurements of axial length and SER. Paper III demonstrated a within-session standard deviation (SD) for AL of 0.005 mm (95% confidence interval [CI] of 0.005–0.005, n=83, Supplementary Material Table T1 in paper III) measured with the IOLMaster 700, suggesting a high repeatability, consistent with a previous study.[183] Cycloplegia was used during measurements of refractive errors at baseline, as non-cycloplegic autorefraction can overestimate myopia and underestimate hyperopia,[233] which subsequently could have affected analysis of SER, and when assessing SER group differences for any ocular parameters. Furthermore, cycloplegic autorefraction with the HRK-8000A had an estimated within-session SD of 0.056 D (95% confidence interval [CI] of 0.042–0.073) in paper I (n=35) and 0.07 D (95% CI of 0.065–0.073) in paper III (n=78), which can be considered a high measurement repeatability and deemed sufficient when classifying participants into refractive error groups. The Nvision-K 5001 autorefractor had a higher within-session SD of 0.21 D (95% CI of 0.019–0.023) in paper III (n=81). Inter-rater segmentation for choroidal thickness had a high reliability in papers I and II, as indicated with an intra-class correlation coefficient of ≥0.94 (see methods in both papers for details). This suggests consistent segmentations across observers. Repeatability of ChT measurements, however, was not assessed in papers I–III. The Spectralis OCT has a reported repeatability of 7 µm,[269] which could be considered low since human changes tend to be small.[42] As an example, mild hyperopic 7–8 and 10–11-year-olds had an average change in ChT, from winter to summer, of -11 and +5 µm respectively (paper III). With the low repeatability, wide variety of factors reported to affect ChT (see introduction), and small changes in humans,[42] it can be inferred that ChT is not a robust measurement in humans, which introduces an uncertainty that needs to be considered along with the findings in papers I–III and other studies involving humans.

Third, the experimental designs in the three papers allowed for collection of a large amount of ocular and biological data from each participant. These designs give an in-depth comprehension of each individual's ocular diurnal rhythm (papers I and II) and the seasonal variation of these rhythms (paper I) by scheduling all measurements to individual's wake and sleep times to capture both early and late risers, and those that are early and late to bed.

The experiments conducted for papers I and II required extensive planning and coordination for the research staff in order to be executed, where the data collections started from 0700–0800, and lasting often until 0400–0500 in the following morning. To avoid overlap during the measurement day on day 8, the measurement days were spread out across 14 non-consecutive days for papers I–II. Having too many participants within one measurement day could increase the risk of too much overlap during measurements. This could have the potential consequence of creating a delay in the taken measurement which would then deviate from the actual scheduled epoch estimated from the hour relative to habitual wake or sleep time. Another challenge with obtaining a larger sample was recruitment, as the experiments required a substantial time commitment from each participant. This involved: 1) a screening examination for ocular biometry, posterior segment imaging and cycloplegic autorefraction; 2) ocular measurements and information the week prior to the experiment; 3) eight epochs of measurements on Day 8 where the participants also were confined to the lab at least for 6 hours, and deprived of certain substances known to affect saliva samples or melatonin.[173, 174] The measurements described at points 2) and 3) were then repeated in the summer for paper I. Although larger amounts of data per participant were prioritized than having a larger sample size and potentially less data per participant, an increased sample size in papers I–III would have been more ideal for improving model fitting and estimates.

Participants in papers I and II did not sleep in the lab and, therefore, no sleep-disrupting measurements were taken. This is a different practice from previous studies assessing ocular diurnal rhythm where participants slept in the lab and were woken up several times in the night for measurements.[57, 58, 116] While it is acknowledged that more datapoints are useful to get a more complete view across a 24-hour period and to improve model fits, waking up participants can however affect measurements. Change of postural position, such as when getting out of bed, can suppress melatonin secretion[121] and intraocular pressure.[191, 192] There is also a reported large individual variation in the light sensitivity for melatonin suppression with levels as low as 10 lux,[258] and the choroid has been reported as well to adjust its thickness from varying light intensities.[54] There is also the aspect of consideration of the participants — waking them up in the night several times could expose the participant to unnecessary stress and discomfort.

Another limitation is using a fixed 24-hour period for the diurnal rhythm analysis. The circadian rhythm is described as having an approximate 24-hour period, but can vary individually.[270] These variations could have improved modelling and estimates if considered. This is potentially an area of interest particularly regarding the choroidal rhythm, as the 24-period could hypothetically be altered from topical atropine, as discussed in paper II. To estimate alternative periodicities of the diurnal rhythm, it would be necessary to conduct several consecutive days of epoch measurements on a participant. This would be out of scope for papers I–II, as it would require considerably more resources to implement and time from the participants into an already exhaustive data collection, and would thus be more suitable for a separate study.

Personal light exposure was not measured in papers I and III, which is discussed further in each respective paper. It can be assumed from the recess time and the reported average outdoors time for Norwegians aged 9–15,[92] that the children and adolescents (paper III) met the minimum threshold of 45 minutes outdoor time suggested to have a protected effect,[88, 89] even in winter. Additionally, there have been reported small regional variations within Norway in terms of time spent outdoor,[92] suggesting that the measured children, adolescents, and young adults in Kongsberg (papers I and III) could be representative for the general population in in Norway in terms of outdoor time. Nonetheless, measurement of personal light exposure is important and recommended for future studies assessing associations between ocular growth and daylight exposure.

Additional details about strengths and limitations are enclosed in papers I–III.

5.4 Future perspectives

The aim of this thesis was to investigate and contribute to the existing knowledge about physiological ocular growth and refractive error development regarding environmental (seasonal variations of daylight availability) and biological factors (diurnal rhythms and ocular dimensions) on a population which has a low reported prevalence of myopia.[17] The findings suggest that the seasonal variations of daylight influence the rate of physiological ocular growth in children and adolescents (paper III). The associations between change in AL and the phase shift in AL across seasons indicate that a seasonal adaptation to daylight changes may be involved with ocular growth (paper I).

More work is however needed to elucidate the role of ocular diurnal rhythms and seasonal adaptation. One suggestion to carry out further research, is to conduct a study on children and adolescents and assess to which degree physiological ocular growth is regulated by shifts in the diurnal rhythms as during seasonal variations over a period of a year. Myopia onset is typically at school age from 8–14 years,[84] which makes this an important population to investigate further. That physiological ocular growth shows seasonal variations in children and adolescents (paper III) is another indication that such investigations are of potential interest. From an ethical perspective, the experimental longitudinal design of studying the effects of large seasonal variations on individuals has the advantage that all individuals will have to undergo the same environmental changes, as opposed to, for example, having a control group where certain individuals will have less outdoor time than others, and thereby potentially inducing more unwanted ocular growth in this group. As there is also a behavioural aspect of daylight exposure with time spent outdoors, it is imperative to measure personal light exposure. The study could also involve a control group with an age-matched sample from a location near the equator which experiences less seasonal variations of daylight availability. Such a study could expand on the current findings of seasonal

adaptation from paper I and III, which potentially also can be transferrable to adaptation to behavioural factors related to circadian disruption. Based on the findings from paper II, a longitudinal study assessing ocular diurnal rhythms and use of topical atropine would be warranted to shed light on whether the in-phase relationship between AL and ChT would be associated with AL change or ocular growth.

Recent studies have reported monochromatic light (red light and ultraviolet/violet light) to slow myopia progression in animals[271, 272] and humans.[273-276] Although the findings from these studies are interesting, there is an ethical concern because of the risk of retinal damage[277] due to harmful levels of light.[278] This highlights the need for following safety standards and for conducting well-designed research studies to better the understanding of the protective mechanisms of monochromatic light, or perhaps rather the protective mechanisms of natural daylight in general.

6 Conclusion

This thesis contributes to the existing knowledge on physiological ocular growth regarding the rate at different ages and developmental stages, and the influences from environmental factors and biological factors. Specifically, the research provides insights on physiological ocular growth in a Norwegian population with a low prevalence of myopia[17] at a location with considerable differences in daylight availability across seasons.[132] These insights include that physiological ocular growth has a seasonal variation in children, adolescents, and young adults, with a faster rate during winter than in the summer (section 5.2.2). Compared to myopic growth (as reported in previous studies), physiological ocular growth was observed to have a slower seasonal rate (for both seasons) and slower annual rate (sections 5.2.2 and 5.2.1). The rate for physiological ocular growth appears to vary by age, developmental stage and season, while myopic growth appears to be dependent on age of onset and season.[37]

Furthermore, Norwegian children aged 7–8 years had comparable AL and SER to similar-aged European and Chinese children, but these differences increased with age at 10–11-years and increased even further at 17–25 years (section 5.2). These observations were in line with European vs. Asian children having increasing differences with age,[30, 215] and with previous studies reporting school myopia onset being typically at the ages 8–14 years.[84] Choroidal thickness in the sample was found to be comparable to other European countries, but thicker overall than to Chinese children even at 7–8-years. From the seasonal and cross-sectional data, it was observed that ChT thinned in the Norwegian children undergoing emmetropization, and thickened in the stage of maintaining emmetropia, and was stable into young adulthood (section 5.2.3).

This thesis lend support to that ocular diurnal rhythms play a role in physiological ocular growth. This is implied from the findings of a seasonal adaptation as observed with AL, and from how the ocular diurnal rhythms are altered with topical atropine 1%. Both findings show similarities to how ocular diurnal rhythms have been implicated with ocular growth, as reported in chick studies.[9, 10] While being an unexplored area, the prominent role of the crystalline lens during emmetropization and maintaining emmetropia (section 5.2.4),[27, 32] findings from animal studies,[36] raises the question whether light or near-work could have a time-of-day effect on the rhythm of the crystalline lens, its long-term development and potential role in myopia (section 5.2.4).

The findings of this thesis point to a future where optometrists need to have a person-centred approach with a broad understanding of the patient's biological, behavioural and genetic aspects. This can be used for tailored treatment along with shared decision-making with the patient and parents to determine which treatment option is the most appropriate in terms of efficacy, the patient's interests, and the likelihood of good compliance.

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Supplementary Material

Supplementary Table 1. *Overview of axial length (AL), change in AL (∆AL), SER, ∆SER and change in model estimations of ∆AL acrophase. All data is from paper I.*

	AL [mm]	∆AL [mm]			SER [D]	∆SER [D]	∆AL acrophase (h)
Participant	**W1**	**W1–S**	**S–W2**	**W1–W2**	**W1**	**W1–W2**	**W1–S**
1	22.427	-0.011	-0.051	-0.062	-0.48	-0.17	1.19
2	22.968	0.068	-0.045	0.023	-0.23	-0.18	0.72
3	25.003	0.050	-0.023	0.027	0.22	-0.14	1.05
4	23.596	0.021	0.010	0.031	-0.91	-0.22	1.28
5	23.746	0.044	-0.009	0.035	1.06	-0.19	1.21
6	24.892	0.067	-0.015	0.052	0.20	-0.05	1.12
7	23.325	0.049	0.010	0.059	0.67	-0.54	1.01
8	21.938	0.046	0.022	0.068	0.25	-0.25	0.70
9	24.898	0.125	0.020	0.145	-4.08	-0.04	0.38
10	24.879	0.110	0.105	0.215	-0.923	-0.58	0.20

Papers

Paper I

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Seasonal Variation in Diurnal Rhythms of the Human Eye: Implications for Continuing Ocular Growth in Adolescents and Young Adults

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Purpose. To investigate the diurnal rhythms in the human eye in winter and summer in southeast Norway (latitude 60°N).

Methods. Eight measures (epochs) of intraocular pressure, ocular biometry, and optical coherence tomography were obtained from healthy participants (17–24 years of age) on a mid-winter's day ($n = 35$; 6 hours of daylight at solstice) and on a day the following summer ($n = 24$; 18 hours of daylight at solstice). Participants wore an activity monitor 7 days before measurements. The epochs were scheduled relative to the individual's habitual wake and sleep time: two in the day (morning and midday) and six in the evening (every hour until and 1 hour after sleep time). Saliva was collected for melatonin. A linear mixed-effects model was used to determine significant diurnal variations, and a sinusoid with a 24-hour period was fitted to the data with a nonlinear mixed-effects model to estimate rhythmic statistics.

Results. All parameters underwent significant diurnal variation in winter and summer ($P < 0.002$). A 1-hour phase advance was observed for melatonin and ocular axial length in the summer ($P < 0.001$). The degree of change in axial length was associated with axial length phase advance ($R^2 = 0.81$, $P < 0.001$) and choroidal thickening ($R^2 = 0.54$, $P < 0.001$) in summer.

Conclusions. Diurnal rhythms in ocular biometry appear to be synchronized with melatonin secretion in both winter and summer, revealing seasonal variation of diurnal rhythms in young adult eyes. The association between axial length and seasonal changes in the phase relationships between ocular parameters and melatonin suggests a between-individual variation in adaptation to seasonal changes in ocular diurnal rhythms.

Keywords: circadian rhythm, melatonin, choroid, ocular growth, myopia

Daylight is the strongest zeitgeber to entrain an individual's master circadian clock. Maintaining a regular diurnal rhythm (i.e., one that is of an approximately 24-hour period) is known to have general health benefits.[1] The components and parameters in the eye also undergo diurnal rhythms.[2–6] Experimental animal studies have shown that disruptions of ocular diurnal rhythms are implicated in accelerated ocular growth (axial elongation that leads to a more myopic refractive error).[7,8] First, it has been reported that during normal growth there is a near-antiphase (9-hour) relationship between ocular axial length (AL) and choroidal thickness (ChT). In eyes undergoing stimulated growth, this shifts to an exact antiphase (12-hours) relationship during accelerated growth and an in-phase relationship during recovery from either form-deprived or lens-induced myopia.[9,10] Second, both constant light and constant darkness have been associated with accelerated growth,[11,12] implying that maintaining a balanced light/dark cycle is important for coordinated growth (axial elongation without a change in refractive error).[13,14] In humans, it has been reported that myopic defocus reverses the phase relationship between AL and ChT,[15] whereas hyperopic defocus leaves the phase relationship unaltered, with only short-term increases in AL and AL amplitude.[16] In animal models, thinning versus thickening of the choroid has been associated with accelerated versus decelerated ocular axial elongation.[17] Choroidal thickening has been linked to decreased scleral matrix remodeling,[10,18] with regulation of ocular growth through prevention of mechanical stretching of the sclera.[17,18] In humans, thinner choroids are reported to be associated with longer eyes.[7]

Daylight exposure, through outdoor activity during daylight hours, is the behavioral factor reported to have the strongest protective role against accelerated ocular growth.[19,20] The mechanisms by which daylight regulates eye growth are not fully understood.[19] Evidence from

both human and animal studies show that light exposure, as well as imposed defocus, affect the ocular structures differently depending on time of day; light therapy has the largest effect on thickening the human choroid in the morning,[21] whereas in chicks myopic defocus inhibits ocular growth more effectively in the evening than in the morning.[22] These differential effects of the time of day raise the question of whether daylight exposure could be linked with improvements not only in overall bodily diurnal rhythm[1] but also in ocular diurnal rhythm and subsequently the diurnal interrelationship among the different ocular structures.

Scandinavian countries seem to be defying the expected worldwide increase in the prevalence of myopia,[23–25] with considerably lower myopia prevalence than that reported in southeast Asian countries, such as China.[26] Scandinavia is located at the subarctic Northern Hemisphere where there are large seasonal variations of daylight length, intensity, and temperature.[27] Even in southeast Norway, daylight duration can vary between 6 hours in the winter and 18 hours in the summer.[28] Because of the low number of available daylight hours in the winter, the low myopia prevalence leads to a supposition of whether seasonal variations in daylight play a role in promoting coordinated growth.[23] Slowing of eye growth and less myopia progression have been reported in the summer due to a higher number of available daylight hours.[29,30] However, effects of seasonal variation of daylight on ocular diurnal rhythms, and its relation to eye growth have not yet been properly explored. To date, the only study looking at daily variations of AL and ChT across seasons was conducted in northeastern Australia, where there are minimal seasonal variations of daylight duration.[31] If daylight plays a prominent role for maintaining healthy (normal growth regulation) phase relationships among ocular structures,[3–6] we hypothesized that the availability of daylight hours in winter compared with summer should have a measurable effect on these phase relationships. Furthermore, the diurnal variations of the ocular structures (except for photoreceptors in the retina which are driven by intrinsic oscillators)[32] might be synchronized to the master circadian clock (located in the suprachiasmatic nucleus), as measured by saliva melatonin secretion.[33] With this in mind, we investigated diurnal variations of melatonin secretion and ocular structures in southeast Norway (latitude 60°N) in winter and the following summer in healthy high school and university students.

Methods

Recruitment

Thirty-five healthy young adults (19 females), 17 to 24 years of age, participated in this study. The study was approved by the Regional Committee for Medical and Health Research Ethics South East Norway and was carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from the individuals prior to participation. Baseline cycloplegic autorefraction (HRK-8000A; Huvitz Co., Ltd., Gyeonggi-do, Korea) was measured 20 minutes after instillation of topical 1% cyclopentolate hydrochloride (Minims single-use; Bausch + Lomb UK Ltd, Kingston upon Thames, UK). One drop of cyclopentolate was used if the participant's irides were blue to green and two drops if they were green to brown. The HRK-8000A incorporates an automatic three-axis eye tracker that optimally repositions the sensor to compensate for the participant's eye movements between measurements, intended to give high measurement repeatability. All participants had best-corrected distance visual acuity of ≤0.00 logMAR (TestChart 2000; Thomson Software Solutions, London, UK); anisometropia of <1.00 diopters (D); stereo acuity of ≤120 seconds of arc (TNO Stereotest; Laméris Ootech, Ede, Netherlands); and no errors on color vision tests, including the Ishihara, 24-plate edition (Kanehara Trading Inc., Tokyo, Japan) and Hardy–Rand–Rittler Pseudoisochromatic Plate Test, 4th edition (Richmond Products, Albuquerque, NM, USA). None of the participants had systemic or ocular disease, used melatonin supplements, or were diagnosed with sleep or mental disorders.

Data Gathering Protocol, First Winter and Summer Measurements

On day 1, self-reported habitual wake time (HWT) and habitual sleep time (HST) were recorded. The participants were given an ActiGraph GT3X wrist-mounted activity monitor (ActiGraph Corp., Pensacola, FL, USA) to wear for the subsequent 7 days and nights to objectively measure their habitual get-out-of-bed times and sleep-onset times (Fig. 1). On day 8, the participants returned to the lab for eight epochs of measurements. The first two epochs (HWT+1 and HWT+4) were 1 and 4 hours after the participant's self-reported wake

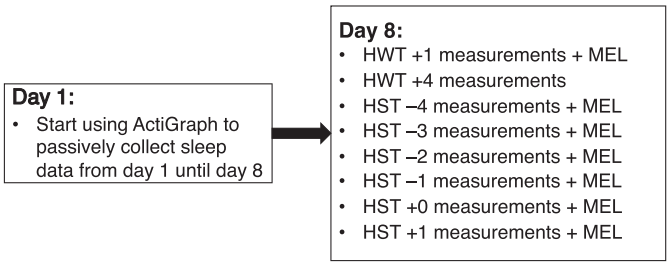

Figure 1. Sequence of measurements performed on the 2 days of data collection. The same protocol was used for both winter and summer seasons. In the figure, MEL indicates that a saliva sample was taken for melatonin analysis.

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times, respectively (the ActiGraph cannot measure wake times). The remaining six epochs were every hour from 4 hours before the participant's HST (as determined by the ActiGraph, using only weekday averages) until 1 hour after (HST–4 to HST+1), ensuring that all measurement epochs were aligned to the individual's chronotype. Participants did not sleep during the measurement period, including the HST+1 epoch.

Throughout each epoch, the participants remained seated on a wheeled chair to avoid the influence of postural changes on measurements.[34–36] Thereafter the participants relaxed their accommodation by watching a movie binocularly at 5 meters for 15 minutes (accommodation washout),[16] using their habitual distance correction if necessary. Each epoch entailed retinal and choroidal imaging by optical coherence tomography (OCT; SPECTRALIS OCT2 EDI; Heidelberg Engineering, Heidelberg, Germany); measurements of central corneal thickness, corneal radius, anterior chamber depth (ACD), crystalline lens thickness (LT), and AL (IOLMaster 700; Carl Zeiss Meditec AG, Jena, Germany); and intraocular pressure (IOP) (iCare; Tiolat Oy, Helsinki, Finland). The IOLMaster 700 is known to have high measurement repeatability.[37] The recorded IOP measurements were the average of three sets where each consisted of six single measures for each eye. Saliva samples (Salivette saliva collection kit; Sarstedt, Nümbrecht, Germany) for analysis of melatonin (MEL) were collected during the last 5 minutes of the accommodation washout, at HWT+1 and at the six evening epochs. The saliva samples were immediately centrifuged at −4°C, 4400 rpm, for 5 minutes (Centrifuge 5702R; Eppendorf SE, Hamburg, Germany) and stored in a −25°C freezer. The samples were analyzed by VITAS Analytical Services (Oslo, Norway) using an ELISA kit (Bühlmann Laboratories, Schönenbuch, Switzerland).

In accordance with previously described guidelines, the participants did not consume or drink certain substances (such as NSAIDs, nicotine, bananas, chocolate) 36 hours prior to—or during—day 8 to avoid any undesirable effects on saliva samples or melatonin.[38,39] Similarly, participants did not consume alcohol or drinks containing caffeine or artificial additives 24 hours prior to or during the experiment. None reported having traveled across more than two time zones within the month prior to the experiments. They were encouraged to wake and sleep as they normally would between day 1 and day 8. Ambient light levels, including the movie displayed on the television, were kept below 20 lux to avoid suppression of melatonin secretion[39] or affecting the choroid.[40]

Data Gathering Protocol, Second Winter Measurements

Ten of the participants (seven females), 19 to 24 years of age, returned for a third measurement of cycloplegic autorefraction and ocular biometry (same instruments and methods as described above) in the following winter between November and January.

Winter and Summer Solstice

Experiments were conducted in the southeast of Norway (latitude 60°N, longitude 9°E) over a 2-year period. Participants were examined in either the 2018/2019 winter or the 2019/2020 winter and the following summer. The aim was to measure the participants as near as possible to both winter and summer solstices to observe the effects of daylight exposure at their most extreme. The winter solstice occurred on December 21, and participants were measured from 32 days to 4 days prior to this date (from 7 hours and 19 minutes down to 6 hours of available daylight). The summer solstice was June 21, and participants were measured from 39 days prior to the solstice and up to 5 days after (from 16 hours and 55 minutes up to 18 hours and 43 minutes of available daylight).[28] From the 35 participants measured in the winter, 24 returned and completed the summer measurements. Although measurements were scheduled to each individual's habitual sleep pattern (Fig. 1), local clock time (UTC+1 during winter, UTC+2 during summer) was also recorded for each measurement. Henceforth, all clock times are reported in standard time (UTC+1).

OCT Measurement Protocol and Segmentation Details

The OCT scan protocol consisted of six radial scans centered on the fovea with 100 B-scans averaged at each orientation and with enhanced depth information enabled. The first measurement was used as the reference image for all subsequent epochs using the retinal tracking system of the instrument. A semiautomatic active contour method was used to segment the retinal and choroidal layers, as described previously.[41,42] The lateral scale of the OCT scans was corrected for each individual's ocular biometry (from an IOLMaster 700 measurement at the same time point) using a four-surface schematic eye model.[43,44] The anterior edge of the inner limiting membrane, posterior boundary retinal pigment epithelium (RPE)–Bruch's membrane band and the inner border of the sclera were segmented by two experienced operators.[45,46] Only horizontal and vertical scans were used for analysis. Mean values were extracted for the central 1-mm retinal thickness (RT) and ChT (Fig. 2).

