Short-Term Exposure to Blue Light Shows an Inhibitory Effect on Axial Elongation in Human Eyes Independent of Defocus

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PURPOSE. Given the potential role of light and its wavelength on ocular growth, we investigated the effect of short-term exposure to the red, green, and blue light on ocular biometry in the presence and absence of lens-induced defocus in humans.

METHODS. Twenty-five young adults were exposed to blue (460 nm), green (521 nm), red (623 nm), and white light conditions for 1-hour each on 4 separate experimental sessions conducted on 4 different days. In each light condition, hyperopic defocus (3D) was induced to the right eye with the fellow eye experiencing no defocus. Axial length and choroidal thickness were measured before and immediately after the light exposure with a non-contact biometer.

RESULTS. Axial length increased from baseline after red light (mean difference ± standard error in the defocussed eye and non-defocussed eye = 11.2 ± 2 μm and 6.4 ± 2.3 μm, \(P < 0.001\) and \(P < 0.01\), respectively) and green light exposure (9.2 ± 3 μm and 7.0 ± 2.5 μm, \(P < 0.001\) and \(P < 0.001\)) with a significant decrease in choroidal thickness (\(P < 0.05\), both red and green light) after 1-hour of exposure. Blue light exposure resulted in a reduction in axial length in both the eyes (\(-8.0 ± 3 μm, P < 0.001\) in the defocussed eye and \(-6.0 ± 3 μm, P = 0.11\) in the non-defocussed eye) with no significant changes in the choroidal thickness.

CONCLUSIONS. Exposure to red and green light resulted in axial elongation, and blue light resulted in inhibition of axial elongation in human eyes. Impact of such specific wavelength exposure on children and its application in myopia control need to be explored.

Keywords: axial length, wavelength, chromaticity, defocus, myopia
Lou and Ostrin\textsuperscript{34} reported significant choroidal thinning and increase in axial length after 1-hour of direct exposure to red light compared to blue light. However, there is no information on how the human eye responds to simultaneous cues that are known to alter ocular biometry (i.e. the different wavelengths of light in the presence of optical defocus). Given that viewing the real-world natural scenes would involve both defocus (say hyperopic defocus during near viewing due to lag of accommodation) and exposure to different spectrum of ambient light (colors in the visual scene or target), it will be imperative to know how the human eye responds to simultaneous cues. This will lay foundation for the future experiments to improve the understanding on how the visual system utilizes chromatic cues to regulate the ocular growth in presence of strong optical defocus.

With this background, this study aimed to investigate how short-term exposure to different narrowband monochromatic (blue, green, and red) and broadband white light influences the ocular biometry in the presence or absence of a hyperopic defocus. Based on the literature, if LCA is associated with ocular growth in humans, we expect that the presence of a negative lens shifting the focal plane of all the three wavelengths (red, green, and blue) behind the retina, should lead to greater increase in axial length with red light followed by green and the least in blue light.

**Methodology**

A total of 29 young adults (16 women) aged 20 to 32 years were approached to participate in the study. Participants were primarily the optometry students and staff of L V Prasad Eye Institute, Hyderabad, India. The study was approved by the Institutional Review Board (IRB) of L V Prasad Eye Institute, India (LEC 05-19-256) and followed the tenets of the Declaration of Helsinki. Informed written consent was obtained from each participant after a detailed explanation of the nature and possible consequences of the study. All participants had best-corrected visual acuity of 0.0 logMAR or better with their habitual correction. No participants had astigmatism >1 diopter (D), any systemic illness, such as diabetes or hypertension, ocular pathologies, or any history of ocular injury or surgery. None of the participants reported intake of caffeine on the day of experiment in any form (i.e. tea, coffee, and beverages), which has been shown to affect the choroidal thickness.\textsuperscript{35}

**Experimental Set-up**

A total of four experimental sessions were designed for every participant with each session, including 1-hour exposure to different monochromatic wavelengths of light (Fig. 2): red light (peak at 620 nm, average irradiance = 0.00013 W/nm/m$^2$, and half maximum width = 35 nm), green light (peak at 523 nm, 0.00021 W/nm/m$^2$, and 37 nm), blue light (peak at 455 nm, 0.000174 W/nm/m$^2$, and 25 nm), or broadband white light.

