PRELIMINARY STUDY OF THE EFFECT OF NITRIC OXIDE ON CHOROIDAL THICKNESS IN YOUNG ADULTS

A thesis presented to the graduate faculty of
New England College of Optometry in partial fulfillment
of the requirements for the degree of Master of Science

Sharon Qiu

May 2022

PRELIMINARY STUDY OF THE EFFECT OF NITRIC OXIDE ON CHOROIDAL THICKNESS IN YOUNG ADULTS

Sharon Qiu

This manuscript has been read and accepted by the
Thesis Committee in satisfaction of the
thesis requirement for the degree of Master of Science

4/15/2022
Date

DocuSigned by:
Jane Gwiazda
3012B96081414B5...
Jane Gwiazda, PhD
Graduate Faculty Advisor

DocuSigned by:
78A8AD972399447...
Thanasis Panorgias, PhD
Graduate Faculty Advisor

DocuSigned by:
Debora Nickla
9FE5318DFA53497...
Debora Nickla, PhD
Thesis Committee Member

DocuSigned by:
AF550B737FF34DA...
Xiaoying Zhu, OD, PhD
Thesis Committee Member

4/15/2022
Date

DocuSigned by:
Jane Gwiazda
3012B96081414B5...
Jane Gwiazda, PhD
Director of Graduate Studies

New England College of Optometry

Table of Contents

Abstract

Myopia is a prevalent refractive error that affects one third of the world's population. Alarmingly, the prevalence of myopia is increasing, and it is predicted that almost half of the population worldwide will be myopic by 2050. To decrease the economic burden caused by the myopia boom at the society and the individual levels, researchers have been investigating different ways to slow myopia progression. One possible method in human eyes involves the use of nitric oxide (NO), a potent systemic vasodilator and multifunctional neuromodulator. In human studies, the choroidal thickness was found to be significantly thicker in emmetropes than in myopes. An increase in choroidal thickness induced by NO has been shown to be associated with decreased ocular growth rate in animal studies. Animal studies have also shown that NO may enhance contrast sensitivity by modulating horizontal cells in the retina through the lateral inhibition process. In the current study, we used beetroot juice, a nitrate-rich dietary supplement, to induce an increase in NO production in the body and to subsequently study its effect on choroidal thickness, axial length, blood pressure and contrast sensitivity in healthy young adults. Placebo juice was also used in a double-masked, repeated-measures design. Our results showed no significant effect of beetroot juice ingestion on choroidal thickness, axial length, blood pressure or contrast sensitivity in 24 healthy young adults. The lack of significance may be due to insufficient concentration of NO that had reached ocular tissues via systemic circulation and/or poor instrument resolution.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to Drs. Jane Gwiazda and Thanasis Panorgias for their mentorship and guidance throughout the project. Thank you for your encouragement, your insights and your constructive feedback, all of which has made me a better scientist at the conclusion of the Master's program. I will apply everything that I have learned from you to my future research ventures as a resident and beyond.

I would like to thank Dr. Janine Mendola at McGill Vision Research for her mentorship since my undergraduate years. Thank you for always offering your insights and for encouraging me to continue pursuing my passion of research beyond university.

I would like to thank Dr. Edward Boshnick at the Global Vision Rehabilitation Center for his encouragements during the writing phase of this thesis, especially towards the end. It has been a distinct privilege to learn from you and have you as a mentor.

I am grateful for the financial support I received from the College Work Study in the department of Graduate Studies for the first two years of the program. Thank you, Dr. Gwiazda for all her assistance throughout the process.

Lastly, I would like to thank all my friends, my optometry colleagues and my family for their continuous support throughout the completion of this degree. A special thank you to my parents and my uncle and aunt for encouraging my love of science and research. To my partner Alex – thank you for your boundless support, for brewing coffee for my long writing days and for being a perfectly average psychophysics subject. This thesis stands as a testament to your unconditional love and encouragement.

List of Figures and Tables

1. Introduction

1.1 Myopia as a public health problem

Myopia (defined in this study as a spherical equivalent refractive error ≤ −0.50 diopters (D)) is a prevalent refractive error that has raised significant public health concerns and garnered great research interest in recent years (Holden et al., 2016; Resnikoff et al., 2019; Wolffsohn et all, 2019).

The prevalence of myopia is on the rise worldwide (Sankaridurg et al., 2021). A review paper by a group of experts representing the international myopia institute (IMI) estimated that myopia affected 34% of the world's population in 2019 (Sankaridurg et al., 2021), an increase from the 22.9% prevalence in 2000 (Holden et al., 2016). The prevalence of myopia is predicted to continue to increase in the upcoming decades: 4.7 billion people, or half of the population worldwide, are predicted to be myopic by 2050 (Francisco et al., 2015). The prevalence of myopia is especially high in East Asia, with more than 50% of urban-dwelling population current myopic in China, Japan, and Korea (Francisco et al., 2015; Kim et al., 2013; Li et al., 2017; Nakamura et al., 2018). The most recent epidemiological study conducted on the US population showed a myopia prevalence of 41.6% in persons aged 12 to 54 years in 1999-2004, a significant increase when compared to the 1971-1972 period (25%) (Vitale et al., 2009). This significant increase in myopia prevalence was seen across racial groups and for all levels of severity of myopia (Vitale et al., 2009).

Myopia is a costly burden to society and the individuals affected by this condition. Globally, uncorrected refractive error, especially myopia, is estimated to cause an economic loss of 202 billion U.S. dollars a year (Fricke et al., 2012). Myopia, if uncorrected, is known

to affect academic performance in children (Sankaridurg et al., 2021). For example, randomized control studies conducted in rural China showed that children who received spectacle correction for myopia demonstrated subsequent improvement in academic performance, especially in mathematics test scores (Ma et al., 2014; Ma et al., 2018). Myopia also impacts the overall quality of life in children and adults (Sankaridurg et al., 2021). Results from several questionnaires showed that myopes scored lower on multiple measures of quality of life than emmetropes and hyperopes, including economic well-being, emotional well-being and social well-being (Pesudovs, 2010; Kandel et al., 2017; Kandel et al., 2018). Another important implication of the increasing prevalence of myopia and in particular, high myopia (spherical equivalent refractive error ≤ -6.00 D), is the increased risk of myopia-related complications at the population level (Fricke et al., 2018; Sankaridurg et al., 2021; Yokoi and Ohno-Matsui, 2018). Myopia is linked to increased risk of early cataract formation and various posterior-segment ocular conditions, such as open-angle glaucoma, posterior staphyloma, retinal detachment and myopic macular degeneration (MMD, also called myopic retinopathy) (Guo et al., 2015; Marcus et al., 2011; Sankaridurg et al., 2021; Tang et all, 2016; Tanaka et al., 2019). MMD is considered a fast-growing leading cause of blindness in East Asia and other areas of the world where myopia prevalence is high (Guo et al., 2015; Sankaridurg et al., 2021). In 2015, the worldwide prevalence of MMD was estimated to be around 2%, and about 10 million people (0.13% of world's population) had vision impairment as a result of MMD (Fricke et al., 2018). The latter number is expected to increase to around 56 million (0.6% of world's population) by 2050 according to the modelling work by Fricke et al. (2018). In part because of the public health implications

described above, research into risk factors for myopia and methods to slow progression has gained significant traction in recent years (Chakraborty et al., 2020; Morgan and Jan, 2022; Sankaridurg et al., 2021; Vagge et al., 2018).

1.2. Myopic eyes

1.2.1. Key anatomical differences

Recently, IMI White Papers (Flitcroft et al, 2019; Jong et al., 2021) consolidated the many definitions of myopia used in research and proposed a comprehensive qualitative definition to the World Health Organization that highlights the anatomical features of myopia: "Myopia: a refractive error in which rays of light entering the eye parallel to the optic axis are brought to a focus in front of the retina when ocular accommodation is relaxed. This usually results from the eyeball being too long from front to back, but can be caused by an overly curved cornea and/or a lens with increased optical power." (Flitcroft et al, 2019; Jong et al., 2021).

Myopia can also be seen as a result of the failure of emmetropization, the developmental process that matches an eye's optical power to its axial length so that parallel light rays entering the eye are focused on the retina when ocular accommodation is relaxed (Flitcroft et al, 2019; Troilo et al., 2019). Human and animal studies have shown that axial elongation is the major contributor to myopia development and progression (Flitcroft et al, 2019).

Axial elongation during emmetropization is a process that involves changes in many ocular components, including the cornea and other anterior segment structures, the retina, the

retinal pigmented epithelium (RPE), the choroid and the sclera. Choroidal thickness has been found to significantly differ in myopes and emmetropes; specifically, choroidal thinning in myopes has been demonstrated in a number of studies involving animals and human subjects (Deng et al., 2018; Ho et al., 2013; Nickla et al., 2013).

1.2.2. The choroid and ocular elongation

The choroid is part of the uveal tract that is situated behind the RPE and in front of the sclera (Summers, 2013). It is a highly vascular tissue that serves multiple roles in the eye (Nickla and Wallman, 2010).

Traditionally, the main contribution of the choroid to ocular homeostasis was its robust blood supply to the outer retina in humans (Nickla and Wallman, 2010). The choroid has the highest blood flow of any tissue in the body per unit tissue weight, about ten times higher than that of the brain and about four times greater than that of the kidney (Urs et al., 2018; Lavinsky and Lavinsky, 2016). One additional unique feature is that, unlike blood flow in most tissues of the body, the high blood flow in the choroid is thought to be constant and not completely autoregulated, meaning that changes in perfusion pressure do not cause a compensatory restriction/dilation response in blood vessels that change the rate of blood flow (Delaey and Van De Voorde J, 2000).

The high choroidal blood flow guarantees a constant supply of oxygen and nutrients to and removal of metabolic waste products from the RPE and photoreceptors, with the latter being one of the most metabolically active cells in the human body (Jaroszynska et al., 2021; Linsenmeier and Braun, 1992). This equilibrium maintains the health of the outer retina, and

when it becomes faulty, many disease states can manifest, with one example being age-related macular degeneration (ARMD) (Zarbin, 2004). The high choroidal blood flow is also thought to provide temperature regulation for the retina, counteracting the damage that may result in the retinal tissues with high temperature (under high illumination) and low temperature (under low illumination) (Nickla and Wallman, 2010; Parver et al., 1980).

A body of research has demonstrated that the choroid's functions are not just limited to those associated with its high blood flow (Troilo et al., 2019), It was found that choroidal thickness showed compensatory changes in response to retinal defocus, which may be linked to the emmetropization process and thus myopia development (Wallman et al., 1995; Wildsoet and Wallman, 1995). In animal models, myopic defocus, where the image was purposely focused in front of the retina through plus lenses, is a well-established technique to induce reduced axial elongation or hyperopic refraction (Schaeffel and Feldkaemper, 2015). Similarly, hyperopic defocus or form deprivation are well-established techniques to induce axial elongation or myopic refraction (Schaeffel and Feldkaemper, 2015). Studies have found that, with experimental manipulations that involved myopic defocus, there was a compensatory increase in choroidal thickness (Wallman et al., 1995; Wildsoet and Wallman, 1995). Conversely, with experimental manipulations that involved hyperopic defocus or form deprivation, there was a compensatory decrease in choroidal thickness (Wallman et al., 1995; Wildsoet and Wallman J, 1995). The compensatory choroidal responses were "rapid (within hours), bidirectional, and highly precise" (Troilo et al., 2019). The changes in choroidal thickness occur before changes in the sclera that result in changes in axial length, which leads to the hypothesis that changes in choroidal thickness may regulate axial length elongation

during the emmetropization process. This hypothesis, if true, would have significant translational implications in human myopia control research (Troilo et al., 2019). Specifically, interventions that increase choroidal thickness may potentially slow myopia development in children.

The hypothesis that there exists a causal relationship between choroidal changes and axial length growth remains controversial. One study (n = 70) by Nickla and Totonelly (2015) showed that in chicks that received no experimental manipulations, the initial choroidal thickness predicted the rate of axial length growth, where eyes with thicker choroids had a slower axial elongation rate than eyes with thinner choroids. This effect only existed under natural conditions, and it disappeared when experimental manipulations were applied, such as lens-induced defocus or form deprivation (Nickla and Totonelly, 2015). However, larger studies involving more than 500 chicks provided different results, where it was shown that the initial choroidal thickness failed to predict the rate of axial length growth both under natural conditions and with form deprivation (Chen et al., 2011; Guggenheim et al., 2011). Furthermore, other studies have demonstrated the decoupling of the choroidal response and the scleral response, where a certain experimental intervention caused either the choroidal response or the scleral response in isolation but not together, suggesting that choroidal changes were not necessarily a prerequisite for axial length growth (Nickla and Schroedl, 2012; Nickla et al., 2013; Winawer and Wallman, 2002).

Although more evidence points against the causal link between choroidal changes and myopia development, more research is needed to understand the role of the choroid in emmetropization and the signaling pathways that are involved. One approach is to modify the

concentration of signaling molecules that may play a role in the emmetropization process in the ocular tissues, either by external pharmacological manipulations or promotion/inhibition of intrinsic production, and to study the resulting effect on the choroid and ocular elongation (Nickla and Wallman, 2010). Three such pharmacological agents have been widely studied, including dopamine, atropine and NO (Nickla and Wallman, 2010). Section 1.4 will provide more details on the effect of NO on the regulation of choroidal thickness and axial length growth.

1.2.3. Key differences in psychophysical dynamics

Besides anatomical differences, myopic eyes also show abnormalities in spatial and temporal vision processing, evidenced by poorer performance in psychophysical tasks, for tasks performed both centrally and peripherally. When comparing myopes and emmetropes who are correctable to 20/20 central visual acuity, the central spatial contrast sensitivity and temporal contrast sensitivity function are impaired in myopes, especially in high myopes (Chen et al., 2000; Collins and Carney, 1990; Fiorentini and Maffei L, 1976; Liou and Chiu, 2001; Vera-Diaz et al., 2018). Blur sensitivity, the ability to detect blur caused by retinal defocus, is also found to be impaired in central vision in myopes compared to emmetropes (Maiello et al., 2017; Rosenfield and Abraham-Cohen, 1999). Understanding the psychophysical dynamics in the myopic central vision is important, because it helps us understand the functional characteristics and the underlying mechanisms of the visual system in myopes (Stoimenova, 2007), and it also helps us understand various disease states associated with myopia. For example, spatial contrast sensitivity loss may serve as an early

marker for open-angle glaucoma. Critical flicker frequency (CFF), one aspect of the temporal contrast sensitivity function, may potentially be investigated as an early indicator for MMD (Chen et al., 2000; Ichhpujani et al., 2020).

Healthy myopic eyes correctable to 20/20 also behave differently when it comes to peripheral psychophysics tasks, where myopes showed overall poorer performance. Myopes showed worse peripheral visual acuity and contrast sensitivity than emmetropes at the same retinal eccentricity, which may be due to the "stretching" effect of an elongated eyeball (Chui et al., 2005; Ehsaei et al., 2013). Myopes showed lower peripheral spatial contrast sensitivity, an effect worsened by central attentional load (Kerber et al., 2016). Lower peripheral temporal contrast sensitivity was also found in the nasal retina of myopes than in hyperopes (Hathibelagal et al., 2021). Furthermore, the visual system in myopes processes blur differently than in emmetropes. Myopes showed a greater degree of both blur adaptation and defocus tolerance in the periphery (Ghosh et al., 2017; Rosén et al., 2012). As clear image quality free of defocus at all eccentricities of the retina is thought to be crucial in the emmetropization process, this differential response to blur in myopes may play a role in driving the emmetropization process awry (Ghosh et al., 2017; Logan et al., 2021; Rosén et al., 2012).

1.3. Current interventions for prevention of myopia onset and progression

Currently, multiple interventions of myopia control are shown to clinically prevent the onset and/or progression of myopia. Theses interventions can be roughly categorized as behavioral, optical or pharmacological (Jonas et al., 2021; Wildsoet et al., 2019).

Out of all the behavioral modifications tested in clinical studies, increased time spent outdoors has consistently shown to be an effective strategy for myopia control (Jonas et al., 2021). Recent meta-analyses have shown that increased time spent outdoors is protective against the onset of myopia and the progression of myopia (Cao et al., 2020; Sherwin et al., 2012), although the effect seems to be more pronounced for the prevention of myopia onset rather than progression (Xiong et al., 2017). One meta-analysis reported that for each additional hour of time spent outdoors per week, the odds of myopia development were decreased by 2% (Sherwin et al., 2012). There is currently no unified theory for how increased time outdoors contributes to lower incidence of myopia and slower myopia progression (Jonas et al., 2021). Multiple factors have been proposed to play a role, including the different properties of the outdoor environment (higher light intensity, different chromatic light composition and different dioptric topographies), less indoor near work and decreased accommodative demand (He et al, 2015; Flitcroft, 2012; Vander Veen et al., 2019). Many countries with high prevalence of myopia, such as Singapore and China, have launched educational programs to encourage school-age children to spend more time outdoors (Ang et al, 2020; Jonas et al., 2021). Coupled with physical exercises, these programs aimed to promote lower incidence of myopia and the overall well-being of children (Ang et al, 2020; Jonas et al., 2021).

