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ORIGINAL RESEARCH

The Macular Choroidal Thickness in Danish Children with Myopia After Two-Year Low-Dose Atropine and One-Year Wash-Out: A Placebo-Controlled, Randomized Clinical Trial

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ABSTRACT

Introduction: Our aim in this work was to investigate the macular choroidal thickness (ChT) changes in 6–12-year-old Danish children with myopia during 2 years of low-dose atropine treatment and 1-year wash-out vs. placebo in an investigator-initiated, placebo-controlled, double-blind randomized clinical trial.

Methods: Ninety-seven participants were randomized to either 0.01% for 2 years, 0.1% loading dose for 6 months followed by 0.01% for 18 months, or placebo, then a 1-year wash-out. The primary outcome was ChT in the sub-foveal and inner and outer superior, nasal, inferior, and temporal sectors. The secondary outcome was axial length (AL). Outcomes were measured at baseline and 6, 12, 24, and 36 months. One-way analysis of variance was used to detect baseline ChT differences between AL-stratified groups (< 24 mm, 24–25 mm, or > 25 mm). To determine the longitudinal changes in ChT and its effect on AL, all eyes were included in linear mixed modeling with individual eyes nested in the study ID as a random effect.

Results: Longer eyes had significantly thinner ChT in all choroidal sectors (adj-$P < 0.01$) at baseline. There was no statistically significant change in any ChT sector after 3 years in the placebo group. Sub-foveal and nasal ChT in the 0.1% loading dose and 0.01% group were not significantly different from placebo after 2-year treatment. In the placebo group, a 1-mm increase in AL was significantly associated with a 47-µm thinner nasal ChT after 3 years (95% confidence interval (CI): – 55; – 38, adj-$P < 0.001$). A 10-µm thicker nasal choroid at baseline was associated with 0.13 mm (95% CI: 0.009; 0.017, adj-$P < 0.001$) less 3-year axial elongation.

Conclusions: The ChT in Danish children with myopia remained stable over the 3-year follow-up. A thinner choroid at myopia onset might predispose to increased axial elongation. Treatment with 0.01% atropine did not change the ChT. We speculate that low-dose atropine does not primarily reduce myopia progression via a choroidal mechanism.

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Keywords: Atropine; Axial length; Choroid; Choroidal thickness; Eye drops; Macula; Myopia; Myopia control

Key Summary Points
The choroid has been found to thin in myopes during childhood, and a thinning choroid is thought to play a part in myopia development.
While higher concentrations of atropine promisingly have been found to thicken the sub-foveal choroid, less is known about the effects of low-dose atropine and choroidal thickness (ChT) changes in other macular sectors.
This study examined whether low-dose atropine treatment affected the macular ChT after a 2-year treatment followed by a 1-year wash-out in placebo-controlled Danish children with myopia.
The ChT remained stable over a 3-year follow-up and treatment with 0.01% low-dose atropine did not significantly increase the ChT compared to placebo.
Speculatively, low-dose atropine does not seem to primarily reduce myopia progression via a choroidal thickening mechanism.

INTRODUCTION

The choroid is the vascularized layer between the sclera and retina of the eye and serves as an important structure responsible for blood supply to the outer retina [1, 2]. The thickness of the choroid is dynamic and has been found to vary depending on diurnal rhythm [3], exertion [4], and accommodation of the eye [5]. The choroid in patients with myopia is generally thinner than in emmetropes [2, 6, 7], but whether the choroid just thins passively as a result of axial elongation-induced stretching, or the choroid actively contributes to faster myopia progression, is not clear. Thinning of the choroid might lead to impaired choroidal perfusion, which could potentially increase the risk of myopia-related complications later in life, such as sight-threatening chorioretinal atrophy [8].