The light sources consisted of 6 light-emitting diode smart bulbs (12-Watt, Wipro Enterprises Ltd., Shenzhen, People's Republic of China, China) mounted in a room measuring 3 × 2 × 3.2 meters (length X width X height). All the bulbs were set up with “Wipro Next Smart Home” and “Google Home” smartphone application integrated with Google voice assistant, which synchronized all the six light bulbs and allowed to switch the color of all the bulbs simultaneously. The brightness level (kept at 100%) was matched for all the bulbs using the aforementioned application. During 1-hour exposure to red, green, blue, and white lights, participants watched a movie (same genre) of their choice on a laptop (Apple MacBook Air 2017, screen size = 13.3-inch, and display resolution = 1440 × 900 pixels) placed at a viewing distance of 3 meters, to ensure relaxed accommodation. To avoid interference of other spectra from the laptop

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Effect of monochromatic light exposure on refraction in various species.}
\end{figure}
FIGURE 2. Flow diagram of the experimental procedure including experimental setup for different light conditions. The upper row of line graphs (A, B, C, D) represents the spectral profile of an ambient light in the experimental room measured at an eye level (combined light from LEDs and laptop screen covered with cellophane sheet) and the lower row of line graphs (A1, B1, C1) represents the transmission spectra obtained when red, green, and blue colored cellophane sheets were placed against white light.

A cellophane sheet was applied over the screen matching the experimental light conditions (i.e. red color sheet, green color sheet, blue color sheet, and no sheet for the red, green, blue, and white light exposure, respectively). The transmission spectra of these red (peak wavelength = 610 nm), green (525 nm) and blue (450 nm) colored cellophane sheet closely matched with the spectral profile of the lighting condition (as shown in Fig. 2). The spectral profile of an ambient light in an experimental room (combined light from LEDs and laptop screen covered with cellophane sheet) was measured at the plane of participant's eye using a handheld portable spectrometer that is calibrated to the visual V lambda per the manufacturer (Photonfy SP-01-BLU, LEDMO-TIVE, Spain). A similar spectral profile of experimental light at the eye level was noted with and without cellophane on the laptop's screen, indicating minimal influence of the cellophane sheet on altering the wavelength of light entering the eye. The luminance of laptop screen in red (37 cd/m²), green (47 cd/m²), and blue (9 cd/m²) lighting condition with the cellophane sheet covering the screen was measured using a photometer (LS-110, Konica Minolta, Japan).

Experimental Protocol

Figure 2 shows the flow of experimental procedures and the experimental setup. All the participants were given a “resting period” of 10 minutes before starting each experimental session where they were instructed to relax, refrain from using their smartphones, and fixate at a plain wall situated at a distance of 3 meters from the eye. This resting period is aimed to minimize the effect of any previous visual tasks or activities on axial length and choroidal thickness. The order of light exposure sessions was randomized for each participant, and only one session
was conducted for a participant on any given day. All the sessions were completed by all the participants in 4 separate visits within 10 days from the start of the first session. To minimize the potential effect of diurnal variations in axial length and choroidal thickness, the experiment for all the sessions was conducted between 8:00 AM to 11:00 AM India Standard Time (IST). For each light condition, participants wore a large aperture trial frame with their best corrected distance sphero-cylindrical distance correction, if any, in both eyes with an extra −3.00 DS lens placed only in front of the right eye to impose hyperopic defocus condition. Considering that the monocular defocus condition paradigm ensures relaxed accommodation in the fellow eye without defocus, we followed this protocol to investigate the effect of different monochromatic light on lens-induced hyperopic defocus. The right eye served as a defocused eye (i.e. observe the influence of different monochromatic light on hyperopic defocus induced ocular biometry), and the left eye was considered as the control (i.e. to observe the influence of different monochromatic light on ocular biometry alone).

Ocular biometry measurements for both eyes were recorded before and immediately after 1 hour of light exposure in each session under the same experimental lights using Lenstar LS-900 non-contact biomter (Haag Streit AG, Koeniz, Switzerland). An average of five ocular measurements was considered for analysis for each participant. The biometer was placed in close proximity to the participants requiring their minimal movement such that it was allowed to obtain measurements rapidly (<1 min) after participants removed the defocus lens. The ocular biometry measurement was always first performed in the right eye (i.e. eye experiencing defocus and light exposure). After 20 minutes of discontinuing light exposure and removing the defocus lens, the biometry was repeated to assess the recovery of changes in axial length. During this “recovery period,” participants followed the same protocol as implemented during the resting period.