Data Analysis

The statistical analysis was performed with R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria)[47] with the nlme package.[48] Parametric tests were used when the data were normally distributed; otherwise, a non-parametric alternative was used. Statistical significance level was set at $\alpha = 0.05$. Spherical equivalent refraction (SER) was calculated as sphere $+ \frac{1}{2}$ cylinder. A random-effects ANOVA model was used to estimate within-session repeatability for the five autorefractor measures of cycloplegic SER, with a nested random-effects structure for "participant" and "eye" using the lme4 package.[37,49] The within-session SD was estimated by profiling the likelihood.[50] The mean of the five measures of SER were used for further analysis. Vitreous chamber depth (VCD) was calculated as AL – (ACD + LT + RT). The right eye was arbitrarily chosen for analysis, as there were no difference between OD and OS for any of the ocular biometry measures. Myopia was defined as SER ≤ -0.50 D, emmetropia as -0.50 D $<$ SER $< +0.50$ D, and hyperopia as SER $\geq +0.50$ D. Interrater reliability for segmentation of the choroid was assessed by calculating the intraclass correlation (ICC) with the irr package[51] in R using a one-way model. ICC was 0.95 (95% confidence interval [CI], 0.929–0.965). The ActiGraph data were processed using ActiGraph ActiL-

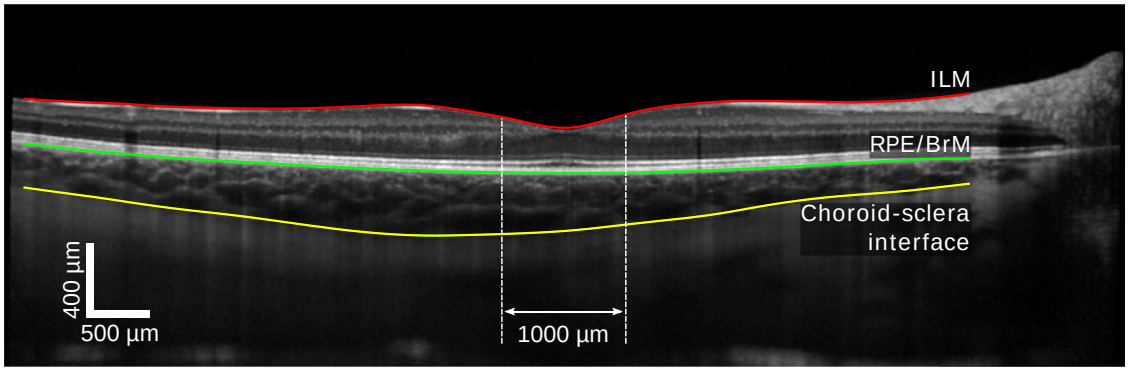

Figure 2. An OCT B-scan annotated with segmented retinal and choroidal layers. Retinal thickness and choroidal thickness were defined as the average area between the inner limiting membrane (ILM) and RPE layers and between the RPE and choroid layers, respectively, averaged over the central 1 mm and over both horizontal and vertical B-scans (horizontal shown).

ife software. Wear time validation was checked with the Choi algorithm,[52] and sleep periods were scored with the Sadeh algorithm as recommended for the age group included in this study.[53] The ActiGraph GT3X has been found to be comparable to polysomnography.[54]

The epochal data were modeled with a sinusoid with a fixed 24-hour period[55]:

$$y(t) = M + A\sin(2\pi(t+\varphi)/24) \quad (1)$$

using a nonlinear mixed-effects (NLME) model, where M, A, and φ are the midline estimating statistic of rhythm (MESOR), amplitude, and phase of the sine wave, respectively; $y(t)$ was the measured value at epoch hour (t) relative to the participant's HST (e.g., $t = -3$ represents the HST–3 measurement). Acrophase is the timing of changes of amplitude within a 24-hour period and indicates how melatonin and ocular parameters co-vary in amplitude with each other throughout a single day. MESOR is the average magnitude in a 24-hour period and can be used to represent co-variation across seasons. For melatonin only, the sinusoid was fitted to the logarithm of the melatonin measurement, $\log(y(t))$, which better captures the profile of melatonin secretion throughout the day.[56] In order to capture the onset of melatonin secretion, dim light melatonin onset (DLMO), with a threshold of 3 pg/mL,[38,57,58] was derived for each individual by solving Equation 2 and substituting $\log(y(t))$ with $\log(3)$:

$$\text{DLMO} = \frac{24\sin^{-1}\left(\frac{\log(3)-M}{A}\right)}{2\pi} - \varphi \quad (2)$$

A linear mixed-effects model with epoch as a repeated factor and SER group as a fixed effect was used to identify significant diurnal variations in the ocular parameters and MEL.[31] Repeated-measures ANOVA was used to study the differences between SER groups across seasons for different ocular parameters and MEL.

Results

Table 1 shows the winter baseline measurements for the 35 participants. The within-session SD for the Huvitz HRK-8000A for these 35 participants was estimated to be 0.056 D (95% CI obtained by profiling the likelihood, 0.052–0.061). This compares favorably with the median value (0.054 D; interquartile range, 0.042–0.073) of the values estimated from each individual using t-statistics. The maximum of these individual values gives a worst case of ±0.176 D. Thirteen participants had hyperopia, 11 had emmetropia, and 11 had myopia. Mean AL was 23.83 mm, and mean cycloplegic SER was –0.50 D. Myopes were significantly older than hyperopes ($P = 0.010$), and there was a significant difference in AL and cycloplegic SER among the SER groups (all $P < 0.001$; Supplementary Table S1).

Table 2 shows the winter baseline and summer measurements for the 24 participants who were evaluated both seasons. Nine participants had hyperopia, six had emmetropia, and nine had myopia. Mean AL increased significantly from winter to summer ($P = 0.003$). Morning MEL (HWT+1) did not vary across seasons, but evening MEL (HST+0) was significantly higher in the summer ($P = 0.001$). There was no season by SER group effect for MEL measurements (Supplementary Table S2). Mean central corneal

Table 1. Winter Demographics and Measurements for All Participants and SER Groups

	All Participants ($n = 35$) (M/F, 19/16)		Emmetropia ($n = 11$) (M/F, 6/5)		Hyperopia ($n = 13$) (M/F, 4/9)		Myopia ($n = 11$) (M/F, 9/2)		
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P****
Age, y	19.83	2.2	20.09	1.92	18.54	1.9	21.09	2.12	<0.012
AL, mm	23.83	1.35	23.31	1.00	23.22	0.91	25.06	1.20	<0.001*
Cycloplegic SER, D	−0.50	2.58	−0.06	0.28	1.36	1.46	−3.14	2.61	<0.001*

AL mean is the group average from the individual average of all measurements at day 8.
* P values are by SER group and were adjusted by applying the Holm–Bonferroni method.

Table 2. Seasonal Comparison for the 24 Participants (13 Males) Who Participated in Winter and Summer

	Winter		Summer		
Parameter	**Mean**	**SD**	**Mean**	**SD**	***P* Value by Season***
Age, y	19.58	2.39	20.04	2.56	<0.001
AL, mm	23.91	1.4	23.96	1.40	0.001
Morning MEL, pg/mL	12.07	10.25	9.35	8.92	0.120
Evening MEL, pg/mL	16.14	7.92	22.64	7.24	< 0.001

Mean values for AL and MEL are the group average from the individual average calculated for each respective season, except for morning MEL and evening MEL, which are the group average of MEL 1 hour after habitual wake-up time (HWT+1) and at habitual sleep time (HST+0), respectively.

*P values were adjusted by applying the Holm–Bonferroni method.

Table 3. Linear Mixed-Effects Model Results Used to Determine Significant Diurnal Variations in the Ocular Parameters and MEL

	Winter (n = 24)		Summer (n = 24)	
Parameter	***F* Statistic**	***P***	***F* Statistic**	***P***
MEL	49.13	<0.001	48.30	<0.001
IOP	61.68	<0.001	45.72	<0.001
ACD	9.37	<0.001	13.74	<0.001
LT	5.04	<0.001	4.99	<0.001
VCD	61.01	<0.001	40.74	<0.001
AL	32.67	<0.001	27.88	<0.001
RT central 1 mm	17.56	<0.001	10.22	<0.001
ChT central 1 mm	6.65	<0.001	3.48	<0.002

thickness and corneal radius exhibited negligible changes from winter to summer (2.4 µm and 0.01 mm, respectively) and are not discussed further in this paper.

Diurnality of Ocular Parameters and MEL: Seasonal Comparisons

Figure 3 shows the diurnal variations of the different ocular parameters and melatonin including the sinusoidal function fitted to the data for each season (each y-axis in the right panels is rescaled for clarity). Figures 3A and 3B show comparisons between the 35 winter-only participants versus the 24 winter and summer participants. There was no difference between the 24 participants when comparing them with the remaining 11 for any ocular parameter or MEL ($P >$ 0.05 for all), indicating that the 24 participants who returned for the summer measurements were representative of the original 35 participants. Figures 3C to 3F show the data for the 24 participants for winter (Figs. 3C, 3D) and summer (Figs. 3E, 3F). Melatonin, IOP, ACD, LT, VCD, AL, RT, and ChT exhibited significant diurnal variation in each season (all $P \leq$ 0.002) (Table 3). The near in-phase relationship between MEL and LT, ACD, and ChT and the near 12-hour antiphase relationship between MEL and IOP, AL, VCD, and RT are apparent (Fig. 3).

HST occurred at the same standard clock time in winter as summer; thus, the diurnal characteristics of the ocular parameters are listed relative to HST in Table 4, with acrophases repeated in standard clock time in Table 5. DLMO, similarly to MEL acrophase, occurred nearly 1 hour earlier in the summer than in the winter. A similar phase advance was observed for ACD and AL, whereas the phase

Table 4. Population Estimates for MESOR, Amplitude, and Acrophase for Ocular Parameters, and MEL for Winter and Summer Calculated from the NLME Model

	Winter						Summer					
	MESOR		Amplitude		Acrophase, h		MESOR		Amplitude		Acrophase, h	
Parameter	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
MEL, log(pg/mL)	0.87	0.66–1.07	3.155	2.92–3.39	3.99	3.62–4.30	1.04	0.82–1.25	2.952	2.71–3.2	3.14*	2.67–3.54
IOP, mm Hg	13.36	12.24–14.47	2.810	2.38–3.32	−10.10	−10.62 to −9.57	13.09	11.89–14.3	2.760	2.33–3.29	−10.27	−10.8 to −9.72
ACD, mm	3.73	3.62–3.84	0.019	0.011–0.028	2.44	1.82–2.99	3.74*	3.63–3.84	0.026	0.019–0.035	1.64	1.04–2.19
LT, mm	3.55	3.46–3.65	0.015	0.01–0.022	2.64	1.53–3.51	3.56	3.47–3.65	0.013	0.008–0.021	3.18	1.98–4.05
VCD, mm	16.41	15.85–16.97	0.055	0.048–0.063	−9.00	−9.43 to −8.55	16.44*	15.88–17	0.063	0.053–0.074	−9.53*	−9.91 to −9.15
AL, mm	23.92	23.37–24.47	0.019	0.015–0.024	−8.79	−9.24 to −8.34	23.96*	23.41–24.52	0.022	0.017–0.028	−9.81*	−10.24 to −9.37
RT central 1 mm, µm	261	256–267	2	1–3	−9.47	−10.30 to −8.60	263*	257–268	2	1–2	−11.32*	−12.52 to −9.97
ChT central 1 mm, µm	354	310–397	2	1–3	3.10	2.42–3.66	343*	302–385	4*	2–7	3.42	2.96–3.82

Acrophase is relative to habitual sleep time.
*Significant seasonal effect on MESOR, amplitude, or acrophase set at $P < 0.05$.

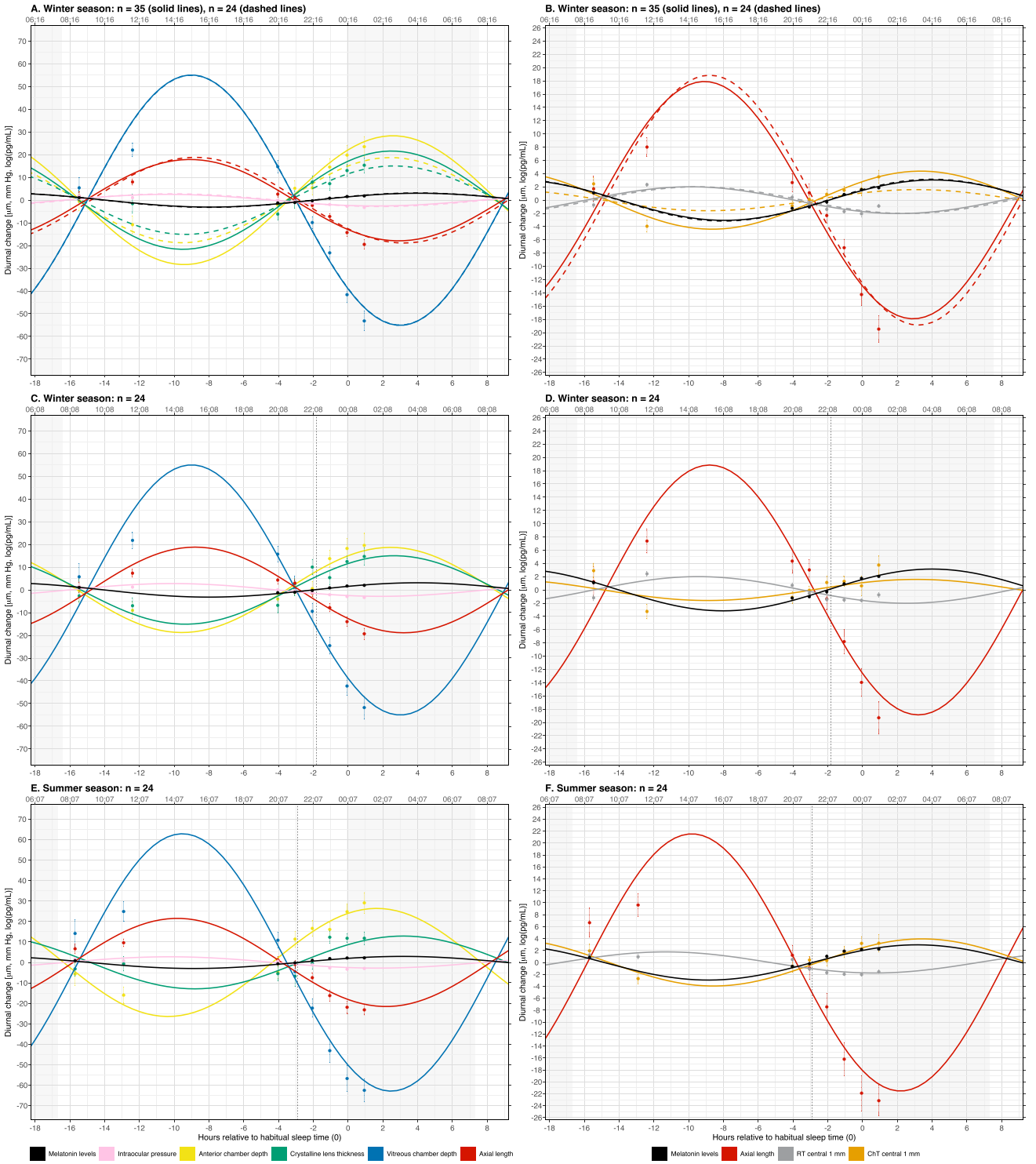

Figure 3. Group averages (±SE *error bars*) for measured ocular parameters or MEL, normalized to each individual's MESOR to emphasize relative differences. *Solid lines* show the sinusoidal model fits from the population estimate based on the fixed-effect estimates from the NLME model. The *gray areas* represent the averaged estimated sleep period. The *x*-axis is relative to habitual sleep time, where 0 is HST (the upper *x*-axis shows standard clock time for easier comparison with other studies). (**A, B**) Fitted models for all 35 participants in the winter study (*solid lines*) and for the 24 participants who returned for summer testing (*dashed lines*) showing that the latter are representative of the former group. **A** shows MEL, IOP, ACD, LT, VCD, and AL; **B** shows AL again (for reference), MEL, RT, and ChT with a rescaled *y*-axis for clarity. (**C, D**) The dashed sinusoids from **A** and **B** reproduced as *solid lines* for easier comparison to **E** and **F**. (**E, F**) Sinusoidal fits for the 24 participants in the summer study, to be compared directly with the same participants' winter data in **C** and **D**. In **C**, **D**, **E**, and **F,** the *dashed vertical line* indicates the timings of DLMO relative to HST. The larger amplitude in VCD compared with AL is mainly accounted for by the antiphase changes in ACD, LT, and ChT.

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Table 5. Relative Acrophase From Estimates Given in Table 4 Converted to Standard Clock Time in Winter and Summer

	Acrophase				
	Winter		Summer		
Parameter	Standard Clock Time	95% CI	Standard Clock Time	95% CI	Seasonal Change
ACD	02:34	01:57–03:07	01:46	01:10–02:19	−00:48
LT	02:46	01:40–03:38	03:02	02:01–04:10	+00:16
ChT central 1 mm	03:14	02:33–03:47	03:32	03:05–03:56	+00:18
MEL	04:07	03:45–04:26	03:16	02:47–03:40	−00:51
IOP	14:02	13:31–14:34	13:51	13:19–14:25	−00:11
RT central 1 mm	14:40	13:50–15:32	12:49	11:37–14:09	−01:51
VCD	15:08	14:42–15:35	14:35	14:13–14:58	−00:33
AL	15:20	14:53–15:47	14:19	13:53–14:45	−01:01
DLMO	22:20	21:55–22:44	21:17	20:53–21:42	−00:47

The – and + signs indicate phase advance and delay, respectively. DLMO (not an acrophase) is included for comparison.

advance for VCD and IOP was smaller. A relative minor phase delay was observed for LT and ChT. As a result, in summer, ACD maintained its relationship with MEL, whereas IOP, LT, VCD, and ChT became more in phase with MEL and AL became more antiphase with MEL. There was a significant change in the phase relationship between AL and ChT, which moved from exact antiphase in the winter to being nearly antiphase in summer: 12.1-hour versus 10.7-hour difference in acrophase (t_{23} = 13, P < 0.001) (Table 4).

Seasonal Changes in Diurnal Characteristics and Phase Relationships

There was a significant change in MESOR from winter to summer for several ocular parameters: deeper ACD and VCD, longer AL, thickening of the retina, and thinning of the choroid (Table 4). There was also a significant increase in amplitude for ChT from winter to summer. To assess the association between change in AL (ΔAL) and ChT (ΔChT) from winter to summer, we divided the participants into three groups based on previous reports of expected ΔAL when there was coordinated ocular growth (axial elongation without a change in refractive error) over a 2-year period at the same latitude[13] and calculated the expected ΔAL for the winter to summer period (7 months). Nine participants had an increase in AL that was deemed to be coordinated growth (0.017 mm ≤ ΔAL < 0.052 mm), 10 participants had an increase in AL that was more than expected from coordinated growth (accelerated ΔAL = 0.052–0.169 mm), and five participants had a minor increase or a decrease in AL that was less than expected from coordinated growth (decelerated ΔAL = −0.113–0.017 mm). Fisher's exact test revealed that the SER groups and the three ΔAL groups were independent from each other (P = 0.7). Figure 4A shows that there was a significant difference among these three ΔAL groups ($F_{2,21}$ = 17.8; P < 0.001; all P < 0.015, Tukey's honestly significant difference [HSD] test). Figures 4B to 4D show that ΔChT (MESOR) across seasons differed significantly among the groups; ChT increased in the group with decelerated ΔAL, whereas it decreased in the two other groups ($F_{2,21}$ = 9.83; P = 0.001; all P < 0.004, Tukey's HSD test). Figure 4C show that ΔAL was not fully accounted for by ΔChT ($F_{1,22}$ = 25.7; P < 0.001; R^2 = 0.54).

Figure 4D shows the strong association between ΔAL and AL phase shift. The group with decelerated ΔAL had a significantly larger phase advance for both VCD and AL across seasons ($F_{2,21}$ = 5.28; P = 0.014; P = 0.011, Tukey's HSD test) compared with the group with accelerated ΔAL ($F_{2,21}$ = 8.68; P = 0.0018; P = 0.001, Tukey's HSD test). There were no differences between the other group combinations. In summer, there was a significant difference between the decelerated ΔAL group and the accelerated ΔAL group for RT ($F_{2,21}$ = 3.76; P = 0.04; P = 0.032, Tukey's HSD test). There were no other differences of MESOR, acrophase, or amplitude for other ocular parameters, evening, morning, or mean MEL nor DLMO between ΔAL groups nor SER groups. There were no associations between chronotype, neither MEL acrophase nor DLMO, and ΔAL or SER (Supplementary Table S3).

Annual Versus Seasonal Changes in AL

For the 10 participants who returned the following winter, we assumed there were no differences in HST between the first winter (W1) and the second winter (W2). The W2 measurements did not occur at the same time (in standard time, local clock time, or habitual sleep time) as the W1 times, so the former were re-expressed relative to the individual's habitual sleep time and used to derive AL values for the W1 and summer (S) seasons, using their respective sinusoid models. Using time-matched AL measurements in this way minimized any influence of diurnal changes arising from differences in measurement times between the three seasons.

As for the original 24 participants, there was a significant increase in mean AL from winter to summer for these 10 participants (pairwise comparison $F_{1,9}$ = 20.90; P = 0.001). There was no change in mean AL from S to W2 (pairwise comparison $F_{1,9}$ = 0.028; P = 0.87); however, from W1 to W2 there was significant annual change in mean AL (pairwise comparison, $F_{1,9}$ = 6.26; P = 0.034). Nine of the participants had a positive annual change in AL. To assess the degree of annual change in AL we calculated expected ΔAL over 12 months and grouped participants in the same manner as described above. Table 6 shows that the annual change (W1 to W2) in AL differed among participants. Eight participants (Table 6, participants 2–9) had seasonal variation in ΔAL, with an increase in AL from W1 to S followed by a smaller

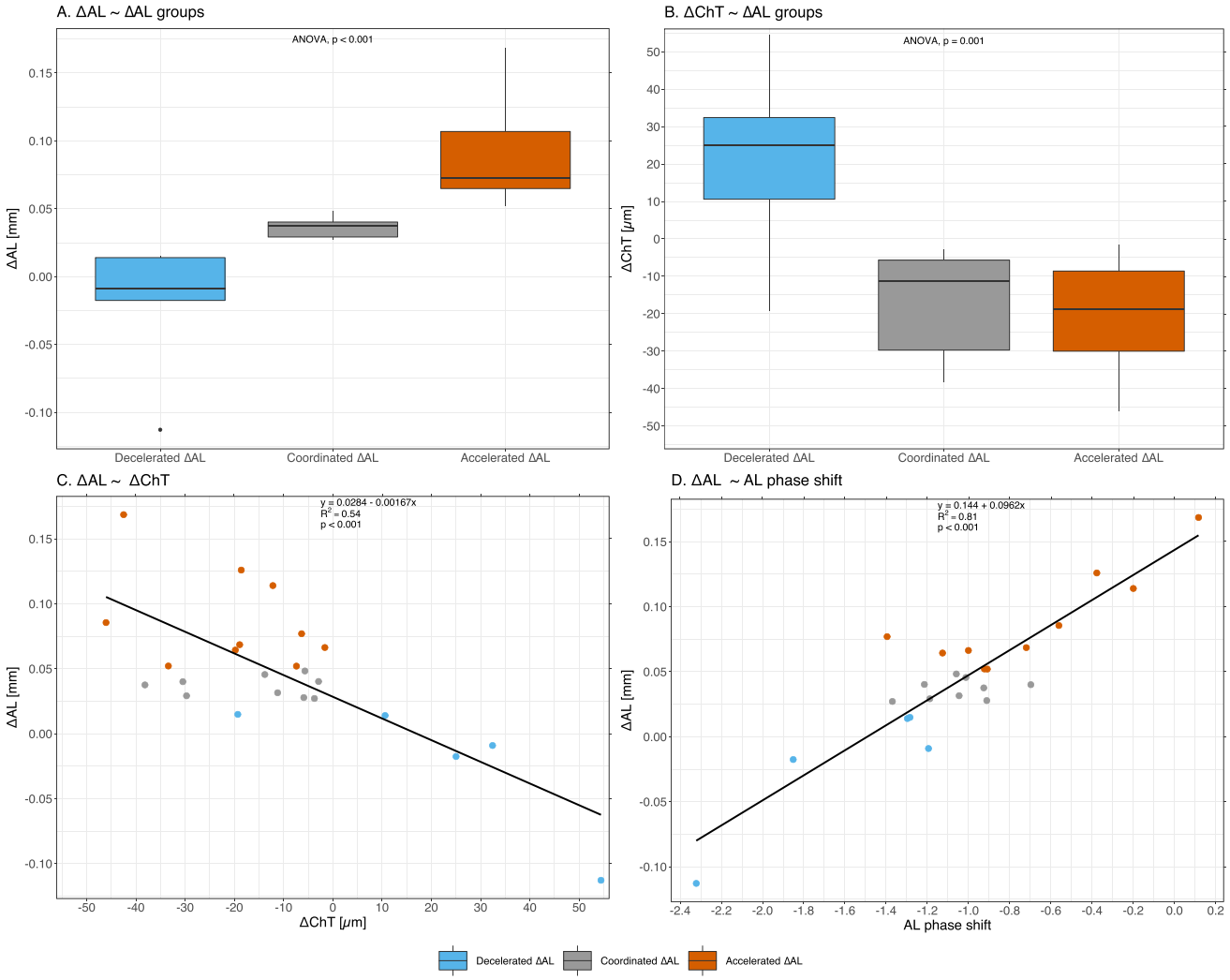

Figure 4. Associations between seasonal change in AL (ΔAL and AL phase shift) and seasonal change in choroidal thickness (ΔChT). Participants have been grouped according to the rate of ΔAL—decelerated ΔAL (*blue*), coordinated ΔAL (*gray*), and accelerated ΔAL (*red*)—using definitions of ΔAL based on expected ΔAL after 7 months[15] (see text for details). (**A**) Boxplot of AL seasonal change for the ΔAL groups. (**B**) Boxplot of ΔChT for the ΔAL groups. (**C**) Scatterplot and linear regression (*solid line*) of ΔAL as a function of ΔChT. (**D**) Scatterplot and linear regression (*solid line*) of ΔAL as a function of AL phase shift. Significance at $P < 0.05$ for one-way ANOVA.