Myopia control methods, such as orthokeratology lenses, have been found to counteract choroidal thinning by a thickening effect [9–11]. This choroidal thickening effect has also been observed in treatments with higher concentrations of atropine eye drops [12–14]. Atropine is a muscarinic receptor antagonist [15] and a recent study indicates that atropine might partially exert its myopia control effect via inhibition of serotonergic receptors [16]. Atropine has a proven dose-dependent reductive effect on myopia progression and few side effects at the lower concentrations that are most commonly prescribed [17, 18]. However, the effect on the choroidal thickness (ChT) of lower dosages of atropine is uncertain. While some studies have found a choroidal thickening effect of low-dose atropine [13, 19], the choroidal thickening effect of atropine has not been reproduced in all studies [20, 21], and a recent meta-analysis failed to find a choroidal thickening effect of 0.01% low-dose atropine [9].

As the eye elongates, the choroid is thought to stretch preferentially in a horizontal direction, increasing the optic disc–fovea distance [22]. One cross-sectional study found that with increasing diopters, the most marked choroidal thinning was observed in the nasal region [23]. Nevertheless, studies on the choroidal thickening effect of atropine have primarily examined the sub-foveal sector of the choroid [13, 19–21, 24], whereas the effect of atropine on the peri- and para-foveal regions of the macula, including the nasal region, have received less attention. Of the atropine studies examining ChT changes across all sectors [12, 19, 21], one study found that peri- and para-foveal ChT increases [19], in contrast to another study that found peri- and para-foveal thinning after 0.01% atropine treatment compared to baseline [21]. Most studies examining the effect of atropine on

ChT in sectors apart from the sub-foveal region lack placebo-control groups. All examinations were seemingly performed after eye dilation, which is known to increase the ChT [25], and all have follow-up times of 6 months or less. In addition, all of the studies examined Asian children with myopia, where choroidal uptake of atropine might be reduced compared to other ethnicities because of more pigmented irises [26, 27]. Since ChT changes could plausibly be used as a measure of myopia control efficacy [13], mapping of atropine-induced sectoral ChT changes is relevant for all ethnicities.

In this study, we examined baseline differences in ChT between groups of 6 to 12-year-old Danish children with myopia stratified by axial length (AL) at baseline. We also examined the 3-year follow-up sectoral changes in ChT in these participants for both the active intervention groups receiving low-dose atropine treatment, and participants receiving placebo.

METHODS

Study Design, Eligibility Criteria, and Settings

The study was an investigator-initiated, double-blind, randomized clinical trial with a 1:1:1 allocation ratio examining macular ChT sectoral changes in 6 to 12-year-old children with myopia receiving low-dose atropine treatment during a 2-year follow-up and 1 year of subsequent wash-out. Eligibility criteria were a spherical power of < − 1 diopter (D) in one eye for children below 9 years of age and a spherical power of < − 2 D for children between 9 and 12 years. Children with astigmatism more severe than < − 1.5 cylindrical D were excluded. Previous use of myopia control or other ocular pathologies present at screening were also reasons for exclusion. Examinations were performed at the ophthalmological departments at Aarhus University Hospital, University Hospital of Southern Denmark – Vejle Hospital, and Rigshospitalet-Glostrup, located in different parts of Denmark. A full list of exclusion criteria, and a more thorough description of the study design, are available in our previous analysis [28].

Ethics Approval and Trial Registration

Trial approval was obtained from the Danish Medicines Agency (reference number: 2018040088), the Danish Data Protection Agency via the Capital Region of Denmark (reference: P-2022-85), and the Committees on Health Research Ethics in the Capital Region of Denmark (reference number: H-18043987). The study adhered to the Declaration of Helsinki. All parents of the study participants provided written informed consent and study participants consented verbally. Good Clinical Practice (GCP) units at the individual hospitals monitored the quality of the study and adherence to GCP standards throughout the study period. Before commencement, the trial was registered in both the European Clinical Trials Database (EudraCT, 2018-001286-16) and at clinicaltrials.gov (NCT03911271).

Intervention

Participants were randomized to either 0.1% loading dose for 6 months followed by 18 months of 0.01% vs. 0.01% for 2 years vs. placebo for 2 years. All groups then underwent 1-year wash-out. Randomization was accomplished via a computer algorithm. Eye drops were applied once each night before bedtime and application was assured by parents filling out a daily checklist.

