Light Exposure, Refractive Error, and Red and Blue Light-Driven Pupillary Responses

THESIS

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By

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Abstract

Myopia is a large public health concern both in the United States and worldwide. Previous research has shown that time spent outdoors is protective against the development of myopia. Intrinsically photosensitive retinal ganglion cells (ipRGCs) encode ambient light levels and help regulate a variety of functions including pupil constriction. The purpose of the study was to examine different methods of measuring time outdoors and to examine the association between exposure to outdoor light, spherical equivalent refractive error, and presumed ipRGC input to the pupil responses to red and blue light.

Subjects were 20 young adults (mean age 24.9±1.8 years, 13 female) who wore a personal visible light monitor (Daysimeter) and an ultra-violet light monitor on an armband for an average of 7.1 ± 0.43 days. Time outdoors was measured by both the Daysimeter and UV badge, as well as a survey completed at a follow up visit. For the purposes of the study, time outdoors was defined as exposure to >1000 lux or any non-zero UV value. Light exposure over the previous 1, 3, 12, and 24 hours, 3 days, and 5 days was calculated as was total light exposure in log(lux-minutes). Using the RAPDx, pupilometer, pupil responses to 0.1Hz flashes were tested under 3 stimulus conditions: 1) alternating red and blue, 2) red-only, and 3) blue-only. The alternating trial lasted for 2 minutes and the single color trials lasted 1 minute with 5 minutes of dark adaptation prior to each trial.

The study showed that the Daysimeter was a good method for the measurement of time outdoors because it agreed best with the activity survey (slope = 0.68, $R^2 = 0.46$). Pupil constriction to red tended to increase during the trial when alternated with blue. There were significant associations between this increasing pupil constriction to red when alternated with blue for longer-term, greater light exposure ($r = -0.46$, $p = 0.042$ at 3 days; $r = -0.51$, $p = 0.021$ at 5 days) but not for the shorter time periods (1, 3, 12, and 24 hours). The difference in blue pulses during the alternating color protocol and blue pulses during the single color protocol were significantly related to spherical equivalent refractive error ($r = 0.58$, $p = 0.008$).

The Daysimeter was validated in the study as a good objective measurement for time outdoors. The association between light exposure over several days and differences in the pupil response suggests that there is an ocular pathway that is modified by long-term light adaptation. The interaction between preceding blue light pulses and red light-driven pupil constriction is consistent with a role for blue-light-sensitive ipRGCs mediating this adaptive effect. Greater photopotentiation shown by larger differences in alternating and single color blue values were associated with less myopic refractive error. This is consistent with the theory that time outdoors is beneficial due to greater exposure to visible light and therefore an increase in ipRGC activity.

Acknowledgments

I would like to thank Drs. Mutti and Hartwick for all of their help and guidance, Drs. Shorter and Satiani for help with data analysis, and Dr. Walline for serving on my committee.

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Chapter 1: Introduction

Myopia occurs when an eye has an axial length that is greater than the focal length of the refractive components of the eye. This is to say a myopic eye is one where parallel rays of light are brought to a focal point in a location that is in front of the retina. This focusing of light in front of the retina causes the experience of blur for distant objects for the patient with this condition. The amount of blur experienced is directly proportional to the distance from the focal point of eye to the retina. Because the focal point is in front of the retina, accommodation does not allow young myopes to bring distant images into focus. Myopes may only achieve clear vision without correction when the object of regard is placed at the far point of the eye or closer. It is at this point that the object of regard is conjugate with the retina. Therefore myopes experience blurred distance vision that must be corrected with spectacles, contact lenses, or in some cases refractive surgical correction.

Myopia is a public health concern in many countries, including the United States. In the United States 33.1% of the population has myopia (Vitale, Ellwein, Cotch, Ferris and Sperduto, 2008). According to the National Eye Institute, the number of myopic people is projected to grow to over 44 million people by 2050. In some countries such as Singapore, that percentage is as high as nearly 80% (Wu, Seet, Yap, Saw, Lim and Chia, 2001). Correcting refractive error creates $3.8 billion nationally in direct costs (Vitale,

Cotch, Sperduto and Ellwein, 2006). Uncorrected refractive error is the leading cause of moderate to severe visual impairment globally (Bourne, Stevens, White, Smith, Flaxman, Price, Jonas, Keeffe, Leasher, Naidoo et al., 2013).

Many studies have been conducted to help elucidate the underlying cause of this epidemic. Myopia is a multifactorial condition that has both a hereditary and an environmental component. Studies have shown that the odds of becoming myopic increase with one myopic parent and increase further with two myopic parents (Mutti, Mitchell, Moeschberger, Jones and Zadnik, 2002). There are a number of genetic loci that have been shown to be associated with myopia. Analysis completed by the CREAM study group shows that 24 genetic loci have been identified as associated with an increased risk of development of myopia (Verhoeven, Hysi, Wojciechowski, Fan, Guggenheim, Hohn, MacGregor, Hewitt, Nag, Cheng et al., 2013). The study indicated that subjects that carried more of the alleles in question experienced a greater risk of developing myopia. Subjects with the highest risk increased their chance of developing myopia by tenfold. Another group of researchers identified 22 loci that were associated with myopia development (Kiefer, Tung, Do, Hinds, Mountain, Francke and Eriksson, 2013). Of those 22, 20 were novel loci that had not previously been identified. Two of these loci identified, the ZIC2 and the SFRP1 loci, are associated with retinal ganglion cells. The studies completed by both Verhoeven et al. and Kiefer et al. both identified the LAMA2, GJD2, and RASGRF1 gene loci as being relevant for association with myopia. Overall the variance explained by all of the significant genes was small in both studies, 3.4% in Verhoeven et al. and 2.9% in Kiefer et al. This means that the many genetic

markers associated with myopia have minimal impact on the phenotype of subjects. Either major genes responsible for myopia have not been identified or subjects may be more susceptible to environmental factors than previously thought.

Besides hereditary factors, environment may also play a large role in the development of myopia. The most longstanding and widely publicized environmental factor associated with myopia is near work. It has been theorized that children who spend more time doing near tasks, such as reading, become more nearsighted, or myopic, as they grow. This stems from the observation that many myopic children and adults do large amounts of near work. There have been studies as recent as 2015 that make the connection between near work and myopia (Huang, Chang and Wu, 2015). This meta-analysis demonstrates that children who perform increased near work are more likely to be myopic. However, the analysis did not support the idea that near work causes the myopia to develop as it did not show that a strong correlation between near work and either incidence or the rate of progression of myopia.

Some studies have examined hyperopic defocus as a cause of myopia development. Early studies in animal models showed that hyperopic defocus could cause the development of myopia. Negative lenses caused hyperopic defocus and an increase in myopic refractive error in chick models (Schaeffel, Glasser and Howland, 1988). Rhesus monkeys also developed greater amounts of myopia when exposed to hyperopic defocus-inducing minus lenses (Smith and Hung, 1999). This caused researchers to look into accommodative lag as a potential cause for myopia in humans. One study showed that

myopic children do not accommodate as strongly to induced blur compared to emmetropic children (Gwiazda, Thorn, Bauer and Held, 1993). Accommodative lag would cause some residual hyperopic defocus. Though this lag could be a mechanism to promote myopia development, another study showed that lag was not predictive for myopia development (Mutti, Mitchell, Hayes, Jones, Moeschberger, Cotter, Kleinstein, Manny, Twelker, Zadnik et al., 2006). Instead, this study showed that accommodative lag was found to be a feature of myopes after the onset of myopia. Further studies showed that foveal lag treatments, such as wearing progressive addition lenses, were not effective in reducing the rate of myopia progression (Gwiazda, Hyman, Hussein, Everett, Norton, Kurtz, Leske, Manny, Marsh-Tootle and Scheiman, 2003). Other studies that followed showed that peripheral retinal defocus was important for affecting central refractive error. Smith et al. used a rhesus monkey model to test peripheral defocus and found that peripheral hyperopic defocus caused an increase in central myopia (Smith, Hung and Huang, 2009). The study also found that the effect persisted when the foveal tissue was ablated, thereby convincingly demonstrating that central myopia could be driven by the peripheral hyperopic defocus. Peripheral myopic defocus induced using orthokeratology lenses and multifocal soft contact lenses has provided some initial success in the reduction of myopia development (Anstice and Phillips, 2011; Walline, Jones and Sinnott, 2009).