Table 6. Baseline and Annual Change in SER and AL

	AL, mm	ΔAL, mm			SER, D	ΔSER, D
Participant	**W1**	**W1–S**	**S–W2**	**W1–W2**	**W1**	**W1–W2**
1	22.427	−0.011	−0.051	−0.062	−0.48	−0.17
2	22.968	0.068	−0.045	0.023	−0.23	−0.18
3	25.003	0.050	−0.023	0.027	0.22	−0.14
4	23.596	0.021	0.010	0.031	−0.91	−0.22
5	23.746	0.044	−0.009	0.035	1.06	−0.19
6	24.892	0.067	−0.015	0.052	0.20	−0.05
7	23.325	0.049	0.010	0.059	0.67	−0.54
8	21.938	0.046	0.022	0.068	0.25	−0.25
9	24.898	0.125	0.020	0.145	−4.08	−0.04
10	24.879	0.110	0.105	0.215	−0.92	−0.58

ΔAL values are from winter (W1) to summer (S), summer to the second winter (W2), and W1 to W2 (annual change). ΔSER values are from W1 to W2. Table is ordered by increasing ΔAL from W1 to W2.

increase or, for some, a decrease, in AL from S to W2. Two participants had a change that indicated no growth ($0.000 < \Delta AL \leq 0.030$ mm); five, coordinated growth ($0.030 < \Delta AL \leq 0.089$ mm); and one, accelerated growth ($\Delta AL > 0.089$ mm). Focusing on those who had a change in SER $\leq$ –0.50 (which is more than the actual range of repeatability for the worst case, 0.36 D, for the HRK-8000A), we see that participant 7 (who had low hyperopia at baseline and had seasonal variation in axial elongation) exhibited an annual elongation that led to a more myopic SER ($\Delta SER = -0.54$). Participant 10, a myope at baseline, had equal axial elongation across seasons and the largest change in SER of all participants ($\Delta SER = -0.58$). Participant 1 had no change from W1 to S followed by a decrease in AL from S to W2, indicating AL shortening ($\Delta AL = -0.062$ mm) with no meaningful change in SER. As was shown for the original 24 participants, there was no significant difference in mean ΔAL between SER groups from W1 to S for these 10 participants either, from neither S to W2 nor W1 to W2.

Discussion

In this study of young adults living at latitude 60°N, seasonal variation in the diurnal rhythms of melatonin secretion and ocular parameters were observed. Specifically, AL exhibited an earlier acrophase in the summer compared with winter. This followed suit with MEL, with an earlier DLMO and earlier acrophase in the summer, as reported by others.[58–60] Choroidal thickness exhibited a slight delay in acrophase, resulting in a significant seasonal shift in the relationship between AL and ChT.

The reported 1-hour phase advance in summer for MEL[60] and DLMO,[58] also observed in this study, coincided with an earlier acrophase for VCD and AL (Table 5). This alludes to the suggestion that these are synchronized to a master clock (that changes with season) and that our methods are sensitive enough to detect this. A previous study conducted in northeast Australia failed to observe seasonal variation in diurnal rhythms of ocular structures, in either AL or any other ocular structure.[31] This could be due to the considerably smaller seasonal difference between the two study locations, with only 2 hours (northeast Australia) more daylight in the summer than winter[28] (c.f. 12 hours in southeast Norway). In other chronobiology studies, a seasonal shift is attributed to the increased number of daylight hours in the summer combined with changes in social behavior (e.g., spending more time outdoors when it is warmer) and daylight saving time.[27,58,60] Seasonal variation of ocular diurnal rhythms may therefore only be observable at latitudes with sufficient differences in number of daylight hours between seasons.[61]

The observed diurnal variation of measured ocular structures supports previous findings.[3–6] Here, we show that changes in AL are significantly associated with changes in the phase relationships between AL and ChT across seasons (Fig. 4D). In the 7 months between the measurements in winter and summer, both increases and decreases in AL were observed, with 22 of 24 participants having an AL change that was larger than the reported repeatability limit (±0.014 mm) for the IOLMaster 700.[37] Interestingly, eyes that were measured to be shorter had a larger phase-advance of AL and a thickening of the choroid, whilst the opposite was observed in eyes that were measured to be longer (Fig. 4D). The association between a near antiphase (rather than exact antiphase) relationship of AL and ChT and AL shortening and ChT thickening is a novel finding in humans. It is analogous to experimental animal models where a near antiphase (rather than exact antiphase) relationship between AL and ChT has been associated with normal ocular growth.[9,10]

Experimental studies with both animals[62,63] and humans[21] indicate that increased ambient light exposure can result in choroidal thickening. Exposure to daylight has been implicated to play a protective role in ocular growth in children,[19,64] with less growth during summer compared with winter.[29,65 66] This growth pattern was also evident in six of the participants who returned the following winter and who were deemed to have maintained their refractive error in the 12 months between the first and the third set of measurements. Five had increases in AL that were compatible with coordinated growth ($0.023 \text{ mm} \leq \Delta AL \leq 0.052 \text{ mm}$) (Table 6, participants 2–6), and one presented with annual AL shortening ($\Delta AL = -0.062$). The decrease in AL in either season observed in some participants and throughout the year for this one participant could be indicative of axial elongation being reversible—as previously reported in animals[67] and humans (children,[68–72] as well as adolescents and young adults[13,31,73,74]). Nonetheless, as reported from animal studies,[67] the amount of choroidal thickening does not equal the amount of eye shortening. Here, only 54% of the variation in AL was explained by the change in ChT (Fig. 4C); thus, further research is needed to understand what AL shortening entails.

Daylight exposure has also been associated with the size of decrease in AL measured at midday and then again in the evening in humans, with less daylight exposure being associated with a longer AL at midday and an associated larger decrease in AL and more AL elongation.[31] We did not find such differences; that is, AL amplitude was not associated with ΔAL regardless of season or grouping. This is reminiscent of findings in chicks.[22,75] Furthermore, there was no association between AL MESOR and AL amplitude for either season. It would not be unreasonable to assume that our participants' exposure to daylight was considerably lower during the autumn–winter period with few available daylight hours compared with the spring–summer period at this latitude. Unlike other studies,[31,76] we did not measure the participants' daylight exposure. In the Australian study (mentioned above), they did not control for their participants' habitual sleep times, ignoring their individual chronotypes, which could mean that they incorrectly calculated average epochal measurements from different points in each individual's rhythm. Moreover, their only evening measures were at 18:00 and 21:00 and, consequently, may have missed their participants' true AL minima. There is also the possibility that our participants are susceptible to other myopigenic factors.[77]

Neither habitual sleep time nor single measures of MEL (such as DLMO) differed among the SER groups or ΔAL groups. Although the phase of MEL did not differ among the SER groups (as reported by others),[78] we also failed to find a significant association between MEL concentration and SER. Similarly, neither we nor others[5] have found that circadian rhythms of ocular parameters differed among refractive error groups. Furthermore (and in line with reporting standards in chronobiology studies),[36,79] our results indicate that the relationship between MEL and SER or AL alone is not sufficient to explain the influence of circadian synchronization on ocular growth. Instead, it must be combined with knowledge about the changes in phase relationships among ocular parameters.

The diurnal rhythm and acrophase of ACD was nearly 3 hours past HST (Table 5), in agreement with most studies.[3–5] Contrary to existing studies,[4–6] however, we found that the diurnal rhythms of ACD and LT were in phase, increasing in depth and thickness throughout the evening with maximum diurnal changes of +38 and +30 µm, respectively (Table 4, Fig. 3). This behavior differs from accommodation, where an increase in lens thickness (i.e., increase in the curvature of the anterior and posterior surfaces of the crystalline lens) leads to a shallower ACD, with the anterior lens surface accounting for most of the LT change.[80,81] An accommodative stimulus of 3 D has been reported to alter LT by +138 µm and, subsequently, ACD by –106 µm.[82] The most parsimonious explanation is that the diurnal changes of the crystalline lens primarily occur at the posterior surface,[4] which is compatible with the diurnal rhythm of VCD being in antiphase to LT. These changes differ from the biomechanical changes associated with accommodation and are substantiated by the in-phase diurnal rhythms of LT and MEL (in both winter and summer) (Fig. 3).

It is known that there are large between-individual variations in foveal shape[83] and metrics,[84] but neither has been found to correlate with refractive error or AL.[85] The diurnal rhythm of central RT was not associated with SER or AL and appears to be like that reported previously.[5] That the advance in acrophase in RT is nearly an hour more than MEL and the other measures is a novel finding, and more work is needed to understand this.

Strengths and Limitations

There are three key strengths to the methodology presented here. First, all measurement epochs were relative to the individual's habitual sleep time (measured objectively with actigraphy). The results imply that rhythmic changes in melatonin and ocular parameters are regulated by an internal bodily mechanism, and making measurements relative to that internal mechanism has demonstrably delivered robust measurements of those rhythms. In contrast, studies where measurements were made relative to wall-clock time[3,4,31] may not yield meaningful population measures unless the population was carefully selected to have the same wake–sleep cycle as one another.[5] Second, the per-individual timing of the epochs encompassed a full waking day—from 1 hour after habitual wake time to 1 hour after habitual sleep time—and thus minimally impacted the participant's normal rhythms. Such a wide range of measurements improves model fitting when data samples are collected at time points spanning both the peaks and troughs of the fitted sinusoids. Other studies have reported ACD,[5] LT, and RT[4] not exhibiting diurnal variation or LT barely reaching significance.[6] These variations may be explained by the different measurement intervals varying from six times every 3 to 7 hours[3] or five times every 2.5 to 3 hours.[4] Even a regimented and exhaustive measurement routine (every 4 hours for 24 hours[5,6]) would involve disturbing participants' sleep patterns and, consequently, their ocular biometry measurements. On a related matter, fitting actual sinusoidal functions to the data and utilizing the MESOR (rather than the arithmetic mean) of the data points also improved the robustness of the results, as the MESOR is not biased by the timing of the measurements. Third, our data collection methodology controlled or nullified as many confounding variables as possible during data collection, including strict exclusion criteria for visual function when selecting participants; carefully controlled lighting conditions during and between measurement epochs; ensuring that the participants remained seated throughout all epochs (to avoid possible effects of postural changes on measurements)[34–36]; and preventing participants from engaging in any near-distance tasks or exposure to bright light sources between measurement epochs.

A limitation of our study is the small number of participants and that we measured the participants on weekdays only and not during a weekend; sleep behavior has been found to vary throughout the weekend, when DLMO can be delayed.[86] In terms of daily variations of ocular parameters, however, a previous study found no differences in AL and ChT between weekdays and weekends.[31] Another limitation was that we did not measure participants' light exposure. Ambient light levels were controlled for in the laboratory 15 to 20 minutes before the first measurement session and throughout the remaining evening; however, we do not know the amount of light the participants were exposed to before arriving at the laboratory. At the latitude studied here, there are considerable differences in daylight availability and intensity between winter and summer.[27] Whether it is the duration of daylight exposure or its intensity or temperature, or a combination thereof, that is important for ocular growth requires further study.[27] Regrettably, only 10 of the original 24 participants were available for a measurement at W2 which limits the robustness of the annual analysis. Including them, however, emphasizes the importance of understanding annual variations in eye growth among individuals.

Conclusions

We report significant seasonal variation in the diurnal rhythms of MEL, VCD, and AL. These rhythms appear to be synchronized in both winter and summer, suggesting that the overall rhythm of the eye expressed through ocular AL is synchronized with the master circadian clock, as measured by saliva melatonin secretion. Additionally, the direction and degree of AL change were significantly associated with the degree of AL phase advance in the summer, alluding to between-individual variation of the eye in adaptation to seasonal changes in diurnal rhythms (at least at latitudes where there are notable seasonal changes in daylight exposure). The finding that the crystalline lens and anterior chamber underwent significant in-phase diurnal variations, both increasing in size in the evening, renews interest in the role that time-of-day exposure of near-distance activities may play in eye growth.[87] It indicates that other behavioral modifications, in combination with daylight exposure, may contribute to delay myopia onset and development.

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Paper II

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The effect of topical 1 % atropine on ocular dimensions and diurnal rhythms of the human eye

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A B S T R A C T

The effect of topical 1 % atropine on the diurnal rhythms of the human eye was investigated. Participants wore an activity monitor on Days 1–7. A set of measures (epochs) encompassing intraocular pressure (IOP), ocular biometry, and retinal imaging were obtained on Day 7 (baseline), followed by eight epochs on Day 8, and one on Day 9 from both eyes of healthy participants (n = 22, 19–25 years). The sleep time of participants (collected via actigraphy) was used as a reference in scheduling epochs. Topical 1 % atropine was instilled in the dominant eye on Day 8, 2 h after habitual wake time, using the fellow eye as control (paired-eye design). Sinusoids with a 24-h period were fitted to the data, and a non-linear mixed-effects model was used to estimate rhythmic statistics. There were no interocular differences in any of the measured parameters at baseline. Comparing pre- versus post-atropine in treated eyes revealed lower IOP, deeper anterior chamber (ACD), decreased crystalline lens thickness and shorter axial length (AL). The same trends were observed when comparing atropine-treated versus fellow control eyes, except for IOP and AL (no differences). Both atropine-treated and fellow control eyes showed significant diurnal variations in all ocular parameters, with atropine-treated eyes revealing larger AL and retinal thickness amplitudes, smaller vitreous chamber depth (VCD) amplitudes, and a significant phase advancement for ACD and VCD. There were no interocular differences in choroidal thickness rhythms. In conclusion, while ocular diurnal rhythms persisted after instillation of 1 % atropine, many rhythmic parameters were altered.

1. Introduction

Atropine, a non-selective anti-muscarinic agent, is now commonly used as a myopia control therapy in children and adolescents, in a low concentration, topical formulation. However, the mechanism of action by which atropine slows myopia progression (Chia et al., 2012; Upadhyay and Beuerman, 2020) remains poorly understood. While muscarinic receptors are present in both the human ocular iris sphincter and ciliary muscles (i.e., mediating pupil constriction and ocular accommodation), atropine's anti-myopia action appears to be unrelated to its cycloplegic effects, as supported by findings from myopia-related studies involving chicks whose accommodation is unaffected by atropine (McBrien et al., 1993; Wildsoet, 2003; Schaeffel et al., 1990). Other potential sites of action for atropine, based on the premise that its action is mediated by interactions with muscarinic receptors, include the retina, retinal pigment epithelium (RPE), choroid and sclera, as all contain muscarinic receptors.

There is mounting evidence tying short-term choroidal thickness (ChT) changes to axial length changes (Ostrin et al., 2023). The choroid also represents a plausible site of action for topical atropine. For example, subfoveal choroidal thickening and axial length (AL) shortening have been reported in humans 60 min after instillation of either 0.01 % atropine (Sander et al., 2019), or of its close relative, homatropine, in a 2 % concentration (Sander et al., 2014; Sander et al., 2018). Atropine has also been reported to inhibit the choroidal thinning induced by imposed hyperopic defocus in similar short-term studies (Chiang & Phillips, 2018; Chiang, Turnbull, & Phillips, 2020). In a longer term (2-year) myopia control clinical trial of topical atropine, concentration-dependent thickening of the choroid was reported (Yam et al., 2022). These observations are consistent with earlier studies in animal models linking the choroid with ocular growth modulation and emmetropization (Marzani and Wallman, 1997), first in the chick (Nickla et al., 2001) and more recently in other animal models (Nickla and Wallman, 2010).

Topical atropine, when used as a myopia control therapy in humans, is typically given just prior to bedtime, to allow partial recovery from its secondary inhibitory effects on accommodation and the pupil's light response, and so minimize its impact on daytime visual function (Chua et al., 2006; Azuara-Blanco et al., 2020; Larkin et al., 2019). This practice raises the question of whether this therapy may affect one or

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more of the ocular diurnal rhythms, some of which are known to be differentially altered during myopia development (Ostrin et al., 2023). For example, significant diurnal variations have been reported in intraocular pressure (IOP) and several ocular dimensions, including AL and ChT, in both humans and experimental animals (Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011; Ostrin, Jnawali, Carkeet, & Patel, 2019; Read, Collins, & Iskander, 2008; Stone, Quinn, & Francis, 2004; Troilo, Smith, & Nickla, 2019). In human eyes, the acrophase (peak) for AL, IOP and retinal thickness (RT) rhythms is around mid-day (from 11:30 to 16:00), while for anterior chamber depth (ACD), crystalline lens thickness (LT), and ChT, it is around midnight (from 21:30–04:00) (Burfield, Carkeet, & Ostrin, 2019; Burfield, Patel, & Ostrin, 2018; Nilsen et al., 2022; Ostrin, Jnawali, Carkeet, & Patel, 2019). Furthermore, an early study involving myopia induction and recovery from the same in chicks, revealed changes in the phase relationship of AL and ChT rhythms from being nearly anti-phase during normal growth (≈9h), to become fully anti-phase during accelerated ("myopic") growth (≈12 h), and fully in-phase during the recovery period (≈0h) (Nickla et al., 1998). The possibility that the phase relationship between AL and ChT rhythms is a biomarker of the rate of eye elongation, as suggested by such animal model studies (Nickla, 2013), raises the question of whether the anti-myopia action of topical atropine may be tied to changes in one or more ocular diurnal rhythms. For example, might atropine mimic the changes observed during decelerated AL elongation in chicks (Nickla et al., 1998) and marmosets (Nickla et al., 2002) — a phase-advance in the ChT rhythm (c.f. Fig. 12 in Ref. (Nickla et al., 1998), with the AL and ChT rhythms shifting from anti-phase to becoming more in-phase after the instillation of atropine.

To date, there have been no investigations in humans, into the effect of topical atropine on ocular diurnal rhythms, and potential effects on the diurnal phase relationship between AL and ChT rhythms. The primary aim of this study was to correct this deficiency by investigating the short-term effects of topical 1 % atropine on ocular diurnal rhythms, building on our previous work on untreated eyes (Nilsen et al., 2022). The secondary aim was to investigate whether our previous observation of crystalline lens thickening in the evening (Nilsen et al., 2022) remains in the absence of any accommodative demand, by way of understanding its origin. Specifically, in our earlier study, the diurnal rhythms of ACD and LT were observed to be in phase, increasing in depth and thickness throughout the evening (Nilsen et al., 2022). This behaviour is different from that seen during accommodation, where increases in LT leads to a shallowing of ACD (anti-phase behaviour) (Kaufman et al., 2011; Xiang et al., 2021). To this end, the short-term effects of topical 1 % atropine on ocular diurnal rhythms over a 26-hour period in healthy young adults were investigated, utilizing the same methodologies as in our previous study, which also served as a source of additional control (reference) data (Nilsen et al., 2022).

2. Methods

Twenty-two young healthy adults (19 females, 3 males, 19–25 years of age) participated in this study. While there were no exclusion criteria related to refractive errors, all participants were required to have best corrected visual acuity of $\leq$ 0.00 logMAR (TestChart 2000, Thomson Software Solutions, London, UK), stereo acuity of $\leq$ 120 s of arc (TNO Stereotest, Laméris Ootech, WC Ede, Netherlands), and make no errors in either of two colour vision tests (Ishihara, 24-plate edition, Kanehara Trading Inc., Tokyo, Japan; Hardy–Rand–Rittler Pseudoisochromatic Plate Test, 4th edition, Richmond Products, Albuquerque, NM, USA). Other exclusion criteria included current or previous myopia control therapy, systemic and/or ocular disease, sleep disorders and/or mental disorders known to affect sleep, and the use of melatonin supplements. The study was approved by the Regional Committee for Medical and Health Research Ethics (Southern Norway Regional Health Authority) and was carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from the participants prior to their participation.

2.1. Study design and data collection protocols

This study extends our previous study investigating seasonal variations in diurnal rhythms of the eye (Nilsen et al., 2022), using a similar methodology, with data collection at similar time-of-year, at the same geographical location, and involving participants of a similar age-group, to allow inter-study comparisons as appropriate. The current study spanned 9 days, with ocular data collection limited to the last 3 days of this period, when the dominant eyes of all participants underwent topical 1 % atropine treatment (minims single dose; Bausch + Lomb, Bridgewater, NJ 08807). Fig. 1 shows the sequence and timing of data collection. On Day 1, participants were given an Actigraph GT3X wrist-mounted activity monitor (Actigraph Corp, Pensacola, USA) to wear for the following seven days and nights to objectively measure their sleep-onset times. For each participant, weekday data were averaged to determine their habitual sleep time (HST). Self-reported habitual wake time (HWT) data (based on estimates by participants of their morning wake-up time) were gathered for the preceding month, and the average used in combination with HST to schedule the various sets of measurements (epochs).

At each epoch, ocular biometry (IOLMaster 700, Carl Zeiss Meditec AG, Jena, Germany), tonometry (iCare, Tiolat Oy, Helsinki, Finland) and posterior segment SD-OCT imaging (Spectralis OCT2-EDI, Heidelberg Engineering, Heidelberg, Germany) were undertaken and diurnal ocular rhythms were derived from collected data. During each epoch, participants remained seated on a wheeled chair and were pushed between instruments, to avoid the potential influence of postural changes on measurements (Lockley, 2020; De Bernardo et al., 2019). From ocular biometric measurements, pupil size, central corneal thickness (CCT), corneal curvature (CR), anterior chamber depth (ACD), crystalline lens thickness (LT), retinal thickness (RT) and axial length (AL) data were extracted, with vitreous chamber depth (VCD) derived as AL − (ACD + LT + RT). IOP data represents the average of three sets of readings, where each set represents six single measurements. Refractions were measured for both far and near fixation distances (6 m and Maltese cross at 30 cm; Nvision-K 5001 Open-field autorefractor, Shin-Nippon, Tokyo, Japan), and used to derive refractive errors and accomm odation amplitudes.