Outcomes

The primary outcome of this post hoc analysis was macular segmental ChT measured via optical coherence tomography (OCT) scan at baseline, and at 6, 12, 24, and 36 months on both eyes without the use of dilating eye drops. Macular choroidal sectors measured were the sub-foveal and peri- and para-foveal superior, nasal, inferior, and temporal segments. Secondary outcome was the effect of the macular nasal ChT on axial elongation after 3 years.

Measurements

A 7.0 × 7.0 mm horizontal macular OCT scan was performed by swept-source OCT (Topcon Europe Medical BV, Capelle aan den Ijssel, The Netherlands). The choroid was automatically segmented via the built-in application into an ETDRS grid with a 1.0-mm central sub-foveal, 3.0-mm peri-foveal, and a 6.0-mm para-foveal ring consisting of both a peri-foveal and para-foveal sector in the superior, nasal, inferior, and temporal segments (see Fig. 1). In cases of mis-segmentation, the choroid was manually segmented by experienced examiners (A.H–H. and N.C.H.) masked to randomization status. OCT examinations were conducted principally between 9:30 am and 1:30 pm to minimize the known diurnal variation in ChT [3]. AL measurements were obtained via an optical biometric device (IOLMaster 700, Carl Zeiss AG, Oberkochen, Germany). Examinations were performed without administration of cycloplegic agents (apart from the nightly administration of 0.01% atropine) to avoid confounding since cycloplegic agents have been found to significantly increase the ChT [14]. The refractive error was measured by autorefraction (Right group, Retinomax K-plus 3, Tokyo, Japan) in cycloplegia at the screening visit to determine whether the inclusion criteria of myopia in at least one eye were met.

Sample Size

Since the main trial outcome was to examine the efficacy and safety of low-dose atropine for reducing myopia progression, the power calculation was based on the reported SER