In the mid 2000s, an alternative theory arose out of near work's shadow. A study by the CLEERE group showed that time spent outdoors is protective against the

development of myopia (Jones, Sinnott, Mutti, Mitchell, Moeschberger and Zadnik, 2007). The study examined survey data from the Orinda Longitudinal Study of Myopia that was collected from 1989 to 2001. 514 children were included in the final analysis, 111 of whom went on to become myopic (21.6%). Subjects were examined as non-myopic third graders and then again as eighth graders to determine the number who became myopic. Activity data were provided via a parental survey. This was used to determine time spent doing near work and time spent outdoors. The study showed that children spending at least 14 hours outdoors doing sports or other activities were less likely to develop myopia. Subjects who went on to remain non-myopic participated in 11.65 hours per week of sports and outdoor activity, while subjects who went on to become myopic participated in an average of 7.98 hours per week. This finding was a fundamental shift from the previously established train of thought. The study put near work head-to-head with time outdoors and time outdoors held up in a multivariate analysis. Time outdoors was protective against myopia ($p<0.00001$, OR = 0.91, 95% CI = 0.87–0.95) and reading hours had no effect ($p = 0.26$ OR = 1.03, 95% CI = 0.98–1.10). This means that time spent outdoors is protective itself and not just representative of time spent without near visual demands. When time outdoors was combined with genetic risk, children with equal genetic risk developed myopia at lower rates with increased time spent outdoors.

Not only is there a difference in direct amount of time spent outdoors, but there is a seasonal variation in myopia progression as well. Myopia progression was greater during the winter months than the summer months (Fulk, Cyert and Parker, 2002). This

supports the notion that myopia was slowed during the time that the children presumably spent more time outdoors.

Another study from the CLEERE group showed that although time outdoors affects the incidence rate of myopia, it does not the affect the rate of progression (Jones-Jordan, Sinnott, Cotter, Kleinstein, Manny, Mutti, Twelker and Zadnik, 2012). The reduction in progression found due to every 10 hours per week of outdoor activity was 0.03 D less progression per year (99% CI = -0.03, 0.08). This supports the idea that once a child becomes myopic they are likely to continue to progress in myopia regardless of time spent outdoors. Other clinical trials that have been completed have shown that students who spend extra time outdoors are less likely to go on to develop myopia versus students who spent more time indoors, and have further confirmed that effects of outdoor exposure on myopia progression are minimal to none (Wu, Tsai, Wu, Yang and Kuo, 2013; He, Xiang, Zeng, Mai, Chen, Zhang, Smith, Rose and Morgan, 2015).

The question regarding the mechanism through which time outdoors provides its benefit still remains unanswered. One theory is that a smaller pupil, due to greater exposure to light, produces a retinal image with better optical quality. Two more attractive theories hinge on the increase in exposure to sunlight when someone spends time outdoors. The first theory suggests that time outdoors may be beneficial due to the increased exposure to ultraviolet wavelengths of light. This may be related to increased vitamin D production with increased sun exposure. Several studies have shown that vitamin D is decreased in myopic subjects (Choi, Han, Park and La, 2014; Yazar, Hewitt,

Black, McKnight, Mountain, Sherwin, Oddy, Coroneo, Lucas and Mackey, 2014; Guggenheim, Williams, Northstone, Howe, Tilling, St Pourcain, McMahon and Lawlor, 2014). Choi et al. showed a positive relationship between serum vitamin D levels and spherical equivalent after adjustment for age ($r = 0.067$, $p = 0.012$) in a Korean population. In the myopic group, there was a significant positive relationship between spherical equivalent and vitamin D ($p = 0.020$), while the non-myopic group did not show any correlation ($p = 0.599$). Yazar et al. showed that vitamin D levels were lower in myopes in an Australian population. In the study, myopic subjects had lower serum vitamin D concentrations compared to non-myopic participants ($p = 0.003$). Guggenheim et al. found that children who spent less time outdoors have lower levels of serum vitamin D concentration ($p = 0.001$). They did not find any association between vitamin D levels and incident myopia ($p = 0.11$). They did confirm the association of time outdoors with incident myopia ($p = 0.001$), concluding that any association between refractive error and vitamin D was merely the effect of time outdoors. These studies support the idea that myopes spend less time outdoors, have less available vitamin D, but that time outdoors may be the more relevant variable to refractive error.

The hypothesized mechanism for the benefit of increased vitamin D involves the ciliary muscle. Previous studies on rat bladder have demonstrated that decreased vitamin D decreases the elasticity of smooth muscle cells (Schroder, Colli, Maggi and Andersson, 2006). It is possible that a similar mechanism may be causing a decreased elasticity in the smooth ciliary muscle cells. The CLEERE study group described changes to the crystalline lens before and after myopia onset (Mutti, Mitchell, Sinnott, Jones-Jordan,

Moeschberger, Cotter, Kleinstein, Manny, Twelker and Zadnik, 2012). The study showed that myopes had thinner lenses in all age groups than emmetropes ($p = 0.0003$). The study found that lens thinning in myopes slows one year before onset of myopia. The study establishes that there is a disconnection in myopes between axial elongation and compensatory lens changes. This may be due to a mechanical restriction in the equatorial dimension of the eye. Less elasticity in ciliary muscle would reduce the compensatory thinning and cause a more prolate eye shape (Atchison, Jones, Schmid, Pritchard, Pope, Strugnell and Riley, 2004). The involvement of the ciliary muscle is consistent with previous studies that show AC/A ratio is increased in subjects with myopia (Mutti, Jones, Moeschberger and Zadnik, 2000).

The other popular theory is related to exposure to visible light. This theory relies on the retinal signaling molecule dopamine. Dopamine is released in the retina by dopaminergic amacrine cells. These amacrine cells are stimulated to release more dopamine as the retina is exposed to increased levels of visible light. The dopamine that is released is utilized by the retina as a local signaling molecule for light adaptation (Do and Yau, 2010). In addition to local signaling, dopamine has also been shown to be an inhibitor of axial growth. Less axial elongation means that the patient is less likely to become myopic. Studies have shown that increased visible light levels have successfully reduced the development of myopia in a chick model (Ashby, Ohlendorf and Schaeffel, 2009). The group used both a form deprivation model and a lens-induced model to demonstrate the effect with the greater inhibitory effect of light being seen in the deprivation model. Ashby and co-workers also made an argument that this effect was due

to dopamine. In eyes where a dopamine inhibitor is injected, the effect is eliminated. Another group showed a similar effect with rhesus monkeys and the form deprivation model (Smith, Hung, Arumugam and Huang, 2013). High ambient lighting in that model reduced myopia. When the group tried to replicate the results in a lens-induced model, the model more dependent on vision compared to form deprivation, the results were negative (Smith et al., 2013). The high ambient lighting did not prevent negative lens induced myopia in rhesus monkeys. Although the bright visible light and dopamine release theory is the predominant one currently, it is not without limitations.

The theory of visible light and growth inhibiting dopamine release may be mediated by an important subset of retinal ganglion cells that act as photoreceptors within the retina. These cells are known as intrinsically photosensitive retinal ganglion cells or ipRGCs. These cells are located in the ganglion cell layer of the inner retina. There are five known subtypes of ipRGC that are named M1-M5 with M1 being the most common subtype. Overall, ipRGCs only make up 0.2% of the ganglion cells in the retina (Munch and Kawasaki, 2013). These cells contain the photopigment melanopsin and are sensitive to shorter, blue wavelengths of light (Guler, Ecker, Lall, Haq, Altimus, Liao, Barnard, Cahill, Badea, Zhao et al., 2008). These cells project to different areas in the brain. One of their primary projections is to the suprachiasmatic nucleus where the information they provide is used to help set the body's natural circadian rhythm (Munch et al., 2013). The ipRGCs also project to the olivary pretectal nucleus to play a role in the pupillary light response (Munch et al., 2013). The ipRGCs act as photon counters to assess exposure to ambient light. They produce a delayed and sustained firing in response to light (Park,

Moura, Raza, Rhee, Kardon and Hood, 2011). This visible sign of pupil response is a non-invasive way to assess ipRGC function within a test subject.