Currently accepted optical interventions of myopia control include multifocal spectacle lenses, multifocal contact lenses and orthokeratology (ortho-k). The first two optical interventions often employ the simultaneous vision design, where the central vision is fully corrected and progressive addition (Add) power is built into the periphery of the lens

through concentric annular zones (Jonas et al., 2021). The peripheral addition power within the annular zones does not compromise central visual acuity, and it was thought that the peripheral myopic defocus induced by the peripheral Add power inhibited axial elongation, a hypothesis supported by animal studies (Arumugam et al., 2014; Liu and Wildsoet, 2011; Tse et al., 2007). Defocus incorporated multiple segments (DIMS) spectacle lenses are one type of multifocal spectacle lenses that were shown to significantly halt myopia progression (Lam et al., 2014; Lam et al., 2020; Lu et al., 2020). One double-masked randomized trial including 160 myopic Chinese children between eight to thirteen years old found that over a two-year period, myopia progression in the DIMS group was significantly less than in the single-vision spectacle group in terms of cycloplegic refraction (−0.41 ± 0.06 D vs. −0.85 ± 0.08 D) and axial elongation (0.21 ± 0.02 mm vs. 0.55 ± 0.02 mm) (Lam et al., 2020). Multifocal soft contact lenses for myopia control have also been studied extensively. In 2019, MiSight® (CooperVision Inc.) became the first soft multifocal contact lens to FDA-approved for slowing myopia progression (Jonas et al., 2021). A three-year randomized multi-center clinical trial showed that the MiSight group had significantly slower progression in cycloplegic refraction (−0.51 ± 0.64 vs. −1.24 ± 0.61 D) and axial elongation (0.30 ± 0.27 vs. 0.62 ± 0.30 mm) when compared to the control group wearing regular soft contact lenses (Chamberlain et al., 2019). Furthermore, the recently published results from the BLINK study showed that a high Add in the multifocal optical design more effectively slowed myopia progression than a medium Add (Walline et al., 2020). Lastly, Ortho-K temporarily reduces the dioptric value of myopia by flattening the central cornea and steepening the peripheral cornea (Smith et al., 2009). Two large randomized clinical trials have also found

that Ortho-K has a myopia control effect by significantly reducing axial elongation by 43% to 63% (Charm and Cho, 2013; Cho and Cheung, 2012). The reduction was greater in younger, rapidly progressing myopic children (Cho and Cheung, 2012; Swarbrick et al., 2015).

For pharmacological interventions, the most widely used agent is low-dose atropine drops. A meta-analysis found that when compared to placebo drops, low-dose atropine drops showed reduced myopia progression in myopic refraction by 1.00 D, and reduced myopia progression in axial length elongation of 0.35 mm (Walline et al., 2020). Two large randomized control trials, the ATOM-1 study and the ATOM-2 study, compared the effect of different atropine concentrations on the reduction of myopia progression (Chia et al., 2012; Chua et al., 2006). It was found that among the studied atropine concentrations of 0.1%, 0.05%, 0.025% and 0.01%, 0.05% atropine was twice as effective as the 0.01% atropine and considered the optimal concentration (Chia et al., 2012). To our knowledge, no human study has investigated the myopia control effect of NO.

1.4. NO

1.4.1. NO overview

NO is classified as a gasotransmitter, a gaseous signaling molecule that is endogenously produced and acts on its receptors to modulate multiple physiological processes in the body (Ahmad et al., 2018). In the body, NO is produced from a reaction involving the NO precursor L-arginine and oxygen, catalyzed by the enzyme nitric oxide synthase (NOS) (Ahmad et al., 2018). Three types of isoforms of NOS have been found in

the body: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). Compared to the effect of eNOS and nNOS, the effect of iNOS is more long-term (Förstermann and Sessa, 2011). In the body, NO is endogenously produced and immediately released in response to internal and external stimuli, including stress, hormones and cellular injury (dos Santos Baião, et al., 2021; Gow et al., 2000; Xia and Krukoff, 2004). The production of NO can also be externally promoted via oral supplementation of nitrate (NO_3^-) through the enterosalivary NO_3^--NO_2^-/NO pathway (dos Santos Baião, et al., 2021). Nitrate is commonly found in leafy greens, beets and various supplement formulations (dos Santos Baião, et al., 2021; Lidder and Webb, 2013).

NO is ubiquitous in the body, and it is a signaling molecule for numerous physiological processes and disease states (Chen et al., 2008). NO mainly exerts its effect through the guanylyl cyclase/cGMP signaling pathway to relax smooth muscle cells in blood vessels (Ahmad et al., 2018; Siervo et al., 2013; Yu et al, 2019). Systemically, NO is a potent vasodilator and act on multiple body systems, including the cardiovascular system, the reproductive system, the nervous system and the immunological system (Ahmad et al., 2018; Siervo et al., 2013; Davies, 2015; dos Santos Baião, et al., 2021). NO supplementation through oral ingestion has been shown to have a multitude of health benefits, including improving cardiovascular health, reducing high blood pressure in preeclampsia, improving sports performance through enhanced oxygen delivery and treating erectile dysfunction (Davies, 2015; Morita et al., 2014; Rytlewski et al., 2005; Shephard, 2012). Furthermore, NO in inhaler form has been shown to be a safe treatment for cardiopulmonary conditions in patients of all ages (Yu et al, 2019).

1.4.2. The effect of NO on the choroid

As a ubiquitous gasotransmitter, NO is also found in the vertebrate eyes as a neuromodulator (Moreno-Lopez et al., 2002). It has been found in RPE, retinal cells, intrinsic choroidal neurons (a group of neurons in the choroid whose function is still largely unknown) and in the terminals of the pterygopalatine ganglia and the parasympathetic ciliary ganglia (Bergua et al., 1996; Cuthbertson et al., 1997; Goldstein et al., 1996; Nilsson, 1996; Schrodl et al., 2000). All three types of NOS isoforms were found in the eye.

Pharmacological manipulations in animals involving NO have been shown to regulate the retinal defocus-induced changes in choroidal thickness and axial length. NO was hypothesized to be part of the signaling cascade that regulates the choroidal thickening and the subsequent inhibition of axial elongation in response to hyperopic defocus (Nickla and Wallman, 2010; Troilo et al., 2019). Studies in chicks receiving myopic defocus (for more hyperopic refraction) or recovering from form deprivation showed that injections of L-NAME or L-NMMA, inhibitors of all NOS isoforms and therefore NO production, blocked both the increase in choroidal thickness and the subsequent inhibition of axial elongation that should follow choroidal thickening (Nickla and Wildsoet, 2004; Nickla et al. 2006; Nickla et al., 2009). If the injection was done after the choroidal thickening had already occurred, axial elongation was not disinhibited (Nickla et al. 2006). On the contrary, studies using promoters of NO production, such as L-arginine (precursor of NO) and sodium nitroprusside (NO donor), promoted choroidal thickening and the subsequent inhibition of axial elongation and prevented the effect of form deprivation (Carr and Stell, 2016). Carr and Stell (2016) also found that simultaneous application of L-NMMA with L-arginine reversed the effect of the

latter and form deprivation myopia developed as a result. The effect of NO on choroidal thickness was shown to last less than 24 hours in chicks (Nickla and Wildsoet, 2004).

The exact biochemical mechanism of how NO regulates choroidal thickness and axial elongation is still unclear (Nickla and Wallman, 2010; Troilo et al., 2019). Additional studies showed that nNOS may be the isoform that is relevant to this process, where only the nNOS-specific inhibitor, but not the eNOS- or iNOS-specific inhibitors, showed a similar effect as L-NAME or L-NMMA (inhibitor of all three isoforms) in preventing the choroidal thickening and inhibition of axial elongation in response to myopic defocus (Nickla et al., 2009). Furthermore, NO may exert its effect downstream of other signaling molecules dopamine and atropine. It has been shown that NOS inhibitors blocked the respective effects that dopaminergic or cholinergic drugs would have had on choroidal thickness/axial length (Nickla et al., 2013; Carr and Stell, 2016).

Overall, research shows that NO plays a role in choroidal thickening and the related axial elongation inhibition in animal models. As a gasotransmitter that is ubiquitous in the body whose systemic production is easily upregulated through oral supplementation, NO appears to be an attractive candidate for its potential role as a novel intervention of myopia control in humans.

1.4.3. The effect of NO on the retina

In the retina, all three types of NOS isoforms exist, but nNOS is the isoform that modulates the majority of processes of visual processing related to NO (Dawson et al., 1991; Vielma et al., 2012; Tsumamoto et al., 2002). NO is produced by virtually all cells in the

retina, including photoreceptors, horizontal cells, bipolar cells, amacrine cells and ganglion cells (Vielma et al., 2012). NO synthesis is regulated by both increased illumination and other neurotransmitters (Vielma et al., 2012). Both flickering light and continuous light were shown to increase NO synthesis and release by multiple retinal cells in animal models (Haamedi and Djamgoz, 2002; Neal et al., 1998; Sekaran et al., 2005). NO synthesis is also modified by other neurotransmitters both in vivo and in vitro (Vielma et al., 2012). Specifically, acetylcholine, glutamate and dopamine upregulation drives nNOS activity, and melatonin dampens nNOS activity to reduce NO synthesis (Borda et al., 2005; Sáenz et al., 2002; Sekaran et al., 2005; Tsumamoto et al., 2002). Similar to other parts of the body, NO in the retina activates the guanylyl cyclase/cGMP signaling pathway, with receptors for guanylyl cyclase on virtually every retinal cell type (Sáenz et al., 2002; Vielma et al., 2012).

NO serves diverse functions in the retina. It has been well-established that NO regulates retinal responses to light. Light responses in animal models can be obtained by electrophysiology recordings or ERG (Vielma et al., 2012). For example, light responses in turtle cones were amplified by NO and L-arginine, and dampened by NOS inhibitors (Levy et al., 2004). NO also affected light response in horizontal cells, although the effect was species dependent: NO promoted light responses in turtle horizontal cells, but inhibited light responses in carp horizontal cells (Levy et al., 2004; Ye et al., 1997). For bipolar cells, NO promoted responses to dim light stimuli but not bright light stimuli (Snellman and Nawy, 2004). NO was also found to promote the light/dark adaptation process by modifying the responses of ganglion cell ON/OFF responses (Nemargut and Wang, 2009; Wang et al., 2003).

Another important function of NO in retina is its gap junction decoupling effect on retinal cells, especially horizontal cells (Vielma et al., 2012). It was found that administration of NO, L-arginine or dopamine promoted the decoupling of horizontal cells (He et al., 2000; Miyachi et al., 1990; Weiler et al., 2000). The decoupling of gap junctions between horizontal cells may reduce the horizontal cell receptive field size and enhance the responsiveness of horizontal cells to light (Pottek et al., 1997). Horizontal cell response plays an important role in lateral inhibition underlying light adaptation and enhancement of contrast sensitivity in light-adapted conditions (Xin and Bloomfield, 2000). Therefore, NO may promote increased contrast sensitivity through horizontal cell-related mechanism. This hypothesis was supported by a study done by Shi et al. (2000), although the exact signaling pathway is still largely unknown.

Besides directly modulating cell physiology through the guanylyl cyclase/cGMP signaling pathway, NO can also be converted into reactive nitrogen species that participate in posttranslational protein modification through the processes of S-nitrosylation and nitration (Martínez-Ruiz et al., 2011; Pacher et al., 2007). These posttranslational protein modifications would then start a cascade of biochemical signaling events that mediate the proliferation, growth and death of retinal cells (Cheung et al., 2000; Koriyama et al., 2010; Vielma et al., 2012).

1.5. Clinical relevance of research goals

Although no clear causal link between choroidal thickening and inhibition of axial length has been proven, it is well established that choroidal thickness significantly differs in

myopes and emmetropes; specifically, choroidal thinning in myopes has been demonstrated in studies involving human subjects (Ho et al., 2013; Deng et al., 2018). As a result, some researchers believe that a thin choroid is a risk factor for, whereas a thick choroid is protective against myopia progression (Zhou et al., 2021). Choroidal thickening, either through pharmacological agents or dietary supplements, can therefore be investigated as a way of preventing or slowing myopia progression.

NO's choroidal thickening effect is well documented, which makes NO a potential candidate for a novel intervention of myopia control. Upregulation of systemic NO production is easily achieved through oral supplementation, such as beetroot juice, a safe nitrate-rich dietary supplement that has been used in other human studies (Siervo et al., 2013). A plausible first step in investigating the possibility of NO as a novel intervention of myopia control would be to administer beetroot juice to young adults. A few studies investigating the effect of pharmacological agents on human choroidal thickness have used both topical and systemic medications (Yeung et al., 2022), but none has included NO. Increased choroidal thickness was observed following the topical administration of glaucoma drops and atropine drops, and following the oral administration of beta-blockers and ethanol (Yeung et al., 2022). If the oral administration of beetroot juice induced a significant increase in choroidal thickness, this would mean that increased NO concentration in systemic circulation has the ability to penetrate the blood-brain barrier and exert a significant choroidal thickening effect on ocular tissues.

Since beetroot juice has a documented blood pressure-lowering effect, we also recorded blood pressure measurements periodically during the study to monitor for hypotension and any related complications.

Lastly, animal studies showed that NO may promote the lateral inhibition process of horizontal cells and therefore enhance contrast sensitivity. To our knowledge, our study would be the first to investigate the effect of NO on contrast sensitivity in human subjects.

1.6. Hypothesis and prediction

This study was designed to test the hypothesis that NO through oral supplementation has a significant effect on choroidal thickness, axial length, blood pressure and contrast sensitivity in a cohort of young adults. Our prediction is that nitrate-rich beetroot juice would cause significant effects of choroidal thickening, axial length reduction, blood pressure lowering and contrast sensitivity enhancement in human subjects.

2. Methods

2.1. Subjects

In this preliminary study, we recruited 15 myopes and 15 emmetropes from the NECO community. Previous studies on the systemic effect of beetroot juice have shown that significant statistical power can be reached with 15 subjects (Siervo et al., 2013). The study followed the doctrines of Declaration of Helsinki for research involving human subjects and was approved by the institutional review board of NECO.

2.2. Inclusion criteria

Subjects were between 18 and 30 years old to reduce the possibility of any confounding retinal and/or systemic diseases that affect the visual system.

- Myopes: myopia between -1.00 D and -6.00 D of spherical equivalent in each eye, and less than 1.00 D of spherical equivalent difference between the two eyes
- Emmetropes: refraction between -0.50 D and +0.50 D of spherical equivalent in each eye, and less than 1.00 D of spherical equivalent difference between the two eyes

2.3. Exclusion criteria

- Self-reported retinal or systemic disease affecting the visual system
- SE < -6.00 D, SE > +0.50 D, or -0.50 D < SE < -1.00 D in either eye exclusively
- Anisometropia ≥ 1.00 D
- Allergy to beets
- Hypotension (systolic blood pressure of less than 100 mmHg or diastolic pressure of less than 70 mmHg), due to the blood pressuring-lowering effect of NO
- Unmanaged hyperlipidemia (total cholesterol level higher than 200 mg/dL)
- Known coronary artery disease or heart failure
- Pregnant or nursing

2.4. Equipment/Supplies/Facilities/Location

The experiment was conducted at 424 Beacon Street, Boston, MA, USA. The equipment and supplies that were used included:

- Optovue RTVue-100 OCT (Optovue Inc., Fremont, CA)
- LENSTAR Optical Biometer (Haag-Streit, OH, USA)
- Grand Seiko open field of view autorefractor (Grand Seiko Co Ltd, Hiroshima, Japan)
- Blood pressure cuff
- Beetroot juice and placebo juice: Regular beetroot juice and placebo juice were purchased from James White Drinks Limited, a company that has offered beetroot juice and placebo juice for a number of studies (Siervo et al., 2013), and the two types of juice could only be distinguished by expiration dates
- HumanN® Nitric Oxide Indicator strips

2.5. Overall experimental design

1) The recruited subjects visited NECO three times: one 30-minute screening session, and two 2-hour test sessions scheduled on two different days with a maximum interval of two weeks in between.

2) Double-masked procedure: the subjects were randomly assigned one type of juice for the initial session and the other for the second visit. The assignment was determined by an unmasked examiner (Dr. Thanasis Panorgias), while the researcher as a masked examiner did not know the assignment until the beginning of the data analysis phase.

2.6. Screening session

1) The subject read the consent form, asked any questions to the researcher and signed the consent form.

2) The researcher measured the subject's uncorrected refractive error in each eye using the Grand Seiko autorefractor without cycloplegia, measured the subject's blood pressure using a blood pressure cuff and distributed a screening questionnaire to determine the subject's eligibility based on either their refractive error, blood pressure level or answers to the questionnaire.

- If the subject was found to be ineligible for the study, the researcher notified the subject and provided $10 compensation to the subject.
- If the subject was found to be eligible for the study, the subject was given a recommended low-nitrate and low-nitrite dietary protocol (no leafy greens or beets) to follow for 24 hours before each of the test sessions.