Along with the axial length, other parameters, such as central corneal thickness and lens thickness were extracted and analyzed. Sub-foveal choroidal thickness (SFCT) was determined using the method described by Read et al. through manual analysis of the A-scan peaks from the Lenstar. An experienced observer (masked to the participant’s refractive error and lighting conditions) first used a magnified view of the selected A-scan region to determine the peaks origination from the retinal pigmented epithelium and chorio-scleral interface. The distance between these two peaks gives the measurement of choroidal thickness. This method has been validated previously to determine the foveal retinal thickness and subfoveal choroidal thickness, and the outcomes are in good agreement with that of optical coherence tomography. To evaluate the intra-grader variability for the choroidal thickness, the analysis was repeated twice for five random participants. The intraclass correlation coefficient was found to be 0.98 and 0.81 for the right and left eye, respectively, based on which one time analysis was performed for the remaining individuals. As photosensitivity is known to be triggered by different light conditions, all participants were asked a battery of questions before the experiment related to their sensitivity or discomfort to any of these conditions (flashing or flickering light/pattern; white light followed by darkness; certain color lights; visual effects in movies and video games; light viewed through fast-moving ceiling fan; environment flicker, such as sunlight; to Venetian blinds, escalators and striped fabrics; or history of sleep deprivation, and general fatigue).

**Participants**

Of the 29 individuals who were approached to participate in the study, one individual who was sensitive to light was not recruited and 3 individuals did not complete exposure to all the light conditions due to their unavailability for all the sessions. Therefore, data of 25 participants (15 emmetropes = SER ≤ 0.50 D, range = 0.50 to −0.25 D; 10 myopes = SER ≥ 0.75 D, and range = −1.75 to −7.00 D) was used for the final analysis.

**Statistical Analysis**

The sample size calculation was performed using the G*Power software. Considering the findings of axial length reported by Lou and Ostrin, to obtain 95% power to detect a 6 μm difference (9 μm SD) in the axial length after 15 minutes, a sample of 25 was required (combining 2 refractive groups). Statistical analyses were performed with IBM SPSS Statistics version 21.0.0 (SPSS, Inc., Chicago, IL, USA). Results are expressed as mean ± standard error of the mean (SEM). For axial length and choroidal thickness, a 2-way repeated-measures ANOVA was performed with two within-subject factors (i.e. light exposure condition [blue, green, red, and white] and time [pre and post defocus]). The post hoc t-test was used to assess the differences in values between before and after 1 hour to compare changes in axial length, and choroidal thickness. All post hoc tests were corrected for multiple comparisons using the Bonferroni correction and P < 0.05 was considered statistically significant. Changes in corneal thickness, lens thickness, anterior chamber depth from baseline were assessed using a paired t-test. One-way ANCOVA was performed for defocused and non-defocused eyes separately, to determine any significant difference in axial length and SFCT changes between two refractive groups (main effects) after adjusting for the baseline measurements.

**Results**

Mean spherical equivalent refraction between right and left eyes were not significantly different (−1.60 ± 0.49 diopters versus −1.21 ± 0.40 diopters, P = 0.59). The baseline axial length recorded before the start of the experiment was similar with all the four experimental light sessions in both the right eye (P = 0.25) and left eye (P = 0.93). The mean axial length of the right and left eyes of all participants was 23.67 ± 0.21 mm and 23.64 ± 0.20 mm, respectively.

**Axial Length and Choroidal Thickness Changes in the Defocused Eye**

Changes in axial length and choroidal thickness after 1-hour exposure to white light, red light, green light, and blue light are shown in Figure 3. Repeated measure ANOVA revealed a significant main effect of time (before and after light exposure; F(1,24) = 5.77, P = 0.02), and a significant interaction of the effect of light condition with time (F(0.72) = 17.04, P = 0.001) on changes in axial length. Post hoc analyses revealed significant increase in axial length from baseline in the defocused eye after 1-hour exposure to red light.
(11.2 ± 2.0 micrometer [μm], \( P = 0.001 \)), green light (9.2 ± 2.5 μm, \( P = 0.001 \)), and the white light (4.0 ± 2.0 μm, \( P = 0.04 \)). Exposure to blue light alone inhibited the effect of hyperopic defocus leading to a significant reduction in axial length (−8.0 ± 2.7 μm, \( P = 0.001 \)). Comparing the changes in axial length between the light conditions revealed that changes in the axial length in red and green light conditions were similar (\( P = 0.09 \)) after 1-hour of exposure and were significantly different compared to blue light exposure (red versus blue: \( P = 0.001 \) and green versus blue: \( P = 0.002 \); see Fig. 3). Axial length measurements obtained after 20-minutes of recovery period were not significantly different to the baseline values in all the light exposure conditions (red, \( P = 0.51 \); green, \( P = 0.84 \); blue, \( P = 0.11 \); and white light, \( P = 0.08 \)).