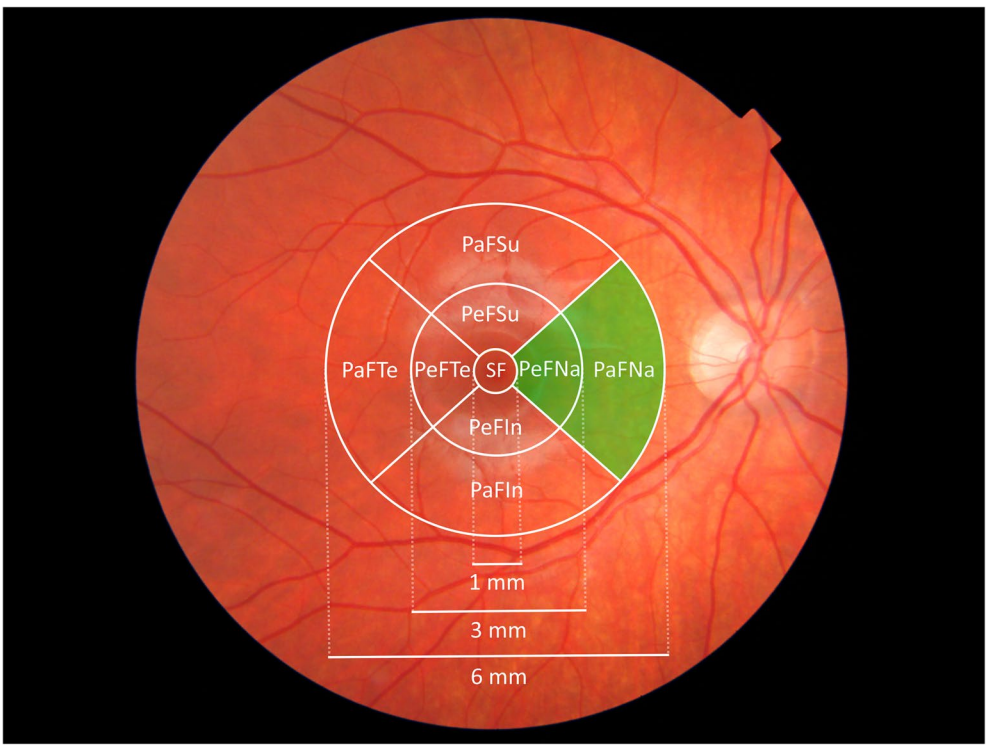

Fig. 1 Example of 6-mm ETDRS grid. An average of the two nasal sectors (green sectors, PeFNa + PaFNa) was used for the linear mixed-model analysis examining the association between axial length and choroidal thickness. *PaFIn* para-foveal inferior sector, *PeFIn* peri-foveal inferior sector, *PaFNa* para-foveal nasal sector, *PeFNa* peri-foveal nasal sector, *PaFSu* para-foveal superior sector, *PeFSu* peri-foveal superior sector, *PaFTe* para-foveal temporal sector, *PeFTe* peri-foveal temporal sector, *SF* sub-foveal sector

progression in a previous study on Danish school children with myopia [29], and not ChT, the primary outcome of this post hoc analysis. To detect a 50% reduction in myopia progression after 36 months of treatment with a significance level of 0.05 and a power of 80%, 21 participants needed to be enrolled in each interventional arm. We accounted for an, at the time, unknown effect size of low-dose atropine treatment in Danish children, study length, and potential drop-out by recruiting extra study participants.

Statistical Methods

Baseline same-sector ChT measurements as a mean of both eyes were compared between three AL-stratified groups by one-way analysis of variance (ANOVA) to examine differences in ChT based on myopia degree. The AL intervals were < 24 mm vs. 24–25 mm vs. > 25 mm. A constrained linear mixed model (LMM) was used to examine how 0.01% and 0.1% loading dose low-dose atropine affected separate macular sectors over 3 years. We assumed an unstructured covariance to achieve the best possible model fit. The treatment site was added as a random effect to account for potential differences between sites. Individual study eyes were nested by participant ID as unique samples and added as a random effect to be able to include both eyes in the linear mixed-model analysis. A second constrained LMM was constructed to analyze how changes in the nasal ChT affected AL progression over 3 years. Analyses were performed with intention-to-treat. Statistical modeling was performed using the R statistical software version 4.2.0 [30] (R Program for Statistical Computing, Vienna, Austria) and LMMstar addon package [31]. *P* values were adjusted for multiple testing using the false discovery rate [32]. Effect estimates with an adjusted *P* value (adj-*P*) < 0.05 were considered statistically significant.

RESULTS

A total of 124 children were screened for eligibility (Fig. 2). Of these, 97 participants were randomized to 0.01% atropine ($N = 32$), 0.1% loading dose ($N = 33$), or placebo ($N = 32$). After randomization, six participants were excluded: Two participants wanted to use another myopia control measure, one participant emigrated to another country after the 1-year visit, one participant was lost to follow-up after the 18-month visit, and two participants withdrew consent. Recruitment and follow-up occurred between 2019 and 2024 (see Table 1 for baseline characteristics).

Baseline Sectoral Choroidal Thickness and Differences Between Axial Length Groups

At baseline, the choroidal sectors were thinnest at the nasal, then the sub-foveal, inferior, superior, and temporal sectors, in that order (Table 1). When comparing participants with < 24 mm, 24–25 mm, and > 25 mm AL at baseline, the macular ChT in all sectors were significantly different from each other between groups, with the > 25 mm group universally having the thinnest sectors (Table 1 – Choroidal thickness by AL group; one-way ANOVA, adj-$P < 0.01$ for all same-sector comparisons).

Change in Sectoral Choroidal Thickness over 3 Years

Sectoral ChT changes over 3-year follow-up compared to baseline in all groups are presented in Fig. 3. There were no statistically significant differences from baseline in any of the sectors. Adjusting the model for baseline age did not change the sectoral effect sizes or significance levels.