2.7. Test session 1

1) The subject was asked to attend each test session between 8 am and 10 am to control for diurnal variation in choroidal thickness and avoid any interactions between NO and food. The subject was asked not to ingest any food or drink, except for water, for 8 hours prior to each test session.

2) The researcher performed the following procedures:

a) Baseline measurements

- The subject's gross baseline systemic NO level in saliva: the subject transferred his or her saliva onto a HumanN® Nitric Oxide Indicator strip. The researcher immediately compared the resulting color to the color chart on the product bottle to determine the subject's baseline systemic NO level (see Figure 1). The researcher disposed of the strip in the biohazard bin located in NECO's animal facilities immediately after the test session.
- The subject's baseline choroidal thickness using OCT: for each eye, three horizontal B-scans through the macula were taken, and the average baseline choroidal thickness was determined using the manual tracing method described in section 2.9.1. For each subject, only one eye was selected for measurements based on the visibility of the chorioscleral border.
- The subject's baseline axial length using LENSTAR: for each eye, the baseline axial length was averaged from five A-scan measurements using LENSTAR. LENSTAR measures axial length from the front of the cornea to the retinal pigment epithelium, not including the choroid.
- The subject's baseline blood pressure using a blood pressure cuff: the digital blood pressure monitor automatically displayed the baseline systolic and diastolic blood pressure. The measurement was taken with the subject sitting and rested.

Figure 1: HumanN® Nitric Oxide Indicator strip and color chart. The shade of pink on the indicator strip after application of saliva suggests a subject's systemic NO level at a given time: depleted, low or optimal. From HumanN (2022)

b) Distribution of beetroot juice or placebo following the double-masked protocol: the subject drank the 7 cl beetroot juice or placebo all at once.

- Following juice ingestion, the subjects were asked not to perform near tasks, since accommodation is known to affect choroidal thickness (Kim et al., 2013). While waiting for the next set of measurements, subjects either relaxed or talked with other subjects to pass time.

c) Measurements 0.5 h post juice ingestion

- The subject's choroidal thickness using OCT: for each eye, three horizontal B-scans through the macula were taken, and the average baseline choroidal thickness was determined using the manual tracing method described in section 2.9.
- The subject's axial length using LENSTAR: for each eye, the axial length was averaged from five A-scan measurements using LENSTAR

- The subject's blood pressure using a blood pressure cuff: the digital blood pressure monitor automatically displayed the baseline systolic and diastolic blood pressure. The measurement was taken with the subject sitting and rested.

d) Measurements 1.0 h post juice ingestion

- The subject's choroidal thickness using OCT: for each eye, three horizontal B-scans through the macula were taken, and the average baseline choroidal thickness was determined using the manual tracing method described in section 2.9.
- The subject's axial length using LENSTAR: for each eye, the axial length was averaged from five A-scan measurements using LENSTAR
- The subject's blood pressure using a blood pressure cuff: the digital blood pressure monitor automatically displayed the baseline systolic and diastolic blood pressure. The measurement was taken with the subject sitting and rested.

e) Measurements 1.5 h post juice ingestion

- The subject's choroidal thickness using OCT: for each eye, three horizontal B-scans through the macula were taken, and the average baseline choroidal thickness was determined using the manual tracing method described in section 2.9.
- The subject's axial length using LENSTAR: for each eye, the axial length was averaged from five A-scan measurements using LENSTAR
- The subject's blood pressure using a blood pressure cuff: the digital blood pressure monitor automatically displayed the baseline systolic and diastolic blood pressure. The measurement was taken with the subject sitting and rested.

- The subject's gross systemic NO level in the saliva using NO Indicator Strips and procedures described above

3) At the end of the test session, the subject was asked to perform a short psychophysics task using the Visual Psychophysics Engine (VPE) program. Myopic subjects were fully corrected with spectacles. Contrast sensitivity to a single low spatial frequency grating (0.3 c/deg) was measured at a viewing distance of 1 m using the method of constant stimuli combined with a 2-alternative forced-choice (2AFC) paradigm. 11 levels of contrast were used (20 to 50% contrast), and each contrast level was presented for 250 ms and 19 times total. On each stimulus presentation, the subject had to indicate the orientation of the grating (left or right) using a response box. At the end of the experiment, the percentage of correct responses for all the different contrast levels was extracted from the software for further analysis. A Weibull logistic function was used to fit the psychometric function on each subject's data. The subject's threshold corresponded to a criterion level of 75 % correct responses

4) After the test session, the subject was compensated for their time and provided a $5 gift card from the campus café for breakfast.

2.8. Test session 2

The subject came back for a second test session on a different day within two weeks to be tested for the other condition (placebo or beetroot juice), and all other experimental steps were the same.

2.9. Data analysis

2.9.1 Overall description of data analysis

First, we tabulated the actual data at each of the four time points, and a paired-samples t-test was used to determine whether there was a significant difference in the baseline measurements at the beginning of the active session and at the beginning of the placebo session. Table 1 details the actual measurements at each time point for subfoveal choroidal thickness, nasal choroidal thickness, temporal choroidal thickness, axial length, systolic blood pressure and diastolic blood pressure.

Next, we calculated the difference in the actual measurements at the later three time points (0.5 h after baseline, 1.0 h after baseline and 1.5 h after baseline) as compared to the baseline measurement by subtracting the baseline measurement from the later measurements. Table 2 details the difference in measurements at the later three time points as compared to the baseline measurement for subfoveal choroidal thickness, nasal choroidal thickness, temporal choroidal thickness, axial length, systolic blood pressure and diastolic blood pressure.

For contrast sensitivity, data was recorded at the end of the active session and at the end of the placebo session. We did not analyze the change of contrast sensitivity across two time points.

Measurement	Active - baseline	Active - 0.5 hr after baseline	Active - 1.0 hr after baseline	Active - 1.5 hrs after baseline	Placebo - baseline	Placebo - 0.5 hr after baseline	Placebo - 1.0 hr after baseline	Placebo - 1.5 hr after baseline
Subfoveal choroidal thickness (μm)	251.07 ± 1.24	251.72 ± 1.31	253.79 ± 1.38	259.45 ± 1.30	251.74 ± 1.19	257.23 ± 1.39	256.08 ± 1.36	260.42 ± 1.40
Nasal choroidal thickness (μm)	244.02 ± 1.24	244.83 ± 1.29	247.95 ± 1.34	251.79 ± 1.31	248.11 ± 1.20	251.65 ± 1.39	249.61 ± 1.34	254.44 ± 1.42
Temporal choroidal thickness (μm)	247.72 ± 1.24	248.48 ± 1.29	252.08 ± 1.28	256.90 ± 1.30	249.94 ± 1.25	254.36 ± 1.33	253.67 ± 1.26	257.30 ± 1.35
Axial length (mm)	24.24 ± 0.06	24.24 ± 0.06	24.25 ± 0.06	24.25 ± 0.06	24.25 ± 0.06	24.25 ± 0.06	24.25 ± 0.06	24.25 ± 0.06
Systolic blood pressure (mmHg)	109.25 ± 0.47	106.38 ± 0.40	107.38 ± 0.46	104.75 ± 0.35	107.88 ± 0.30	104.25 ± 0.28	107 ± 0.31	103.83 ± 0.32
Diastolic blood pressure (mmHg)	74.42 ± 0.35	72.42 ± 0.41	73.17 ± 0.38	73.96 ± 0.37	72.54 ± 0.29	70.17 ± 0.27	72.00 ± 0.24	70.63 ± 0.22

Table 1: Actual measurements at each time point. Each data point was averaged from all subjects and accompanied by the standard error of the mean (SEM). "Active" represents "active session." "Placebo" represents "placebo session."

Difference in actual measurements	Active - 0.5 hr after baseline	Active - 1.0 hr after baseline	Active - 1.5 hrs after baseline	Placebo - 0.5 hr after baseline	Placebo - 1.0 hr after baseline	Placebo - 1.5 hr after baseline
Subfoveal choroidal thickness (μm)	0.65 ± 0.47	2.72 ± 0.60	8.38 ± 0.48	5.49 ± 0.56	4.34 ± 0.70	8.69 ± 0.84
Nasal choroidal thickness (μm)	0.81 ± 0.45	3.93 ± 0.63	7.77 ± 0.51	3.53 ± 0.58	1.50 ± 0.62	6.33 ± 0.80
Temporal choroidal thickness (μm)	0.76 ± 0.59	4.36 ± 0.57	9.18 ± 0.46	4.42 ± 0.57	3.74 ± 0.67	7.36 ± 0.80
Axial length (mm)	0.0058 ± 0.0005	0.0075 ± 0.0006	0.0079 ± 0.0008	-0.0033 ± 0.0009	-0.0050 ± 0.0010	-0.0042 ± 0.0012
Systolic blood pressure (mmHg)	-2.88 ± 0.34	-1.88 ± 0.42	-4.50 ± 0.32	-3.63 ± 0.14	-0.875 ± 0.23	-4.04 ± 0.25
Diastolic blood pressure (mmHg)	-2.00 ± 0.32	-1.25 ± 0.33	-0.458 ± 0.29	-2.38 ± 0.25	-0.542 ± 0.28	-1.92 ± 0.26

Table 2: Difference in actual measurements at the later three time points. Each data point was averaged from 24 subjects and accompanied by SEM. “Active” represents “active session.” “Placebo” represents “placebo session.”

2.9.2. Choroidal thickness

Choroidal thickness in each B-scan image was measured using digital calipers (Harb et al., 2016). This was done at three retinal locations (fovea, one nasal location 250 μm from fovea, and one temporal location 250 μm from fovea) for each of the twelve B-scan images (three B-scan images at each measurement time point for four measurements in total). Measurements were performed by two masked measurers: the researcher and another experimenter. If the measurements from the two experimenters differed by more than 15% or 30 μm (Harb et al., 2016), they were adjudicated: both experimenters individually re-measured the choroidal thickness until the difference in the two measurements fell within 15% or 30 μm (Harb et al., 2016). Otherwise, the results were averaged between the two experimenters. Overall, we obtained choroidal thickness of one eye at four time points of a test session (baseline measurement, 0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement) for each subject. Each subject had two test sessions, one with active juice and one with placebo juice.

Using SPSS, a paired t-test was used to compare the baseline choroidal thickness at each retinal location at the beginning of the active session and at the beginning of the placebo session in each subject. The goal is to ensure that the baseline choroidal thickness was not significantly different at the beginning of each session for the same subject.

For the rest of the analysis, we looked at difference over time in choroidal thickness compared to the baseline measurement. For each of the three later time points of a test session (0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement), the difference in choroidal thickness compared to the

baseline measurement was calculated (see data in Table 1 and Table 2). Using Excel, the results from all subjects were averaged to produce a graph of the change in the difference in choroidal thickness over time for the active test session and the placebo test session at each retinal location.

Using SPSS, three-way repeated-measures analysis of variance (ANOVA) and appropriate statistical corrections (i.e. Bonferroni for multiple corrections) were applied to investigate differences among different experimental conditions with three within-subjects factors: retinal location (subfoveal vs. nasal vs. temporal), juice type (active vs. placebo) and time (0.5 hour after baseline vs. 1.0 hour after baseline vs. 1.5 hours after baseline). We performed separate exploratory analysis for the two refractive error groups for the active and the placebo sessions at the subfoveal retinal location at 1.5 hours after baseline only, due to the lack of overall statistical significance (see Section 3.2.4).

2.9.3. Axial length

For each subject, only the eye selected for choroidal thickness analysis was used for axial length analysis. Overall, we obtained axial length of the chosen eye at four time points of a test session (baseline measurement, 0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement) for each subject. Each subject had two test sessions, one with active juice and one with placebo juice.

Using SPSS, a paired t-test was initially used to compare the baseline axial length at the beginning of the active session and at the beginning of the placebo session in each subject. The goal is to ensure that the baseline axial length was not significantly different at the

beginning of each session for the same subject. However, the assumption of normality was violated, as assessed by the Shapiro-Wilk's test. As a result, the nonparametric Wilcoxon signed-rank test was selected instead. However, the symmetrical distribution assumption of the Wilcoxon signed-rank test was violated, and the exact sign test was ultimately chosen to compare the baseline axial lengths.

For the rest of the analysis, we looked at difference over time in axial length compared to the baseline measurement. For each of the three later time points of a test session (0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement), the difference in axial length compared to the baseline measurement was calculated (see data in Table 1 and Table 2). Using Excel, the results from all subjects were averaged to produce a graph of the change in the difference in axial length over time for the active test session and the placebo test session.

Using SPSS, two-way repeated-measures ANOVA and appropriate statistical corrections (i.e. Bonferroni for multiple corrections) were applied to investigate differences among different experimental conditions with two within-subjects factors: juice type (active vs. placebo) and time (0.5 hour after baseline vs. 1.0 hour after baseline vs. 1.5 hours). We performed separate exploratory analysis for the two refractive error groups for the active and the placebo sessions at 1.5 hours after baseline only, due to the lack of overall statistical significance (see Section 3.3).

2.9.4. Blood pressure

Overall, we obtained systolic blood pressure and diastolic blood pressure at four time points of a test session (baseline measurement, 0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement) for each subject. Each subject had two test sessions, one with active juice and one with placebo juice. The systolic blood pressure and the diastolic blood pressure were separately analyzed.

Using SPSS, a paired t-test was used to compare the baseline systolic or diastolic blood pressure at the beginning of the active session and at the beginning of the placebo session in each subject. The goal is to ensure that the baseline blood pressure was not significantly different at the beginning of each session for the same subject.

For the rest of the analysis, we looked at difference over time in systolic or diastolic blood pressure compared to the baseline measurement. For each of the three later time points of a test session (0.5 hour after baseline measurement, 1.0 hour after baseline measurement and 1.5 hours after baseline measurement), the difference in either systolic or diastolic blood pressure compared to the baseline measurement was calculated (see data in Table 1 and Table 2). Using Excel, the results from all subjects were averaged to produce a graph of the change in the difference in either systolic blood pressure or diastolic blood pressure over time for the active test session and the placebo test session.

Using SPSS, two-way repeated-measures ANOVA and appropriate statistical corrections (i.e. Bonferroni for multiple corrections) were applied to investigate differences among different experimental conditions with two within-subjects factors: juice type (active vs. placebo) and time (0.5 hour after baseline vs. 1.0 hour after baseline vs. 1.5 hours).

2.9.5. Contrast sensitivity

Using the VPE program outputs at the end of the active session and at the end of the placebo session, the researcher used a Weibull logistic function to fit the psychometric function on each subject's data. The subject's threshold corresponded to a criterion level of 75 % correct responses. Contrast sensitivity is then calculated as the inverse of the threshold.

Using Excel, contrast sensitivity was averaged by refractive error group (myope vs. emmetrope) and by juice type (active vs. placebo). A graph was made to show contrast sensitivity across different refractive groups at the end of the active session and the placebo session.

Using SPSS, two-way mixed ANOVA and appropriate statistical corrections (i.e. Bonferroni for multiple corrections) were applied to investigate differences among different experimental conditions with two factors: juice type (active vs. placebo) and refractive error (myopia vs. emmetropia).

3. Results

3.1. Subjects and exclusion of data

At the screening session, six subjects were ineligible for the study based on inclusion and exclusion criteria. Thirty subjects were eligible and enrolled in the study, including twenty-two females and eight males. In terms of refractive error, fifteen subjects were emmetropes and fifteen subjects were myopes. All subjects met all the other inclusion/exclusion criteria described above.

Three subjects had more than 33% of their choroidal measurements that required adjudication, defined by a difference of more than 15% or 30 μm in the measurements from the 2 measurers (Harb et al., 2016). When measuring choroidal thickness using digital calipers on OCT images, during the adjudication process, the two masked measurers had significantly different results on three subjects. Upon closer examination, the discrepancy in measurements between the two measurers suggested poor visibility of the chorioscleral border, and therefore increased difficulty in accurately measuring choroidal thickness. Data from these three subjects were excluded from all analyses to ensure accurate results.

At the beginning and at the end of each test session, the HumanN® Nitric Oxide Indicator strip was used to determine the systemic NO level in saliva. The NO level was classified as "depleted" or "optimal," based on the color scale provided by the manufacturer. At the beginning of each test session, due to the fasting effect, all subjects showed a "depleted" NO level. At the end of each test session, the subject's NO level should correspond to the type of juice ingested. In other words, all subjects who received placebo juice should continue to show a "depleted" NO level, whereas all subjects who received active juice should show an "optimal" NO level. At the beginning of the data analysis stage, after the experimenter was unmasked, twenty-seven subjects showed NO levels that matched the juice given at each test session. However, three subjects showed NO levels that did not match the juice given at each test session – they showed a "depleted" NO level when active juice was given, and they showed an "optimal" NO level when placebo juice was given. This mismatch could be due to malfunctioning NO strips, labeling error of juice, measurement

error, or any combination thereof. Data from these three subjects were excluded from all analyses to ensure accurate results.

Overall, out of the thirty subjects recruited for the study, data from twenty-four subjects were used for analysis, including eighteen females and six males. The mean age was 24.8 ± 2.13 years. The ethnic distribution was as follows: eleven subjects were Asian (including East Asian and Southeast Asian), ten subjects were Caucasian, one subject was Hispanic, one subject was Middle Eastern, and one subject identified as mixed. In terms of refractive error, twelve subjects were emmetropes and twelve subjects were myopes.