The first set of ocular measurements (first epoch, baseline data) was made on Day 7, approximately 4 h after the self-reported habitual wake time (HWT+4). On Day 8, atropine was instilled in the dominant eye, 2 h after HWT (HWT+2), with additional epochs scheduled 1 and 4 h after HWT (HWT+1 and HWT+4), and at 9, 4, 3, 2, 1 and 0 h before the participant's habitual sleep time (HST: HST-9, HST-4, HST-3, HST-2, HST-1, HST +0). A final measurement session was carried out on Day 9 (two days after the collection of baseline data) at HWT+4. Thus, for each participant, the timing of the epochs was aligned with their respective chronotype. This measurement schedule was chosen to capture the sinusoidal characteristics of observed parameters, including the descriptive melatonin onset (DLMO) from which rhythm statistics were estimated (Lockley, 2020). Atropine dosing was adjusted based on iris colour (Mackey et al., 2011), with one drop of atropine instilled in those with lightly pigmented (blue to green) irides and two drops instilled in those with more heavily pigmented (green-with-brown-iris-ring-to-brown) irides (Salazar et al., 1976). To relax accommodation in untreated (fellow control) eyes prior to the start of each measurement session, participants watched a movie (binocularly) at 5 m for 15 min through their habitual distance correction as necessary (Chakraborty et al., 2013). On all occasions, right eyes were measured before left eyes, irrespective of ocular dominance.

Radial OCT scans (6 orientations with 100 B-scans averaged at each orientation, all with enhanced-depth information enabled) were collected at each epoch, using the baseline scan as the reference image for the instrument's retinal tracking system. The method for correction

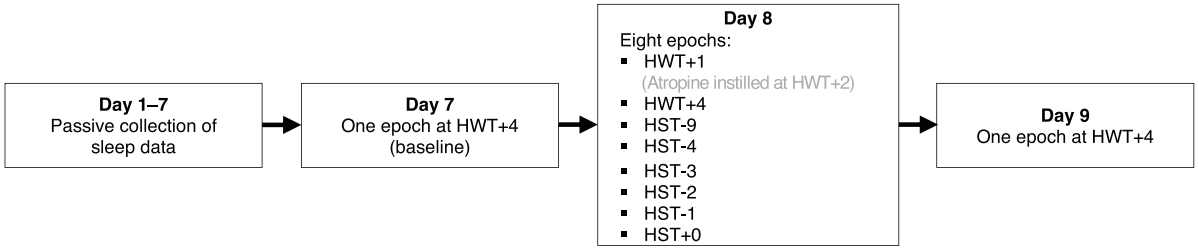

Fig. 1. Data collection schedule showing timing of measurements (epochs) from Day 1 to Day 9. Habitual wake time (HWT) represents individual self-reported average time for the past month; habitual sleep time (HST) was determined for each individual from actigraphy data collected on Day 1–7.

for lateral magnification and segmentation was the same as that described previously (Nilsen et al., 2022). Only horizontal and vertical scans were analysed, with mean thicknesses derived for the central 1 mm of retina and underlying choroid (Fig. 2).

Circulating melatonin (MEL) levels were evaluated from collected saliva samples (Salivette Saliva examination kit, Sarstedt, Nümbrecht, Germany). The first sample was self-collected at wake time before participants got out of bed (HWT+0), and thereafter samples were collected during the last 5 min of the accommodation washout period, at HWT+1, HST-4, HST-3, HST-2, HST-1 and HST+0. Upon collection, saliva samples were immediately centrifuged at −4°C, 4400 RPM for 5 min (Centrifuge 5702R, Eppendorf SE, Germany), and stored in a −25 °C freezer. The samples were analysed by VITAS Analytical services (Oslo, Norway), using appropriate ELISA kits (Bühlmann Laboratories, Schönenbuch, Switzerland).

During all measurements, independent of time of day, ambient light levels were kept below 20 lx to minimize the risk of suppressing evening melatonin secretion, including levels emanating from the TV that participants watched prior to measurements (Crowley, Suh, & Molina, 2017). Ambient light levels remained below 20 lx between evening measurements (HST-4 to HST + 0), when the participants were confined to the laboratory. In accordance with published recommendations for similar studies (Benloucif, Burgess, & Klerman, 2008; Crowley, Suh, & Molina, 2017), participants were also instructed to avoid consuming NSAIDs, nicotine, bananas, and chocolate for 36 h prior to and during data collection to minimize their potentially confounding effects on saliva melatonin levels. For similar reasons, participants were also asked to avoid alcohol and drinks containing caffeine or artificial food colorants for 24 h prior to and during the data collection. While measurement schedules did not require participants to sleep in the laboratory at any time during the study, all were encouraged to maintain their habitual wake and sleep schedules over the study period. Also, none reported travelling across more than 2 time zones within the month preceding the study, ruling out such influences on diurnal rhythms.

2.2. Data analysis

Statistical analyses made use of R statistical software, version 3.6.3, with the NLME package (R Core Team, 2016). Parametric tests were used where the data were confirmed to be normally distributed; otherwise non-parametric alternative tests were used. Statistical significance was set at α=0.05. Participants were characterized by the refractive error of their atropine-treated eye based on the autorefractor data from the Day 8 HWT+4 measurement of their atropine-treated eye. Spherical equivalent refractive errors (SER) were calculated as sphere + ½ cylinder, with myopia being defined as SER $\leq$ −0.50 diopters (D), emmetropia as −0.50 D < SER < +0.50 D, and hyperopia as SER $\geq$ +0.50 D. Accommodation responses were calculated as differences between autorefractor data (expressed as SER), captured under distant and near viewing conditions. Inter-rater reliability for segmentation of the choroid has been reported to be high (intraclass correlation = 0.95, 95 % confidence interval = 0.929–0.965) (Nilsen et al., 2022). To provide a between-session repeatability for OCT derived choroidal thickness for the central 1 mm, the images obtained from the fellow control eye on Day 7 HWT+4 and Day 8 HWT+4 were compared. The coefficient of variation and the limits of agreement were determined with Bland-Altman analysis (Supplementary Fig. S1) (Bland and Altman, 1986). Data processing and analyses of Actigraph data have been described elsewhere (Nilsen et al., 2022).

A non-linear mixed-effects model was used to estimate rhythm statistics (Midline Estimating Statistic of Rhythm, MESOR), amplitude and phase, and dim-light melatonin onset (DLMO), as described previously (Nilsen et al., 2022). To better capture the floor effect typically observed with daytime melatonin measurements, a sinusoid was fitted to the log of the measured melatonin levels (Kennaway, 2019). One-way repeated measures ANOVAs, with the epoch as the within-subject factor and refractive error group as the between-subjects factor, were used to identify significant diurnal variations in saliva melatonin levels and/or one of the measured ocular parameters (Burfield, Patel, & Ostrin, 2018; Ostrin, Jnawali, Carkeet, & Patel, 2019; Read, Collins, & Iskander, 2008). One-way ANOVAs were also used to examine differences between refractive error groups and the effect of topical atropine on

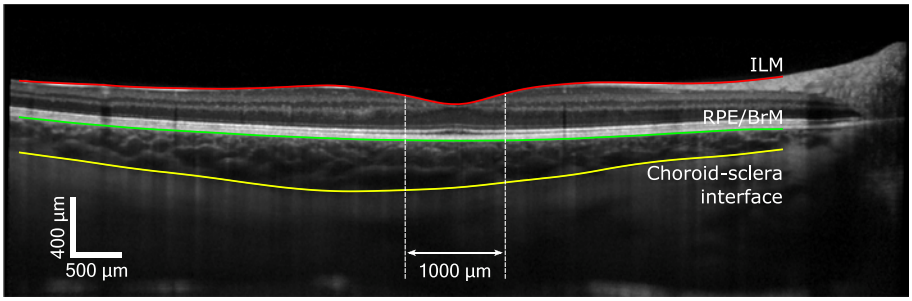

Fig. 2. A horizontal OCT B-scan annotated with segmented retinal and choroidal layers. Retinal and choroidal thicknesses were derived as the distances between the inner limiting membrane (ILM) and retinal pigment epithelium (RPE), and between the RPE and choroid-scleral interface respectively, averaged over the central 1 mm, with reported values representing averages derived from both horizontal and vertical B-scans.

rhythm statistics for a given ocular parameter by calculating the difference between the atropine-treated eyes with the fellow-control eyes. For these comparisons, effect size η^2 (eta-squared) was calculated to be ≥ 0.40, indicating a large effect size (Cohen and Cohen, 1983). Diurnal analyses made use of data collected during the 7 epochs on Day 8 after atropine instillation (HWT+4 until HST+0) in all cases except the case of choroidal thickness, for which the HWT+4 data were uncharacteristically noisy (possibly due to variable patterns of atropine uptake between participants), which prevented convergence of the NLME model.

3. Results

Of the 22 participants, nine were myopic, five were emmetropic, and eight were hyperopic. Table 1 summarizes baseline refractive errors and ocular dimensions of both of their eyes as measured on Day 7, HWT+4, and the corresponding data collected on Day 8, HWT+4 (2 h after instillation of atropine in the dominant eyes). The refractive errors of participants (SER) ranged between −4.13 and +1.82 D (mean ± SD: −0.26 ± 1.60 D), with no anisometropia ≥ 0.75 D. All but one participant had astigmatism ≤ 1.25 D, the one exception being a myope with astigmatism of −2.25 D in both eyes.

3.1. Acute ocular effects of topical atropine, 2 h after atropine instillation

While there were no significant interocular differences in any ocular parameters at baseline on Day 7 ($p > 0.05$), the monocular instillation of atropine introduced significant interocular differences in SER, ACD and LT after just 2 h, reflecting the changes in atropine-treated eyes ($p < 0.001$, Table 1, pairwise statistics provided in Supplementary Table S1–S2). Specifically, treated eyes showed a more positive SER, deeper ACD and reduced LT compared with their fellow control eyes (Day 8 HWT+4; $p < 0.001$). Similar trends are evident in the changes from baseline profiles in treated eyes (Day 7 HWT+4 versus Day 8 HWT+4; all $p < 0.001$); in addition, the instillation of atropine resulted in decreases from baseline in IOP, AL and VCD (all $p \leq 0.005$). While there was no statistically significant atropine-induced change in ChT at this 2 h time point (interocular differences, Day 7 HWT+4 versus Day 8 HWT+4), there were notable individual variations, unrelated to the refractive error groups to which participants belonged (Supplementary Table S3). Based on independent t-tests, there were no differences between the group receiving 1 atropine drop ($n = 14$) versus the group receiving 2 drops ($n = 8$) for any ocular parameters (IOP, CCT, ACD, LT, VCD, AL, RT, ChT, and accommodation amplitude), at day 8 HWT + 4 ($p \geq 0.061$), and likewise, no group-related difference between day 7 HWT+4 and day 8 HWT+4 ($p \geq 0.178$).

3.2. Time-course of changes induced by topical atropine

3.2.1. Effects on ocular biometric parameters

For all ocular biometric parameters (ACD, LT, VCD, AL, RT, and ChT), instillation of atropine resulted in persistent, measurable changes in atropine-treated eyes (Fig. 3). ACD initially deepened, while LT, VCD and AL all decreased. Additionally, ChT showed a small, measurable increase (HWT+1 to HWT+4) at the time-point where a diurnal decrease was expected, based on the change in fellow control eyes (Nilsen et al., 2022). In all cases, the opposite behaviour, i.e., barely-noticeable change, was observed of these parameters in fellow control eyes. On the other hand, increases in RT were observed in both treated and fellow control eyes, albeit larger in the former. For fellow control eyes, the patterns of change across the day for all parameters were generally consistent with those recorded from untreated right and left eyes in our previous study, which involved a different study population (Nilsen et al., 2022), with the one exception being ChT and the HST-2 evening epoch, when a decrease was observed in fellow control eyes, as well as treated eyes.

3.2.2. Enduring (26 h post instillation) effects of atropine

Accommodation and pupillary responses were assessed to confirm the expected ocular effects of atropine. As expected, accommodation responses to a target at 30 cm were significantly reduced by atropine ($t(20) = 9.15$, $p < 0.001$), with this effect persisting over the 26-hour monitoring period, with only a slight increase from 0.09 ± 0.19 D at 2 h to 0.24 ± 0.19 D across subsequent epochs ($R^2 = 0.11$, $p = 0.001$, Supplementary Fig. S2). Accommodative responses in fellow control eyes remained relatively stable and unaffected over the same period ($R^2 < 0.01$, $p = 0.75$), i.e., +2.12 ± 0.84 D and +2.15 ± 0.81 D, respectively. The pupils of treated eyes became dilated with the instillation of atropine, with minimal recovery over the 26-hour monitoring period, as reflected in the lack of any significant differences in pupil size across epochs ($R^2 < 0.01$, $p > 0.20$ for both treated and fellow untreated eyes).

On Day 9 (HWT+4), most of the atropine-induced changes, i.e., as reflected in pre- (Day 7) versus post-atropine treatment, remained; ACD was deeper, LT smaller, VCD shorter and SER more positive (Supplementary Table S4). Also of these parameters, only changes in ACD showed refractive error-related differences at this last time point ($F(2, 17) = 6$, $p = 0.009$).

3.3. Diurnal rhythms and the effects of topical atropine

For each of the measured ocular parameters, data were fitted with sine waves, from which estimates of MESORs, amplitudes and

Table 1
Mean ocular parameters for fellow control and atropine-treated eyes, measured pre- and post-instillation of 1 % atropine (i.e., Day 7 HWT + 4 and 2 h after instillation Day 8 HWT+4). Significance was calculated by pairwise comparisons of Day 7 versus Day 8 for fellow control eyes and atropine eyes (*), respectively, and for atropine versus fellow control eyes on Day 8 (†). Differences between Day 8 HWT+4 and Day 7 HWT+4 data are also shown for both control and atropine-treated eyes.

	Fellow (control) eyes					Atropine treated eyes				
	Day 7 HWT+4		Day 8 HWT+4		Difference	Day 7 HWT+4		Day 8 HWT+4		Difference
Parameter	Mean	SD	Mean	SD	Mean	Mean	SD	Mean	SD	Mean
SER [D]	−0.56	1.57	−0.62	1.54	−0.06	−0.60	1.48	−0.26*	1.56	0.34†
IOP [mm Hg]	14.52	3.70	13.36	4.23	−1.16	15.09	3.61	13.56	4.70	−1.53†
CCT [μm]	548	25	548	25	0	547	25	548	25	1
ACD [mm]	3.588	0.249	3.589	0.240	0.001	3.590	0.262	3.714*	0.244	0.124†
LT [mm]	3.665	0.267	3.673	0.270	0.008	3.661	0.283	3.595*	0.262	−0.066†
VCD [mm]	16.115	0.876	16.106	0.880	−0.009	16.118	0.838	16.050	0.847	−0.068†
AL [mm]	23.593	0.798	23.591	0.801	−0.002	23.592	0.754	23.583	0.758	−0.009†
RT‡ [μm]	254	19	253	19	−1	253	21	254	21	1
ChT‡ [μm]	332	78	330	78	−2	335	81	335	81	0

* Significant pairwise difference between atropine-treated and fellow control eyes at Day 8 HWT+4; $p < 0.05$.
† Significant pairwise difference between values measured pre- and post- atropine instillation in treated eyes; $p < 0.05$.
‡ Averaged over the central 1 mm.

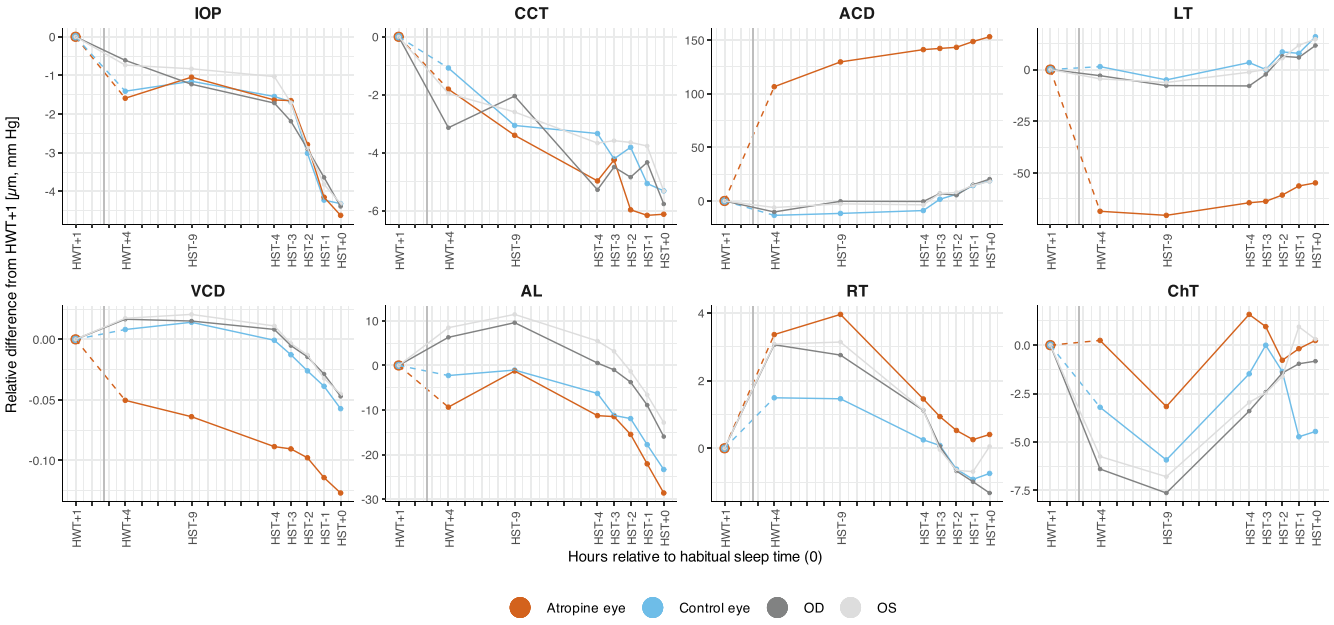

Fig. 3. The time-course of changes in ocular parameters in atropine-treated (orange) and fellow control eyes (blue), pre- and post-instillation of 1 % atropine; also included for comparison are data from a previous study for which both eyes remained untreated (grey, OD, OS; n = 35) (Nilsen et al., 2022) for intraocular pressure (IOP), central corneal thickness (CCT), anterior chamber depth (ACD), crystalline lens thickness (LT), vitreous chamber depth (VCD), axial length (AL), retinal thickness (RT) and choroidal thickness (ChT, central 1 mm for RT and ChT). Change is calculated as the relative difference from HWT+1 (left-most point in each graph), i.e., measurements taken 1–2 h prior to atropine instillation. The average time of atropine instillation (between HWT+2 and HWT+3) is indicated by solid vertical lines and the dashed orange/blue lines link the pre- and first post-instillation measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acrophases were obtained. These derived data are summarized in Table 2 andTable 3.

3.3.1. *Effects of atropine on ocular rhythms*

Both atropine-treated and fellow control eyes exhibited significant diurnal variations in the following parameters: IOP, CCT, ACD, LT, VCD, AL, RT and ChT ($p < 0.05$, Supplementary Table S5). Compared to the rhythms in fellow control eyes, treated eyes had a smaller VCD amplitude, but larger AL and RT amplitudes ($p < 0.049$ for all), as well as phase advances in their ACD and VCD rhythms ($p < 0.001$ for all) (Fig. 4 A–D). Nonetheless, for both eyes, ACD and LT rhythms were in phase (<1h versus ≈5 h phase-difference in the fellow control and atropine-treated eye, respectively), and in the case of both AL and ChT rhythms, they were nearly in-phase (≈5–6 h phase-difference, Table 2). Also, during the evening, the crystalline lens thickened and the ACD increased, despite the absence of any accommodative demand (mean raw time course data in Fig. 3 and sinusoidal model fits in Fig. 4). Overall, MESORs showed similar trends to values recorded 2 h after the instillation of atropine, with treated eyes having significantly increased ACDs on average, but smaller LTs and VCDs compared to their fellow control eyes (Table 2, statistics provided in Supplementary Table S6). These trends are also reflected in the interocular differences in MESORs ($p < 0.038$ for all).

3.3.2. *Melatonin*

Saliva melatonin levels (MEL) also showed significant diurnal variation (MESOR: 1.23 log pg/mL, amplitude 3.17 log pg/mL, acrophase 3.34 h; 95 % CI: 1.01–1.45; 2.82–3.52, 3.02–3.64, respectively).

3.4. *Differences between refractive error groups*

3.4.1. *Differences in rhythm characteristics between refractive error groups*

When, for a given ocular parameter, there was significant individual variation in MESORs, amplitudes and/or acrophases in the NLME model, a random component representing them was retained in the model. This procedure yielded individual estimates, which were used to assess differences between SER groups (Fig. 5 A–D). Such cases included MESORs for all parameters, amplitudes for ACD, LT, VCD, AL and RT, and acrophases for MEL, ACD, LT and VCD. In the case of fellow control eyes, hyperopes exhibited an earlier acrophase for ACD compared to emmetropes and myopes ($F(2, 19) = 6.54$, $p = 0.007$, Tukey's honestly significant difference (HSD) test $p \leq 0.036$). Differences between atropine-treated and fellow control eyes for MESOR and acrophase were calculated by way of assessing the effects of atropine and differences between the refractive error groups. In the case of MESORs and the effect of atropine, hyperopes also showed greater deepening of ACD ($F(2, 19) = 18.5$, $p < 0.001$, Tukey's HSD test $p < 0.001$) and reduction in LT ($F(2, 18) = 6.85$, $p = 0.006$, Tukey's HSD test $p \leq 0.023$) compared to emmetropes and myopes. All three refractive error groups exhibited atropine-induced phase advances in their ACD rhythms, with hyperopes exhibiting a significantly larger phase-advance compared to the other two groups ($F(2, 19) = 11.6$, $p < 0.001$, Tukey's HSD test $p \leq 0.003$). No other derived parameter showed difference across the refractive error groups.

3.4.2. *Accommodation and pupillary responses*

Myopes showed smaller mean accommodation responses compared to emmetropes and hyperopes, as reflected in significant intergroup differences in results for fellow control eyes, as well as in interocular difference between atropine and fellow control eyes (i.e., $F(2, 19) = 7.83$, $p = 0.003$, Tukey's HSD test $p < 0.0443$, $F(2, 19) = 6.22$, $p = 0.008$, Tukey's HSD test $p = 0.010$, respectively). On the other hand, no refractive error-related differences in either the pupil size of fellow control eyes or atropine-induced pupil size changes were observed.

3.5. *Inter-study comparisons*

Table 4 and Fig. 6 show data collected in the current study (E–F) and in our previous study [where both eyes were untreated, for winter (C) and summer (D)] (Nilsen et al., 2022). Comparison of Fig. 6 E and C

Table 2
Population estimates (Est.) of MESORs, amplitudes and acrophases derived from sine wave fits to data from fellow control and atropine-treated eyes, calculated from the NLME model. Acrophase is estimated relative to habitual sleep time. 95% confidence intervals (95% CI) are included in the table.

	Fellow control eye					Atropine eye				
	MESOR		Amplitude		Acrophase (hour)	MESOR		Amplitude		Acrophase (hour)
Parameter	**Est.**	**95 % CI**	**Est.**	**95 % CI**	**Est.**	**95 % CI**	**Est.**	**95 % CI**	**Est.**	**95 % CI**
IOP [mm Hg]	12.13	10.57–13.69	2.19	1.72–2.77	−8.77	−9.46—8.06	12.37	10.69–14.05	2.45	1.97–3.05	−8.49	−9.07—7.89
CCT [μm]	546	536–556	2	1–2	−12.90	−15.26—9.33	546	536–556	2	1–3	−12.47	−14.49—9.76
ACD [mm]	3.603	3.501–3.706	0.023	0.018–0.03	3.190	2.12–3.99	3.736*	3.636–3.835	0.021	0.018–0.025	−1.820*	−3.07—0.67
LT [mm]	3.678	3.563–3.794	0.014	0.010–0.021	3.54	2.46–4.48	3.600*	3.488–3.712	0.009	0.006–0.014	3.05	1.41–4.43
VCD [mm]	16.079	15.722–16.436	0.045	0.038–0.053	−8.78	−9.25—8.32	16.017*	15.671–16.362	0.034*	0.028–0.041	−11.08*	−11.71—10.4
AL [mm]	23.585	23.242–23.928	0.012	0.009–0.017	−9.45	−9.97—8.91	23.571	23.245–23.896	0.016*	0.012–0.020	−8.81	−9.23—8.38
RT† [μm]	252	245–260	1	1–2	−10.73	−11.94—9.38	252	244–261	2*	2–2	−11.32	−12.3—10.25
ChT† [μm]	325	292–357	7	5–12	−4.37	−5.14—3.60	331	298–365	4	2–9	−3.29	−5.23—1.34

* Significant effect of atropine on MESOR, amplitude or acrophase set at $p < 0.05$.
† Averaged over the central 1 mm.

reveals a notable phase difference in ChT, consistent with a more in-phase relationship between AL and ChT in atropine-treated eyes (E).