The pupillary response can be exploited as a direct measure of ipRGC activity and potentially as an indirect measure of retinal dopamine. The ipRGCs have a very slow response with a longer latency period and a long response duration or post-illumination pupil response (Munch et al., 2013). When stimulated with light, the ipRGCs continue to fire long after the stimulus has gone off. This can cause differences in the amount of pupil redilation that occurs after stimulation with bright blue light, as compared to bright red light, when the blue and red stimuli are photopically matched (designed to evoke equal photon absorptions in cone photopigments). With blue light stimuli, the pupil does not redilate as quickly as when stimulated with photopically-matched red light, and this difference has been directly attributed to the prolonged light responses that persist in ipRGCs after blue light stimulation (Gamlin, McDougal, Pokorny, Smith, Yau and Dacey, 2007). In a study by Park et al. redilation following blue stimuli was prolonged by variable amounts depending on stimulus duration and intensity (Park et al., 2011).

An important question is whether human ipRGC light responses are influenced by prior exposure to varying degrees of ambient light levels encountered during daily living. The primary purpose of this study is to determine the relationship between myopia development, a person's own light exposure history, and the response of ipRGCs as estimated by the pupil response to red and blue oscillating light. A secondary purpose is to evaluate the validity of different methods for assessing the amount of time an individual spends outdoors.

Chapter 2: Materials and Methods

Subject Recruitment

Subjects were recruited from the student population at The Ohio State University College of Optometry. Subjects were selected on a first come, first served basis provided that the subjects met the inclusion criteria for the study. Study participation was voluntary. Subjects had to be willing to participate in the study and had to have a best-corrected visual acuity of 20/25 or better in the better eye with no unexplained decrease in acuity in the fellow eye. Subjects could be from either gender but could not have active ocular disease or systemic health condition that would affect systemic vitamin D. No exclusions were made based on refractive error; subjects were included in the study regardless of previous or current refractive error state.

Subject Consent

Subjects provided written informed consent prior to agreement to partake in the research study. This study followed the tenets of the Declaration of Helsinki on medical protocol and ethics and was reviewed and approved by the Biomedical Institutional Review Board of The Ohio State University. The purpose and the procedures of the study were explained to each subject including the benefits and risks of participation. Subjects were also informed of the use of personal identifying information. Personal subject information was not shared and was protected according to the Health Insurance

Portability and Accountability Act (HIPAA). Subjects were informed during the consent process that each subject would be compensated $20.00 upon the completion of the study, with $10.00 given at the end of the initial visit as well at the follow up visit. Subjects were instructed that they could drop out of the study at any time for any reason without repercussions.

Subject Statistics

Subjects were 20 young adults (mean age 24.8 ± 1.8 years, 13 female). The average cycloplegic spherical equivalent refractive error of the subjects was -2.29D ± 1.96D with the range from -6.33D to +1.69D. There were 17 subjects who were myopic and three subjects that were emmetropic or hyperopic. For the purpose of this study, myopia was considered to be a subject with a refractive error of at least -0.50D of myopia in each principal meridian. Subjects were given a survey to assess the status of parental refractive error. Questions for each parent included: Does your birthmother/birthfather wear glasses or contact lenses? How old was he/she when he/she began wearing glasses? Does he/she need glasses primarily for viewing things clearly in the distance, reading/working at a computer or other close work, or equally for distance and close work? Parents were classified as myopic if glasses were worn for distance only, or if glasses were equally important for distance and near if they were prescribed before age 17 years (Walline, Zadnik and Mutti, 1996). There were 7 subjects who had two myopic parents, 8 subjects had one myopic parent, and 5 subjects had no myopic parents.

Initial visit

At the initial visit subjects were introduced to the study and guided through informed consent. Once consent was given, visual acuity was measured. Subjects stood 10 feet away from a Bailey-Lovie acuity chart and completed acuities for each eye under monocular conditions with correction and without correction. After acuity measurement, subjects' refractive errors were measured using a Grand Seiko WR-5100K autorefractor without cycloplegia but with a Badal optometer target to relax accommodation. Subjects covered one eye with an eye patch and autorefraction was done monocularly. Subjects were then given a short medical history questionnaire to ensure the subject met the inclusion criteria. After completing entrance testing, subjects were given light sensitive badges to wear for one week.

Badges

Two light sensitive badges were affixed to an elastic armband. One badge was designed to monitor ultraviolet wavelengths of light, specifically UV-B rays. This small white badge was designed and produced by the National Institute of Water and Atmospheric Research in New Zealand. This badge took readings of the current amount of UV exposure of a subject at that moment in time. The badge took this measurement six times per minute and date and time stamped the data values. The six values produced for each minute were averaged to find the average amount of UV over that minute. The UV badges were calibrated against a factory-calibrated Kipp and Zonen UVS-B-T UV radiometer (Table 1). The UV sensitive badge provided raw counts that were converted to

irradiance values in watts per square meter. The equation for this conversion was a third order polynomial equation.

Badge	Third order	Second order	First order	Intercept
9	1.50E-10	3.70E-07	1.81E-03	3.24E-02
10	5.92E-09	3.35E-06	2.42E-03	2.22E-02
11	-2.70E-09	2.31E-06	1.30E-03	8.43E-02
15	1.77E-09	2.07E-06	2.83E-03	2.51E-02
16	2.84E-09	1.60E-06	2.15E-03	2.78E-02

Table 1. UV calibration coefficients used in the third order polynomial equation to convert raw UV badge counts to irradiance values.

The other badge affixed to the armband measured exposure to wavelengths in the visible light spectrum. The Daysimeter, produced and calibrated by the Lighting Research Center at the Rensselaer Polytechnic Institute in Troy, New York, is a personal visible light monitor that was able to measure lux values over one-minute intervals. This device integrated the total exposure of lux over that minute rather than just recording a snapshot of a moment in time within a one minute interval. This badge also date and time stamped the data points.

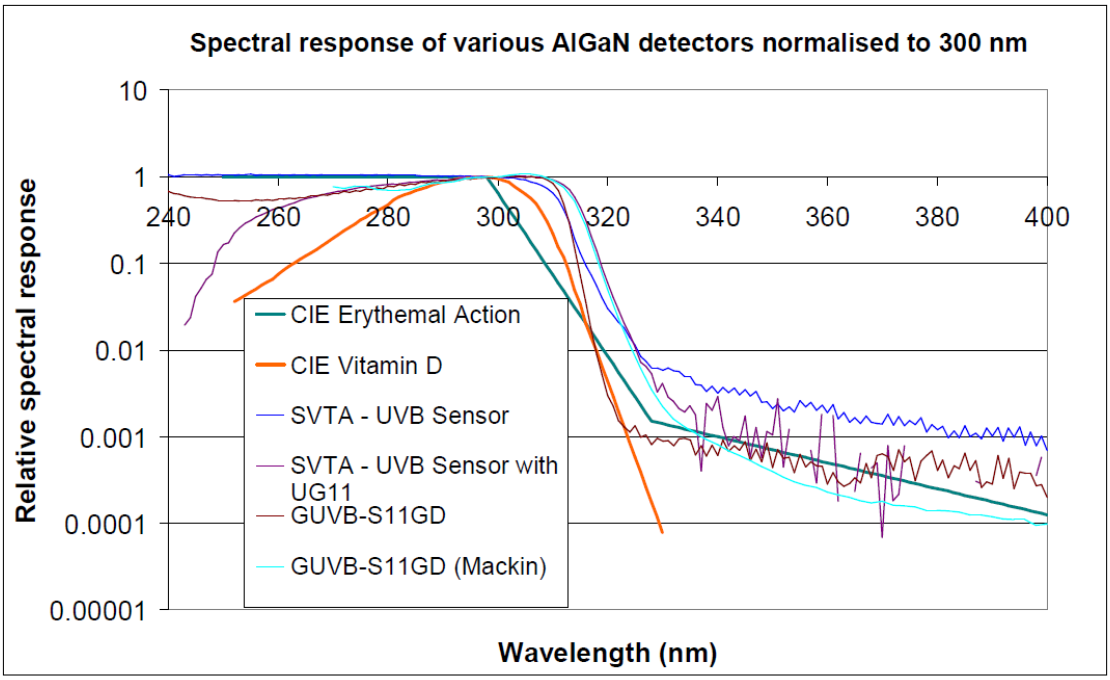

Figure 1. Spectral sensitivity to UVB in NIWA badges (GUVB-S11GD) (Swift, Hamlin, Nield and McKenzie, 2010). There is a strong falloff after 315 nm in close approximation with vitamin D production sensitivity.