3.2. Choroidal thickness

3.2.1. Subfoveal choroidal thickness

The baseline subfoveal choroidal thickness for the active and the placebo sessions was not significantly different, $t(23) = -0.272$, $p = 0.788$. As shown in Table 1, the baseline subfoveal choroidal thickness for the active session was 251.07 ± 29.77 μm, and the baseline subfoveal choroidal thickness for the placebo session was 251.74 ± 28.57 μm.

Figure 2 illustrates the difference over time in choroidal thickness compared to the baseline measurements for the active and the placebo test sessions at the subfoveal retinal location for all 24 subjects. In the active session, the trend was a monotonic increase in subfoveal choroidal thickness over time. In the placebo session, the trend was an increase in subfoveal choroidal thickness at all times, with the smallest increase at 1.0 h after baseline. The choroid in both the active and placebo conditions ended up thicker by about the same amount, 8 microns, though the temporal patterns of increase differed.

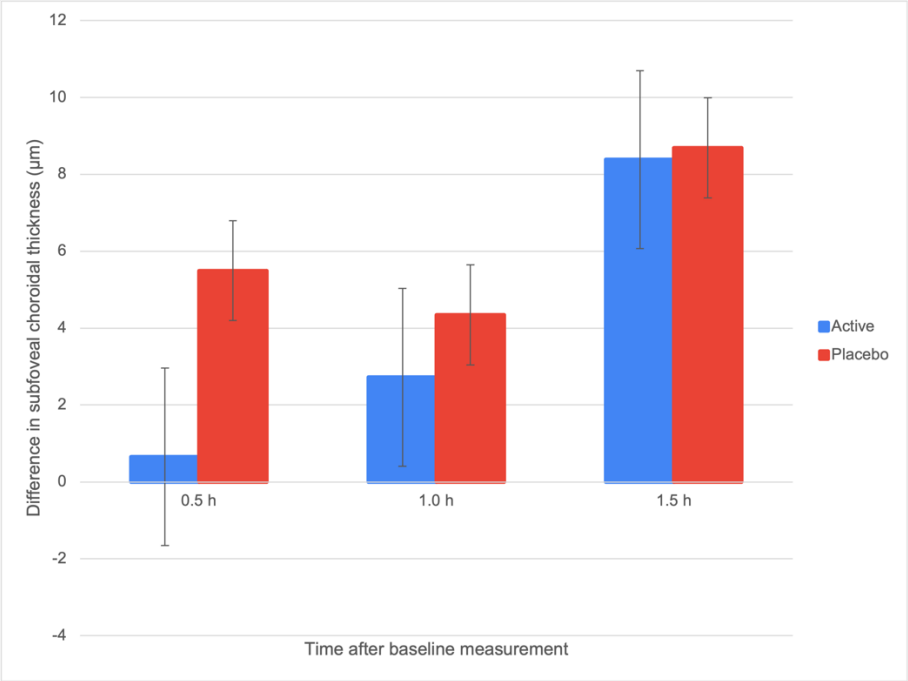

Figure 2: Change in the difference in choroidal thickness from baseline over time for the active and the placebo test sessions at the subfoveal retinal location. Error bars indicate ± 1 SEM. Values greater than 0 mean that the choroid thickened over time relative to baseline

Figure 3 illustrates the relationship between the difference in subfoveal choroidal thickness and refractive error at 1.5 hours after baseline during the active session. Figure 4 illustrates the relationship between the difference in subfoveal choroidal thickness and refractive error at 1.5 hours after baseline during the placebo session. Overall, no correlation was found between the difference in subfoveal choroidal thickness and refractive error group for either the myope group or the emmetrope group. In the placebo session, both the myope group and the emmetrope group exhibited a wider range of values than in the active session (see Figure 4).

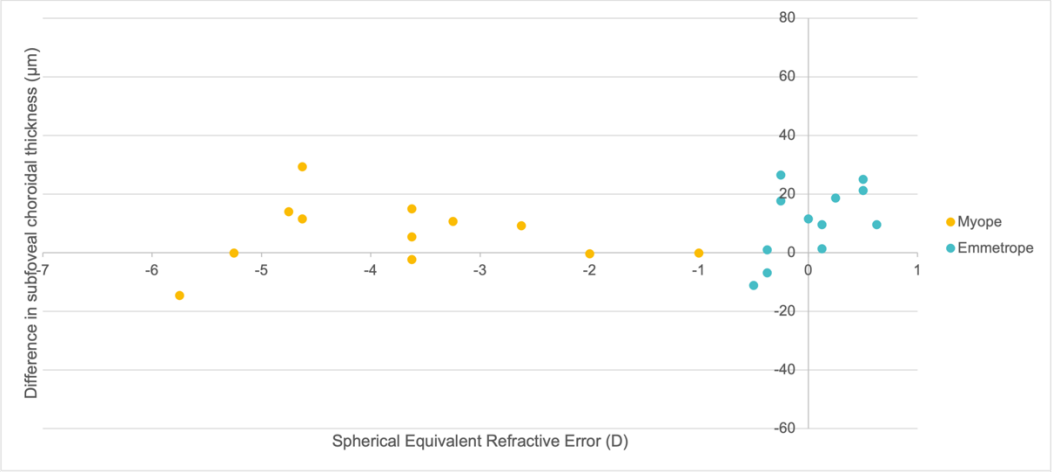

Figure 3: Scatter plot of the difference in subfoveal choroidal thickness against refractive error at 1.5 hours after baseline during the active session. Values greater than 0 mean that the choroid thickened over time relative to baseline.

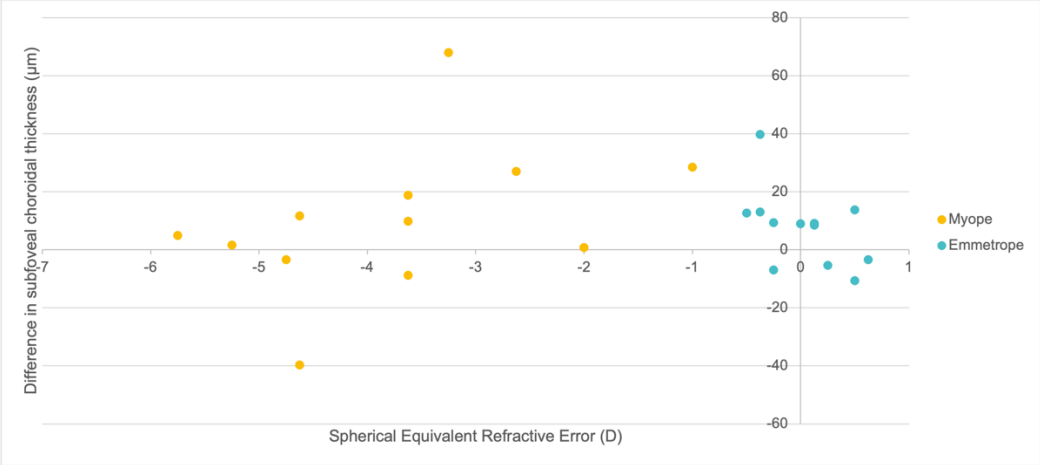

Figure 4: Scatter plot of the difference in subfoveal choroidal thickness against refractive error at 1.5 hours after baseline during the placebo session. Values greater than 0 mean that the choroid thickened over time relative to baseline.

3.2.2. Nasal choroidal thickness

The baseline nasal choroidal thickness for the active and the placebo sessions was not significantly different, $t(23) = -0.272$, $p = 0.788$. As shown in Table 1, the baseline nasal

choroidal thickness for the active session was 244.02 ± 29.82 µm, and for the placebo session was 248.11 ± 28.86 µm.

Figure 5 illustrates the difference over time in choroidal thickness compared to the baseline measurements for the active and the placebo test sessions at the nasal retinal location for all 24 subjects. In the active session, the trend was a monotonic increase in nasal choroidal thickness over time. In the placebo session, the trend was an increase in nasal choroidal thickness at all times, with the smallest increase at 1.0 h after baseline. The choroid in the active condition ended up slightly thicker than in the placebo condition.

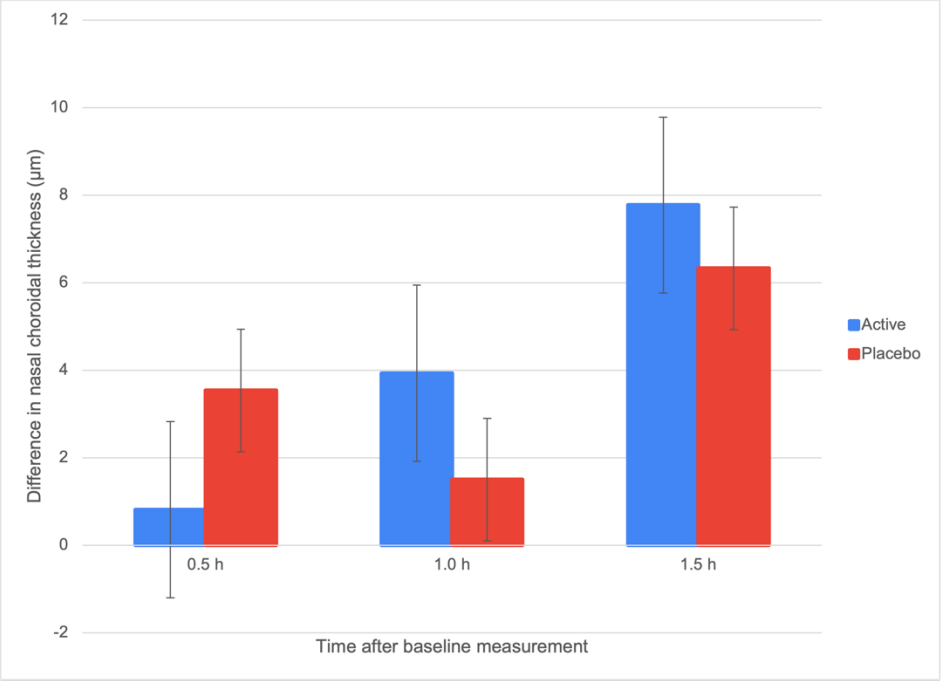

Figure 5: Change in the difference in choroidal thickness from baseline over time for the active and the placebo test sessions at the nasal retinal location. Error bars indicate ± 1 SEM

3.2.3. Temporal choroidal thickness

The baseline temporal choroidal thickness for the active and the placebo sessions was not significantly different, $t(23) = -0.746$, $p = 0.463$. As shown in Table 1, the baseline temporal choroidal thickness for the active session was 247.72 ± 29.72 μm, and the baseline temporal choroidal thickness for the placebo session was 249.94 ± 30.01 μm.

Figure 6 illustrates the difference over time in choroidal thickness compared to the baseline measurements for the active and the placebo test sessions at the temporal retinal location for all 24 subjects. In the active session, the trend was a monotonic increase in temporal choroidal thickness over time. In the placebo session, the trend was an increase in temporal choroidal thickness at all times, with the smallest increase at 1.0 h after baseline. The choroid in the active condition ended up slightly thicker than in the placebo condition.

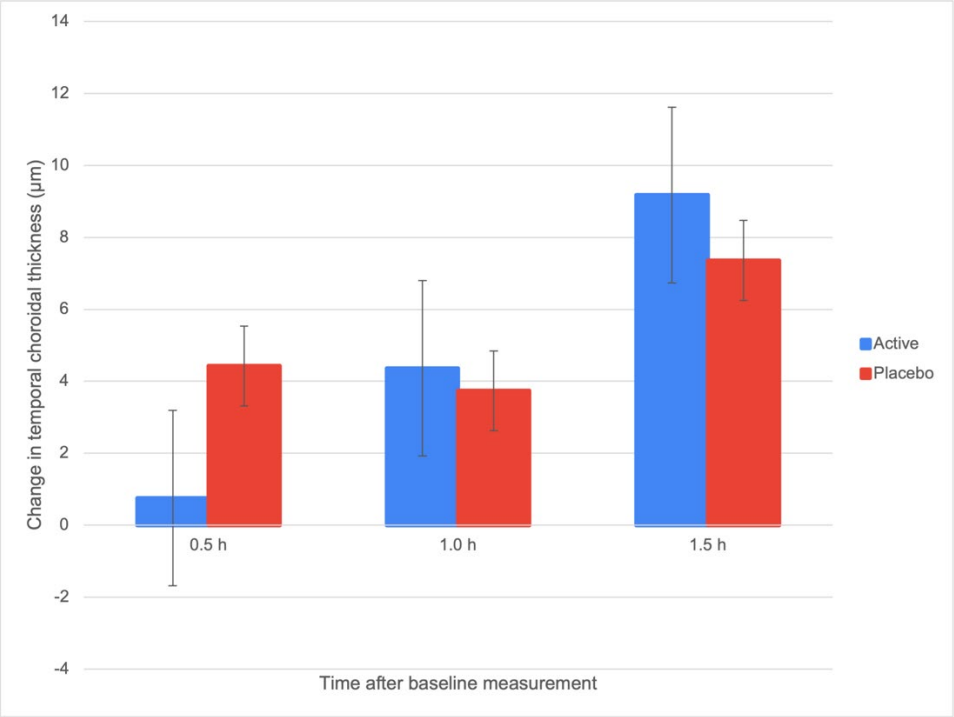

Figure 6: Change in the difference in choroidal thickness from baseline over time for the active and the placebo test sessions at the temporal retinal location. Error bars indicate ± 1 SEM

Overall, there was a similar trend in the changes in choroidal thickness over time across three retinal locations: In the active session, there was a monotonic increase in choroidal thickness over time. In the placebo session, there was an increase in choroidal thickness at all times, with the smallest increase at 1.0 h after baseline.

3.2.4. Interaction between retinal location, juice type and time for choroidal thickness

For choroidal thickness, there was no statistically significant three-way interaction between retinal location, juice type and time, $F(4, 92) = 0.443$, $p = 0.777$. Further analysis revealed no statistically significant two-way interaction between condition*location ($F(2, 46)$

= 1.339, p = 0.272), condition*time (F(2, 46) = 0.841, p = 0.438), or location*time (F(4, 92) = 0.384, p = 0.819).

3.3. Axial length

There was no statistically significant difference in the median axial length between the active session (24.24 mm) and the placebo session (24.25mm) at baseline, p = 0.134.

Figure 7 illustrates the difference over time in axial length compared to the baseline measurements for the active and the placebo test sessions for all 24 subjects. The differences in axial length values were converted from mm to μm for better graphic presentation. In the active session, there was a monotonic increase in axial length over time. In the placebo session, there was a decrease in axial length at all times relative to baseline.

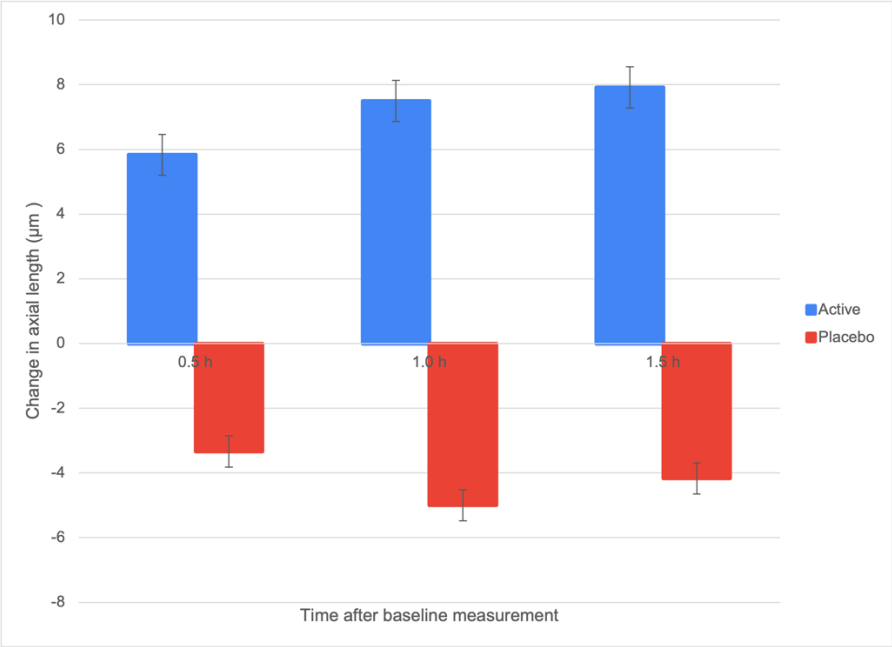

Figure 7: Change in the difference in axial length from baseline over time for the active and the placebo test sessions. Error bars indicate ± 1 SEM. Values above zero represent an increase in AL and those below zero a decrease

Figure 8 illustrates the relationship between the difference in axial length and refractive error at 1.5 hours after baseline during the active session. Figure 9 illustrates the relationship between the difference in axial length and refractive error at 1.5 hours after baseline during the placebo session. Overall, no correlation was found between the difference in axial length and refractive error group for either the myope group or the emmetrope group. The emmetrope group exhibited a wider range of values in the difference in axial length during both the active session and the placebo session; however, the variability was driven by a few outliers that showed shortening of axial length at 1.5 hours after baseline (see Figure 8 and Figure 9).