4. Discussion

This study investigated the short-term effects of a one-time instillation of topical 1 % atropine on various diurnal ocular rhythms, including IOP, with specific interest in whether some or all persisted after its instillation and to what extent their amplitudes and/or their phase relationships are affected. In all cases, the various diurnal ocular rhythms were found to persist in atropine-treated eyes (see below), although significant differences from those of untreated fellow control eyes were found, in terms of MESORs [ACD (deeper), LT (thinner), VCD (shallower)], amplitudes [AL (larger), RT (larger), VCD (smaller)], and acrophases [≈5h and 2 h 20 min phase-advance for ACD and VCD, respectively]. In the case of both AL and ChT rhythms, treated and fellow-untreated eyes showed approximately the same acrophases, which nonetheless, become more in-phase in comparison with our previous study (Table 4) (Nilsen et al., 2022). In the case of ACD and LT rhythms, they were fully in phase for fellow-untreated eyes, and near in phase for treated eyes, with the anterior chamber deepening, and the crystalline lens thickening over the course of the evening (Figs. 3–4).

Overall, eyes treated with topical 1 % atropine did not show any change in choroidal thickness after instillation of atropine, contrasting with reports of thickening in children undergoing myopia-control treatment (Yam et al., 2022; Xu et al., 2023). However, perhaps noteworthy, the choroids of treated eyes did not thin when diurnal thinning was expected (Fig. 3, c.f. HWT+1 pre-instillation of atropine with HWT + 4). This finding is also consistent with a report of choroidal thickening 30 and 60 min after day-time instillation of 0.01 % atropine, i.e., within a time window of 09:00–14:00 (Sander et al., 2019). In the current study, this "relative choroidal thickening" effect of atropine also altered the phase relationship between choroidal thickness and axial length rhythms, to become more in-phase, in atropine-treated eyes, and curiously also in fellow control eyes (Fig. 4 and 6E–F). That the effect is similar in both eyes is consistent with a cross-over effect of atropine, as evident in accommodation data (discussed further below), and highlights the importance of having available for comparison, data from other studies involving healthy, untreated eyes (Burfield, Carkeet, & Ostrin, 2019; Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011; Nilsen et al., 2022). That the changes induced by atropine brought the AL and ChT rhythms more in phase, here by advancing the ChT rhythm by approximately 6 h compared to our previous study (c.f. Fig. 6 C and E) (Nilsen et al., 2022), whilst leaving the AL rhythm unchanged, has a parallel in observations from another human study involving imposed myopic defocus and in which the choroid was found to thicken and also undergo phase-advance in its rhythm, by 9 h, accompanied by a phase-delay in the AL rhythm by 6 h—resulting in AL and ChT rhythms also becoming more in-phase (Chakraborty et al., 2012). Both optical and atropine treatment, when applied longer term, have been linked to slowed myopia progression (Yam et al., 2022; Xu et al., 2023). Interestingly, in our previous study using the same methodologies to track seasonal changes in ocular diurnal rhythms in untreated eyes (Fig. 6 C–D), an anti-phase relationship between AL and ChT rhythms was observed in winter (~12 h phase-difference, Fig. 6C), with these rhythms becoming more in-phase in summer (1–3 h phase-shift, Fig. 6D), but only in eyes with coordinated or decelerated growth. Thus, when comparing these two studies we can see that the AL and ChT phase relationship was related to a change in the phase of ChT only, with the phase of AL remaining the same as for the same time of year and also reported in other studies (Burfield, Patel, & Ostrin, 2018; Chakraborty, Read, & Collins, 2011). Importantly, in related studies involving experimental animal models, AL and ChT rhythms are typically found to be fully in anti-phase (≈12 h phase-difference) during periods of accelerated growth (Fig. 6A), and more in-phase during periods of normal growth (≈9h phase-difference, Fig. 6B) (Nickla et al.,

Table 3
Estimates of relative acrophase for listed ocular parameters and both fellow control and atropine-treated eyes (Table 2) converted to standard clock time (ST). Interocular differences in acrophase are listed in the right most column. Phase advance is indicated with a – sign, and phase delay is indicated with a + sign.

	Fellow control eye		Atropine eye		
Parameter	**Acrophase (ST)**	**95 % CI**	**Acrophase (ST)**	**95 % CI**	**Acrophase difference**
IOP	16:01	15:19–16:43	16:18	15:43–16:54	+0:17
CCT	11:53	09:31–15:27	12:19	10:18–15:01	+0:26
ACD	03:58	02:54–04:46	22:58	21:43–00:07	-5:01*
LT	04:19	03:15–05:16	03:50	02:12–05:13	-0:29
VCD	16:00	15:32–16:28	13:42	13:04–14:23	-2:18*
AL	15:20	14:49–15:52	15:58	15:33–16:24	+0:38
RT†	14:03	12:51–15:24	13:28	12:29–14:32	-0:35
ChT†	20:25	19:39–21:11	21:30	19:33–23:27	+1:05

* Significant effect of atropine on acrophase set at $p < 0.05$.
† Averaged over the central 1 mm.

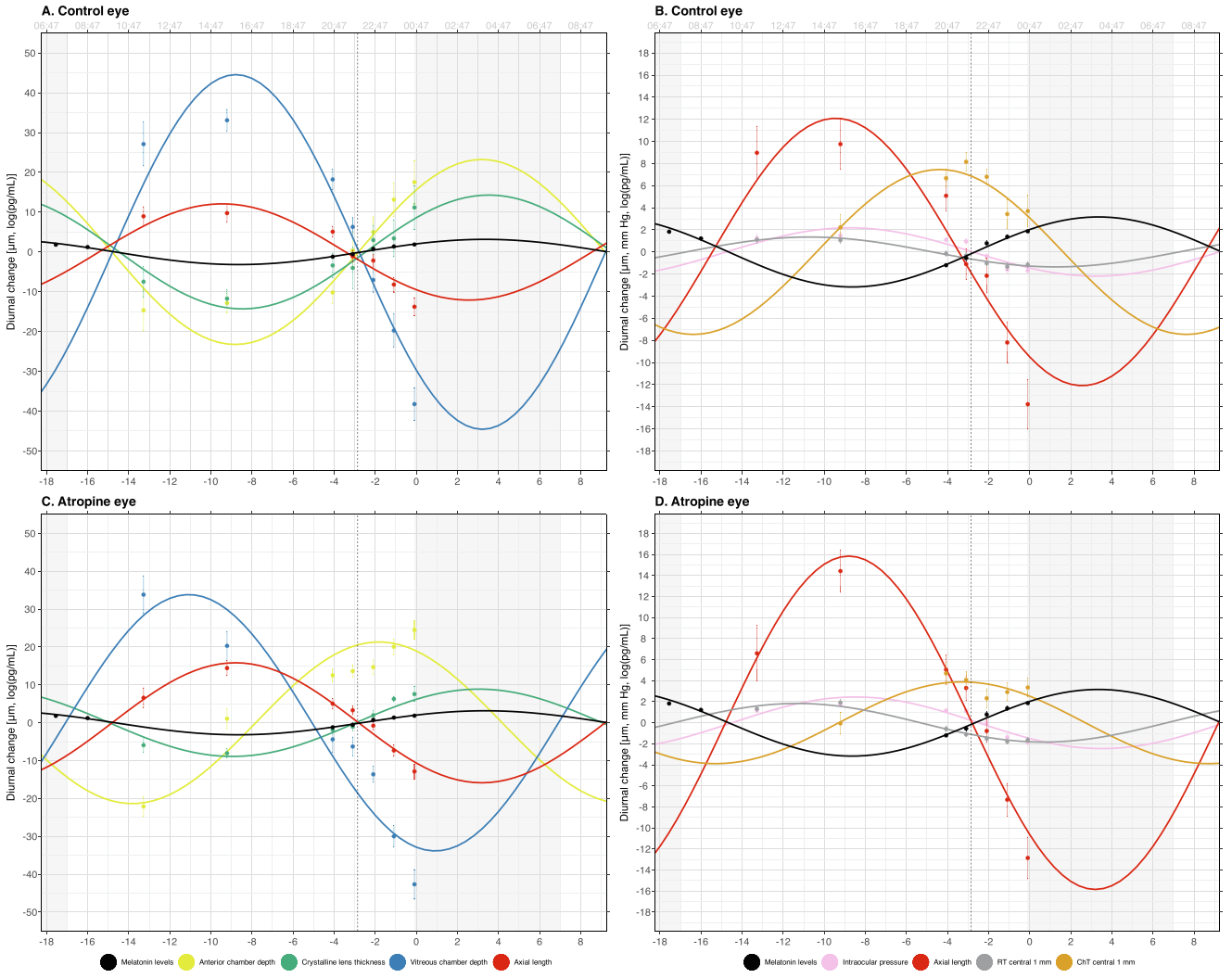

Fig. 4. A-D. Group means (±SE error bars) for measured ocular parameters and saliva melatonin level (MEL), normalized to each individual's MESOR. The curves represent sinusoidal model fits from the population estimate based on the fixed effect estimates from the non-linear mixed effects model. The grey areas represent the averaged estimated sleep period. The x-axis is relative to habitual sleep time (HST) where 0 is HST (the upper x-axis shows standard clock time for easier comparison with other studies) (A, C). Fitted models for melatonin (MEL), anterior chamber depth (ACD), crystalline lens thickness (LT), vitreous chamber depth (VCD) and axial length (AL) for fellow control eyes (A) and atropine-treated eyes (C) Fitted models for MEL, intraocular pressure (IOP), AL, retinal thickness (RT) and choroidal thickness (ChT) (central 1 mm for RT and ChT), with a rescaled y-axis for clarity for fellow control eyes (B) and atropine-treated eyes (D). The dashed vertical lines indicate the timings of dim light melatonin onset (DLMO) relative to HST. The first MEL measurement taken when awakening at Day 8 differed in timing from the self-reported habitual wake time.

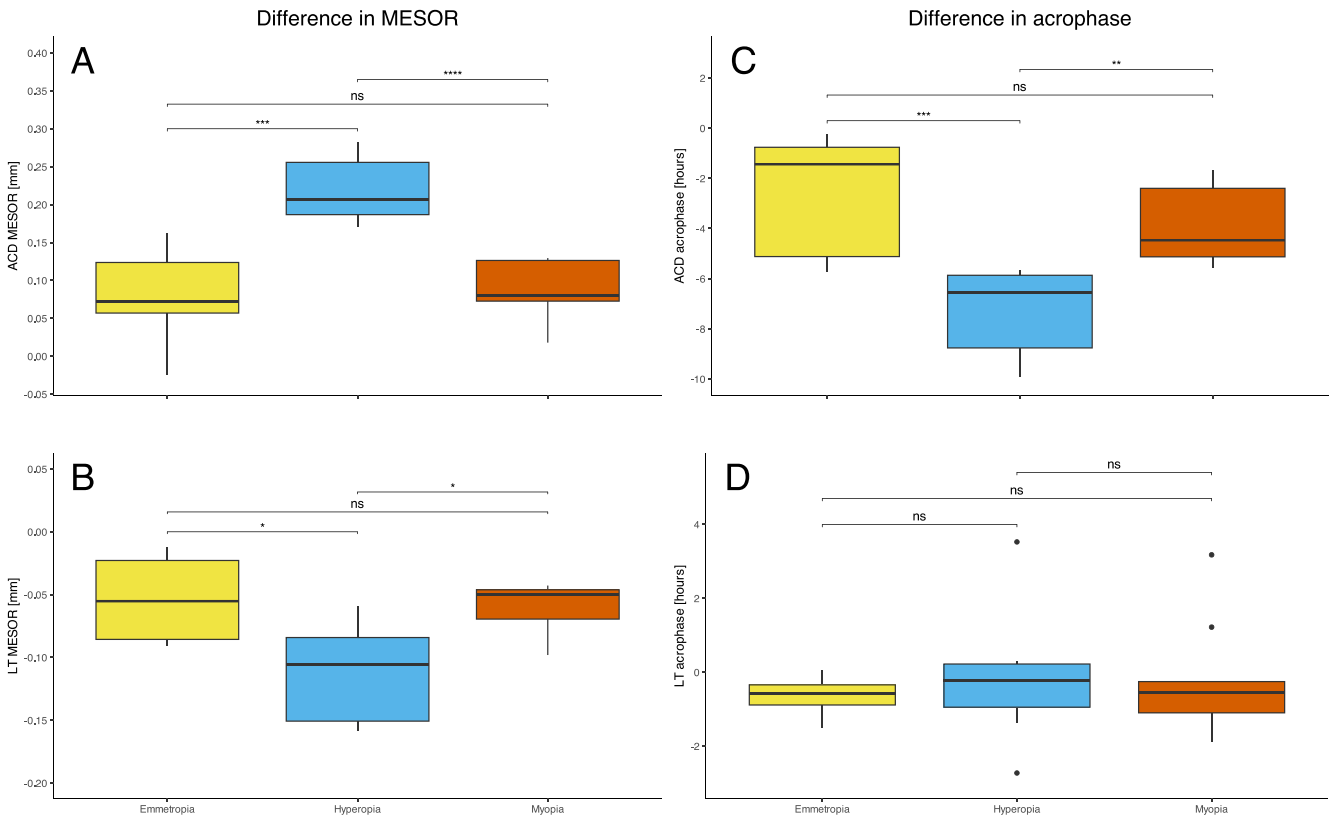

Fig. 5. A–D. Differences in MESOR (A–B) and acrophase (C–D) between atropine-treated and fellow control eyes for anterior chamber depth (ACD, top row) and crystalline lens thickness (LT, bottom row) for emmetropes, hyperopes and myopes. Statistical significance for all panels estimated using one-way ANOVAs, p-values shown in figure are Tukey HSD adjusted: *p < 0.05, **p < 0.01, ***p < 0.001).

Table 4
Comparisons between untreated eyes (previous study) (Nilsen et al., 2022) with atropine-treated eyes for data collected in November (winter), but not in the same year. Time is shown in standard clock time.

	Untreated eyes	Atropine treated eyes	Differences
AL acrophase	15:20	15:58	38 mins
ChT acrophase	03:14	21:30	-5 h 44 mins
AL and ChT phase relationship	11 h 54 mins	5 h 32 mins	6 h 22 mins

1998). On the other hand, such changes in acrophase were not observed in a study involving chicks treated with atropine, although in this case, delivered by intravitreal injection and limited to eyes undergoing myopia induction using negative lenses (Nickla et al., 2019). Together, these observations lend support to the notion that retinal signals arising from behavioural, optical and/or pharmacological interventions have potential to interact with, and perturb, the eye's natural diurnal rhythms to alter eye growth (Nickla, 2013; Chakraborty et al., 2018).

4.1. Accommodation and cross-over effects

The observed differences between the atropine-treated eyes and their fellow eyes (deeper anterior chambers, thinner lenses, and shallower vitreous chambers) are consistent with an inhibitory effect of atropine on accommodation and on the order of magnitude of that expected, based on the difference in residual accommodation between the two eyes (≈2 D). Nonetheless, that AL shows the same trend for both eyes and that atropine-treated and fellow eyes show similar ChT rhythm in the current study, yet the findings for the latter untreated eyes differ from results in our previous study where both eyes were untreated, were unexpected and warrant explanation.

The possibility of a cross-over (contralateral) effect warrants consideration in this context. Such effects have been documented with tropicamide and phenylephrine in humans (Kara et al., 2014; Patsiopoulos et al., 2003), as well as with topical atropine in a study involving rabbits (Wang et al., 2019). Another alternative explanation for similar effects on AL and ChT rhythm in untreated eyes is interocular yoking, which describes interactions between the two eyes that are presumed to be mediated by central neuronal pathways (Zhu, McBrien, & Smith E.L., Troilo D., Wallman J., 2013). Although this has been reported in several paired-eye animal studies, this would seem, however, to be the least plausible explanation for our findings, given the many other influences on the choroid and its very high blood flow (Wang et al., 2019; Nickla and Schroedl, 2019). Among other possibilities that cannot be ruled out as contributing factors leading to the observed altered phase relationship, is that it is a by-product of the changes occurring secondary to the monocular reduction in accommodation and/or decreases in cholinergic stimulation of non-vascular smooth muscle in the choroid (Meriney and Pilar, 1987; Poukens et al., 1998; Flügel-Koch et al., 1996). That choroidal thickness is affected by accommodation has been reported in several studies (Ghosh, Collins, Read, Davis, & Chatterjee, 2014; Kaphle, Schmid, Suheimat, Read, & Atchison, 2023; Woodman, Read, & Collins, 2012; Woodman-Pieterse, Read, Collins, & Alonso-Caneiro, 2015), with attribution to changes in the tone of the choroidal nonvascular smooth

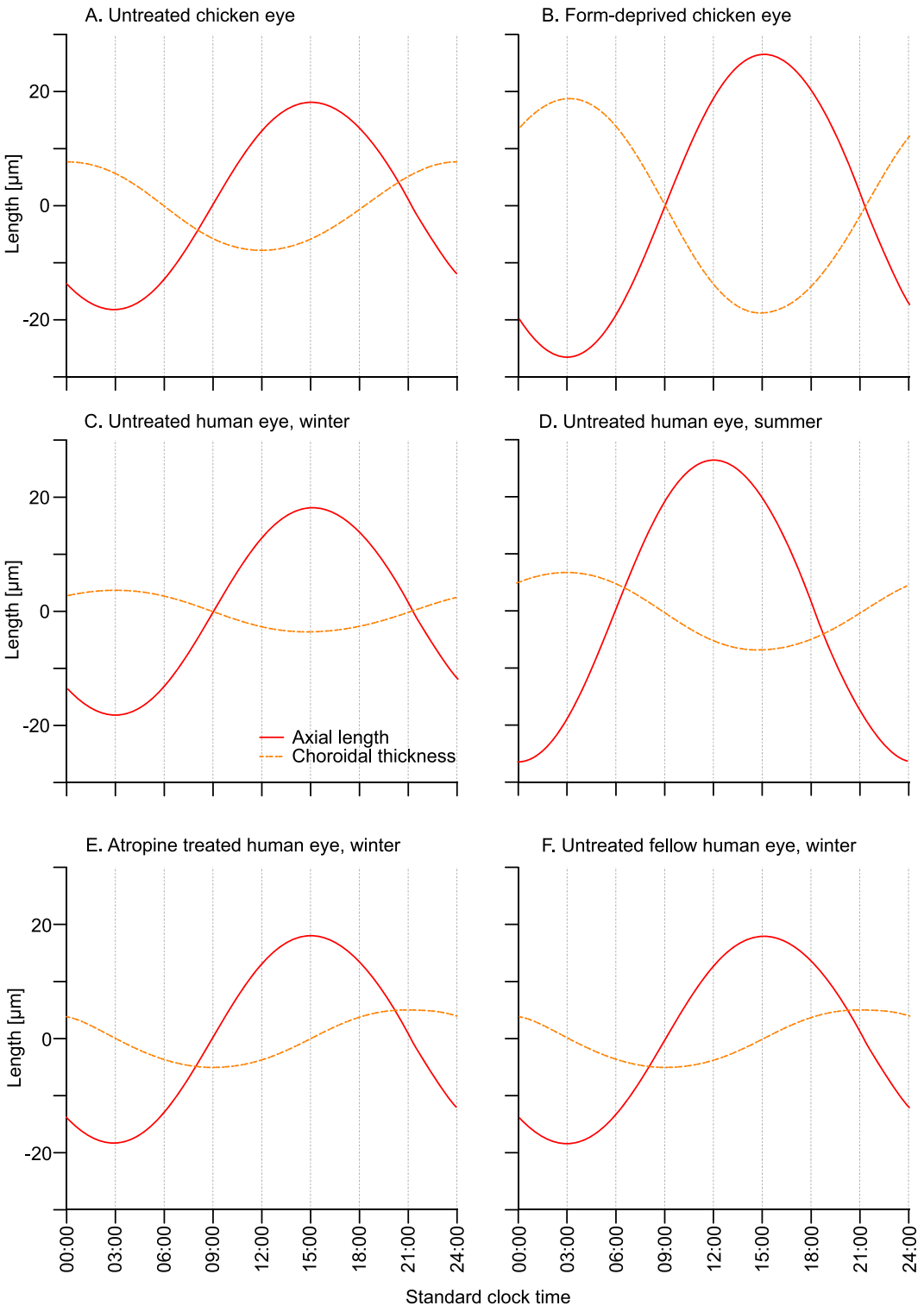

Fig. 6. A–F. Phase relationships between axial length and choroidal thickness rhythms as a function of standard clock time. In chicks, the two rhythms were found to be approximately 9 h out-of-phase in normal (untreated) eyes (A: top left) while the choroid rhythm shifted this to 12 h out-of-phase in form-deprived eyes (B: top right). A and B are adapted from Figure 12 in Ref. (Nickla et al., 1998). In our previous study on humans (C, D), where both eyes remained untreated (Nilsen et al., 2022), the two rhythms were found to be approximately 12 h out-of-phase in winter (C: middle left), with AL advancing to become a few hours more in-phase in summer (9 h out-of-phase), for those who had experienced coordinated or decelerated growth (D: middle right). In this study, the two rhythms were found to be even more in-phase (6 h out-of-phase) both in the atropine (E: bottom left) and control (F: bottom right) eyes. The difference was related to a 6 h phase advance of choroidal thickening, while AL phase remained the same when comparing the atropine treated eye with the untreated human eye in winter (E versus C) and untreated chicken eyes (E versus A).

muscle and/or choroidal blood flow (Woodman-Pieterse et al., 2015).

4.2. Diurnal changes in crystalline lens thickness

The diurnal rhythm in crystalline lens thickening persisted, despite accommodation being paralyzed by atropine, and atropine-treated eyes maintained the same acrophase as fellow control eyes (Table 2). This finding parallels our previous observation of evening thickening of the crystalline lens that was mechanistically different from accommodation-related thickness changes (Nilsen et al., 2022). That the rhythms of ACD and LT were also in-phase implies that the changes were largely confined to the posterior part of the lens (Chakraborty et al., 2011). This interpretation is also corroborated by the observed antiphase relationship of VCD and LT rhythms, and in contrast to accommodation-related changes in the curvature of the anterior lens (and, to a lesser extent, the posterior lens), which lead to shorter ACD and VCD (Kaufman et al., 2011).

The mechanism (or mechanisms) underlying the diurnal changes of crystalline lens thickness is not known, although it is tempting to speculate that changes in ciliary muscle tonus and independent metabolic changes within the crystalline lens may be involved (Burfield, Patel, & Ostrin, 2018). That the diurnal changes persist in atropine-treated eyes tend to rule out the former, as tonic accommodation is minimized in these eyes yet the LT acrophase persists (Choi and Cho, 1963), whilst also the anterior lens surface flattens and LT is reduced (Bartlett & Jaanus, 2008; Hashemi, Asharlous, & Khabazkhoob, 2020) Furthermore, melatonin appears to be synthesized by the crystalline lens (Alkozi et al., 2017), in addition to the pineal gland and retina (Ostrin, 2019), with levels of melatonin reported to increase in the evening in the rabbit lens (Abe et al., 1999). There is a noteworthy similarity in the timing of evening thickening in the human crystalline lens and increases in melatonin levels (Fig. 4 A–D).

ACD and VCD exhibited significant phase differences between the fellow control and atropine-treated eyes (Table 2). Since these ocular parameters are interdependent and most likely reflect the combined effects of the crystalline lens moving and changing its thickness (no interocular differences in neither CCT nor RT, Table 2), the phase differences could be explained by increased noise, as reflected in the less optimal curve fitting (c.f. ACD and VCD in Fig. 4 A and C).