Badge selection was made by looking for good consistency between badges. To test this, badges were placed outside in an area that receives consistent sunlight without any shadow interference. The data for each badge were then plotted (Figure 2 and Figure 5). Badges were selected from among groups of badges that performed similarly and were at the center of the distribution of badges. Choosing badges that performed in a similar way reduced inter-subject data variability due to badge performance.

Figure 3 shows the amount of available ultra-violet light during a typical day as measured by Kipp and Zonen UVS-B-T UV radiometer (pictured in Figure 4). The

oscillations from passing clouds in Figure 3 are the cause of the oscillations measured by the badges in Figure 2.

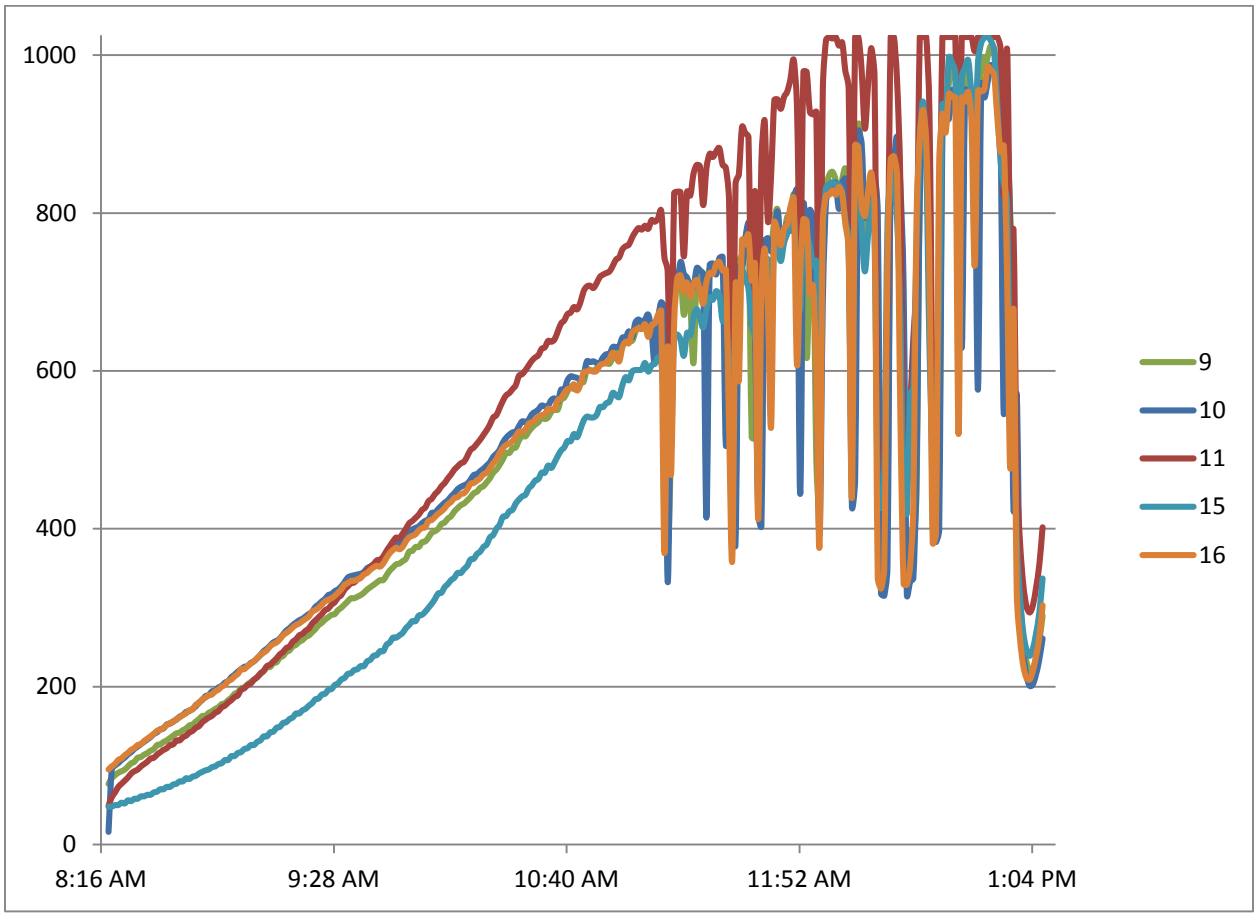

Figure 2. This figure represents the calibration of the ultra-violet badge numbers that were used in the study. The maximum amount of counts the badge will register is 1025. Badges may reach the maximum amount of counts due to the gain control on the badges. To calculate the actual amount of ultra-violet light present, the badge counts were converted using a third order polynomial calibration equation. Consistency among badges is seen even through oscillations due to cloud cover.

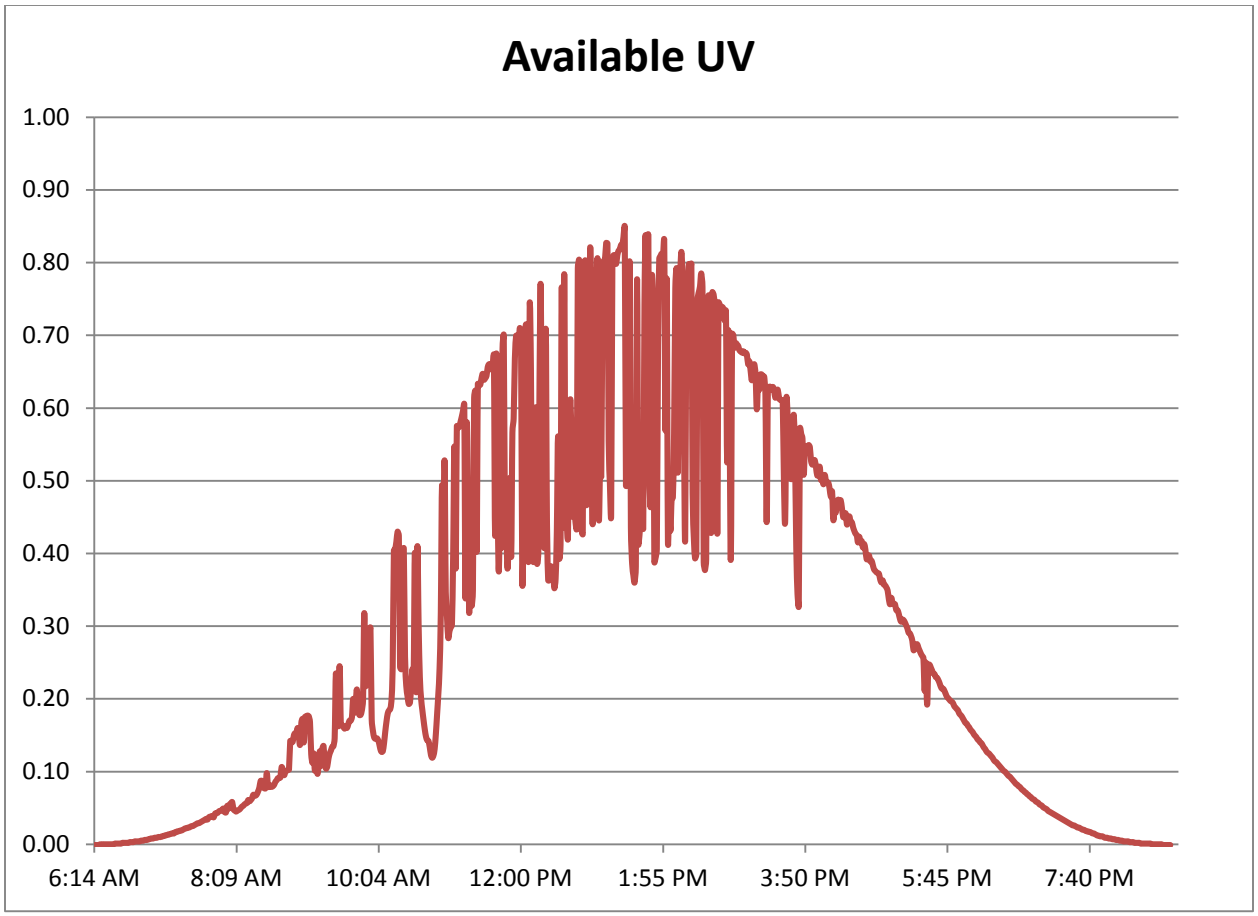

Figure 3. This figure is a representative graph that shows the amount of available ultra-violet light during a typical day as measured by Kipp and Zonen UVS-B-T UV radiometer. The many oscillations are due to cloud cover. The cloud cover can be variable throughout the day. This validates the performance of the ultra-violet badges and demonstrates that the oscillations are due to available ultra-violet light and not badge malfunctions.