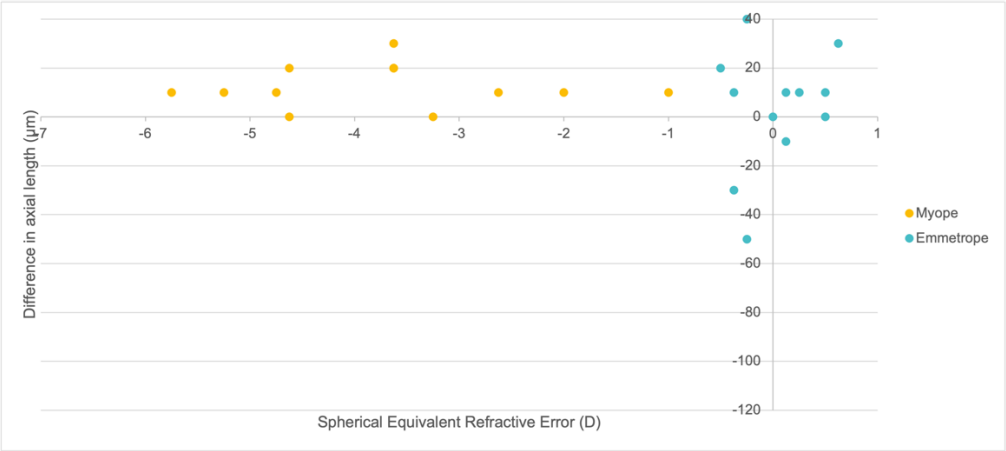

Figure 8: Scatter plot of the difference in axial length against refractive error at 1.5 hours after baseline during the active session. Values greater than 0 mean that the axial length increased over time relative to baseline.

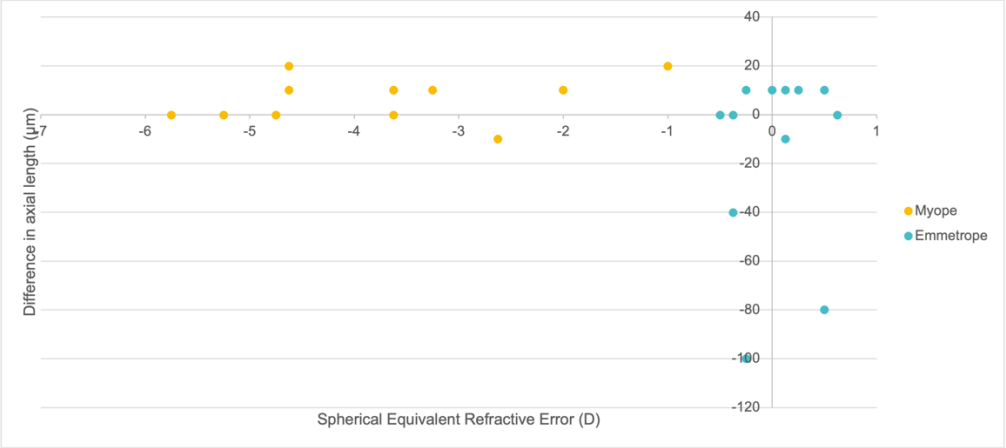

Figure 9: Scatter plot of the difference in axial length against refractive error at 1.5 hours after baseline during the placebo session. Values greater than 0 mean that the axial length increased over time relative to baseline.

There was no statistically significant two-way interaction between juice type and time, $F(2, 46) = 0.603$, $p = 0.552$. The main effect of time showed no statistically significant difference in axial length between trials, $F(1.321, 30.378) = 0.028$, $p = 0.921$. However, the main effect of juice type showed a statistically significant difference in axial length between

trials, $F(1, 46) = 5.321$, $p = 0.03$. The axial length increased with the active juice and decreased with the placebo, an unexpected finding.

3.4. Blood pressure

3.4.1. Systolic blood pressure

The baseline systolic blood pressure for the active and the placebo sessions were not significantly different, $t(23) = 0.891$, $p = 0.382$. The baseline systolic blood pressure for the active session was 109.25 ± 11.25 mmHg, and the baseline systolic blood pressure for the placebo session was 107.88 ± 8.44 mmHg.

Figure 10 illustrates the difference over time in systolic blood pressure compared to the baseline measurements for the active and the placebo test sessions for all 24 subjects. For both the active and the placebo sessions, there was a decrease in the systolic blood pressure at all times after baseline with the smallest decrease at 1.0 h after baseline.

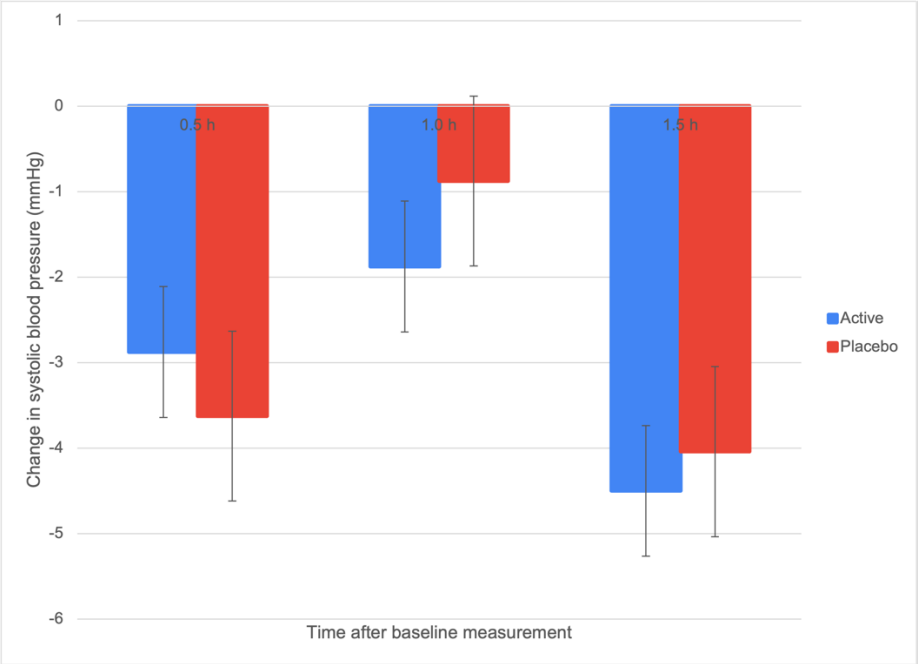

Figure 10: Change in the difference in systolic blood pressure from baseline over time for the active and the placebo test sessions. Error bars indicate ± 1 SEM. Values greater than 0 mean that the systolic blood pressure increased over time relative to baseline.

There was no statistically significant two-way interaction between juice type and time, $F(2, 46) = 0.603$, $p = 0.552$. The main effect of juice type showed no statistically significant difference in systolic blood pressure between trials, $F(1.321, 30.378) = 0.028$, $p = 0.921$. However, the main effect of time showed a statistically significant difference in systolic blood pressure between trials, $F(2, 46) = 5.089$, $p = 0.01$. Systolic blood pressure was statistically different between 1.0 hour after baseline measurement and 1.5 hours after baseline measurement for both juice groups combined, $p = 0.017$, a mean difference of -2.896 (95% CI, -0.440 to -5.352) mmHg.

3.4.2. Diastolic blood pressure

Figure 11 illustrates the difference over time in diastolic blood pressure compared to the baseline measurements for the active and the placebo test sessions for all 24 subjects. For the placebo session, there was a decrease in the diastolic blood pressure at all times after baseline with the smallest decrease at 1.0 h after baseline. For the active session, the amount of decrease in diastolic pressure reduced over time and approached baseline at 1.5 h after baseline. Although there is no statistical significance ($p = 0.14$) via a paired-samples t-test, the diastolic blood pressure for the active session appeared to be higher than the diastolic blood pressure for the placebo session at 1.5 h after baseline.

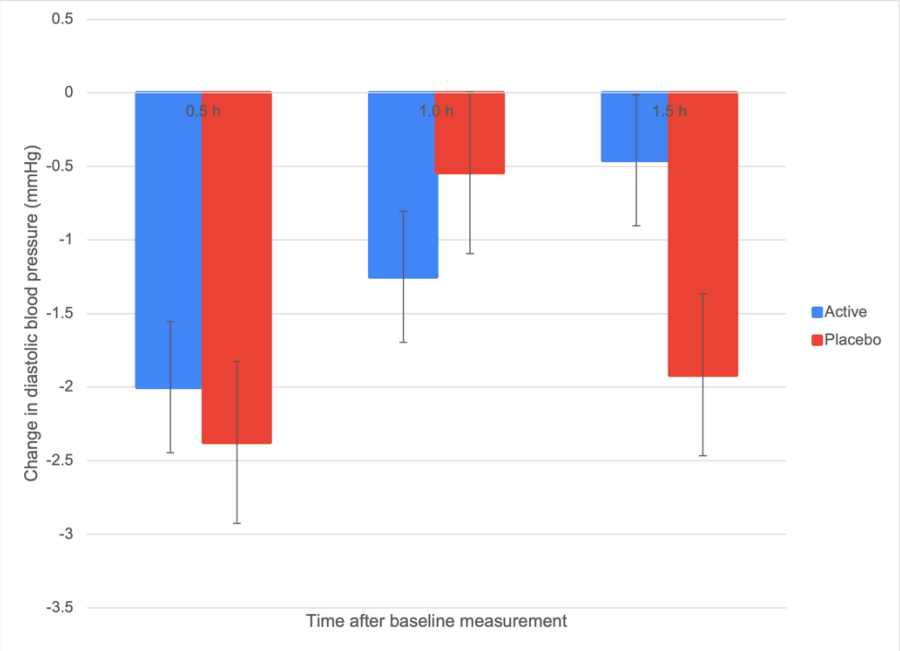

Figure 11: Change in the difference in diastolic blood pressure from baseline over time for the active and the placebo test sessions. Error bars indicate ± 1 SEM. Values greater than 0 mean that the diastolic blood pressure increased over time relative to baseline.

The baseline diastolic blood pressure for the active and the placebo sessions were not significantly different, $t(23) = 1.289$, $p = 0.210$. The baseline diastolic blood pressure for the active session was 74.42 ± 7.25 mmHg, and the baseline systolic blood pressure for the placebo session was 72.54 ± 7.03 mmHg.

There was no statistically significant two-way interaction between juice type and time, $F(2, 46) = 0.913$, $p = 0.408$. The main effect of juice type showed no statistically significant difference in diastolic blood pressure between trials, $F(1,23) = 0.084$, $p = 0.775$. The main effect of time showed no statistically significant difference in diastolic blood pressure between trials $F(2,46) = 1.419$, $p = 0.252$.

3.5. Contrast sensitivity

Figure 12 illustrates contrast sensitivity across different refractive groups at the end of the active session and the placebo session. For myopes, contrast sensitivity appears to be higher at the end of the active session. For emmetropes, contrast sensitivity appears to be higher at the end of the placebo session, although there is no statistically significant difference ($p = 0.22$) via a paired-samples t-test,

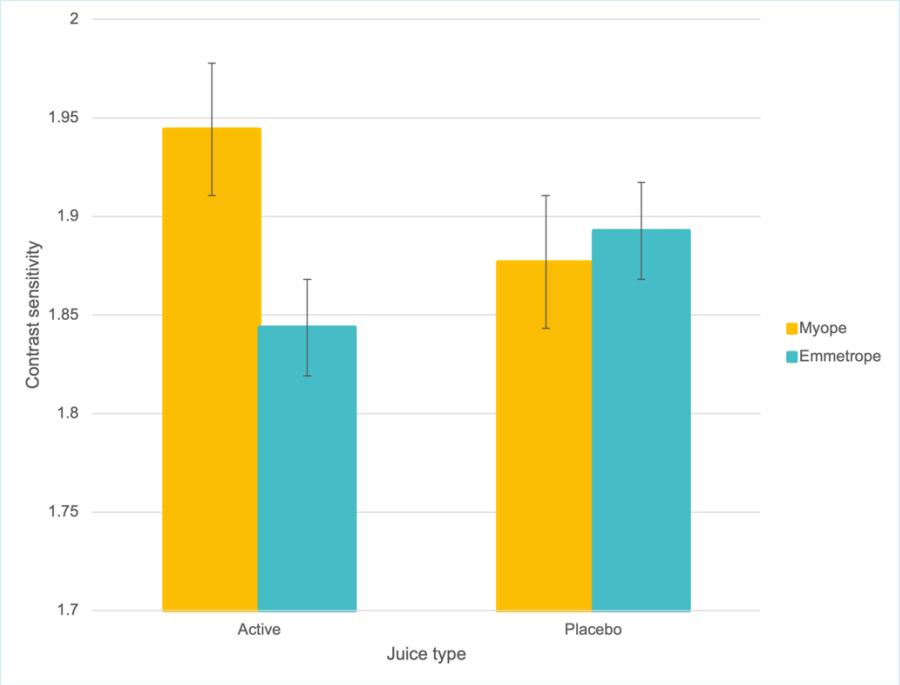

Figure 12: Contrast sensitivity across different refractive groups at the end the active session and the placebo session. Error bars indicate ± 1 SEM

There was no statistically significant interaction between juice type and refractive error group on contrast sensitivity, $F(1, 22) = 0.893$, $p = 0.355$. The main effect of juice type showed no statistically significant difference in contrast sensitivity across different refractive groups, $F(1,22) = 0.022$, $p = 0.883$. The main effect of refractive error group showed no statistically significant difference in contrast sensitivity across different juice types, $F(1, 22) = 0.359$, $p = 0.555$.

4. Discussion and Conclusions

In the current study, we investigated the effect of a dietary supplement, beetroot juice with nitric oxide, on human choroidal thickness, axial length, blood pressure and contrast

sensitivity. Overall, we found no significant changes in these variables over the course of 1.5 hours, although a few trends were found.

4.1. Choroidal thickness

Although there was no statistical significance, a similar trend was observed in the changes in choroidal thickness across three retinal locations: In the active session, there was a monotonic increase in choroidal thickness over time. In the placebo session, there was a decrease in the difference in choroidal thickness at 1.0 h after baseline compared to 0.5 h after baseline, and an increase in the difference in choroidal thickness at 1.5 h after baseline.

To our knowledge, our study was the first to investigate the effect of NO via dietary absorption on human choroidal thickness. Most studies that investigated the effect of pharmacological agents on choroidal thickness have been animal studies. As discussed in the introduction, studies consistently showed that NOS inhibitors inhibited choroidal thickening in chicks and axial elongation shortly followed (Nickla and Wildsoet, 2004; Nickla et al., 2006; Nickla et al., 2009; Nickla and Wallman, 2010), whereas NO or NO precursors promoted choroidal thickening with the inhibition of axial elongation shortly following (Carr and Stell, 2016). However, a few earlier studies did show conflicting results by demonstrating the opposite: injection of L-NAME inhibited axial elongation in the development of form deprivation myopia and hyperopic defocus-induced myopia (Fujikado et al., 1997; Fujikado et al., 2001). The method of aadministration of NO was intravitreal injection in all animal studies (Carr and Stell, 2016; Nickla and Wildsoet, 2004; Nickla et al.,

2006; Nickla et al., 2009; Nickla and Wallman, 2010). Oral administration of NO has not been tested.

Results of the current study did not show significant changes over time in choroidal thickness at any of the three retinal locations with either active or placebo juice. The reasons could be two-fold. For the first reason, we consider the oral route of administration of NO used in the current study. Oral ingestion of nitrite promotes NO production, but for the intervention to have any effect on the choroid, the newly produced NO molecules would need to go through systemic circulation to reach choroidal circulation. One issue with the oral route of administration is that NO has a very short half-life – only two milliseconds in blood and two seconds in tissues (Eroglu E et al., 2018; Park et al., 2020). It is possible that the newly produced NO as a result of beetroot juice ingested had already dissipated before reaching any ocular issue. Future studies could repeat the same study with oral administration of NO, but with increased concentration of nitrate in the beetroot juice and/or more frequent administration of beetroot juice. Future studies could also consider using a different route of administration, such as the topical administration of NO. In glaucoma therapy research, a few compounds have been developed as a combination of a known glaucoma therapy drug and an NO-donor (Erdinest et al., 2021). The goal is to overcome the short half-life of NO by metabolizing the compound in the eye and releasing both the drug and NO to the target tissue (Erdinest et al., 2021). One such promising candidate is 0.024% Latanoprostene bunod (Bausch and Lomb) (Araie et al., 2015; Kaufman et al., 2017; Kawase et al., 2016), which passed the Phase 3 trial and was approved by the FDA to enter the market as the VYZULTA® glaucoma therapy in 2017 (Medeiros, 2016). To our knowledge, no study has

been conducted on the effect of topical agents containing NO-donor moieties on choroidal thickness, including VYZULTA®.