Of the 25 participants, SFCT measurements were available for 22 participants across all the light conditions. Changes in SFCT revealed a significant main effect of time (\( F(1, 24) = 13.15, P = 0.002 \)) with significant interaction of the effect of condition by time (\( F(3, 63) = 3.84, P = 0.01 \)). Post hoc test revealed significant thinning of the choroid with red light (before exposure = 160 ± 6 μm versus after exposure, 152 ± 6 μm, \( P = 0.03 \)), green light (154 ± 8 μm versus 147 ± 8 μm, \( P = 0.02 \)), and white light (158 ± 4 μm versus 153 ± 5 μm, \( P = 0.03 \)), but not with blue light exposure (145 ± 7 μm versus 149 ± 9 μm, \( P = 0.11 \)).

Axial Length and Choroidal Thickness Changes in the Fellow Eye (Non-Defocused)

Repeated measure ANOVA revealed significant main effect of time (\( F(1, 24) = 2.24, P = 0.05 \)), and a significant interaction effect of light condition by time (\( F(3, 72) = 4.20, P = 0.02 \)). Post hoc analysis indicated a significant increase in axial length from baseline in the non-defocused eye after 1-hour of exposure to red light and green light (6.4 ± 2.3 μm, \( P = 0.01 \); and 7.0 ± 2.5 μm, \( P = 0.004 \), respectively), but not in white light (1.6 ± 3.1 μm, \( P = 0.61 \)). Similar to the defocused eye, blue light resulted in a reduction in axial length in the non-defocussed eye; however, the changes were not statistically significant (−6.0 ± 3.6 μm, \( P = 0.11 \)). Although there was significant thinning of SFCT in both the red light (before exposure, 157 ± 8 μm versus after exposure, 146 ± 7 μm, \( P = 0.04 \)) and green light conditions (153 ± 9 μm versus 145 ± 9 μm, \( P = 0.03 \)), the changes were not statistically significant with blue (149 ± 7 μm versus 152 ± 8 μm, \( P = 0.24 \)) and white light (149 ± 5 μm versus 153 ± 6 μm, \( P = 0.58 \)).
The majority of participants showed the trend of increasing in axial length with red and green light exposure and reduction in axial length with blue light exposure (Fig. 4).

Overall, the changes in the central corneal thickness, lens thickness, and anterior chamber depth of both eyes did not vary from baseline in any of the light conditions (Supplementary File S1).

There was no significant difference in the changes in axial length of the defocused eye between emmetropes and myopes, after red light exposure (12.0 ± 3.1 μm versus 10.0 ± 2.6 μm, P = 0.38), blue light (−5.3 ± 3.6 versus −12.0 ± 2.6 μm, P = 0.29), and white light (5.0 ± 2.4 μm versus 4.5 ± 3.2 μm, P = 0.68). However, a significant difference in axial length was found after green light exposure (12.6 ± 3.7 μm versus 4.0 ± 2.6 μm, P = 0.03) indicating a greater change in emmetropes compared to that of myopes. In the non-defocused eye, the changes in axial length between two refractive groups were not statistically significant in any of the light exposure conditions. The changes in choroidal thickness between emmetropes and myopes were similar with all light condition: red light (−9.5 ± 3.1 μm versus −6.0 ± 3.8 μm, P = 0.47); blue light (5.1 ± 4.4 μm versus 3.5 ± 3.4 μm, P = 0.49); green light (−6.9 ± 3.5 μm versus −6.6 ± 4.1 μm, P = 0.45); and white light (−3.01 ± 2.7 μm versus −7.2 ± 2.8 μm, P = 0.85). A similar trend was noticed in the non-defocused eye.

**DISCUSSION**

The current study describes changes in ocular biometry in response to different light conditions in the presence and absence of lens-induced hyperopic defocus in humans. The findings of the current study revealed that the middle and longer wavelength of light resulted in axial elongation, irrespective of the presence of hyperopic defocus. Blue light exposure inhibited the effect of hyperopic defocus, and resulted in a reduction in axial length in both defocused eye and non-defocused eye. The increased axial length in defocused eyes was associated with thinning of choroid in the red and green light conditions.

The findings of this study are in agreement with the outcomes of similar experiments conducted in guinea pigs and chicks. Jiang et al. demonstrated that lens-induced hyperopic defocus under blue light (470 nm) inhibited axial elongation, whereas red light-induced more myopic refraction accompanied by deeper vitreous chamber depth over time. Likewise, Yu et al. reported that guinea pigs with monocular −5 D lenses when reared under blue light developed less myopia and had shorter axial length relative to the animals reared under the white light conditions. A similar phenomenon was observed in chicks where imposing a negative lens under red light caused a decrease in choroidal thickness and no such change with blue light exposure.