Figure 4. Picture of Kipp and Zonen UVS-B-T UV radiometer.

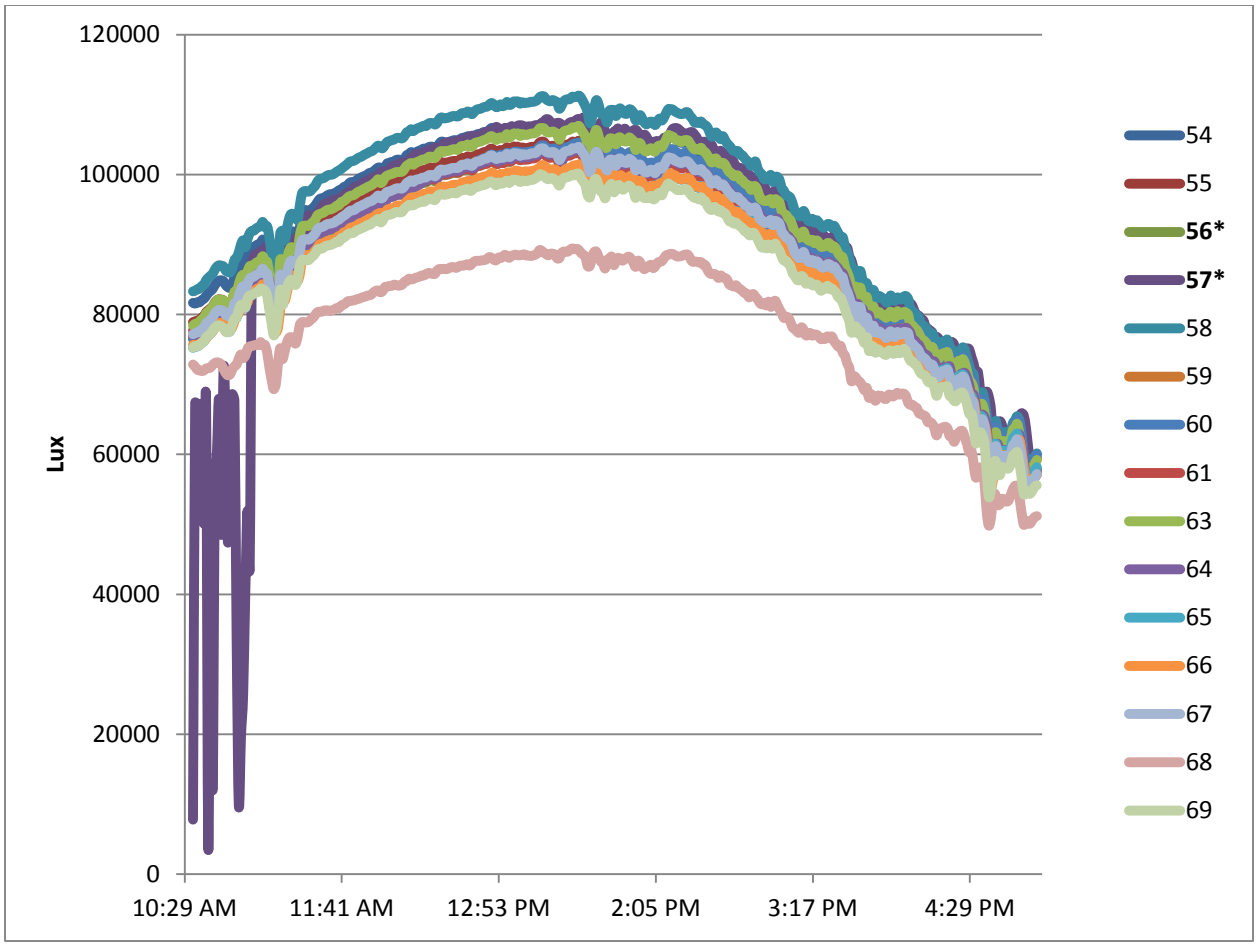

Figure 5. This figure represents the data used to select the Daysimeter visible light badges. The badges demonstrate excellent inter-badge consistency. Badges that were consistent and fell within the center of the center of the distribution were chosen to distribute to subjects first. Badges 57 and 68 were eliminated from use due to inconsistency compared to other badges.

Instructions for the week

Subjects were instructed to place the armband on the outermost layer of clothing on either the left or the right arm. Figure 6 shows the two badges fixed to the elastic armband. The armbands were to be worn from waking to bedtime each day during the time that the subjects had the armband. Subjects were advised that the badges on the

armband were water resistant but not waterproof, therefore subjects did not wear armbands while bathing or doing any water activities but kept the badges in the same vicinity of such activities. Subjects were instructed to continue daily activities as they would normally. No restrictions were put on activities and subjects were able to wear badges during exercise. Subjects were also told to pay particular attention to the food that they ate during the week while wearing the badge as they would be completing a survey on their weekly food intake at the follow up visit.

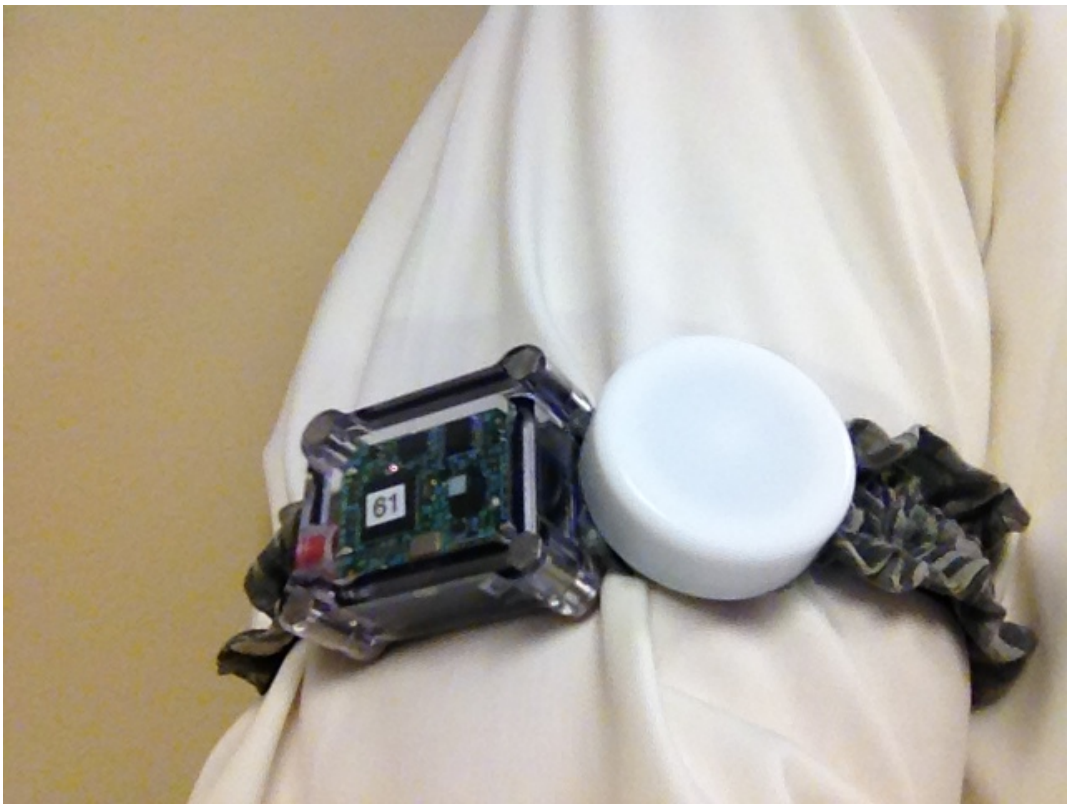

Figure 6. Picture of Daysimeter on left and UVB badge on right.