Another possible reason for non-significant results is measurement errors. The OCT used for the study was a 2004 Optovue RTVue-100 OCT. Despite the researcher's best efforts in image acquisition and data analysis, the choroioscleral border was still hard to distinguish in some subjects. The ambiguous choroioscleral border in OCT images could contribute to the non-significant results. Future studies should consider using an OCT machine with a higher resolution, such as a swept-source OCT (SS-OCT) (Copete et al., 2013; Hirata et al., 2011; Mansouri et al., 2014). One study using SS-OCT and the manual tracing method of determining choroidal thickness found that SS-OCT was able to produce images with clear choriosclera borders and measure choroidal thickness with reproducibility in 100% of eyes, versus only 74.4% of eyes using spectral-domain OCT (SD-OCT) (Copete et al., 2013). Future studies should also consider repeating the same study but using automated segmentation software instead of the manual tracing method for measuring choroidal thickness (Gupta et al., 2015; Zhao et al., 2020). It was found that compared to manual tracing, automated segmentation software helped obtain accurate results faster, especially for inexperienced experimenters (Zhao et al., 2020).

4.2 Axial length

In animal studies, axial elongation follows shortly after and is closely linked to choroidal thickening (Nickla and Wallman, 2010). In the studies previously discussed in this thesis, inhibitors of NO production not only inhibited choroidal thickening, but also induced

ocular elongation (Nickla and Wildsoet, 2004; Nickla et al., 2006; Nickla et al., 2009; Nickla and Wallman, 2010). In the Carr and Stell (2016) study where an agonist of NO production was used, the axial length was significantly shorter than in the control trials.

Very few studies have evaluated the short-term effect of pharmacological agents on axial length in human eyes. One such study used low-dose 0.01% atropine, a mainstay intervention for myopia control, as the pharmacological agent of choice (Sander et al., 2019). To our knowledge, none of the studies has utilized NO. Sander et al. found that low-dose 0.01% atropine induced a statistically significant shortening of axial length at 60 mins post drop administration in young myopic adults, but the amount (-6 ± 5 μm) was likely not clinically significant (Sander et al., 2019).

In the current study, we did not find a significant effect of axial-length shortening in young adults. This corresponds to the lack of significant results in the literature on short-term changes in axial length immediately in response to pharmacological interventions. We did find the unexpected trend that axial length became longer during the active session and shorter during the placebo session, although the overall change was less than 10 μm (see Figure 5), smaller than the estimated diurnal axial length fluctuation of 15-40 μm (Stone et al., 2004). It is possible that the trend observed in our study was due to random fluctuations in axial length, but more studies are needed to investigate if a similar trend is seen.

Similar to choroidal thickness, it is also possible that the non-significance in axial length data was due to the oral route administration of NO used in this study. As shown in the Sander et al., 2019 study, a topical agent would be more likely to exert effects on ocular tissues directly.

4.3. Blood pressure

Overall, there was no statistically significant two-way interaction between juice type and time for either the systolic blood pressure or the diastolic blood pressure. The only statistically significant main effect was time in terms of systolic blood pressure: Systolic blood pressure was statistically higher at 1.0 hour after baseline measurement than at1.5 hours after baseline measurement, $p = 0.017 < 0.05$, a mean difference of 2.9 (95% CI, 0.44 to 5.35) mmHg.

Two recent meta-analyses (Siervo et al. (2013) and Bahadoran et al. (2017)) summarized the effect of beetroot juice on blood pressure: Siervo et al. (2013) found that based on 12 eligible studies, there was a significant effect of beetroot juice supplementation on systolic blood pressure with a mean reduction of 4.5 mmHg when compared to the placebo condition, whereas there was not a significant effect of beetroot juice supplementation on diastolic blood pressure with a mean reduction of 0.9 mmHg when compared to the placebo condition. The effect was similar for both short-term (< 3 days) and long-term (≥3 days) supplementation of beetroot juice (Siervo et al., 2013). Bahadoran et al. (2017) further investigated the effect based on the type of placebo. After analyzing 27 studies using the nitrate-depleted beetroot juice as placebo, the same type of placebo used in the current study, there was a significant effect of beetroot juice supplementation on systolic blood pressure with a weighted mean difference of -2.91 mmHg, whereas there was not a significant effect of beetroot juice supplementation on diastolic blood pressure with a weighted mean difference of -0.71 mmHg when compared to the placebo condition

(Bahadoran et al., 2017). The effect was similar for both short-term (< 2 weeks) and long-term (≥ 2 weeks) supplementation of beetroot juice (Bahadoran et al., 2017).

Our results agree with the meta-analyses above. Although no significance in two-way repeated-measures ANOVA was found in either systolic or diastolic blood pressure, there was a significant reduction in systolic blood pressure at 1.5 hours after baseline compared to 1.0 hour after baseline. The mean difference of 2.9 mmHg was on par with the result shown in Bahadoran et al., 2017. Although not significant, there was a downward trend for systolic blood pressure even in the placebo group. This result also agreed with a prevailing theory in related nutrition research, which stated that in both the active and the nitrate-depleted placebo beetroot juice, biologically active components other than nitrate could potentially regulate reduction of systolic, and to a lesser extent, diastolic blood pressure (Bahadoran et al., 2017; Clifford , 2015).

The limited significance in the blood pressure data in the present study could be due to two reasons. The first reason is that the current study only recruited young, healthy subjects with no chronic systemic conditions. It was found that the reduction in systolic and diastolic blood pressure with beetroot juice supplementation was significantly higher in subjects with chronic systemic conditions than in healthy subjects (Bahadoran et al., 2017; Siervo et al., 2013). The second reason is that the current study only measured blood pressure within a relatively short period of 1.5 hours after juice ingestion, whereas most studies in the literature measured blood pressure over a longer period of time, at least 2 days with daily juice ingestion (Bahadoran et al., 2017; Siervo et al., 2013).

4.4. Contrast sensitivity

Although no statistical significance was found in the contrast sensitivity data, it appears that for the placebo session, the contrast sensitivity was higher for the emmetropic group than the myopic group, which agrees with the finding reported by some studies that the central spatial contrast sensitivity is impaired in myopes (Collins and Carney, 1990; Fiorentini and Maffei L, 1976; Liou and Chiu, 2001). Furthermore, myopes but not emmetropes showed an improvement in contrast sensitivity at the end of the active session when compared to the placebo session, which could support the theory that nitrate-rich beetroot juice may enhance contrast sensitivity in eyes with existing impaired contrast sensitivity.

In the current literature, only a few animal studies investigated the effect of NO on contrast sensitivity. One study in chicks investigated spatial contrast sensitivity under low- and high-photopic illumination and how it was modulated by externally applied dopamine and NO (Shi et al., 2020). It was found that in low-photopic illumination, NO donors and dopamine agonists increased contrast sensitivity to similar levels as found in high-photopic illumination with no pharmacological interventions (Shi et al., 2020). Furthermore, this enhanced contrast sensitivity was abolished and reduced to the level under low-photopic illumination if either a NO production inhibitor or a dopamine antagonist was applied sequentially (Shi et al., 2020). These findings suggested that NO may play a role in contrast sensitivity modulation.

Similar to choroidal thickness and axial length results, it is also possible that the oral administration of NO used in this study affected its availability in ocular tissues and led to the non-significant results.

4.5. Strengths/limitations and future directions

The strengths of the current study include the double-masked, repeated-measures experimental design and the use of the nitrate-depleted placebo juice. The study was also carefully designed to control for any other factors that could potentially affect choroidal thickness other than NO, such as accommodation and diet leading up to the testing session. Furthermore, the study used an established protocol and two experimenters for choroidal thickness measurements, which were taken at the same time of day for the two sessions.

The current study also has a number of limitations. An OCT of low resolution was used to measure choroidal thickness, the main outcome measurement. Moreover, both experimenters who performed manual tracing did not have prior experience with the technique, potentially contributing to human measurement errors. Lastly, we did not consider the amount of juice ingestion by body weight, potentially leading to greater effect of systemic NO upregulation in subjects with lower body weight and less effect of systemic NO upregulation in subjects with higher body weight.

The overall lack of statistical significance in the current study could potentially be attributed to the insufficient level of NO that had reached ocular tissues via systemic circulation and/or poor instrument resolution. Future studies should consider repeating the measurements with increased concentration of nitrate in the beetroot juice per body weight

and/or more frequent administration of beetroot juice. Future studies should also consider using a topical pharmacological agent with NO donating moieties, such as Latanoprostene bunod. Lastly, future studies should consider using an OCT machine with a higher resolution and automated segmentation software to obtain more accurate choroidal thickness measurements.

4.6. Conclusions

In this preliminary study, there was no significant effect of NO-rich dietary supplement beetroot juice on choroidal thickness, axial length, blood pressure or contrast sensitivity over 1.5 hours in a cohort of 24 healthy young adults.

References

Ahmad A, Dempsey SK, Daneva Z, Azam M, Li N, Li PL, Ritter JK (2018). Role of Nitric Oxide in the Cardiovascular and Renal Systems. *International Journal of Molecular Sciences*;19(9):2605.

Aliancy J, Stamer W, Wirostko B (2017). A Review of Nitric Oxide for the Treatment of Glaucomatous Disease. *Ophthalmology and Therapy*;6(2):221-232.

Ang M, Flanagan JL, Wong CW, et al (2020). Review: Myopia Control Strategies Recommendations from the 2018 WHO/IAPB/BHVI Meeting on Myopia. *British Journal of Ophthalmology*;104:1482–1487

Araie M, Sforzolini B, Vittitow J, Weinreb R (2015). Evaluation of the Effect of Latanoprostene Bunod Ophthalmic Solution, 0.024% in Lowering Intraocular Pressure over 24 h in Healthy Japanese Subjects. *Advances in Therapy*;32(11):1128-1139.

Arumugam B, Hung L-F, To C-H, et al (2014). The Effects of Simultaneous Dual Focus Lenses on Refractive Development in Infant Monkeys. *Investigative Ophthalmology & Visual Science*;55:7423–7432.

Bahadoran Z, Mirmiran P, Kabir A, Azizi F, Ghasemi A (2017). The Nitrate-Independent Blood Pressure–Lowering Effect of Beetroot Juice: A Systematic Review and Meta-Analysis. *Advances in Nutrition: An International Review Journal*;8(6):830-838.

Bergua A, Mayer B, Neuhuber W (1996). Nitrergic and VIPergic Neurons in the Choroid and Ciliary Ganglion of the Duck Anis carina. *Anatomy and Embryology*;193(3).

Borda E, Berra A, Saravia M, Ganzinelli S, Sterin-Borda L (2005). Correlations Between Neuronal Nitric Oxide Synthase and Muscarinic M3/M1 Receptors in the Rat Retina. *Experimental Eye Research*;80(3):391-9.

Cao K, Wan Y, Yusufu M, Wang N (2020). Significance of Outdoor Time for Myopia Prevention: a Systematic Review and Meta-analysis Based on Randomized Controlled Trials. *Ophthalmic Research*;63:97–105

Carr BJ, Stell WK (2016). Nitric Oxide (NO) Mediates the Inhibition of Form-Deprivation Myopia by Atropine in Chicks. *Scientific Reports*;6(1).

Carr BJ, Stell WK (2017). The Science Behind Myopia. *Webvision: The Organization of the Retina and Visual System*. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470669/

Chakraborty R., Read S.A., Vincent S.J. (2020) Understanding Myopia: Pathogenesis and Mechanisms. *Updates on Myopia*. Springer, Singapore. https://doi.org/10.1007/978-981-13-8491-2_4

Chamberlain P, Peixoto-de-Matos SC, Logan NS, Ngo C, Jones D, Young G (2019). A 3-year Randomized Clinical Trial of MiSight Lenses for Myopia Control. *Optometry and Vision Science*;96:556–567.

Charm J, Cho P (2013). High Myopia Partial Reduction Ortho-k: a 2-year randomized study. *Optometry and Vision Science*;90:530–539.

Chen K, Pittman R, Popel A (2008). Nitric Oxide in the Vasculature: Where Does It Come From and Where Does It Go? A Quantitative Perspective. *Antioxidants & Redox Signaling*;10(7):1185-1198.

Chen YP, Prashar A, Erichsen JT, To CH, Hocking PM, Guggenheim JA (2011). Heritability of Ocular Component Dimensions in Chickens: Genetic Variants Controlling Susceptibility to Experimentally Induced Myopia and Pretreatment Eye Size are Distinct. *Investigative Ophthalmology & Visual Science*;52:4012–4020

Chen PC, Woung LC, Yang CF (2000). Modulation Transfer Function and Critical Flicker Frequency in High-Myopia Patients. *Journal of the Formosan Medical Association*;99(1):45-8

Cheung WS, Bhan I, Lipton SA (2000). Nitric Oxide (NO.) Stabilizes Whereas Nitrosonium (NO+) Enhances Filopodial Outgrowth by Rat Retinal Ganglion Cells in Vitro. *Brain Research*;868(1):1-13.

Chia A, Chua WH, Cheung YB, et al (2012). Atropine for the Treatment of Childhood Myopia: Safety and Efficacy of 0.5%, 0.1%, and 0. 01% Doses (Atropine for the Treatment of Myopia 2). *Ophthalmology*;119:347–354.

Cho P, Cheung SW (2012). Retardation of Myopia in Orthokeratology (ROMIO) Study: a 2-year Randomized Clinical Trial. *Investigative Ophthalmology & Visual Science*;53:7077–7085.

Chua WH, Balakrishnan V, Chan YH, et al (2006). Atropine for the Treatment of Childhood Myopia. *Ophthalmology*;113:2285–2291.

Chui T, Yap M, Chan H, Thibos L (2005). Retinal Stretching Limits Peripheral Visual Acuity in Myopia. *Vision Research*;45(5):593-605.

Clifford T, Howatson G, West D, Stevenson E (2015). The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Nutrients*;7(4):2801-2822.

Collins J, Carney L (1990). Visual performance in high myopia. *Current Eye Research*;9:217–223.

Copete S, Flores-Moreno I, Montero J, Duker J, Ruiz-Moreno J (2013). Direct Comparison of Spectral-Domain and Swept-Source OCT in the Measurement of Choroidal Thickness in Normal Eyes. *British Journal of Ophthalmology*;98(3):334-338.

Cuthbertson S, Jackson B, Toledo C, Fitzgerald ME, Shih YF, Zagvazdin Y, Reiner A (1997). Innervation of Orbital and Choroidal Blood Vessels by the Pterygopalatine Ganglion in Pigeons. *The Journal of Comparative Neurology*;386(3):422-42. PMID: 9303427.

Davies KP (2015). Development and Therapeutic Applications of Nitric Oxide Releasing Materials to Treat Erectile Dysfunction. *Future Science OA*;1(1):FSO53.

Delaey C, Van De Voorde J (2000). Regulatory Mechanisms in the Retinal and Choroidal Circulation. *Ophthalmic Research*;32(6):249-56.

Deng J, Li X, Jin J, Zhang B, Zhu J, Zou H, Xu X, Xie J, Wang L, Zhu S, He X (2018). Distribution Pattern of Choroidal Thickness at the Posterior Pole in Chinese Children With Myopia. *Investigative Opthalmology & Visual Science*;59(3):1577.

Dawson T, Bredt D, Fotuhi M, Hwang P, Snyder S (1991). Nitric Oxide Synthase and Neuronal NADPH Diaphorase are Identical in Brain and Peripheral Tissues. *Proceedings of the National Academy of Sciences*;88(17):7797-7801.

Dolgin E (2015). The Myopia Boom. *Nature*;519(7543):276-8. doi: 10.1038/519276a. PMID: 25788077.

dos Santos Baião D, Vieira Teixeira da Silva D, Margaret Flosi Paschoalin V (2021). A Narrative Review on Dietary Strategies to Provide Nitric Oxide as a Non-Drug Cardiovascular Disease Therapy: Beetroot Formulations—A Smart Nutritional Intervention. *Foods*;10(4):859.

Duke-Elder S (1978). *Practice of refraction*. Elsevier; p 26.

Ehsaei A, Chisholm CM, Pacey IE, Mallen EAH(2013). Visual Performance Fall-off with Eccentricity in Myopes versus Emmetropes. *Journal of Optometry*;6(1):36–44.

Erdinest N, London N, Ovadia H, Levinger N (2021). Nitric Oxide Interaction with the Eye. *Vision*;5(2):29.

Eroglu E, Bischof H, Charoensin S, Waldeck-Weiermaier M, Graier WF, Malli R (2018). Real-Time Imaging of Nitric Oxide Signals in Individual Cells Using geNOps. *Methods in Molecular Biology.*;1747:23-34.

Fiorentini A, Maffei L (1976). Spatial Contrast Sensitivity of Myopic Subjects. *Vision Research*;16:437–438

Flitcroft D (2012). The Complex Interactions of Retinal, Optical and Environmental Factors in Myopia Aetiology. *Progress in Retinal and Eye Research*;31:622–660.

Flitcroft D, He M, Jonas J, Jong M, Naidoo K, Ohno-Matsui K, Rahi J, Resnikoff S, Vitale S, Yannuzzi L (2019). IMI – Defining and Classifying Myopia: A Proposed Set of Standards for Clinical and Epidemiologic Studies. *Investigative Opthalmology & Visual Science*;60(3):M20.