Effect of 0.01% and 0.1% Loading Dose Low-Dose Atropine on Sub-Foveal and Nasal Choroidal Thickness over 3 Years

Neither sub-foveal or nasal ChT in the 0.01% or 0.1% loading dose groups differed from placebo after 2 years of treatment (sub-foveal: 0.1% loading dose, adj-$P = 0.55$; 0.01%, adj-$P = 0.39$; and nasal: 0.1% loading dose, adj-$P = 0.43$; 0.01%, adj-$P = 0.81$, respectively), or after the final (third) year of wash-out (sub-foveal: 0.1%

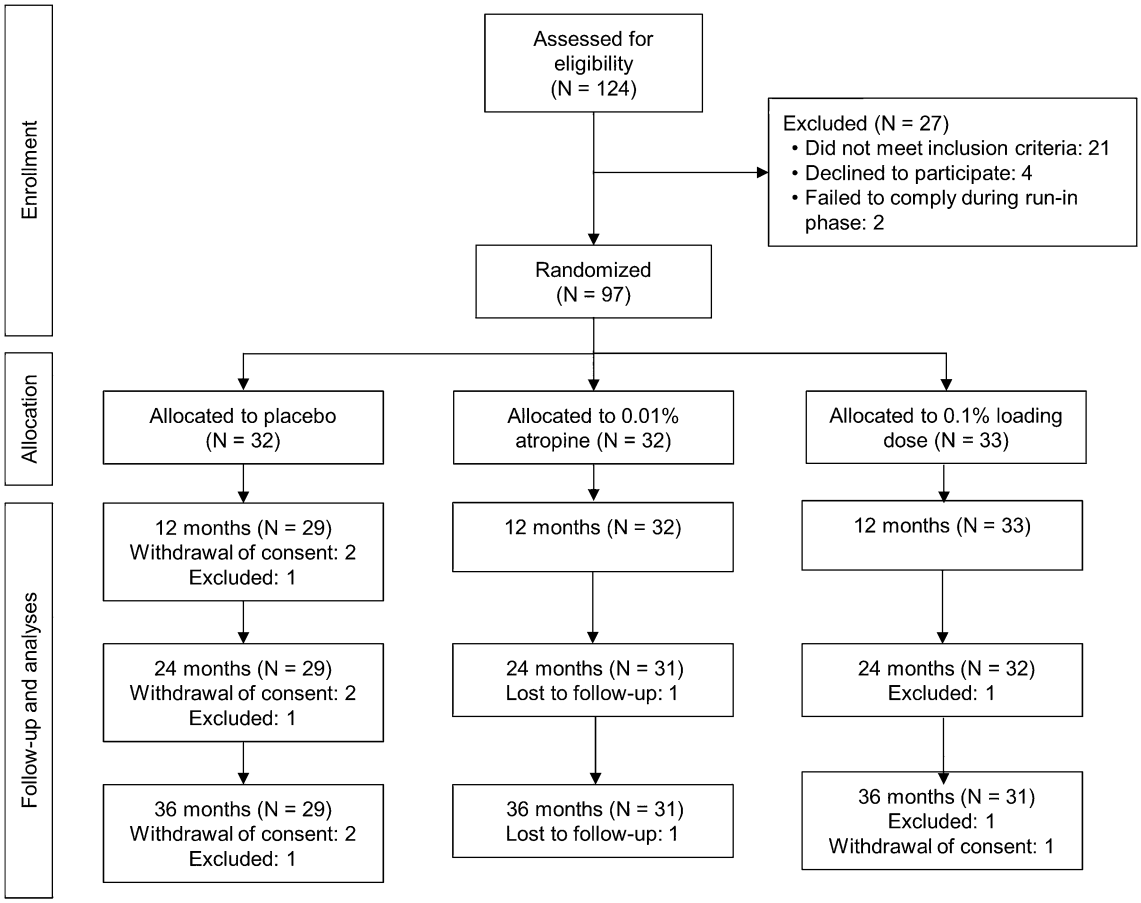

Fig. 2 Consorted standards of reporting flow chart of the study

loading dose, adj-$P = 0.93$; 0.01%, adj-$P = 0.93$; and nasal: 0.1% loading dose, adj-$P = 0.93$; 0.01%, adj-$P = 0.98$, respectively).