Follow up visit

Follow up visits were scheduled as close to 1 week from the first visit as possible. Subjects would turn in the badges at the beginning of the follow up visit and the time that the subject arrived would be recorded. The subject would then immediately undergo pupillometry measurements. After pupil testing, the badge data would be downloaded. Data would be downloaded prior to subjects completing the remainder of the testing to ensure that usable data were captured. Subjects were asked to identify any times that the badge was not worn or times that the subject was exposed to the outdoors but the badge was not. Subjects would then complete a survey on activities that were done during the previous week and a food intake survey. The subject would then produce a saliva and blood sample for analysis.

RAPDx

Pupil testing was done using the RAPDx, a commercially available instrument from Konan Medical. The RAPDx has the ability to provide a variety of full or partial field stimuli to one or both eyes with an LCD screen. While the stimuli are presented, video recording produces a real time pupil diameter that can be analyzed at various time points. The stimulus intensity and flicker frequency can be altered to meet the needs of the desired testing.

Alternating protocol

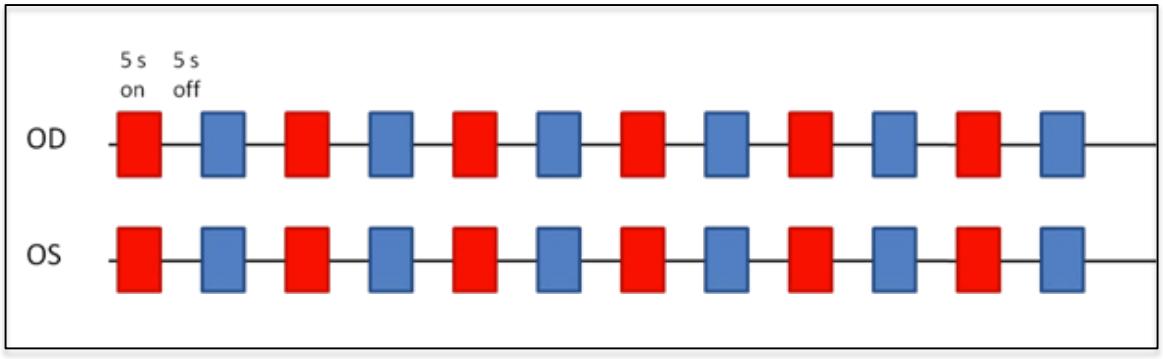

Figure 7. Graphical representation of alternating protocol.

The alternating protocol was the first test stimulus sequence used for each subject. Immediately preceding this testing, the patient was placed in a dark room for five minutes of dark adaptation. Upon completion of the five minute dark period the test began with a full field stimulus of red light presented to both eyes for five seconds. The field would then go dark for five seconds. This period of dark still included the background illumination of the LCD screen. After the five second dark period there was a period of five seconds of blue light. There was five more seconds of dark. The cycle then repeated at this point. This protocol alternated at 0.1 Hz for a total of 2 minutes (12 cycles).

Red protocol

After the alternating protocol, there was another five minute period of dark adaptation. After the adaptation period there was another sequence of stimuli presented to the subject. First a full field red stimulus was presented for five seconds and then a full field dark for five seconds. The sequence then repeated. This sequence continued at 0.1

Hz for one minute. At the end of the protocol there were a total of six presentations of red light and six dark periods.

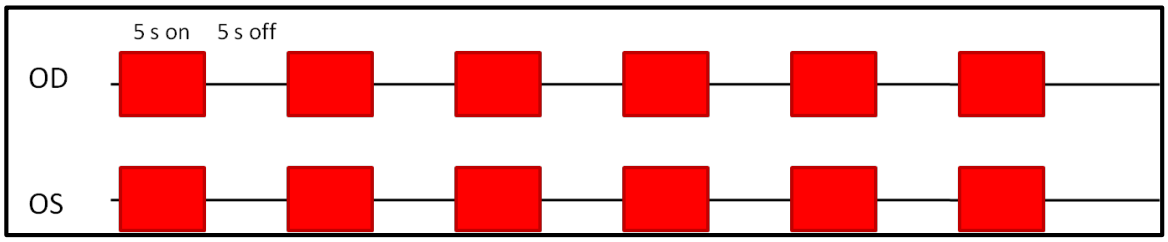

Figure 8. Graphical representation of red only protocol.

Blue protocol

After the red protocol, there was another five minute period of dark adaptation. After the adaptation period there was another sequence of stimuli presented to the subject. First a full field blue stimulus was presented for five seconds and then a full field dark for five seconds. The sequence then repeated. This sequence continued at 0.1 Hz for one minute. At the end of the protocol there were a total of six presentations of blue light and six dark periods.

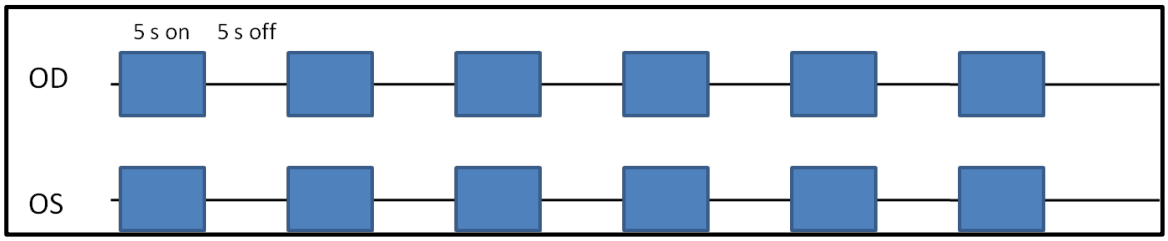

Figure 9. Graphical representation of blue only protocol.

Pupil data analysis

The data analysis used to for the pupil data was a custom Excel macro that was created by Patrick Shorter, OD, PhD. The output from the RAPDx yielded 40 readings of pupil diameter per second in each eye. The data was originally recorded in number of pixels but was converted to millimeters using a standardized equation. The equation was generated by using the instrument to measure solid black disks of known diameters. The results matched closely with manufacturer measurements but the custom equations were used for the purposes of this thesis.

The pupil diameters, now in millimeters, were binned and averaged into 0.25 second intervals. The data points could then be analyzed using raw diameter values or could be transformed to normalized values. To normalize the pupil size, diameters were changed to percent constriction. Each subject's maximum and minimum pupil diameter in either eye over the course of all testing was determined. The smallest pupil size was set to be 100% constriction for that subject. The largest pupil size was set to be 0% constriction for that subject. This normalized pupil size was often preferred for analysis because it eliminated variability due to baseline pupil size.

Activity survey

An activity frequency survey was completed by each subject at each follow up visit. The subject was asked to recall specific activities that they may have completed throughout the week and pick activities from their week listed on the survey. They were also given space to list activities that may not have already been included in the survey.

Subjects were asked how many times they performed a specific activity and the average length of time spent in that activity. There were separate questions for activities done indoors or outdoors. The survey was designed to assess the amount of time that a subject spent outdoors while wearing the light sensitive badges. Once the survey was completed, the frequency of each activity was multiplied by the average time spent doing that activity. That yielded a total amount of time spent completing each activity. The outdoor activities were then added together to produce a total time outdoors for the week. The Compendium of Physical Activities was used along with total time completing each activity to give a complete picture of caloric exertion for the week for each subject (Ainsworth, Haskell, Whitt, Irwin, Swartz, Strath, O'Brien, Bassett Jr, Schmitz, Emplaincourt et al., 2000). The survey also asked questions regarding sun exposure such as sleeve and pant length, hat use, sunscreen use, and sunglass use. The activity survey is included in the Appendix.