Förstermann U, Sessa WC (2011). Nitric Oxide Synthases: Regulation and Function. *European Heart Journal*.;33(7):829-37, 837a-837d.

Francisco B, Salvador M, Amparo N (2015). Oxidative Stress in Myopia. *Oxidative Medicine and Cellular Longevity*;2015:1-12.

Fricke T, Holden B, Wilson D, Schlenther G, Naidoo K, Resnikoff S, Frick K (2012). Global Cost of Correcting Vision Impairment from Uncorrected Refractive Error. *Bulletin of the World Health Organization*;90(10):728-738.

Fricke TR, Jong M, Naidoo KS, et al (2018). Global Prevalence of Visual Impairment Associated with Myopic Macular Degeneration and Temporal Trends from 2000 through 2050: Systematic Review, Meta-analysis and Modelling. *British Journal of Ophthalmology*; 102(7): 855–862.

Fujikado T, Kawasaki Y, Fujii J, et al (1997). The Effect of Nitric Oxide Synthase Inhibitor on Form-deprivation Myopia. *Current Eye Research*;16:992–996.

Fujikado T, Tsujikawa K, Tamura M, Hosohata J, Kawasaki Y, Tano Y (2001). Effect of a Nitric Oxide Synthase Inhibitor on Lens-induced myopia. *Ophthalmic Research*;33:75–79.

Gabriele M, Wollstein G, Ishikawa H, Kagemann L, Xu J, Folio L, Schuman J (2011). Optical Coherence Tomography: History, Current Status, and Laboratory Work. *Investigative Ophthalmology & Visual Science*;52(5):2425.

Ghosh A, Zheleznyak L, Barbot A, Jung HW, Yoon G (2017). Neural Adaptation to Peripheral Blur in Myopes and Emmetropes. *Vision Research*;132:69–77.

Goldstein I, Ostwald P, Roth S (1996). Nitric Oxide: A Review of Its Role in Retinal Function and Disease. *Vision Research*;36(18):2979-2994.

Gow A, Chen Q, Gole M, Themistocleous M, Lee V, Ischiropoulos H (2000). Two Distinct Mechanisms of Nitric Oxide-mediated Neuronal Cell Death Show Thiol Dependency. *American Journal of Physiology-Cell Physiology*;278(6):C1099-C1107.

Grytz R, Siegwart JT Jr (2015). Changing Material Properties of the Tree Shrew Sclera during Minus Lens Compensation and Recovery. *Investigative Ophthalmology & Visual Science*;56:2065–2078.

Guggenheim JA, Chen YP, Yip E, et al (2011). Pre-treatment Choroidal Thickness is not Predictive of Susceptibility to Form-deprivation Myopia in Chickens. *Ophthalmic and Physiological Optics*;31:516–528.

Guo X, Xiao O, Chen Y, He M (2015). Fundus Changes in Highly Myopic Eyes with Different Shapes Identified by High-resolution Three-dimensional Magnetic Resonance Imaging. *Investigative Ophthalmology & Visual Science*; 56(7): 4081.

Gupta P, Jing T, Marziliano P, Cheung C, Baskaran M, Lamoureux E, Wong T, Cheung C, Cheng C (2015). Distribution and Determinants of Choroidal Thickness and Volume Using Automated Segmentation Software in a Population-Based Study. *American Journal of Ophthalmology*;159(2):293-301.e3.

Haamedi SN, Djamgoz MB (2002). Dopamine and Nitric Oxide Control Both Flickering and Steady-light-induced Cone Contraction and Horizontal Cell Spinule Formation in the Teleost (carp) Retina: Serial Interaction of Dopamine and Nitric Oxide. *Journal of Comparative Neurology*;449(2):120-8.

Harb E, Hyman L, Gwiazda J, Marsh-tootle W, Zhang Q, Norton TT, et al (2016). Choroidal Thickness Profiles in Myopic Eyes of Young Adults in the Correction of Myopia Evaluation Trial cohort. *American Journal of Ophthalmology*;160(1):62-71.

Hathibelagal A, Manoharan M, Verkicharla P (2021). Do Myopes Have Deficits in Peripheral Flicker Sensitivity? *Journal of Optometry*;170(4)

He S, Weiler R, Vaney DI (2000). Endogenous Dopaminergic Regulation of Horizontal Cell Coupling in the Mammalian Retina. *Journal of Comparative Neurology*;418(1):33-40.

He M, Xiang F, Zeng Y, et al (2015). Effect of Time Spent Outdoors at School on the Development of Myopia among Children in China: a Randomized Clinical Trial. *JAMA*;314:1142-1148.

Hirata M, Tsujikawa A, Matsumoto A, Hangai M, Ooto S, Yamashiro K, Akiba M, Yoshimura N (2011). Macular Choroidal Thickness and Volume in Normal Subjects Measured by Swept-Source Optical Coherence Tomography. *Investigative Ophthalmology & Visual Science*;52(8):4971.

Ho M, Liu D, Chan V, Lam D (2013). Choroidal Thickness Measurement in Myopic Eyes by Enhanced Depth Optical Coherence Tomography. *Ophthalmology*;120(9):1909-1914.

Holden B, Fricke T, Wilson D, Jong M, Naidoo K, Sankaridurg P, Wong T, Naduvilath T, Resnikoff S (2016). Global Prevalence of Myopia and High Myopia and Temporal Trends from 2000 through 2050. *Ophthalmology*;123(5):1036-1042.

Huang J, Wen D, Wang Q, et al (2016). Efficacy Comparison of 16 Interventions for Myopia Control in Children: a Network Meta-analysis. *Ophthalmology*; 123: 697–708

HumanN (2022). N-O Indicator Strips (25) [Internet]. [cited 2022 Mar 20]. Available from: https://shop.humann.com/products/nitric-oxide-indicator-strips?variant=19861378072687

Ichhpujani P, Thakur S, Spaeth G (2020). Contrast Sensitivity and Glaucoma. *Journal of Glaucoma*;29(1):71-75.

Jaroszynska N, Harding P, Moosajee M (2021). Metabolism in the Zebrafish Retina. *Journal of Developmental Biology*;9(1):10

Jonas J, Ang M, Cho P, Guggenheim J, He M, Jong M, Logan N, Liu M, Morgan I, Ohno-Matsui K, Pärssinen O, Resnikoff S, Sankaridurg P, Saw S, Smith E, Tan D, Walline J, Wildsoet C, Wu P, Zhu X, Wolffsohn J (2021). IMI Prevention of Myopia and Its Progression. *Investigative Ophthalmology & Visual Science*;62(5):6.

Jonas J, Xu L, Wei W, et al (2016). Retinal thickness and axial length. *Investigative Ophthalmology & Visual Science*;57:1791–1797.

Jonas J, Ohno-Matsui K, Holbach L, et al (2017). Retinal Pigment Epithelium Cell Density in Relationship to Axial Length in Human Eyes. *Acta Ophthalmologica*; 95:22–28.

Jonas J, Ohno-Matsui K, Panda-Jonas S (2019). Myopia: Anatomic Changes and Consequences for Its Etiology. *Asia-Pacific Journal of Ophthalmology*;8(5):355-359.

Jong M, Jonas J, Wolffsohn J, Berntsen D, Cho P, Clarkson-Townsend D, Flitcroft D, Gifford K, Haarman A, Pardue M, Richdale K, Sankaridurg P, Tedja M, Wildsoet C, Bailey-Wilson J, Guggenheim J, Hammond C, Kaprio J, MacGregor S, Mackey D, Musolf A, Klaver C, Verhoeven V, Vitart V, Smith E (2021). IMI 2021 Yearly Digest. *Investigative Ophthalmology & Visual Science*;62(5):7.

Kandel H, Khadka J, Goggin M, Pesudovs K. Patient-Reported Outcomes for Assessment of Quality of Life in Refractive Error: a Systematic Review. *Optometry and Vision Science*. 2017; 94(12): 1099–1107

Kandel H, Khadka J, Fenwick EK, et al (2018). Constructing Item Banks for Measuring Quality of Life in Refractive Error. *Optometry and Vision Science*; 95(7): 575–587.

Kaufman P (2017). Latanoprostene Bunod Ophthalmic Solution 0.024% for IOP Lowering in Glaucoma and Ocular Hypertension. *Expert Opinion on Pharmacotherapy*;18(4):433-444.

Kawase K, Vittitow J, Weinreb R, Araie M, Hoshiai S, Hashida S, Iwasaki M, Kano K, Kawase K, Kato T, Kuwayama Y, Muramatsu T, Mitsuhashi M, Matsuzaki S, Nakajima T, Sato I, Yoshimura Y (2016). Long-term Safety and Efficacy of Latanoprostene Bunod 0.024% in Japanese Subjects with Open-Angle Glaucoma or Ocular Hypertension: The JUPITER Study. *Advances in Therapy*;33(9):1612-1627.

Kempen J, Mitchell P, Lee K, Tielsch J, Broman A, Taylor H, Ikram M, Congdon N, O'Colmain B (2004). The Prevalence of Refractive Errors Among Adults in the United States, Western Europe, and Australia. *Archives of Ophthalmology*;122(4):495

Kerber KL, Thorn F, Bex PJ, Vera-Diaz FA (2016). Peripheral Contrast Sensitivity and Attention in Myopia. *Vision Research*;125:49-54.

Kim E, Morgan I, Kakizaki H, Kang S, Jee D (2013). Prevalence and Risk Factors for Refractive Errors: Korean National Health and Nutrition Examination Survey 2008-2011. *PLOS ONE*;8(11):e80361.

Koriyama Y, Chiba K, Yamazaki M, Suzuki H, Muramoto K, Kato S (2010). Long-acting Genipin Derivative Protects Retinal Ganglion Cells from Oxidative Stress Models in vitro

and in vivo through the Nrf2/antioxidant Response Element Signaling Pathway. *Journal of Neurochemistry*;115(1):79-91.

Kramer R, Davenport C (2015). Lateral Inhibition in the Vertebrate Retina: The Case of the Missing Neurotransmitter. *PLOS Biology*;13(12):e1002322.

Labhishetty V, Chakraborty A, Bobier WR (2019). Is Blur Sensitivity Altered in Children with Progressive Myopia? *Vision Research*. 2019;154:142–153.

Laerd Statistics (2015). Paired-samples t-test using SPSS Statistics. *Statistical Tutorials and Software Guides.* Retrieved from https://statistics.laerd.com/

Lagrèze W, Schaeffel F (2017). Preventing Myopia. *Deutsches Aerzteblatt Online*.

Lam CSY, Tang WC, Tse DY-Y, Tang YY, To CH (2014). Defocus Incorporated Soft Contact (DISC) Lens Slows Myopia Progression in Hong Kong Chinese Schoolchildren: a 2-year Randomised Clinical Trial. *British Journal of Ophthalmology*;98:40–45.

Lam CSY, Tang WC, Tse DY, Lee RPK, Chun RKM, Hasegawa K, Qi H, Hatanaka T, To CH (2020). Defocus Incorporated Multiple Segments (DIMS) Spectacle Lenses Slow Myopia Progression: a 2-year Randomised Clinical Trial (2020). *British Journal of Ophthalmology*;104(3):363-368.

Lavinsky F, Lavinsky D (2016). Novel Perspectives on Swept-source Optical Coherence Tomography. *International Journal of Retina and Vitreous*;2(1).

Levy H, Twig G, Perlman I (2004). Nitric Oxide Modulates the Transfer Function between Cones and Horizontal Cells during Changing Conditions of Ambient Illumination. *European Journal of Neuroscience*;20(11):2963-74.

Li Y, Liu J, Qi P (2017). The Increasing Prevalence of Myopia in Junior High School Students in the Haidian District of Beijing, China: a 10-year Population-based Survey. *BMC Ophthalmology*;17(1).

Lidder, S.; Webb, A (2013). Vascular Effects of Dietary Nitrate (as Found in Green Leafy Vegetables and Beetroot) via the Nitrate-nitrite-nitric Oxide Pathway. *British Journal of Clinical Pharmacology*;75, 677–696.

Linsenmeier RA, Braun RD (1992). Oxygen Distribution and Consumption in the Cat Retina during Normoxia and Hypoxemia. *Journal of General Physiology*;99(2):177-97.

Liou S, Chiu C (2001). Myopia and Contrast Sensitivity Function. *Current Eye Research*;22:81–84

Lu Y, Lin Z, Wen L, et al (2020). The Adaptation and Acceptance of Defocus Incorporated Multiple Segment Lens for Chinese Children. *American Journal of Ophthalmology*;211:207–216.

Liu Y, Wildsoet C (2011). The Effect of Two-zone Concentric Bifocal Spectacle Lenses on Refractive Error Development and Eye Growth in Young Chicks. *Investigative Ophthalmology & Visual Science*;52:1078–1086.

Logan N, Radhakrishnan H, Cruickshank F, Allen P, Bandela P, Davies L, Hasebe S, Khanal S, Schmid K, Vera-Diaz F, Wolffsohn J (2021). IMI Accommodation and Binocular Vision in Myopia Development and Progression. *Investigative Ophthalmology & Visual Science*;62(5):4.

Ma X, Zhou Z, Yi H, et al (2014). Effect of Providing Free Glasses on Children's Educational Outcomes in China: Cluster Randomized Controlled Trial. *BMJ*; 349: g5740.

Ma Y, Congdon N, Shi Y, et al (2018). Effect of a Local Vision Care Center on Eyeglasses Use and School Performance in Rural China: a Cluster Randomized Clinical Trial. *JAMA Ophthalmology*; 136(7): 731–737

Maiello G, Walker L, Bex P, Vera-Diaz F (2017). Blur Perception Throughout the Visual Field in Myopia and Emmetropia. *Journal of Vision*;17(5):3.

Mansouri K, Medeiros F, Tatham A, Marchase N, Weinreb R (2014). Evaluation of Retinal and Choroidal Thickness by Swept-Source Optical Coherence Tomography: Repeatability and Assessment of Artifacts. *American Journal of Ophthalmology*;157(5):1022-1032.e3.

Marcus M, De Vries M, Junoy Montolio F, Jansonius N (2011). Myopia as a Risk Factor for Open-angle Glaucoma: A Systematic Review and Meta-analysis. *Ophthalmology*. 2011; 118(10): 1989–1994.

Martínez-Ruiz A, Cadenas S, Lamas S (2011). Nitric Oxide Signaling: Classical, Less Classical, and Nonclassical Mechanisms. *Free Radical Biology and Medicine*;51(1):17-29.

McBrien N (2003). Role of the Sclera in the Development and Pathological Complications of Myopia. *Progress in Retinal and Eye Research*;22:307–338.

Medeiros F, Martin K, Peace J, Scassellati Sforzolini B, Vittitow J, Weinreb R (2016). Comparison of Latanoprostene Bunod 0.024% and Timolol Maleate 0.5% in Open-Angle Glaucoma or Ocular Hypertension: The LUNAR Study. *American Journal of Ophthalmology*;168:250-259.

Miyachi E, Murakami M, Nakaki T (1990). Arginine Blocks Gap Junctions between Retinal Horizontal Cells. *Neuroreport*;1(2):107-10.

Moreno-Lopez B, Escudero M, Estrada C (2002). Nitric Oxide Facilitates GABAergic Neurotransmission in the Cat Oculomotor System: a Physiological Mechanism in Eye Movement Control. *The Journal of Physiology*;540(1):295-306.

Morgan I, Jan C (2022). China Turns to School Reform to Control the Myopia Epidemic: A Narrative Review. *Asia-Pacific Journal of Ophthalmology*;11(1):27-35.

Morita M, Hayashi T, Ochiai M, Maeda M, Yamaguchi T, Ina K, Kuzuya M (2014). Oral Supplementation with a Combination of L-citrulline and L-arginine Rapidly Increases Plasma L-arginine Concentration and Enhances NO Bioavailability. *Biochemical and Biophysical Research Communications*;454(1):53-57.

Nakamura Y, Nakamura Y, Higa A, Sawaguchi S, Tomidokoro A, Iwase A, Araie M (2018). Refractive Errors in an Elderly Rural Japanese Population: The Kumejima study. *PLOS ONE*;13(11):e0207180.

Neal M, Cunningham J, Matthews K (1998). Selective Release of Nitric Oxide from Retinal Amacrine and Bipolar Cells. *Investigative Ophthalmology & Visual Science*;39(5):850-3.

Nemargut JP, Wang GY (2009). Inhibition of Nitric Oxide Synthase Desensitizes Retinal Ganglion Cells to Light by Diminishing Their Excitatory Synaptic Currents Under Light Adaptation. *Vision Research*;49(24):2936-47.

Nickla DL, Damyanova P, Lytle G (2009). Inhibiting the Neuronal Isoform of Nitric Oxide Synthase Has Similar Effects on the Compensatory Choroidal and Axial Responses to Myopic Defocus in Chicks as Does the Non-specific Inhibitor l-NAME. *Experimental Eye Research*;88(6):1092-1099.

Nickla DL, Lee L, Totonelly K (2013). Nitric Oxide Synthase Inhibitors Prevent the Growth-inhibiting Effects of Quinpirole. *Optometry and Vision Science*;90(11):1167-75.