The Association of Choroidal Thickness with Axial Elongation

Over 3 years in the placebo group, a 1-mm increase in AL was associated with a statistically significant 47-µm (95% confidence interval (95% CI): – 55; – 38, adj-$P < 0.001$) decrease in nasal ChT. Having a 10-µm thicker nasal ChT at baseline was associated with 0.13 mm (95% CI: 0.009; 0.017, adj-$P < 0.001$) less axial elongation increase over 3 years.

DISCUSSION

This is to our knowledge the first study examining ChT changes over time in non-dilated children with myopia randomized to low-dose atropine treatment versus placebo. Since the choroid has been found to thicken in response to cycloplegic agents [25], examination in non-dilated eyes is the most accurate way of measuring ChT changes over time. We found that higher AL was associated with a thinner choroid in all macular sectors at baseline. The ChT did not statistically significantly change in any sector over 3 years in the placebo group. The ChT was not statistically significantly different for low-dose atropine-treated eyes compared to placebo after 2 years of

Table 1 Baseline characteristics and baseline axial length group comparison of sectoral choroidal thickness

Baseline characteristics			
Parameter			**Mean (95% CI)**
Age, years			9.4 (range, 6–12)
Axial length, mm			24.6 (24.4; 24.9)
Spherical equivalent refraction, D			− 2.99 (− 3.37; − 2.60)
Sex, boy/girl			42/55
Choroidal thickness			
Sub-foveal, µm			248 (235; 261)
Outer superior, µm			258 (247; 269)
Inner superior, µm			260 (248; 271)
Outer nasal, µm			164 (153; 174)
Inner nasal, µm			212 (200; 225)
Outer inferior, µm			246 (234; 257)
Inner inferior, µm			249 (236; 262)
Outer temporal, µm			258 (247; 269)
Inner temporal, µm			264 (251; 276)
Choroidal thickness by axial length group	**< 24 mm** ***N*** **= 30**	**24–25 mm** ***N*** **= 43**	**> 25 mm** ***N*** **= 24**
Sub-foveal, µm	291 (267; 315)	240 (222; 257)	209 (185; 232)
Outer superior, µm	288 (267; 308)	255 (242; 268)	227 (204; 250)
Inner superior, µm	296 (274; 318)	253 (239; 268)	226 (204; 249)
Outer nasal, µm	192 (171; 212)	159 (146; 173)	136 (117; 155)
Inner nasal, µm	251 (229; 274)	205 (189; 221)	178 (155; 200)
Outer inferior, µm	277 (256; 297)	245 (230; 261)	205 (184; 226)
Inner inferior, µm	286 (264; 308)	245 (228; 263)	209 (187; 231)
Outer temporal, µm	284 (262; 306)	259 (244; 274)	223 (202; 243)
Inner temporal, µm	299 (276; 323)	260 (244; 277)	226 (203; 249)

Data are presented as mean (95% confidence interval) unless otherwise noted
95% CI 95% confidence interval, *D* diopters, *N* number of participants in group

treatment. Axial elongation over 3 years was less in participants with a thicker choroid at baseline and was highly inversely correlated with increased ChT.

Children with myopia as a rule generally have a thinner sub-foveal ChT than emmetropes [33, 34]. Studies indicate that the sub-foveal ChT decreases in children with myopia as the eye elongates during childhood [33, 35, 36], in contrast with the ChT thickening observed during this period in emmetropes [33]. While many studies only report ChT changes over time in the sub-foveal sector,