The changes in the posterior segment of the eye after 1-hour of exposure to red and green light were not just confined to the defocussed eye, but the changes in biometry were also noted in the non-defocused left eye. The findings of axial length and choroidal thickness in the non-defocused eye are in line with the recent study conducted on humans, which showed greater thinning of choroid and increased axial length after 1-hour exposure to red light, broadband light, and darkness compared to blue light exposure. However, this study did not investigate biometry changes under the effect of different monochromatic light in combination with lens-induced hyperopic defocus. In the current study, we found that the changes in axial length after the offset of light exposure and/or removal of defocus returned to baseline value after 20 minutes of the recovery period. Although the change in axial length found under different light conditions is within the repeatability of the biometer that is used in this study (0.01 mm). Individual participant's data (see Fig. 4) shows the majority of the participants had axial length increment in red and green light exposure, and decrement in blue light exposure.

The findings reported here contribute to the current understanding of the involvement of the spectral composition of light in regulating ocular growth in humans. The significant reduction in axial length even in the presence of hyperopic defocus under blue light supports the theory proposed by Rucker and Wallman that chromatic signals associated with LCA may not be essential to regulate short-term or transient growth of eye. If the visual system utilizes chromatic cues to regulate the ocular growth, the presence of a negative lens that shifts the focal plane of all the three-wavelength (red, green, and blue) behind the retina, should have led to an asymmetrical increase in axial length with different light exposure, a greater increase in axial length with red light followed by green and the least in blue light. However, the negative-lens induced hyperopic defocus failed to increase axial length even in presence of hyperopic defocus under blue light condition, that is otherwise known to trigger axial elongation in humans. The short-term exposure to blue light resulted in a significant reduction in axial length accompanied by thickened choroid. Based on the previous findings, it could be considered that blue light inhibits ocular growth through the non-LCA mechanism, such as blue cone-mediated ON-pathway, reduced levels of retinoic acid, role of intrinsically photosensitive retinal ganglion cells (ipRGCs), in-focus/out-of-focus image, and increased depth of focus due to
decreased pupil size,\textsuperscript{28,34} nevertheless, the exact mechanism remains unclear in humans. Given that a positive linear relationship exists between the pupil diameter and the size of blur circle on the retina,\textsuperscript{13} the possible role of blur circle size under different light conditions in altering ocular size cannot be ruled out. In a control experiment conducted in a subset of five individuals, we found that pupil size was larger under the red light compared to the blue light condition (experimental details not stated in the manuscript) contributing to larger blur circle size under the red light compared to the blue light, which may have also contributed in enhancing the eye lengthening with the red light. However, it is difficult to determine if increase in axial length with the red light is primarily due to wavelength, increased pupil/blur circle size, or both. The elucidation of the mechanisms involved was beyond the scope of the study, further research is required to determine whether chromatic signal associated with LCA or any neuronal signaling pathways (associated with blue-cone mediated ON pathway), the role of ipRGCs and melanopsin, reduced level of retinoic acid, and larger retinal blur circle modulates the ocular biometry in humans under monochromatic light conditions.

One of the several factors that differ between indoor and outdoor environments is the spectral composition of ambient light.\textsuperscript{8} The presence of blue-enriched outdoor natural light and the distinctly reduced amount of red light might be one of the potential linkages between the time outdoors and reduced risk of myopia.\textsuperscript{44} However, based on the observed effect of blue light in the current study, we speculate that manipulating the spectra of light under artificial conditions might also influence ocular growth and refractive changes. Czepita and colleagues\textsuperscript{45} reported that the prevalence of hyperopia was higher in children whose houses were lit by fluorescent lighting (emission spectra near to blue light) compared to tungsten-like lighting (emission spectra near to red light). Furthermore, it has been proposed that reading compared to tungsten-like lighting (emission spectra near to blue light) versus fluorescent lighting (emission spectra near to red light). In addition, due to limited sample size, the change in axial length was not adjusted for choroidal thickness.

In conclusion, this is the first report on the combined effect of different monochromatic light conditions and optical defocus on ocular biometry in humans’ eyes. Blue light inhibited the effect of lens-induced hyperopic defocus, and red light increased it. Exposure to red and green light resulted in a significant increase in axial length and thinning of the choroid, irrespective of the presence of defocus. The findings from the present study indicating a significant increase in axial length under the strong hyperopic defocus, improve our understanding of the effect of blue light on axial length in human eyes and could lead to the foundation for the development of an anti-myopia strategy involving blue light exposure.

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