Nickla DL, Schroedl F (2012). Parasympathetic Influences on Emmetropization in Chicks: Evidence for Different Mechanisms in Form Deprivation vs Negative Lens-induced Myopia. *Experimental Eye Research*;102:93–103.

Nickla DL, Totonelly K (2015). Choroidal Thickness Predicts Ocular Growth in Normal Chicks but not in Eyes with Experimentally Altered Growth. *Clinical and Experimental Optometry*;98(6):564-70.

Nickla DL, Wallman J (2010). The Multifunctional Choroid. *Progress in Retinal and Eye Research*;29(2):144-168.

Nickla DL, Wildsoet CF (2004). The Effect of the Nonspecific Nitric Oxide Synthase Inhibitor NG-Nitro-l-Arginine Methyl Ester on the Choroidal Compensatory Response to Myopic Defocus in Chickens. *Optometry and Vision Science*;81(2):111-118.

Nickla DL, Wilken E, Lytle G, Yom S, Mertz J (2006). Inhibiting the Transient Choroidal Thickening Response Using the Nitric Oxide Synthase Inhibitor L-NAME Prevents the Ameliorative Effects of Visual Experience on Ocular Growth in Two Different Visual Paradigms. *Experimental Eye Research*. 2006;83(2):456-464.

Nickla DL, Zhu X, Wallman J (2013). Effects of Muscarinic Agents on Chick Choroids in Intact Eyes and Eyecups: Evidence for a Muscarinic Mechanism in Choroidal Thinning. *Ophthalmic and Physiological Optics*;33(3):245-256.

Nilsson S (1996). Nitric Oxide as a Mediator of Parasympathetic Vasodilation in Ocular and Extraocular Tissues in the Rabbit. *Investigative Ophthalmology & Visual Science*;37(10):2110-9.

Nilsson S (2000). The Significance of Nitric Oxide for Parasympathetic Vasodilation in the Eye and other Orbital Tissues in the Cat. *Experimental Eye Research*;70(1):61-72.

Pasquale L (2016). Vascular and Autonomic Dysregulation in Primary Open-angle Glaucoma. *Current Opinion in Ophthalmology*;27(2):94-101.

Norman RE, Flanagan JG, Rausch SM, et al (2010). Dimensions of the Human Sclera: Thickness Measurement and Regional Changes with Axial Length. *Experimental Eye Research*; 90:277–284.

Norton TT, Rada JA (1995). Reduced Extracellular Matrix in Mammalian Sclera with Induced Myopia. *Vision Research*;35:1271–1282.

Nyakayiru J, Jonvik K, Trommelen J, Pinckaers P, Senden J, van Loon L, Verdijk L (2017). Beetroot Juice Supplementation Improves High-Intensity Intermittent Type Exercise Performance in Trained Soccer Players. *Nutrients*;9(3):314.

Olsen TW, Aaberg SY, Geroski DH, et al (1998). Human Sclera: Thickness and Surface Area. *American Journal of Ophthalmology*; 125:237–241.

Pacher P, Beckman J, Liaudet L (2007). Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Reviews*;87(1):315-424.

Park J, Piknova B, Jenkins A, Hellinga D, Parver L, Schechter A (2020). Potential Roles of Nitrate and Nitrite in Nitric Oxide Metabolism in the Eye. *Scientific Reports*;10(1).

Parver L, Auker C, Carpenter D (1980). Choroidal Blood Flow As a Heat Dissipating Mechanism in the Macula. *American Journal of Ophthalmology*;89(5):641-646.

Pesudovs K (2010). Item Banking: Generational Change in Patient-reported Outcome Measurement. *Optometry and Vision Science*. 2010; 87(4): 285–293

Pottek M, Schultz K, Weiler R (1997). Effects of Nitric Oxide on the Horizontal Cell Network and Dopamine Release in the Carp Retina. *Vision Research*;37(9):1091-1102.

Phillips JR, Khalaj M, McBrien NA (2000). Induced Myopia Associated with Increased Scleral Creep in Chick and Tree Shrew Eyes. *Investigative Ophthalmology & Visual Science*;41:2028–2034.

Qiao-Grider Y, Hung LF, Kee CS, Ramamirtham R, Smith EL III (2010). Nature of the Refractive Errors in Rhesus Monkeys (Macaca mulatta) with Experimentally Induced Ametropias. *Vision Research*;50:1867–1881

Resnikoff S, Jonas J, Friedman D, He M, Jong M, Nichols J, Ohno-Matsui K, III E, Wildsoet C, Taylor H, Wolffsohn J, Wong T (2019). Myopia – A 21st Century Public Health Issue. *Investigative Ophthalmology & Visual Science*;60(3):Mi.

Rosén R, Lundström L, Unsbo P (2012). Sign-dependent Sensitivity to Peripheral Defocus for Myopes due to Aberrations. *Investigative Ophthalmology & Visual Science*;53(11):7176–7182.

Rosenfield M, Abraham-Cohen J (1999). Blur Sensitivity in Myopes. *Optometry and Vision*;76(5):303-307.

Rytlewski K, Olszanecki R, Korbut R, Zdebski Z (2005). Effects of Prolonged Oral Supplementation with L-arginine on Blood Pressure and Nitric Oxide Synthesis in Preeclampsia. *European Journal of Clinical Investigation*;35(1):32-37.

Sander B, Collins M, Read S (2019). Short-Term Effect of Low-Dose Atropine and Hyperopic Defocus on Choroidal Thickness and Axial Length in Young Myopic Adults. *Journal of Ophthalmology*;2019:1-8.

Sáenz DA, Turjanski AG, Sacca GB, Marti M, Doctorovich F, Sarmiento MI, Estrin DA, Rosenstein RE (2002). Physiological Concentrations of Melatonin Inhibit the Nitridergic Pathway in the Syrian Hamster Retina. *Journal of Pineal Research*;33(1):31-6.

Sankaridurg P, Tahhan N, Kandel H, Naduvilath T, Zou H, Frick K, Marmamula S, Friedman D, Lamoureux E, Keeffe J, Walline J, Fricke T, Kovai V, Resnikoff S (2021). IMI Impact of Myopia. *Investigative Ophthalmology & Visual Science*;62(5):2.

Schaeffel F, Feldkaemper M (2015). Animal Models in Myopia Research. *Clinical and Experimental Optometry*;98(6):507-517.

Sekaran S, Cunningham J, Neal MJ, Hartell NA, Djamgoz MB (2005). Nitric oxide Release is Induced by Dopamine during Illumination of the Carp Retina: Serial Neurochemical Control of Light Adaptation. *European Journal of Neuroscience*;21(8):2199-208.

Schmid KL, Robert Iskander D, Li RWH, Edwards MH, Lew JKF (2002). Blur Detection Thresholds in Childhood Myopia: Single and Dual Target Presentation. *Vision Research*;42(2):239–247.

Schrodl F, Brehmer A, Neuhuber W (2000). Intrinsic Choroidal Neurons in the Duck Eye Express Galanin. *The Journal of Comparative Neurology*;425(1):24-33.

Schwiegerling J (2004). *Field Guide to Visual and Ophthalmic Optics*. SPIE Press, Bellingham, WA

Shephard R (2012). The Effect of Nitric-Oxide-Related Supplements on Human Performance. *Yearbook of Sports Medicine*.:176-177.

Sherwin JC, Reacher MH, Keogh RH, et al (2012). The Association Between Time Spent Outdoors and Myopia in Children and Adolescents: a Systematic Review and Meta-analysis. *Ophthalmology*;119:2141–2151

Shi Q, Teves M, Lillywhite A, Pagtalunan E, Stell W (2020). Light Adaptation in the Chick Retina: Dopamine, Nitric Oxide, and Gap-junction Coupling Modulate Spatiotemporal Contrast Sensitivity. *Experimental Eye Research*;195:108026.

Siegwart JT, Norton TT (1999). Regulation of the Mechanical Properties of Tree Shrew Sclera by the Visual Environment. *Vision Research*;39:387–407.

Siervo M, Lara J, Ogbonmwan I, Mathers J (2013). Inorganic Nitrate and Beetroot Juice Supplementation Reduces Blood Pressure in Adults: A Systematic Review and Meta-Analysis. *The Journal of Nutrition*;143(6):818-826.

Snellman J, Nawy S (2004). cGMP-dependent Kinase Regulates Response Sensitivity of the Mouse on Bipolar Cell. *Journal of Neuroscience*;24(29):6621-8.

Smith EL, III, Hung LF, Huang J (2009). Relative Peripheral Hyperopic Defocus Alters Central Refractive Development in Infant Monkeys. *Vision Research*;49:2386–2392.

Stringham J, Stringham N (2015). Nitric Oxide and Lutein: Function, Performance, and Protection of Neural Tissue. *Foods*;4(4):678-689.

Stoimenova B (2007). The Effect of Myopia on Contrast Thresholds. *Investigative Ophthalmology & Visual Science*;48(5):2371.

Stone R, Quinn G, Francis E, Ying G, Flitcroft D, Parekh P, Brown J, Orlow J, Schmid G (2004). Diurnal Axial Length Fluctuations in Human Eyes. *Investigative Ophthalmology & Visual Science*;45(1):63.

Summers J (2013). The Choroid as a Sclera Growth Regulator. *Experimental Eye Research*;114:120-127.

Swarbrick HA, Alharbi A, Watt K, Lum E, Kang P (2015). Myopia Control during Orthokeratology Lens Wear in Children using a Novel Study Design. *Ophthalmology*;122:620–633.

Tanaka N, Shinohara K, Yokoi T, et al (2019). Posterior Staphylomas and Scleral Curvature in Highly Myopic Children and Adolescents Investigated by Ultra-widefield Optical Coherence Tomography. *PLOS ONE*; 14(6): e0218107

Tang Y, Wang X, Wang J, et al (2016). Prevalence of Age-related Cataract and Cataract Surgery in a Chinese Adult Population: The Taizhou Eye Study. *Investigative Ophthalmology & Visual Science*; 57(3): 193–1200

Theophanous C, Modjtahedi B, Batech M, Marlin D, Luong T, Fong D (2018). Myopia Prevalence and Risk Factors in Children. *Clinical Ophthalmology*; Volume 12:1581-1587.

Tkatchenko AV (2006). Whole-mount BrdU Staining of Proliferating Cells by DNase Treatment: Application to Postnatal Mammalian Retina. *Biotechniques*;40:29–30, 32.

Troilo D, Nickla DL, Wildsoet CW (2000). Choroidal Thickness Changes During Altered Eye Growth and Refractive State in a Primate. *Investigative Ophthalmology & Visual Science*;41:1249–1258.

Troilo D, Smith E, Nickla D, Ashby R, Tkatchenko A, Ostrin L, Gawne T, Pardue M, Summers J, Kee C, Schroedl F, Wahl S, Jones L (2019). IMI – Report on Experimental Models of Emmetropization and Myopia. *Investigative Ophthalmology & Visual Science*;60(3):M31.

Tse DY, Lam CS, Guggenheim JA, et al (2007). Simultaneous Defocus Integration During Refractive Development. *Investigative Ophthalmology & Visual Science*;48:5352–5359.

Tsumamoto Y, Yamashita K, Takumida M, Okada K, Mukai S, Shinya M, Yamashita H, Mishima H (2002). In situ Localization of Nitric Oxide Synthase and Direct Evidence of NO Production in Rat Retinal Ganglion Cells. *Brain Research*;933(2):118-129.

Urs R, Ketterling J, Yu A, Lloyd H, Yiu B, Silverman R (2018). Ultrasound Imaging and Measurement of Choroidal Blood Flow. *Translational Vision Science & Technology*;7(5):5.

Vagge A, Ferro Desideri L, Nucci P, Serafino M, Giannaccare G, Traverso CE (2018). Prevention of Progression in Myopia: A Systematic Review. *Diseases*;6(4):92.

Vander Veen DK, Kraker RT, Pineles SL, et al. (2019). Use of Orthokeratology for the Prevention of Myopic Progression in Children: a Report by the American Academy of Ophthalmology. *Ophthalmology*;126:623–636.

Vera-Diaz F, Bex P, Ferreira A, Kosovicheva A (2018). Binocular Temporal Visual Processing in Myopia. *Journal of Vision*;18(11):17.

Vielma A, Delgado L, Elgueta C, Osorio R, Palacios A, Schmachtenberg O (2010). Nitric Oxide Amplifies the Rat Electroretinogram. *Experimental Eye Research*;91(5):700-709.

Vielma A, Retamal M, Schmachtenberg O (2012). Nitric Oxide Signaling in the Retina: What Have We Learned in Two Decades?. *Brain Research*;1430:112-125.

Vitale S, Sperduto RD, Ferris FL 3rd (2009). Increased Prevalence of Myopia in the United States between 1971-1972 and 1999-2004. *Archives of Ophthalmology*;127(12):1632-9. doi: 10.1001/archophthalmol.2009.303. PMID: 20008719.

Walline JJ, Lindsley KB, Vedula SS, et al (2020). Interventions to Slow Progression of Myopia in Children. *Cochrane Database Syst Rev*;1:CD004916.

Walline JJ, Walker MK, Mutti DO, et al (2020). Effect of High Add Power, Medium Add Power, or Single-vision Contact Lenses on Myopia Progression in Children: The BLINK Randomized Clinical Trial. *JAMA*;324:571–580.

Wallman J, Wildsoet C, Xu A, et al (1995). Moving the Retina: Choroidal Modulation of Refractive State. *Vision Research*;35:37–70.

Wang GY, Liets LC, Chalupa LM (2003). Nitric Oxide Differentially Modulates ON and OFF Responses of Retinal Ganglion Cells. *Journal of Neurophysiology*;90(2):1304-13.

Weiler R, Pottek M, He S, Vaney DI (2000). Modulation of Coupling between Retinal Horizontal Cells by Retinoic Acid and Endogenous Dopamine. *Brain Research Reviews*;32(1):121-9.

Wildsoet C, Chia A, Cho P, Guggenheim J, Polling J, Read S, Sankaridurg P, Saw S, Trier K, Walline J, Wu P, Wolffsohn J (2019). IMI – Interventions for Controlling Myopia Onset and Progression Report. *Investigative Ophthalmology & Visual Science*;60(3):M106.

Wildsoet C, Pettigrew JD (1988). Kainic Acid-induced Eye Enlargement in Chickens: Differential Effects on Anterior and Posterior Segments. *Investigative Ophthalmology & Visual Science*;29: 311–319.

Wildsoet C, Wallman J (1995). Choroidal and Scleral Mechanisms of Compensation for Spectacle Lenses in Chicks. *Vision Research*;35:1175–1194.

Winawer J, Wallman J (2002). Temporal Constraints on Lens Compensation in Chicks. *Vision Research*;42:2651–2668.

Wolffsohn J, Flitcroft D, Gifford K, Jong M, Jones L, Klaver C, Logan N, Naidoo K, Resnikoff S, Sankaridurg P, Smith E, Troilo D, Wildsoet C (2019). IMI – Myopia Control Reports Overview and Introduction. *Investigative Ophthalmology & Visual Science*;60(3):M1.

Xia Y, Krukoff T (2004). Estrogen Induces Nitric Oxide Production via Activation of Constitutive Nitric Oxide Synthases in Human Neuroblastoma Cells. *Endocrinology*;145(10):4550-4557.

Xin D, Bloomfield SA (2000). Effects of Nitric Oxide on Horizontal Cells in the Rabbit Retina. *Visual Neuroscience*;17(5):799-811..

Xiong S, Sankaridurg P, Naduvilath T, et al (2017). Time Spent in Outdoor Activities in Relation to Myopia Prevention and Control: a Meta-analysis and Systematic Review. *Acta Ophthalmologica*;95:551–566.

Ye B, Du J, Yang X (1997). Differential Effects of Nitric Oxide on Rod and Cone Pathways in Carp Retina. *Science China Life Sciences*;40(1):71-8.

Yeung S, Park J, Park D, You Y, Yan P (2022). The Effect of Systemic and Topical Ophthalmic Medications on Choroidal Thickness: A Review. *British Journal of Clinical Pharmacology*.

Yokoi T, Ohno-Matsui K (2018). Diagnosis and Treatment of Myopic Maculopathy. *Asia The Asia-Pacific Journal of Ophthalmology*; 7(6): 415–421

Zarbin MA (2004). Current Concepts in the Pathogenesis of Age-related Macular Degeneration. *Acta Ophthalmologica*;122(4):598-614.

Yu B, Ichinose F, Bloch DB, Zapol WM (2019). Inhaled Nitric Oxide. *British Journal of Pharmacology*;176(2):246-255.

Zhao M, Alonso-Caneiro D, Lee R, Cheong A, Yu W, Wong H, Lam A (2020). Comparison of Choroidal Thickness Measurements Using Semiautomated and Manual Segmentation Methods. *Optometry and Vision Science*;97(2):121-127.

Zhou X, Ye C, Wang X, Zhou W, Reinach P, Qu J (2021). Choroidal Blood Perfusion as a Potential "Rapid Predictive Index" for Myopia Development and Progression. *Eye and Vision*;8(1).