we examined ChT change from baseline to 3 years in all macular sectors but did not find a statistically significant change in any sector, including the sub-foveal. Previous studies report conflicting results [33–35], however. Read et al. found that the mean sub-foveal ChT increased over 18 months in their 10- to 15-year-old participants with no statistically significant difference in ChT between myopic and non-myopic participants [34]. However, when participants were stratified according to rate of AL growth, they found that the choroid thickened the least in their group of fast AL progressors. In contrast, Shen et al. similarly observed sub-foveal ChT thickening over 2 years follow-up for persistent non-myopes in a Chinese school but found thinning of the sub-foveal ChT in myopic progressors [33]. Hansen et al. examined participants in the Copenhagen Child Cohort 2000 and found choroidal thickening from age 11 to 16 and a negative correlation between the sub-foveal ChT and increasing negative diopters, i.e., the more myopic, the thinner the choroid [35]. Methodological differences between studies could explain these conflicting results. Myopia degree and age have been found to be negatively correlated with the ChT [34, 37], and they also interact, i.e., myopia progression typically occurs at a faster rate at onset [38, 39]. Our age-inclusion criterium of 6 to 12-year-old children was generally younger than the above-mentioned studies. Our observation of no 3-year change in ChT could therefore be a result of the axial elongation-associated choroidal thinning being confounded by the general choroidal thickening occurring at this childhood stage. However, adjusting the model for age at baseline did not change the sectoral effect sizes or significance levels. In short, ChT changes over 3 years in 6 to 12-year-old Danish children with moderate myopia seem to be modest at best, perhaps because the greatest AL-associated choroidal thinning occurs at myopia onset.

We found that a thinner baseline ChT was highly correlated with increased axial elongation after 3 years. While the causative factors of myopia remain elusive, a popular theory of myopia pathogenesis is that scleral remodeling induces axial elongation [40]. Scleral hypoxia might be a causative factor and is speculated to occur as a result of decreased choroidal blood flow increasing expression of the hypoxia-inducing factor-1α

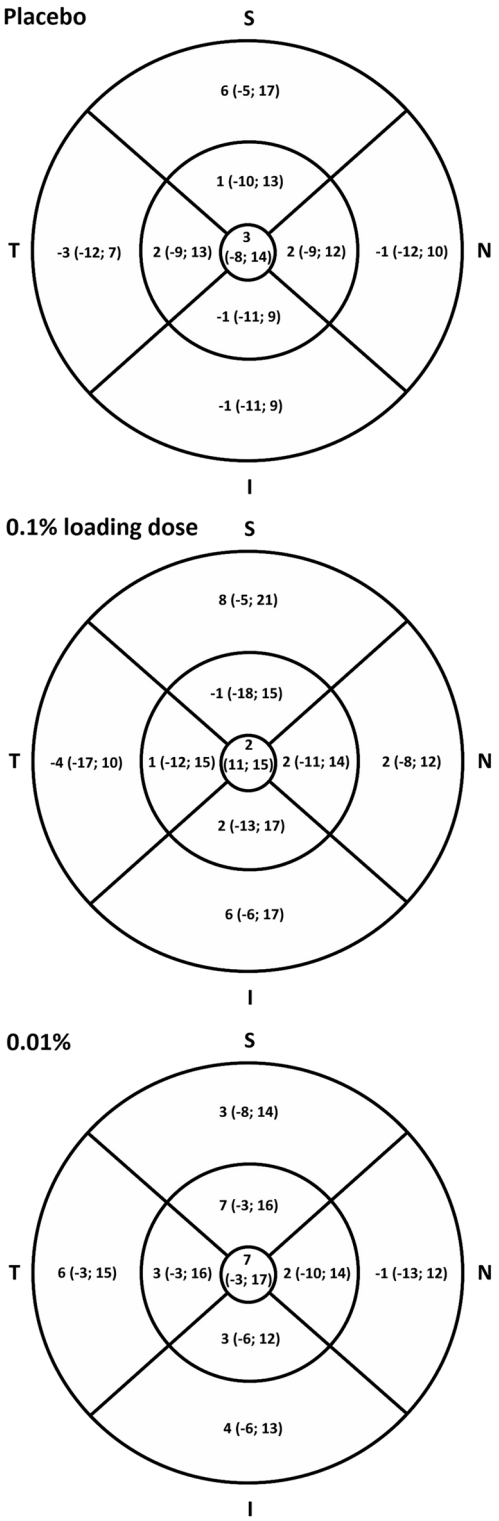

Fig. 3 Mean change in sectoral choroidal thickness at 3-year follow-up in participants receiving placebo, 0.1% loading dose, or 0.01%. Mean change is reported as mean (95% confidence interval). *I* inferior, *N* nasal, *S* superior, *T* temporal

pathway leading to axial elongation by remodeling of the scleral cellular matrix [40]. This is supported by studies finding reduced choroidal blood flow in myopic eyes compared to emmetropic eyes [41, 42]. While our study did not examine choroidal blood flow, decreased ChT must logically interact with a reduced flow, an interaction demonstrated in guinea pigs [43]. While more longitudinal studies examining these factors are needed, it seems plausible that a thinner ChT could contribute to reduced choroidal blood flow causing the aforementioned chain of events leading to scleral remodeling, and ultimately, faster myopia progression.