Food survey

A food intake survey, the Block Kids Food Frequency Questionnaire (FFQ), version 2004 was also completed by each subject at each follow up visit. The questionnaires includes 77 food items and was developed from the NHANES 1999-2002 dietary recall data. The nutrient database was developed from the USDA Nutrient Database for Dietary Studies, version 1.0 (adapted from http://www.nutritionquest.com/products/questionnaires_screeners.htm). The subject was shown various food items and asked to recall what he or she had eaten the week prior,

how frequently a particular food item was eaten, and what portion size was eaten. This survey was used to help determine the dietary intake of vitamin D.

Saliva sample

During the follow up visit subjects provided a saliva sample. This sample was often collected simultaneously while the subject was completing the surveys. Subjects were asked to refrain from eating, drinking or using oral hygiene products for at least 1 hour prior to collection. Then subjects were asked to rinse their mouth with water. Subjects were then asked to produce as much saliva as possible without producing mucus. The sample was collected with the aid of a sterile funnel-shaped collection aid into up to two 1.5 ml centrifugation vials. The vials were centrifuged at 2,600g for 15min at 4°C The liquid portion of the saliva was pipetted into a 2.0 ml cryovial (Salimetrics, Carlsbad, CA) and stored in a -80°C freezer until analysis for vitamin D levels could be completed by the Ohio State University shared pharmacoanalytic laboratory. Results are not yet available and are therefore not included in this thesis.

Blood sample

During the follow up visit subjects provided a blood sample. The sample was obtained using a small finger stick with a 1.5 mm sterile, disposable lancet (Sarstedt, Nümbrecht, Germany. After the area was disinfected with an alcohol pad and the lancet applied to the tip of the finger, blood was obtained and used to fill up to two 300 µl containers (coated with ethylenediamine tetraacetic acid (EDTA) as the anti-coagulant).

The blood samples were then placed on ice for 15 minutes then centrifuged at 2000g until separation of the plasma had occurred. The plasma was then pipetted into a separate container and stored in a -80°C freezer until analysis for vitamin D levels could be completed by the Ohio State University shared pharmacoanalytic laboratory. Results are not yet available and are therefore not included in this thesis.

Chapter 3: Results

UV vs. Lux vs. detailed journal over short course of time

A preliminary study was conducted to preview the sensitivity of the UV sensitive badge and the Daysimeter to outdoor exposure. The investigators kept detailed diary notes in order to compare to the badge recorded values. Diary entries included an extended period outdoors, an early morning walk, and late afternoon yard work.

UV	Lux	Detailed Journal
295.83 min	591 min	595 min

Table 2. Table of time outdoors over one weekend calculated by three methods.

Table 2 depicts the time outdoors in minutes by three methods. The detailed journal was completed over the course of one weekend. This journal recorded to the minute the actual amount of time that was spent outside. Lux values from the Daysimeter over 1000 were considered the threshold to be considered outside for this measurement. 1000 lux has been established in previous research as an appropriate criterion for time outdoors (Dharani, Lee, Theng, Drury, Ngo, Sandar, Wong, Finkelstein and Saw, 2012). There was a very close agreement between the Daysimeter lux badge and the journal tally, however the UV badge severely underestimated the amount of time spent outside.

This suggested that the Daysimeter lux badge might be the preferred method for measurement of time outdoors.

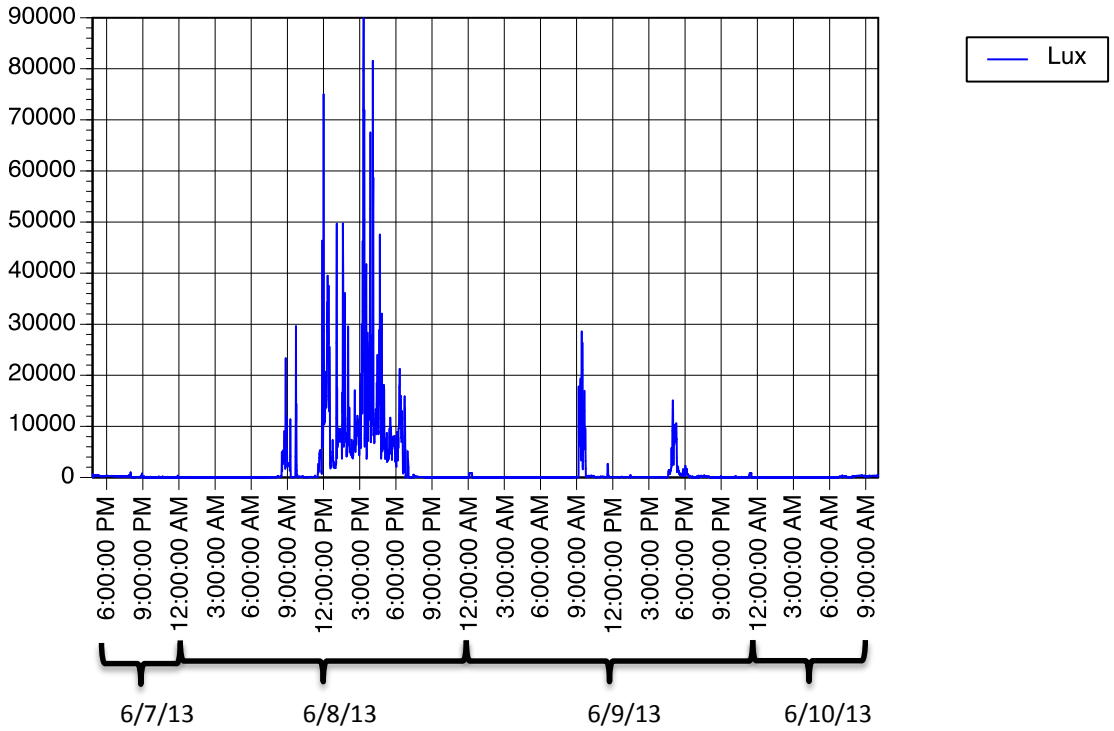

Figure 10. Lux exposure values of a single subject on weekend of June 7, 2013 to June 10, 2013.

Figure 10 displays the lux values of a single subject over the course of one weekend. Periods of high light exposure were considered to be time spent outdoors. The figure displays a large period of time outdoors on June 8, 2013. The maximum lux values were near 90,000 lux on that day. There were also smaller periods of time spent outdoors on June 9, 2013.

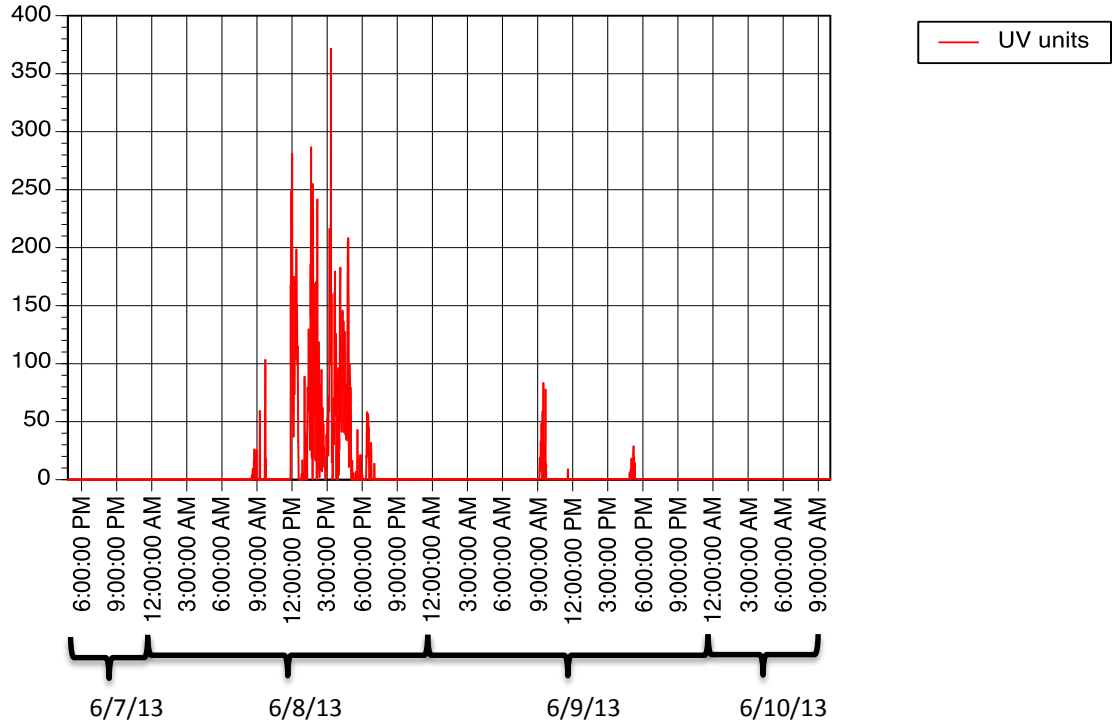

Figure 11. Ultra-violet exposure values of a single subject on weekend of June 7, 2013 to June 10, 2013.