4.3. Differences between refractive error groups

The between-individual intra-ocular variation in ChT for the effect of atropine 1 % was independent of refractive error (Supplementary Table S3). There were no differences between the refractive error groups in terms of the effects of 1 % atropine on IOP, AL, RT and ChT, consistent with previous findings in young adults with 2 % homatropine, another non-selective antimuscarinic drug (Sander et al., 2018). Atropine did, however, induce greater deepening of ACDs and reductions in LTs on average (i.e., MESORs) in the hyperopic group compared with emmetropic and myopic groups. That ciliary muscle tone is likely to be highest in hyperopes and lowest in myopes offers a plausible explanation for differences between these two refractive error groups in the effect of atropine (Zadnik et al., 1999). These findings are also in line with results from a study conducted on children (6–12 years old) involving topical 1 % cyclopentolate (Hashemi et al., 2020).

4.4. Strengths and limitations

Key strengths of this study include the measurement schedule, which was designed to respect individual chronotypes, as well as the nature of the data used to derive them, as described previously (Nilsen et al., 2022). Importantly, wake-up and sleep-onset times of participants were measured objectively (via actigraphy) over the week preceding the lab-based data collection, and were used along with self-reported wake times to create a customized measurement schedules for each individual. Respecting habitual sleep times in this manner allowed diurnal ocular rhythms to be assessed in relation to each individual's chronotype. Melatonin rhythms, derived from assayed saliva samples, were used to further validate derived chronotypes; where the sinusoid was fitted to the logarithm of the measurement to account for the sharp drop and rise in secretion in the morning and evening, respectively (equivalently, a floor effect during the day) (Kennaway, 2019). The participants were confined to the lab for the evening measurements, during which time ambient light levels were kept below 20 lx to avoid artificially suppressing melatonin secretion, as there can be large between-individual variations in sensitivity in suppressing melatonin (Phillips et al., 2019).

When modelling periodic behaviour, it is desirable to sample at regular intervals (say, hourly for a circadian rhythm) for the duration of at least one cycle. There are, however, significant logistical and practical problems with disturbing the sleep patterns of volunteers during their working week and, consequently, we did not attempt any measures after their habitual bedtimes. Further, it has been shown that sleep-disturbing measurements are of questionable value due to the release of stress hormones affecting cardiovascular function, and changes of body posture influencing ocular biometry, intraocular pressure (Liu et al., 1998), and choroidal thickness (Anderson et al., 1985). Instead, we aimed to densely sample parameters around the expected time of dim-light melatonin onset (DLMO), which increased the likelihood of capturing this useful measure of an individual's melatonin cycle (Kennaway, 2019), while also allowing rich sampling in the region of maximum rate-of-change in the other biometric parameters (which is most evident in the early evening measures in Fig. 3). The dense sampling at such an influential part of the rhythm greatly restricted the search space of the sinusoid fitting algorithm, giving confidence to the parameters estimated to assess rhythmic behaviour.

We did not include any measures after habitual bedtime, and while it has been shown that sleep-disturbing measurements are of questionable value in a study like this (Kennaway, 2019), we cannot rule out such influences on our results in the absence of such data.

In the current study, we did not collect a full (day long) set of baseline data before initiating the monocular 1 % atropine treatment, choosing instead to make use as "reference baseline" data, equivalent data from the previous diurnal study, which was conducted at the same time of year the year before. Although these data were collected from different participants, the age range and time of year for these two studies are comparable.

As described, the instillation of atropine affected the MESOR, amplitude and acrophase of various ocular diurnal rhythms, as modelled with a sinusoid with a 24-hour period. Nonetheless, it is plausible that our atropine intervention may have altered the periodicity of the choroid rhythm (Nickla and Schroedl, 2019), which appears to be the more dynamic of choroidal and scleral structures (Read, Fuss, Vincent, Collins, & Alonso-Caneiro, 2019; Zhu, Goto, Singh, Torres, & Wildsoet, 2022). We cannot rule out the possibility that changes (or the absence thereof) in MESOR, amplitude and acrophase of ChT reflect a change in periodicity. Distinguishing between these two possibilities would require significantly more epochal measurements over several days (both pre- and post-atropine-instillation), which would introduce additional questions and challenges, including decisions in relation to atropine dosing.

The potential cross-over and yoking effects are limitations to the paired-eye design used in this study. One approach to circumvent this potentially confounding effect would be to use a "2-day" design, with baseline data collected from both eyes on day 1, followed by repeated measurements on day 2 after instillation of atropine in one (or both) eyes.

The use of different atropine doses, according to iris colour, did not appear to have influenced study outcomes. This outcome is in line with the expected greater inactivation of atropine through binding to melanin in eyes with darker irides, despite their exposure to a higher dose of

atropine (2 drops) (Salazar et al., 1976; Bahrpeyma et al., 2022).

A high concentration (1 %) of atropine was administered once in the morning, in order to give a large – but short-term – measurable effect on rhythmic statistics. While the timing of atropine treatment was found not to influence outcomes in one study in chicks (Nickla et al., 2019), a longer term study is warranted to assess the effects on the various ocular rhythms of repeated daily (evening) atropine treatment with lower concentrations, as currently used for myopia control. Questions of interest in undertaking such a study include whether the observed alteration in the phase-relationship between AL and ChT rhythms persists with long term therapy, and thus its relationship to changes in the rate of eye elongation, with slowed growth being the expected outcome with topical atropine in myopic children. Finally, with drug-eluting contact lenses likely to become available soon, understanding the effect of the timing of atropine instillation may be an especially important factor to investigate in the context of treatment efficacy.

5. Conclusion

During a 24 h period post instillation of topical 1 % atropine, the diurnal rhythms of ocular parameters persisted, although MESORs, amplitudes and/or acrophases were altered in many cases. The rhythmicity of choroidal thickness changes was also affected in both atropine-treated and fellow control eyes, with changes in the phase-relationship between axial length and choroidal thickness rhythms akin to that observed under conditions favouring slowed eye growth. Our results add support to the choroid being a key site for atropine's myopia control effect, while they do not address the question of whether it is the primary site of action as opposed to the down-stream target of an atropine-initiated anti-myopia signal. Additionally, we report for the first time that the crystalline lens undergoes diurnal variation in its thickness even when accommodation is minimized by topical atropine. The significance of this observation for ocular growth regulation and control of myopia progression, along with the effects on all rhythms of more extended use of topical atropine and different concentrations of atropine, warrant further investigation, given increasingly popular use of "low dose" atropine for control of myopia in children.

Disclosure

N.G. Nilsen, **None**; S.J. Gilson, **None**; H.R. Pedersen, **None**; L.A. Hagen, **None**; C.F. Wildsoet, **None**; R.C. Baraas, **None**.

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CRediT authorship contribution statement

Nickolai G. Nilsen: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Stuart J. Gilson:** Methodology, Software, Investigation, Data curation, Formal analysis, Writing – original draft. **Hilde R. Pedersen:** Investigation, Validation, Writing – review & editing. **Lene A. Hagen:** Investigation, Writing – review & editing. **Christine F. Wildsoet:** Conceptualization, Methodology, Writing – review & editing. **Rigmor C. Baraas:** Conceptualization, Methodology, Investigation, Validation, Writing – original draft, Funding acquisition, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be shared on Figshare upon publication if manuscript is accepted

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.visres.2023.108341.

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Paper III

Nilsen NG, Gilson SJ, Lindgren H, Kjærland M, Pedersen HR, Baraas RC. Seasonal and Annual Change in Physiological Ocular Growth of 7- to 11-Year-Old Norwegian Children. Investigative Ophthalmology & Visual Science. 2023;64(15):10-, doi:10.1167/iovs.64.15.10

Seasonal and Annual Change in Physiological Ocular Growth of 7- to 11-Year-Old Norwegian Children

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Purpose. To investigate seasonal and annual change in physiological eye growth in Norwegian school children.

Methods. Measurements of ocular biometry, non-cycloplegic spherical equivalent autorefraction (SER), and choroidal thickness (ChT) were obtained for 92 children (44 females) aged 7 to 11 years at four time points over a year (November 2019–November 2020). Seasons (3- and 5-month intervals) were classified as winter (November–January), winter–spring (January–June), and summer–autumn (June–November). Cycloplegic SER was obtained in January and used to group children. The seasonal and annual changes were tested with a linear mixed-effects model (P values were adjusted for multiple comparisons).

Results. All the children experienced annual ocular growth, irrespective of SER, but less so during the summer–autumn. The baseline SER was lower ($P < 0.001$), axial length (AL) was longer ($P < 0.038$), and choroids were thicker in 10- to 11-year-old than 7- to 8-year-old mild hyperopes ($P = 0.002$). Assuming mild hyperopes ($n = 65$) experience only physiological eye growth, modeling revealed seasonal and annual increases in AL across sex and age ($P < 0.018$), with less change during the summer–autumn than winter–spring. The 7- to 8-year-olds had a larger decrease annually and over winter–spring in SER ($P \leq 0.036$) and in ChT over winter–spring than the 10- to 11-year-olds ($P = 0.006$).

Conclusions. There were significant seasonal and annual changes in AL in children who had physiological eye growth irrespective of age within this cohort. Annual changes in SER and seasonal choroidal thinning were only observed in 7- to 8-year-old children. This indicates continued emmetropization in 7- to 8-year-olds and a transition to maintaining emmetropia in 10- to 11-year-olds.

Keywords: seasonal changes, physiological eye growth, refractive error development, myopia, choroidal thickness

There is growing evidence supporting the theory that time spent outdoors and increased daylight exposure could be major factors for normal emmetropization during childhood and for maintaining emmetropia throughout adolescence and, consequently, delaying or preventing myopia onset.[1,2] Supporting evidence has been provided by cluster randomized trials whereby compulsory outdoor time during recess at school has been tested as the intervention and shown to be successful at decreasing the incidence of myopia.[3–5] Objective measures of light exposure during one of these cluster randomized trials reported a strong association between the protective effect of outdoor time and the duration and intensity of the light,[4] akin to data from animal models of myopia such as rhesus monkeys.[6] Furthermore, when compared with Southeast Asia, considerably lower myopia prevalence has been reported in Scandinavia (<13% in 16- to 19-year-olds, 10% in 12-year-olds),[7,8] where compulsory outdoor time during recess is the norm (irrespective of time of year).[7–9] Data on adolescents and young adults from southeast Norway (latitude 60°N), where there are ≈12 hours more daylight available in summer than in winter,[10] suggest that the delayed onset and low prevalence of myopia could be a result of children's eyes being adapted to seasonal variations in daylight availability.[7,11] There is value in such a suggestion, as there are several reports on seasonal variation in myopia development with progression being slower during summer compared with winter,[12–16] which has been linked to increased availability of daylight in summer rather than fewer school hours,[16] or a combination of both.[15]

Animal studies have consistently shown that choroidal thickness may act as a biomarker of eye growth (for a review, see Troilo et al.[17]). Choroidal thickness has been shown to be affected by light exposure in both animals[18,19] and human adults.[20–23] The reported associations between less thickening or thinning of the choroid, increased axial length, and myopia in human studies of children aged 6 to 18 years[24–26] imply that the choroid may act also as an eye growth biomarker in humans.[27] Human emmetropization is reported to be influenced by visual experience and,

in general, to be completed by 6 years of age,[28] but it is not known if physiological ocular growth follows a similar seasonal pattern as that observed for myopic ocular growth. Physiological ocular growth is defined here as a two-phase process: normal eye growth as experienced by children who successfully emmetropize and, in the second phase, maintenance of emmetropia/mild hyperopia through coordinated growth (i.e., the eye grows in length while its crystalline lens flattens, thins, and loses optical power, but the refractive error remains unchanged).[28–31] An 18-month-longitudinal study reported an inverse relationship between daylight exposure and change in axial length in 10- to 15-year-old children (n = 60 non-myopes)[32] and a potential (but nonsignificant) seasonal variation with larger axial length (AL) changes and less thickening of the choroid in winter.[24] The difference in daylight availability between seasons at the study location (Australia, latitude 27°S) was 3 hours. Taken together, it is reasonable to hypothesize that physiological eye growth follows seasonal variation in availability of daylight, if guided by the same mechanism as that observed for myopic ocular growth,[12–16,28] with larger changes in axial elongation in winter than in summer. Determining to what degree physiological ocular growth and choroidal thickness follow a similar seasonal pattern as that observed for myopic ocular growth is required to better understand what differs between success and failure to maintain emmetropia, with failure leading to myopia.[28] The aim of this study was to investigate seasonal and annual changes in physiological eye growth and choroidal thickness in a cohort of healthy 7- to 11-year-old schoolchildren, who have mandatory outdoor time during recess every school day, irrespective of season, and who live at a location (Norway, latitude 60°N) where there are large differences in daylight availability between winter and summer. Another aim was to shed light on whether children who experienced only physiological ocular growth were undergoing emmetropization or if they had transitioned to maintenance of emmetropia, whereby the refractive error remained unchanged (maintaining emmetropia/mild refractive error).

Methods

Participants

Ninety-two children (44 female; aged 7–11 years), who attended second and fifth grade (7–8 years old and 10–11 years old, respectively) at one primary school in Kongsberg, Norway, were enrolled in this 12-month prospective longitudinal study. The study was approved by the Regional Committee for Medical and Health Research Ethics (Southern Norway Regional Health Authority), and both parents/caregivers provided written consent for their child to participate. The study was carried out in accordance with the tenets of the Declaration of Helsinki. All children included in the study were healthy with no history of ocular disease as reported by their parents. Habitual distance high-contrast visual acuity was in the range −0.18 to 0.70 logMAR (TestChart 2000; Thomson Software Solutions, London, UK) and stereo acuity 15 to 480 seconds of arc (TNO Stereotest; Laméris Ootech, WC Ede, Netherlands). Three myopes (cycloplegic spherical equivalent autorefraction [SER] −1.25 to −0.50 D) and 11 significant hyperopes (cycloplegic SER +2.00 to <+3.00) were uncorrected and referred to the university eye clinic. None of the children who wore prescription correction had received any other optical treatment than single-vision spectacle correction.

Outdoor Time Before and During the COVID-19 Lockdown

The children's weekdays start and end in the before- and after-school (BAS) program that is offered from 07:00 to 17:00. Most children (>63% in this municipality in 2020) attend this program,[33] as primary caregivers are typically in full-time employment (85%).[34] The 7- to 8-year-olds have structured teaching from 08:30 to 13:00 and the 10- to 11-year-olds from 08:30 to 13:45. All have a 15-minute recess in the morning and 30 minutes after lunch, and the older children have an additional 10-minute recess in the afternoon. The children must go outdoors during recess, irrespective of weather or time of year. It is reasonable to assume that most of the children will get 1 to 2 hours of outdoor time every day, even in midwinter (when combining outdoor time during recess and the BAS program), and Table 1 shows that this outdoor time coincides with daylight hours.

The COVID-19 lockdown lasted 6 weeks (March 12–April 20, 2020) for the second graders and 9 weeks (March 12–May 11, 2020) for the fifth graders.[35–37] The rector of the school reported that homeschooling was scheduled as normal schooldays, including outdoor time during recess, but with no BAS program. Each child had their own tablet for participating in online learning, for doing and reporting on their school- and homework. In 2020, 96% of the Norwegian population and 99% of those aged 9 to 79 years had their own smartphone.[38] Norwegian children in the relevant age group reportedly spent close to 4 hours per day online in 2020 (including school activity).[39]

Data-Gathering Protocol

Repeated measures of body height, retinal imaging, ocular biometry, and autorefraction were obtained at baseline in November 2019 (autumn, A1), with follow-up measures obtained in January 2020 (winter, W), June 2020 (spring/summer, S), and the subsequent autumn, November 2020 (A2). Details about number of schooldays and availability of daylight are given in Table 1. The child's height was measured first (without footwear); thereafter, measurements of non-cycloplegic autorefraction (Nvision-K 5001 open-view autorefractor; Shin-Nippon, Tokyo, Japan) at a distance of 600 cm, followed by ocular biometry to measure corneal radius (CR) and AL (IOLMaster 700; Carl Zeiss Meditec AG, Jena, Germany); and lastly, optical coherence tomography (OCT) of the choroid (Spectralis OCT2-EDI; Heidelberg Engineering, Heidelberg, Germany). All measurements were obtained at school.

To minimize the effect of diurnal variations on ocular parameters,[40] the children were measured between 11:00 and 14:30. Instruments were placed at approximately the same locations in a classroom at the school at each study visit with curtains kept closed to maintain similar light levels (measured to be 170–190 lux and 20–50 lux at the headrest of the autorefractor and OCT, respectively) to keep any effect of differing light levels on choroidal thickness (ChT) to a minimum.[20] After biometry measurements and just before OCT imaging, the children watched a movie on a TV for

Table 1. Timing and Number of Months Between Study Visits, Range of Daylight Availability[10] in Hours and Minutes Between Study Visit, Number of School Days and Number of Weekdays With Available Daylight in the Morning When the Children Are Walking to School, and From School Ending Until the Evening Right Up to Typical Bedtime

Season	# Months	Range of Daylight [Hours:Minutes]	# Nonschool Days*	# School Days	# Weekdays With Daylight: When Walking to School	# Weekdays With Daylight: Until Bedtime 20:30†
A1–W Autumn–Winter	2	07:25–06:40	24	25	0	0
W–S Winter–Spring	5	06:44–18:35	64	91	66	41
S–A2 Summer–Autumn	5	18:38–08:08	87	74	45	33
A1–A2 Annual	12		175	190	111	74

The study visits were in November 2019 (A1), January 2020 (W), June 2020 (S). and November 2020 (A2).
* Number of nonschool days includes weekends and holidays
† Recommended bedtime for Norwegian children aged 7 to 11 years of age.[87]

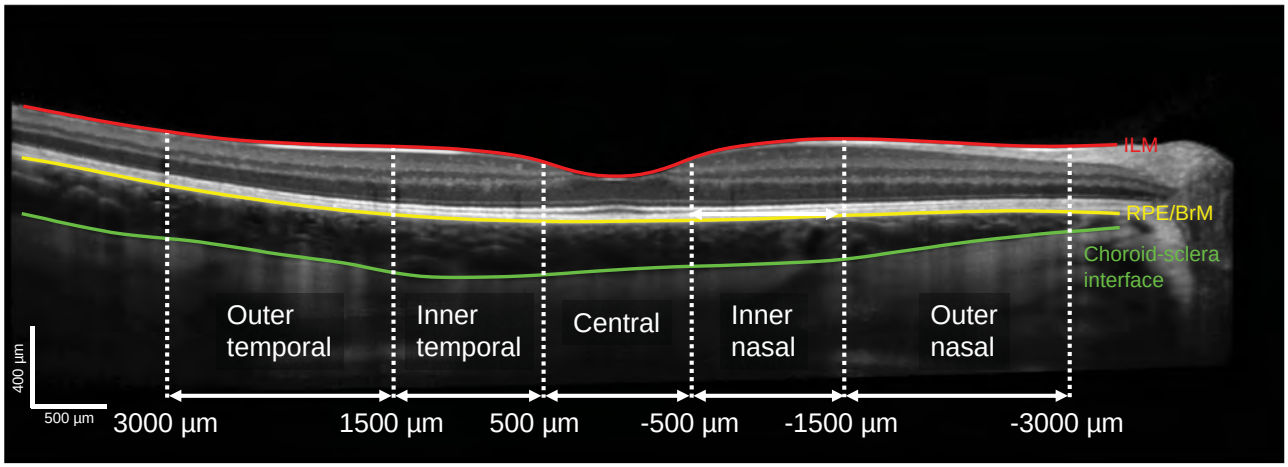

Figure 1. Example of segmentation of the retinal and choroidal thicknesses, which were defined as the area between the inner limiting membrane (ILM) and retinal pigment epithelium (RPE) layers, and the RPE layers and choroid–sclera interface, respectively. Mean thicknesses were extracted for the subfoveal (single A-scan), central (1-mm-wide), and the nasal and temporal inner (1-mm) and outer (1.5-mm) macular areas.

15 minutes at a 6-m distance for "accommodation washout," to minimize the accommodative effect on choroidal thickness from previous near-work.[41] The light levels at the location where the children were seated to watch the movie varied between 90 and 110 lux depending on the brightness of the movie's scenes.

OCT images were not acquired in November 2020 (A2) due to COVID-19 restrictions at the school, preventing the necessary contact time per child. Cycloplegic autorefraction (Huvitz HRK-8000A; Huvitz Co. Ltd., Gyeonggi-do, Korea) was measured once, in January 2020, 2 weeks after the January 2020 follow-up session, as this was the most suitable time for the school. Autorefraction was performed 30 minutes postinstillation of 1% cyclopentolate hydrochloride (Minims single dose; Bausch + Lomb, Bridgewater, NJ, USA). Children with lightly pigmented (blue to green) irides received one drop while those with more heavily pigmented irides received two drops.[42] There were 92 children attending the baseline measures in November 2019; 91 of these attended the repeated measurement session in January 2020 (one child withdrew from the study), of whom 78 also attended on the day we obtained cycloplegic SER. Thirteen of the children were absent from school on the day cycloplegic measures were obtained. A total of 84 children completed the remaining repeated measurement sessions, with OCT images of sufficient quality for analysis obtained for 79 of these 84.

OCT Measurement Protocol and Segmentation

The OCT protocol included a six-line 30-degree radial scan centered at the fovea with 100 B-scans averaged at each orientation, with enhanced depth information. If there were fixation issues or a child could not sit still, the number of scans was reduced to either one (horizontal) or two (horizontal + vertical) line scans. The baseline measurement was set as a reference image for all subsequent measurements, using the instrument's retinal tracking system. For segmentation, a semiautomatic active contour method was fitted to the retinal and choroidal layers (as described previously[43,44]). Interrater reliability was assessed for the segmented subfoveal choroidal thickness (SFChT) by calculating the intraclass correlation (ICC) with a one-way model in R (irr package).[45] ICC was 0.94 (n = 82; 95% confidence interval [CI], 0.91–0.96). Only the horizontal B-scans were used for analysis. The OCT scan's lateral scaling was corrected for each individual's ocular biometry (from an IOLMaster 700 measurement shortly before the OCT imaging) using a four-surface schematic eye model.[46,47] The horizontal line scans were used to extract SFChT as well as mean ChT values for the central 1-mm area, the nasal and temporal inner 1-mm area (0.5–1.5 mm from the foveal center), and the outer 1.5-mm area (1.5–3 mm from the foveal center; Fig. 1). None of the children had any sign of ocular disease.

Data Analysis

Statistical analysis was performed using R statistical software, version 4.3.1.[48] Parametric tests were used where the data had a normal distribution; otherwise, nonparametric tests were used. Statistical significance level was $\alpha = 0.05$. As there were no differences between OD and OS for measured values of ocular biometry (AL and CR) or non-cycloplegic and cycloplegic SER ($P > 0.34$), OD was arbitrarily chosen for analysis. CR was calculated as the average of two main meridians.

The children were classified into three groups: (1) *myopia or risk of myopia*, (2) *mild hyperopia*, and (3) *significant hyperopia*. The *myopia/risk-of-myopia* group was based on Zadnik's cutoff points for cycloplegic spherical refractive error: <+0.50 D for those aged 7 to 8 years and ≤+0.25 D for those aged 9 to 10 years.[49] *Mild hyperopia* was defined as Zadnik's cutoff points above for each age group and ≤+2.00 D, as it was assumed that this group experienced physiological eye growth.[31,49] *Significant hyperopia* was defined as >+2.00 D.

Linear mixed-effects models (LMMs, *lme4*[50] and *lmerTest*[51] R packages) were used to analyze the longitudinal data, using participant ID as a random effect and season, sex, and age groups as fixed effects, with a stepwise approach to assess significant predictors and interactions. A two-way ANOVA was used to examine differences between sex and age groups at baseline for a given ocular parameter or body height. Tukey's honestly significant difference (HSD) test was used to assess the specific significant differences between the groups. The *z*-score analysis with thresholds at ±1.96 (95% CI) were used to determine if the individuals in the *significant hyperopia* group or the *myopia/risk-of-myopia* group differed from those assumed to undergo physiological ocular growth (the *mild hyperopia* group) for changes in AL, SFChT, and body height.