Treatment with higher atropine concentrations (1%<) increases the ChT [9, 12, 13, 21]. However, whether atropine in lower dosages (0.01%) also has a choroidal thickening effect is more controversial [13, 20, 21, 24]. In Asian children with myopia, Wang et al. failed to find a statistically significant difference in ChT thickness between their 0.01% and placebo groups after 3 months [20]. Yam et al. only examined the sub-foveal ChT and found a concentration-dependent thickening of their 0.025% and 0.05% groups, but not of 0.01% atropine [13]. These observations are consistent with our findings of no ChT increases in the sub-foveal and nasal sectors after 3-year 0.01% atropine treatment compared to placebo. In contrast, Li et al. found ChT increases in Asian children with myopia receiving low-dose atropine, but their follow-up was only 8 weeks [19]. Two of the three studies were conducted in cycloplegic eyes [13, 20], while cycloplegic status was not specified for one [19]. Of the three studies, only Yam et al. and Wang et al. employed a placebo group, and for a shorter-term follow-up (1 year and 3 months, respectively). In comparison, our examinations were performed over a 3-year follow-up, in non-cycloplegia and with a placebo group, allowing us to discern between ChT changes as a result of myopia progression vs. a treatment effect. While it seems that 0.01% atropine does not significantly change ChT thickness in children with myopia, 0.01% atropine nevertheless is a moderately efficacious treatment for myopia progression. This could indicate that low-dose atropine exerts its reductive effect on myopia progression largely via another pathway.

This study had some limitations. While it seems paradoxical that ChT is highly inversely correlated with AL, but at the same time remained stable over 3 years in a group of active myopic progressors, this could be confounded by the ChT chiefly thinning at myopia onset combined with the ChT thickening observed as a part of normal childhood development. The power calculation for our trial was calculated with a reduction in SER progression as the main outcome, and not ChT, as this post hoc study examined, and therefore this comparison could be underpowered. A larger sample size would have increased our power and perhaps have shown a statistically significant, albeit potentially clinically insignificant, 3-year ChT change. Another limitation of this study is that the study inclusion period and initial follow-up visits occurred during the first COVID-19 epidemic. Research indicates that COVID-19-related home confinement might accelerate myopia progression [44] and therefore the myopia progression of participants might have been negatively impacted during this period, although COVID-19 restrictions in Denmark were not as severe as reported in some Asian countries (i.e., no government-enforced home confinement). The prospective nature of this trial allows for future follow-up that might contribute to a better understanding of the relationship between myopia progression and changes in the choroid.

CONCLUSIONS

In conclusion, children with more myopia at baseline generally had a thinner choroid, but the mean ChT did not statistically significantly differ over a 3-year follow-up, perhaps because the largest changes in ChT occur at myopia onset. A thinner choroid at baseline was associated with increased axial elongation after 3 years. Treatment with 0.01% atropine does not seem to change the ChT, and therefore we speculate that low-dose atropine does not

primarily exert its myopia-controlling effect via modification of the choroid.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. The authors Niklas C Hansen, Anders Hvid-Hansen, Toke Bek, Flemming Møller, Nina Jacobsen, and Line Kessel declare no conflicts of interest.

Ethical Approval. This trial was approved by the Committees on Health Research Ethics in the Capital Region of Denmark (reference number: H-18043987), the Danish Medicines Agency (reference number: 2018040088), and the Danish Data Protection Agency via the Capital Region of Denmark (reference: P-2022–85). The trial followed the Declaration of Helsinki. All parents of the study participants provided written informed consent and study participants consented verbally.

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