LMM was used to estimate the within-session SD, and profiling the likelihood for the 95% CI, for cycloplegic (Huvitz HRK-8000A) and non-cycloplegic SER (Nvision-K 5001) and for axial length (IOLMaster 700), which were 0.07 D (0.065–0.073), 0.21 D (0.19–0.23), and 0.0048 mm (0.0046–0.0050, respectively; see Supplementary Table S1 for details). The values reported by HRK-8000A, Nvision-K 5001, and IOLMaster are based on the mean of five, five, and six single measurements, respectively.

Results

Baseline Characteristics

Table 2 shows the range of cycloplegic SER for the three SER groups by sex and age groups. Individuals were classified according to *cycloplegic* SER from winter (W), except for the 13 children who did not receive cycloplegic autorefraction, who were classified by a model that predicted cycloplegic SER from non-cycloplegic SER (*adjusted* SER; see Supplementary Material). The adjustment was made for these 13 and for all non-cycloplegic autorefraction measurements obtained at A1, S, and A2. Bland–Altman analysis shows that the mean difference between the *adjusted* and cycloplegic SER was −0.06D; Supplementary Fig. S1).

Table 3 shows baseline measures (A1) of AL, CR, and SFChT by sex- and age-per-SER group. There were no differences between sex groups in AL in the 7- to 8-year-old group, while the 10- to 11-year-old males had longer AL than females in both age groups ($F(1, 66) = 4.58$, $P = 0.036$, Tukey's HSD test $P < 0.003$). The females had, overall, a steeper CR than the males ($F(1, 68) = 9.79$, $P = 0.003$), with no differences between age groups. There were no differences in *adjusted* SER between females and males in the two age groups, but the larger AL in the 10- to 11-year-old group (Tukey's HSD test $P = 0.038$) corresponded with a significantly lower *adjusted* SER (difference of −0.58 D, $z = 4.89$, $r = 0.52$, $P < 0.001$). The choroid was significantly thicker in the subfoveal, central, and temporal (inner) areas compared to the nasal areas (inner and outer), with temporal outer and nasal inner areas being similar ($F(5, 376) = 28.89$, $P < 0.001$, Tukey's HSD test $P < 0.003$). Independent of area, the 10- to 11-year-olds had significantly thicker choroids than the 7- to 8-year-olds (difference of 24 µm, $F(1, 376) = 10.14$, $P = 0.002$), and females had significantly thicker choroids than males (difference of 21 µm, $F(1, 376) = 8.70$, $P = 0.003$). There was a weak but significant association between body height and AL at baseline ($R^2 = 0.09$, $P < 0.006$), and the 10- to 11-year-olds were significantly taller than the 7- to 8-year-olds with no sex differences (ANOVA, $F(1, 68) = 118$, $P < 0.001$). There were no differences in ChT between areas, sex group, or age group. At baseline, there was no difference between the 84 who completed all four measurements and the 92 children who attended only the baseline session, which was not for *adjusted* SER, AL, CR (all $P \geq 0.869$), or SFChT ($n = 79$, $P \geq 0.869$).

Table 2. Range of SER in Winter ($n = 91$) for Each of the Three SER Groups, Subgrouped by Sex and Age

	7- to 8-Year-Olds			10- to 11-Year-Olds		
Characteristic	***n***	**Median**	**Range**	***n***	**Median**	**Range**
Significant hyperopia	9	+2.26	+2.04–+3.49	6	+2.69	+2.01–+5.67
Female	5	+2.26	+2.14–+2.38	3	+2.65	+2.01–+5.18
Male	4	+2.51	+2.04–+3.49	3	+2.73	+2.44–+5.67
Mild hyperopia	31	+1.13	+0.59–+1.95	39	+0.84	+0.30–+1.82
Female	14	+1.00	+0.59–+1.95	21	+0.85	+0.30–+1.35
Male	17	+1.30	+0.65–+1.91	18	+0.80	+0.56–+1.82
Myopia/risk of myopia	3	−0.67	−0.98–+0.14	3	−0.20	−1.14–+0.20
Female	0	–	–	0	–	–
Male	3	−0.67	−0.98–+0.14	3	−0.20	−1.14–+0.20

SER grouping was according to cycloplegic autorefraction ($n = 78$) or by *adjusted SER* where cycloplegic autorefraction was unavailable ($n = 13$). There were no females in the *myopia/risk-of-myopia* group.

Table 3. Baseline (A1) Measures of Body Height ($n = 92$), AL ($n = 92$), CR ($n = 92$), and SFChT ($n = 84$) per Age Group for Females and Males per Refractive Error Group

	7- to 8-Year-Olds					10- to 11-Year-Olds				
Characteristic	***n***	**Mean**	**SD**	**Median**	**Range**	***n***	**Mean**	**SD**	**Median**	**Range**
Height (cm)										
All	43	127.3	5.5	127.9	116.5–140.6	49	143.4	7.5	144.2	114.3–159.8
Female	19	127.1	5.0	125.3	121.0–137.0	25	143.6	6.6	144.4	129.4–159.8
Male	24	127.4	5.9	128.4	116.5–140.6	24	143.1	8.5	144.1	114.3–157.8
AL (mm)										
All	43	22.67	0.67	22.69	21.38–24.15	49	22.94	0.86	23.00	20.29–24.53
Significant hyperopia	9	22.13	0.44	22.11	21.38–22.75	6	22.07	1.10	22.43	20.29–23.23
Female	5	22.33	0.33	22.25	21.97–22.75	3	21.78	1.33	22.25	20.29–22.81
Male	4	21.87	0.47	21.81	21.38–22.48	3	22.36	1.02	22.62	21.24–23.23
Mild hyperopia	31	22.80	0.66	22.78	21.54–24.15	40	22.97	0.72	23.01	21.78–24.20
Female	14	22.65	0.47	22.80	21.54–23.21	22	22.57	0.52	22.54	21.78–23.71
Male	17	22.92	0.77	22.72	21.56–24.15	18	23.45	0.63	23.60	22.12–24.2
Myopia/risk of myopia	3	22.89	0.63	23.17	22.17–23.34	3	24.20	0.29	24.04	24.02–24.53
Female	0	–	–	–	–	0	–	–	–	–
Male	3	22.89	0.63	23.17	22.17–23.34	3	24.20	0.29	24.04	24.02–24.53
CR (mm)										
All	43	7.81	0.24	7.81	7.42–8.27	49	7.76	0.27	7.76	7.21–8.39
Significant hyperopia	9	7.82	0.23	7.86	7.59–8.15	6	7.77	0.23	7.79	7.40–8.11
Female	5	7.93	0.22	7.93	7.60–8.15	3	7.63	0.22	7.67	7.40–7.83
Male	4	7.67	0.14	7.62	7.59–7.87	3	7.91	0.18	7.86	7.76–8.11
Mild hyperopia	31	7.84	0.24	7.81	7.42–8.27	40	7.75	0.28	7.77	7.21–8.39
Female	14	7.81	0.21	7.82	7.42–8.22	22	7.63	0.25	7.69	7.21–8.19
Male	17	7.87	0.27	7.81	7.42–8.27	18	7.90	0.24	7.95	7.35–8.39
Myopia/risk of myopia	3	7.50	0.08	7.47	7.43–7.59	3	7.81	0.28	7.68	7.63–8.13
Female	0	–	–	–	–	0	–	–	–	–
Male	3	7.5	0.08	7.47	7.43–7.59	3	7.81	0.28	7.68	7.63–8.13
SFChT (µm)										
All	36	317	78	318	162–476	48	332	95	321	151–571
Significant hyperopia	7	336	88	325	202–454	6	389	141	379	191–571
Female	4	310	100	297	202–443	3	362	193	325	191–571
Male	3	372	71	338	325–454	3	416	101	432	308–508
Mild hyperopia	26	307	79	305	162–476	39	330	87	321	151–529
Female	12	312	74	285	226–476	21	343	94	371	151–517
Male	14	304	85	318	162–473	18	315	79	303	191–529
Myopia/risk of myopia	3	349	49	362	295–391	3	253	11	247	245–266
Female	0	–	–	–	–	0	–	–	–	–
Male	3	349	49	362	295–391	3	253	11	247	245–266

Seasonal Variations in Physiological Ocular Growth: Sex and Age Group Differences

It was assumed that those in the *mild hyperopia* group ($n = 65$) would experience physiological ocular growth at this age. A linear mixed-effects model was used to assess seasonal (only the two 5-month intervals) and annual changes in physiological ocular growth in this group, in terms of AL, by sex and age groups and any group interactions—that is, winter–spring (W–S), summer–autumn (S–A2), and annually (A1–A2).

There was a significant decrease in *adjusted* SER annually for 7- to 8-year-old males and females, and over winter–spring for the 7- to 8-year-old males ($F(12, 183) = 7.64$, $P < 0.001$, Holm adjusted $P \leq 0.008$), but not for those aged 10 to 11 years. There was a significant interaction for season by age group ($F(3, 189) = 8.22$, $P < 0.001$); the 7- to 8-year-olds had a significantly larger decrease in *adjusted* SER over winter–spring and annually than the 10- to 11-year-olds (Holm adjusted $P \leq 0.036$).

There was a significant elongation in AL over winter–spring, summer–autumn, and annually for each sex in each age group (Fig. 2) ($F(12, 183) = 70.07$, $P < 0.001$, Holm adjusted $P < 0.018$). There was a significant interaction between season and age group ($F(3, 186) = 30.79$, $P < 0.001$); the 7- to 8-year-olds had larger increases than the 10- to 11-year-olds over winter–spring (+0.080 mm) and annually (+0.099 mm, Holm adjusted $P < 0.001$) but not over summer–autumn. The interaction between season and sex was also significant ($F(3, 186) = 2.89$, $P = 0.037$), where males had, overall, larger changes annually than females (+0.034 mm, Holm adjusted $P = 0.032$).

Seasonal Variations in Ocular Growth: Differences Between SER Group. To assess differences in ocular growth between SER groups, AL change in the *myopia/risk-of-myopia* group ($n = 5$) and the *significant hyperopia* group ($n = 14$) were compared with those with expected physiological growth ($n = 65$, *mild hyperopia*). A z-score with a threshold of ±1.96 (95% confidence level) was used. One child in the *myopia/risk-of-myopia* group (SER: −1.14; z-score range: 2.31 to 5.64) and two children in the

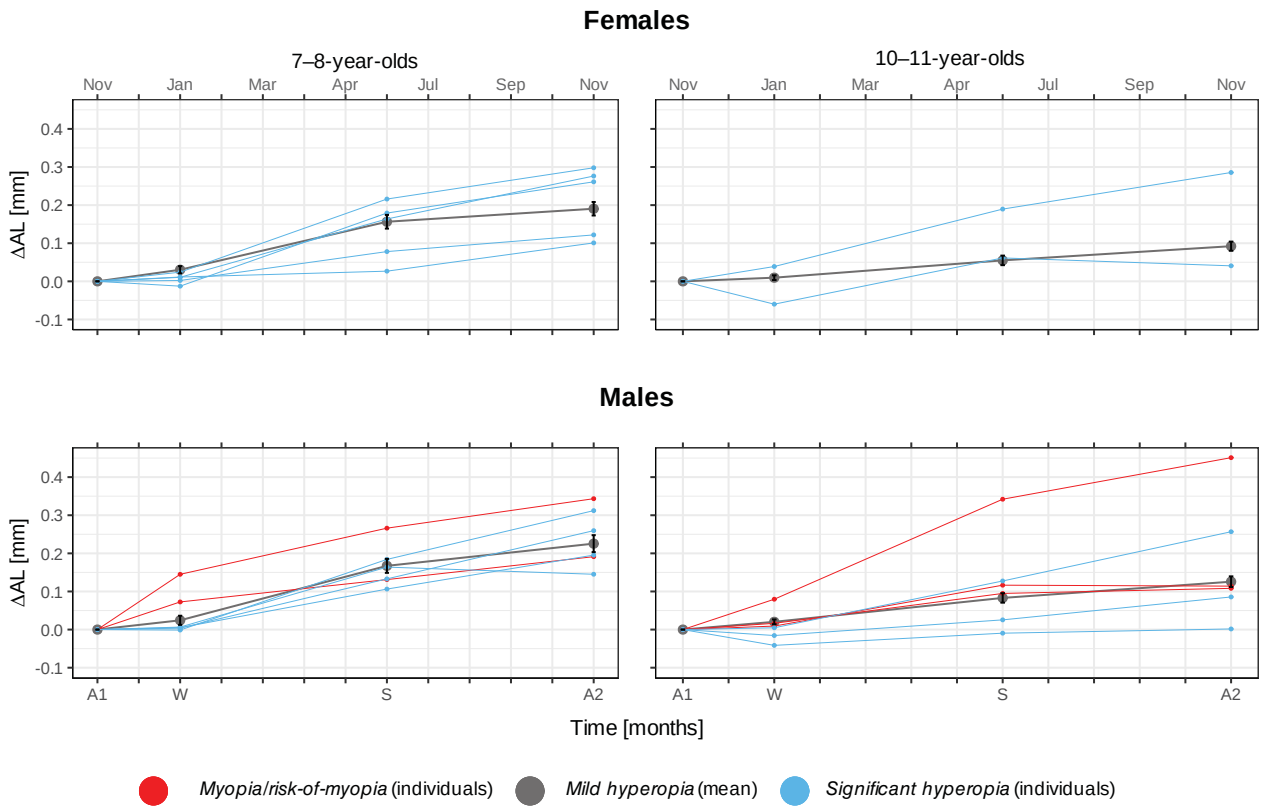

Figure 2. Change in axial length (ΔAL) by sex and age groups across the measurement sessions: autumn 1 (A1), winter (W), summer (S), and autumn 2 (A2), normalized by the baseline value from A1. The *black lines* represent the average change for the *mild hyperopia* group (n = 30: 7- to 8-year-olds and n = 35: 10- to 11-year-olds), with the *error bars* representing the mean ± SE. Each *blue line* represents a child in the *significant hyperopia* group (n = 9: 7- to 8-year-olds and n = 5: 10- to 11-year-olds), while each *red line* represents a child in the *myopia/risk-of-myopia* group (n = 2: 7- to 8-year-olds and n = 3: 10- to 11-year-olds). There were no female children in either age group in the *myopia/risk-of-myopia* group.

significant hyperopia group (SER range: +2.73 to +5.18; z-score range: 2.27 to 3.80) had a larger AL change than those with normal physiological AL change. Three children in the *significant hyperopia* group (SER range: +2.01 to +5.67) had less AL change (z-score range: −3.31 to −2.03). For these six children, the AL change exceeded the threshold between one or more of the follow-up periods (winter–spring, summer–autumn, and annually), with no apparent seasonal effect.

Monthly Rate of Change in Physiological Ocular Growth per SER Group. The monthly rate of change in AL was calculated by dividing ΔAL by the actual number of days within each seasonal-change category (autumn–winter, winter–spring, summer–autumn, and annually) and then multiplying by a nominal 30-day month. For the *mild hyperopia* group, the linear mixed-effects model showed a significant interaction between season and age group (F(3, 188) = 6.18, P = 0.001); the 7- to 8-year-olds had larger monthly increases than the 10- to 11-year-olds over winter–spring and annually (Holm adjusted P < 0.008) but not over autumn–winter or summer–autumn. For both age groups in the *mild hyperopia* group, the highest monthly rate of change was over winter–spring while the lowest was over summer–autumn. For the *significant hyperopia* group and the *myopia/risk-of-myopia group* aged 7 to 8 years, the monthly rate over autumn–winter was the lowest and the highest, respectively (Fig. 3).

Seasonal Variations in Choroidal Thickness: Differences Between Sex, Age, and SER Groups

Differences in ChT Between Sex and Age Groups. The linear mixed-effects model showed no seasonal difference in any of the ChT areas (horizontal scans) between sex or age groups. ChT for each group did, however, significantly vary with area (F(12, 266) = 2.19, P = 0.012, Holm adjusted P < 0.012, Supplementary Table S3). The subfoveal, central 1-mm, and temporal inner and outer ChT areas were all significantly thicker than the nasal inner and outer areas for the 7- to 8-year-old females and males and 10- to 11-year-old males. For these three groups, ChT at the nasal inner area was significantly thicker than the nasal outer area. The subfoveal and central 1-mm areas did not differ, nor did either of them differ from the temporal inner and outer areas. Females aged 10 to 11 years differed from these comparisons for the temporal outer area; the subfoveal and central 1-mm areas were significantly thicker than the temporal outer area, and there was no difference between the nasal inner and temporal outer areas. There was a significant interaction between season and age group (F(1, 675) = 7.75, P = 0.006); the 7- to 8-year-olds had a significantly larger decrease of ChT than the 10- to 11-year-olds over winter–spring (Holm adjusted P = 0.006). There was no interaction between season and choroidal area (Fig. 4).

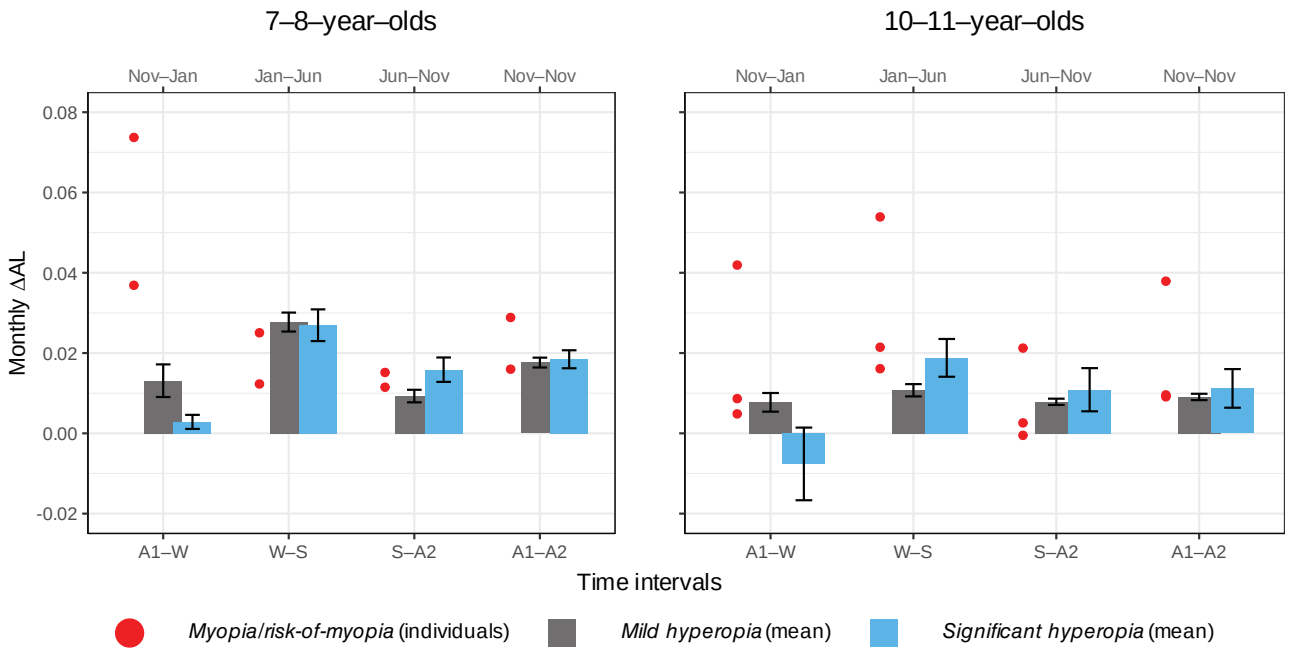

Figure 3. Mean change in AL (ΔAL) per month within each season and annually for the 7- to 8-year-olds (*left panel*) and the 10- to 11-year-olds (*right panel*). *Gray bars* represent the *mild hyperopia* group ($n = 30$ and 35, *left* and *right panels*), *blue bars* represent the *significant hyperopia* group ($n = 9$ and 5, *left* and *right panels*), and *red dots* represent the individuals in the *myopia/risk-of-myopia* group ($n = 2$ and 3, *left* and *right panels*). *Error bars* represent the mean ± SE.

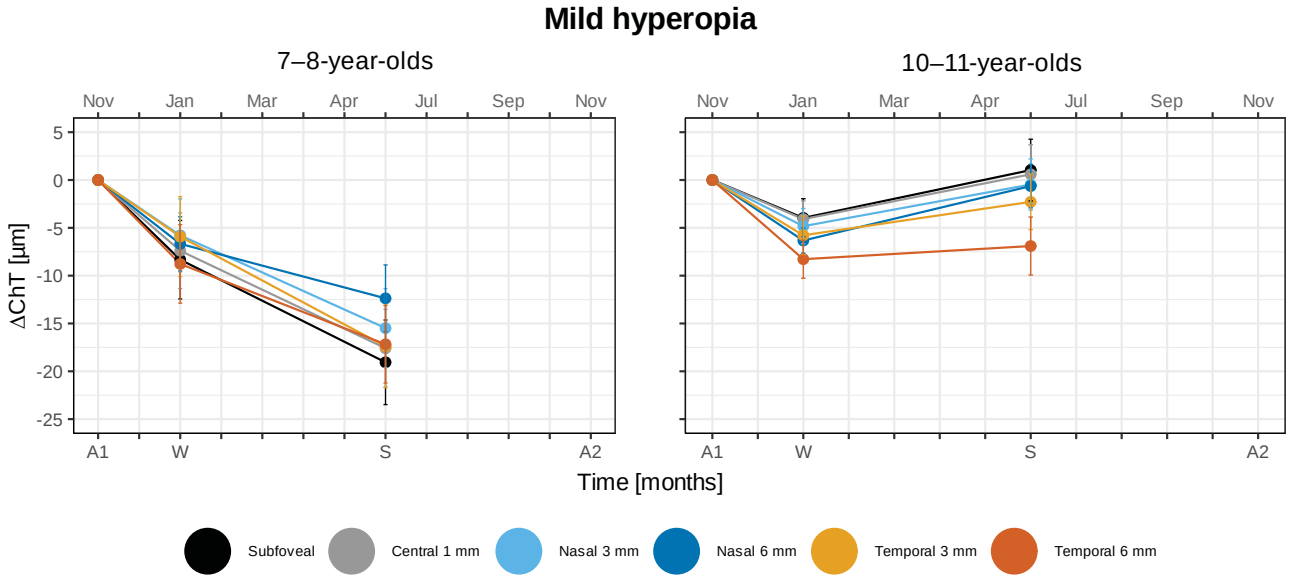

Figure 4. Change in choroidal thickness (ΔChT) by age group across the measurement sessions: autumn 1 (A1), winter (W), and summer (S), normalized by the baseline value from A1. Each area (subfoveal, central 1-mm, nasal and temporal inner [1-mm] and outer [1.5-mm] areas) is indicated by a different color as shown by the legend. The data are for the *mild hyperopia* group ($n = 25$: 7- to 8-year-olds and $n = 37$: 10- to 11-year-olds). *Error bars* represent the mean ± SE.

Differences in ChT Between SER Groups. To assess differences in ChT between SER groups, comparisons were made between those with expected physiological growth ($n = 62$, *mild hyperopia*), *myopia/risk of myopia* ($n = 5$), and *significant hyperopia* ($n = 12$) using a z-score analysis. Only one child in the *significant hyperopia* group had less change in ChT (central 1-mm, temporal inner, and temporal outer areas) over winter–spring than those with physiological growth (z-score range: −2.13 to −1.98). For the remaining 16 children, z-score range was −1.81 to 1.59.

Associations Between Axial Length and Subfoveal Choroidal Thickness. Figure 5 illustrates the significant association between longer AL and thinner SFChT, with a significant interaction between SFChT, sex group, and age groups ($n = 79$, adjusted $R^2 = 0.40$, $P < 0.001$). Figure 6 illustrates the significant association

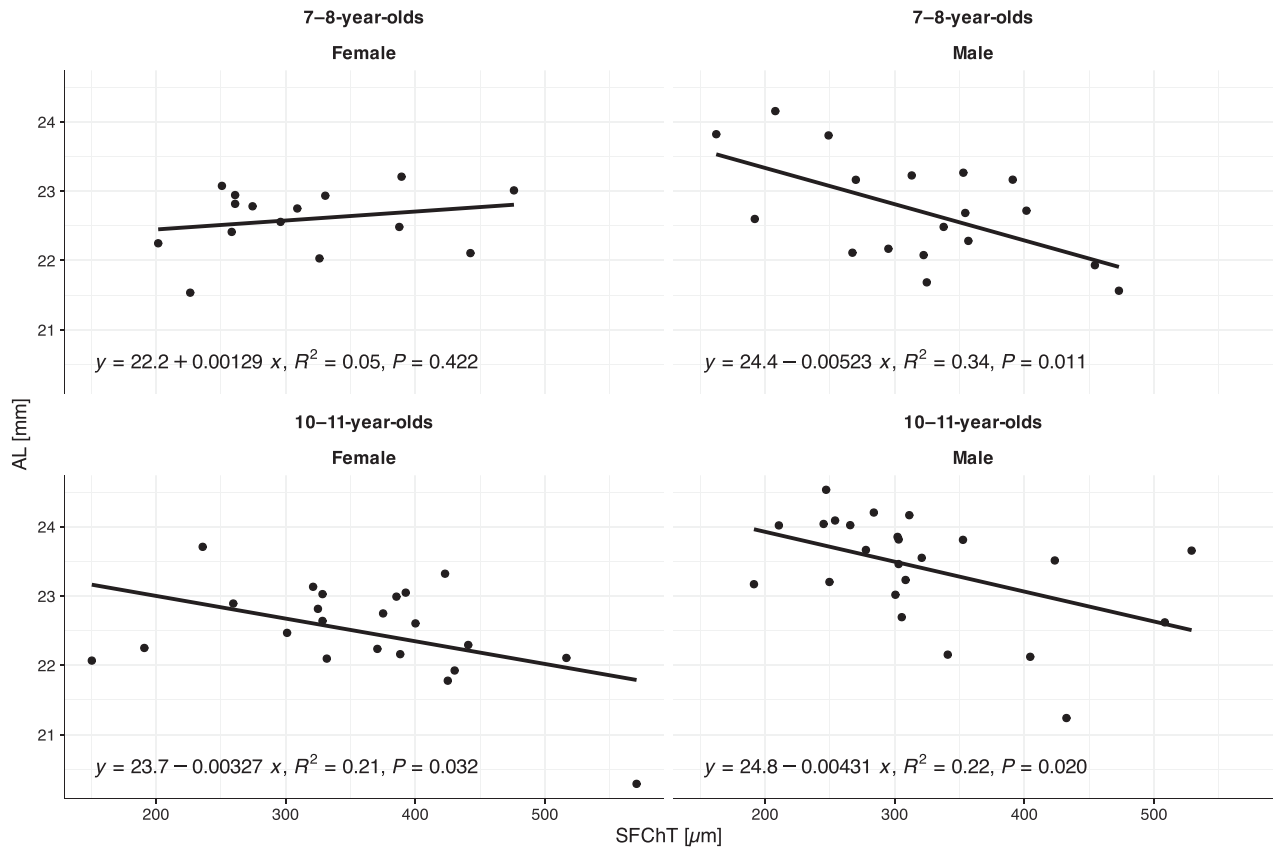

Figure 5. Association between AL and SFChT by sex and age groups in autumn (baseline).

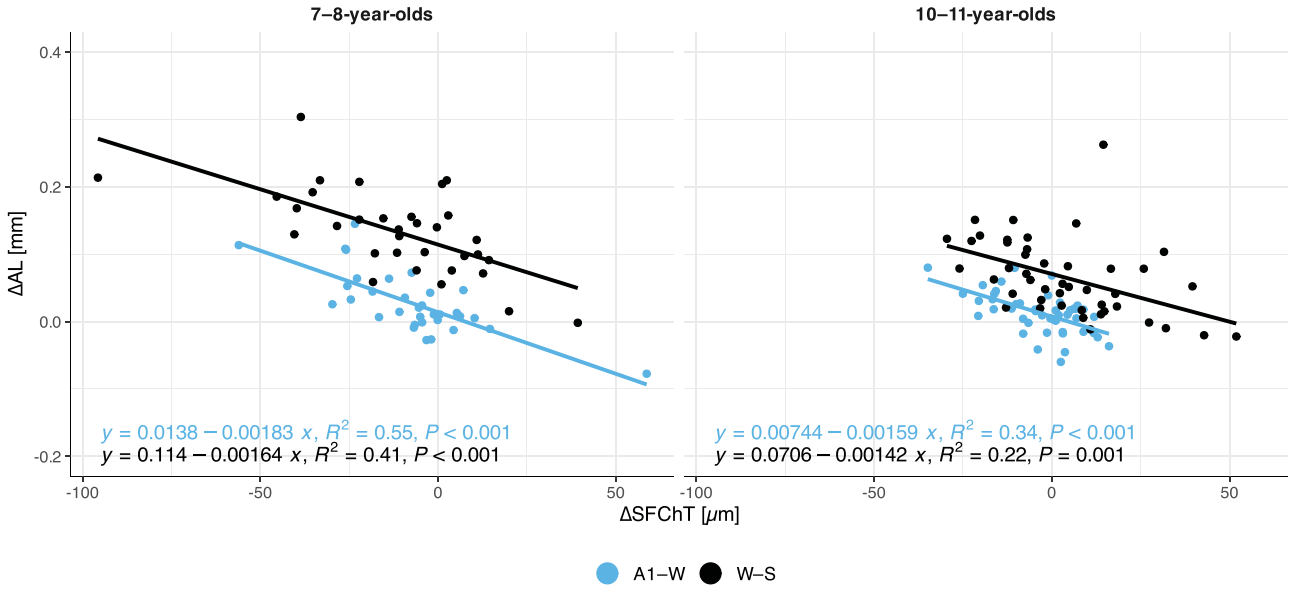

Figure 6. Association between change in axial length (ΔAL) and change in subfoveal choroidal thickness (ΔSFChT) over autumn–winter (A1 to W) and over winter–spring (W to S). Data from autumn 2 were not included since choroidal thickness was not obtained during that data collection (see Methods section).

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between ΔAL and ΔSFChT, with a significant interaction between age group and season ($n = 79$, adjusted $R^2 = 0.65$, $P < 0.001$) over autumn–winter and winter–spring for both age groups. There was no association between ΔAL and SFChT over autumn–winter or winter–spring.

Seasonal Variations in Body Height

The linear mixed-effects model showed a significant increase in body height for both females and males in each age group over all three seasons and annually ($F(12, 182) = 31.78$, $P < 0.001$, Holm adjusted $P < 0.005$), but no interactions between season and sex group or season and age group. There was also no association with change in AL and change in height, for either season or annually.

Discussion

In this study, we measured ocular parameters in school children aged 7 to 11 years to assess differences across three seasons (autumn–winter, winter–spring, and summer–autumn) and annually, at a latitude (60°N) where there are considerable differences in available daylight over the year. The results show that children experience ocular growth throughout the year, but the rate of growth slows down during the summer–autumn season. This was the case not only in children who had *myopia/risk of myopia* but also in those who had *mild* and *significant hyperopia*. It was assumed that *mild hyperopes* (cycloplegic SER +0.50 to +2.00 D 7–8 years and +0.25 to +2.00 D 9–10 years)[31,49] would experience only physiological ocular growth. The results for this SER group show that there were age and sex differences in physiological ocular growth: (1) the 7- to 8-year-olds had a larger annual AL growth than 10- to 11-year-olds, which was associated with faster AL growth over the winter–spring seasons; (2) males had larger annual AL growth than females, independent of age and season; and (3) AL growth was unrelated to an increase in body height. This coincided with 7- to 8-year-old *mild hyperopes* having a mean annual change in *adjusted* SER of −0.29 D and no associated annual change in SER in the older group. The 7- to 8-year-old *mild hyperopes* also experienced the largest decrease in choroidal thickness over the winter–spring seasons. Similar patterns of change in choroidal thickness were observed in children with *myopia/risk of myopia* and *significant hyperopia*.

Seasonal Change in Physiological Ocular Growth

That the rate of physiological ocular growth in mild hyperopes was found to be higher over the winter–spring period compared with summer–autumn resembled that reported for myopic children.[12–16] Slowed growth rate over the summer has been associated with an increase in available daylight hours,[15,16] which can be paralleled with the reported protective effects of outdoor time against myopia development.[1,3–5] The protective effects of daylight have been hypothesized to be related to its different characteristics (e.g., intensity, spectral composition) compared with indoor electric light. Indeed, exposure to high-intensity illumination has been shown to be critical for optimal refractive development in rhesus monkeys.[6] What is noteworthy here is that children experienced more physiological growth during the winter—a period when they continue to experience a minimum of 45 minutes of outdoor time during the school day, and this amount of additional daylight exposure has been reported to have a protective effect against myopia.[4,5] Though the children in our study had 4 days more at school per month in winter (Table 1), at this latitude, the solar elevation angle (α) is $0° < \alpha < 20°$ between 06:30 and 16:00 from November to the end of February.[52] Published data on the spectral composition of daylight from Helsinki, which is at the same latitude as Kongsberg, show that in winter, daylight is both of lower intensity and the spectral composition is blue skewed (blue/green and blue/red ratios >1).[53] From May to the end of August, the daylight intensity is much higher and the spectral composition is balanced over the same time of day when $\alpha > 20°$ (06:30–16:00) and of lower intensity and becoming blue skewed in the evening when $0° < \alpha < 20°$.[52,53] Exposure to high-intensity polychromatic daylight, and in particular the short-wavelength part of the spectrum, activates intrinsically photosensitive retinal ganglion cells,[54–57] positively affecting diurnal rhythms and dopamine release (if exposure is during morning and day).[55,58,59] Normal melanopsin signaling through modulation of dopaminergic activity plays important roles for the development of the retinal clock network in mice[60] and, when disturbed, linked with myopia.[61] In combination with differences in daylight intensity and spectral composition, the children may also spend more time outdoors on nonschool days in summer. As actual outdoor time was not measured in this study, it was not possible to assess to what degree 4 more nonschool days per month (averaged over the summer, Table 1) may also have contributed to the slowed eye growth observed in summer.

The Relationship Between Choroidal Thickness and Axial Length

In agreement with previous reports, the different areas of the choroid varied in thickness and were overall similar to that reported earlier in children.[62] The thickness asymmetries in nasal and temporal choroidal areas, in which thinner temporal areas were associated with more physiological ocular growth (Fig. 4, Supplementary Table S3), is the same as that reported for Chinese myopic 12- to 13-year-olds by Tian et al.[63] They did not find this association for the non-myopes, but the range of refractive errors included in their cohort is not given, so it is difficult to relate directly to our data on mild hyperopes. Furthermore, significant inverse associations were observed between baseline AL and SFChT (Fig. 5) and between ΔAL and ΔSFChT, with the strongest association for the youngest children (Fig. 6).[24–26] The results concur with an association between the choroid and physiological ocular axial elongation, both during emmetropization and for maintaining emmetropia. In the younger group, the choroid continues to thin in parallel with continuous physiological axial elongation. In the older group, the choroid is thicker and physiological axial elongation has slowed down. The choroid appears to undergo a thickening process well into adolescence.[62,64] Here, the SFChT was on average 23 µm thicker in the 10- to 11-year-old mild hyperopes (Table 3), with minimal changes over the winter (Table 4). The difference between the younger and older group amounts to an increase in SFChT of 7 to 9 µm/year, which is comparable to that reported in other studies.[24,25] We surmise that the observed choroidal thickening in 10- to 11-year-old mild

Table 4. Seasonal and Annual Changes in AL, *Adjusted* SER, and Choroidal Thickness by Age Group

	7- to 8-Year-Olds					10- to 11-Year-Olds				
Characteristic	***n***	**Mean**	**SD**	**Median**	**Range**	***n***	**Mean**	**SD**	**Median**	**Range**
ΔAL (mm)										
Significant hyperopia										
A1–W	9	0.006	0.010	0.005	−0.013 to 0.024	5	−0.015	0.039	−0.016	−0.06 to 0.039
W–S	9	0.133	0.060	0.153	0.015 to 0.192	5	0.094	0.053	0.121	0.032 to 0.151
S–A2	9	0.080	0.046	0.082	−0.019 to 0.128	5	0.055	0.061	0.060	−0.021 to 0.129
Annual	9	0.219	0.080	0.260	0.101 to 0.312	5	0.134	0.129	0.086	0.002 to 0.286
Mild hyperopia										
A1–W	30	0.027	0.042	0.025	−0.077 to 0.113	35	0.015	0.026	0.018	−0.037 to 0.08
W–S	30	0.135	0.066	0.137	−0.002 to 0.304	35	0.054	0.046	0.052	−0.022 to 0.146
S–A2	30	0.047	0.043	0.041	−0.054 to 0.148	35	0.040	0.023	0.037	−0.011 to 0.088
Annual	30	0.209	0.080	0.200	0.106 to 0.505	35	0.108	0.056	0.104	0.015 to 0.231
Myopia/risk of myopia										
A1–W	2	0.109	0.051	0.109	0.073 to 0.145	3	0.035	0.039	0.016	0.009 to 0.08
W–S	2	0.090	0.044	0.090	0.059 to 0.121	3	0.149	0.099	0.107	0.078 to 0.262
S–A2	2	0.069	0.012	0.069	0.06 to 0.077	3	0.040	0.060	0.013	−0.002 to 0.109
Annual	2	0.267	0.108	0.267	0.191 to 0.343	3	0.224	0.196	0.114	0.108 to 0.451
Δ*Adjusted* SER (D)										
Significant hyperopia										
A1–W	9	−0.03	0.39	−0.02	−0.86 to 0.42	5	0.01	0.11	0.04	−0.15 to 0.13
W–S	9	−0.08	0.25	−0.09	−0.39 to 0.46	5	−0.05	0.29	0.00	−0.45 to 0.31
S–A2	9	−0.11	0.35	−0.15	−0.86 to 0.41	5	0.44	1.31	0.06	−0.43 to 2.73
Annual	9	−0.22	0.27	−0.25	−0.55 to 0.19	5	0.40	1.37	−0.19	−0.35 to 2.85
Mild hyperopia										
A1–W	30	−0.04	0.27	−0.03	−0.53 to 0.55	35	0.01	0.19	0.04	−0.34 to 0.35
W–S	30	−0.16	0.23	−0.17	−0.71 to 0.58	35	−0.04	0.17	−0.05	−0.31 to 0.32
S–A2	30	−0.09	0.24	−0.04	−0.87 to 0.26	35	−0.04	0.15	−0.03	−0.37 to 0.31
Annual	30	−0.29	0.22	−0.26	−0.82 to 0.07	35	−0.07	0.18	−0.10	−0.46 to 0.37
Myopia/risk of myopia										
A1–W	2	−0.21	0.12	−0.21	−0.3 to −0.12	3	0.07	0.16	0.04	−0.07 to 0.25
W–S	2	0.06	0.48	0.06	−0.28 to 0.4	3	−0.36	0.13	−0.37	−0.48 to −0.22
S–A2	2	−0.23	0.02	−0.23	−0.25 to −0.22	3	−0.08	0.17	−0.10	−0.24 to 0.09
Annual	2	−0.38	0.62	−0.38	−0.82 to 0.06	3	−0.37	0.38	−0.24	−0.8 to −0.07
ΔSFChT (μm)										
Significant hyperopia										
A1–W	6	−1	9	0	−17 to 10	6	1	3	1	−4 to 4
W–S	6	−18	20	−17	−45 to 4	6	−5	17	−7	−29 to 18
Mild hyperopia										
A1–W	25	−8	21	−7	−56 to 59	37	−4	12	−1	−35 to 16
W–S	25	−11	27	−6	−96 to 39	37	5	19	3	−26 to 52
Myopia/risk of myopia										
A1–W	2	−15	11	−15	−23 to −7	3	−4	6	−3	−10 to 1
W–S	2	−4	21	−4	−18 to 11	3	11	17	15	−7 to 26

Refractive error grouping is the same as in Table 3. Only children who completed all four measurements were included for AL ($n = 84$) and *adjusted* SER ($n = 84$) and three measurements for SFChT ($n = 79$).

hyperopic eyes is a signature of continued, but slowed, physiological ocular growth for maintaining the refractive error.[65] Choroidal thickening is associated with an increase in choroidal blood flow and increased levels of oxygen and nutrient supply that alter scleral remodeling and growth.[27] Indeed, it has been shown in animal models of myopia that increased choroidal oxygen supply inhibits the hypoxia inducible factor 1α signaling pathway[66] that is activated during accelerated ocular growth.[67] Thus, if myopia onset is a failure to maintain emmetropia/mild hyperopia, then this may be a failure in coordination between the choroid's developmental process, whereby thinning stimulates and thickening inhibits the choroid's response to visual experience that might stimulate ocular growth and development of the ocular components of the eye required for a lasting emmetropic eye.[65,68]

Annual Changes in Physiological Ocular Growth and Spherical Equivalent Refractive Error

The annual change in AL for those undergoing physiological ocular growth was 0.21 mm and 0.11 mm for 7- to 8-year-olds and 10- to 11-year-olds, respectively (*mild hyperopia* group in Table 4). This is comparable to the average annual axial elongation reported for emmetropic children from age 6 to 9 years in the Netherlands (0.19 mm/year).[69] Corneal radii appear to change very little after early childhood,[68–71] which was similar to our results (average annual change of 0.007 mm, results not shown). The larger annual AL change in the 7- to 8-year-old children corresponded with a significant annual decrease in *adjusted* SER (Table 4). That the annual rate of physiological ocular growth has been reported to slow down from after the age of 9

(cf. Table 4 and Figure 3D in Zadnik et al.[29]) is corroborated by our results for 10- to 11-year-olds. The continued, but slowed, AL growth without an associated change in SER for the older children indicates that they have entered a phase whereby they maintain their emmetropia/mild refractive error (through a coordinated decrease in crystalline lens power[30,72]). When active emmetropization completes and transitions to maintenance of a mild refractive error, however, varies between individuals. First, we observed that the 7- to 8-year-old males had a larger decrease in SER (but same increase in AL) than their female peers. Thus, that females appear to exhibit faster myopic progression at an earlier age (measured by SER) than their male peers[73,74] could be due to emmetropization completing, on average, at an earlier age for females. Second, albeit with a small sample (Table 4 and Fig. 3), some significant hyperopes have a continued decrease in SER and more-than-physiological AL growth at ages 10 to 11 (but their growth pattern appears to be different from that of emmetropes and myopes[28]). Both instances were associated with thinning of the choroid.

Physiological ocular growth is desirable as part of emmetropization (for a review, see Flitcroft[28]) and as part of coordinated growth for maintaining a mild refractive error throughout adolescence.[30] The bulk of this growth happens in winter, and it seems that for emmetropization to complete around mild hyperopia,[31] slowing of growth is needed in summer. To maintain physiological rather than accelerated growth from winter to spring, the slowing of ocular growth needs to be accompanied by development of a thicker and more resilient choroid[27,30,72–74] over the summer–autumn. This resilience appears to decay, as the monthly AL increase was slower over autumn–winter compared with winter–spring (Fig. 3).

Sex Differences in Ocular Biometry

At baseline (Table 3), there were no differences in AL between males and females aged 7 to 8 years, but the 10- to 11-year-old males had almost 1-mm longer eyes than peer females. Males also had significantly flatter corneal radii than females (0.06–0.27 mm), resulting in no differences in SER between sexes. In the Generation R study,[69] males had a significantly longer eye and flatter corneas than females at both 6 years (0.5 and 0.14 mm) and at 9 years (0.52 and 0.13 mm), but their sample included also hyperopes and myopes (cf. their Table 2).

There were no seasonal differences in AL elongation between the sexes, but as observed in a study including children aged 10 to 15,[32] males had a small but significantly larger annual change in physiological ocular growth than females (0.034 mm), independent of age.

Body Height

In line with previous reports,[75–77] a significant but weak association between baseline body height and AL was observed. The annual increase in body height was as expected from reported growth curves for Norwegian 7- to 11-year-old children.[78] There was no association between ΔAL and Δbody height as reported in another study on primary school children.[79]

Strengths and Limitations

The study benefited from the cohort having mandatory outdoor time during recess every school day irrespective of season. Though daylight exposure was not measured, all would have had a minimum of 45 minutes of daylight exposure every school day. Thus, when we use recess time as a proxy, all would have exceeded the 40 minutes of outdoor time per day reported to decrease myopia incidence.[5] Considering additional outdoor time when walking/cycling to and from school and some outdoor time during the BAS program, it is not unreasonable to assume that most children would have had 1 to 2 hours of outdoor daylight exposure every school day throughout the year. It is a limitation that we did not obtain objective measurements of personal light exposure. This would be needed to quantify differences in (1) dose–response (intensity × duration) variation in winter versus summer and (2) exposure to the shorter wavelengths of the spectrum in the evening over spring–summer, like that reported for adults.[80]

Another strength is that each child was measured over a 30-minute period within a 3.5-hour window around midday to account for any diurnal variation. However, prior to measurement, children went about their school day as normal, including outdoor recess. Since, at that time of the day, any child would not have been outdoors for more than 30 minutes and would have spent up to 30 minutes in the measurement room with light levels below 110 lux during the 15 minutes prior to OCT imaging, choroidal thinning as a result of short-term outdoor time should have been neutralized.[23]

The study was limited by only having a single cycloplegic autorefraction measurement. Taking cycloplegic measurements at all time points was considered too disruptive to the children's school day and would have been impractical during COVID-19. Crystalline lens power and refractive errors with cycloplegia are important when assessing changes during ocular development, as uncontrolled accommodation can contribute to measuring more negative values of SER.[81–83] To circumvent this, we modeled *adjusted SER* based on the measured cycloplegic SER with AL/CR, non-cycloplegic SER, and age as predictors. This resulted in reasonable estimates of changes in SER.[68]

Another limitation is the small number of children with *myopia/risk of myopia* and *significant hyperopia*. The frequencies of refractive errors, however, are in line with that reported for children and adolescents in this region of the world.[7–9] Additionally, that 3 of the 6 myopes (−0.50 to −1.25 D) and 11 of the 15 significant hyperopes (+2.00 to +3.00D) were uncorrected during the parts of the study limits the generalizability of the results for these refractive error groups. Previous studies have reported that both accommodation[84] and defocus[41] can influence choroidal thickness, potentially affecting the uncorrected hyperopes and the uncorrected myopes, respectively.

It is unlikely that the short COVID-19 lockdown with homeschooling that included outdoor recess, when compared with lockdowns in other countries,[85] would have affected the measurements in June 2020 and November 2020. Thus, observed changes of the mild hyperopes appear to be related to physiological ocular growth. COVID-19 restrictions did prevent the collection of OCT measurements at the last time point (November 2020), preventing assessment of changes in ChT over the summer–autumn season and annually.

Lastly, we used Zadnik's age-sensitive cutoff points for SER[49] to assess physiological ocular growth (*mild hyperopes*, assuming that this is a more natural endpoint for emmetropization)[31] and to identify children with myopia risk. This resulted in a higher threshold for SER than typically used for emmetropia ($-0.50 < SER < +0.50$), reducing the likelihood that those assumed to experience physiological ocular growth would have been pre-myopic at baseline.[86]

Conclusions

There were significant seasonal and annual changes in AL in children irrespective of refractive error, notably in children assumed to experience only normal physiological eye growth irrespective of age. The results confirm that the time of year and the frequency at which children have eye examinations are important factors when assessing myopia risk and scheduling of any needed myopia control treatment.[15] Annual changes in AL were smaller and the choroid was thicker in 10- to 11-year-old *mild hyperopes*. Annual decline in SER and seasonal ChT thinning were observed in 7- to 8-year-old but not 10- to 11-year-old *mild hyperopes*, supporting the notion that the 7- to 8-year-olds are still undergoing emmetropization, while the 10- to 11-year-olds have transitioned to maintaining emmetropia. That mild hyperopes have more ocular growth over winter suggests that human physiological ocular growth may follow a seasonal cycle linked with the availability and variability in intensity and spectral composition of daylight.

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