Figure 11 displays the NIWA ultra-violet badge counts of a single subject over the course of the same weekend. Any minutes with a non-zero badge count were considered to be time spent outdoors. The figure displays a large period of time outdoors on June 8, 2013. There were also smaller periods of time spent outdoors on June 9, 2013.

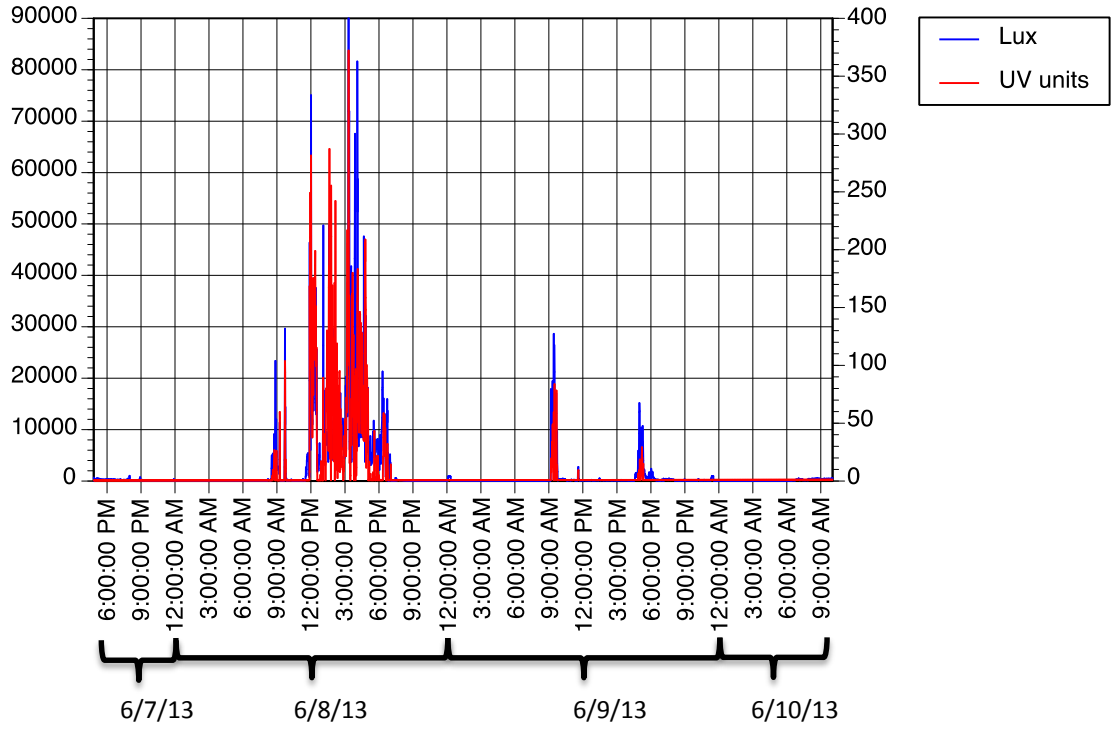

Figure 12. Ultra-violet and lux exposure values of a single subject on weekend of June 7, 2013 to June 10, 2013.

Figure 12 displays the overlay of the comparison of time spent outdoors using the lux and ultra-violet badges of the same single subject over the course of the same weekend. Any minutes with a non-zero UV badge count and more than 1000 lux were considered to be time spent outdoors. The figure displays a large period of time outdoors on June 8, 2013. There were also smaller periods of time spent outdoors on June 9, 2013. The overlay shows a relatively good agreement for periods of time or epochs that were spent outdoors, however a more detailed comparison shows area of disagreement.

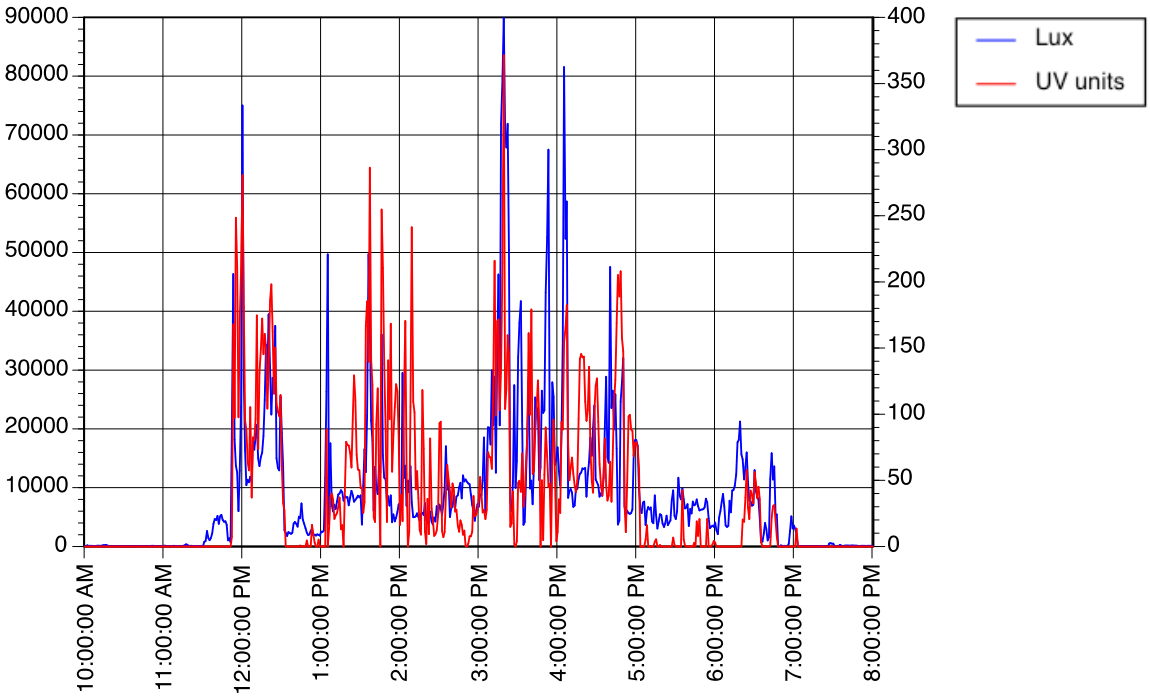

Figure 13. Ultra-violet and lux exposure values of a single subject on June 8, 2013.

Figure 13 displays this more detailed view in an overlay of the comparison of time spent outdoors using the lux and ultra-violet badges of the same single subject over the course of one day of the weekend. Any minutes with a non-zero UV badge count and 1000 lux were considered to be time spent outdoors. The figure highlights the difference of the UV badge and the lux badge. While both badges show a similar beginning and end to this epoch outdoors, there is much more variability in the reading of the UV badge. The UV badge demonstrates a number of minutes within the epoch that had a zero count while the visible light Daysimeter badge was still registering 1000 lux or more. This pattern is reflected in the lower number of minutes recorded by the UV badge.

Badge results vs. activity survey results within study population

Survey	Daysimeter	UVB Badge
285 minutes per week (median)	685 minutes per week (median)	203 minutes per week (median)
495 minutes per week (mean)	730 minutes per week (mean)	268 minutes per week (mean)
Range 105-1680	Range 66-1593	Range 3-768
Interquartile range 212-743	Interquartile range 417-993	Interquartile range 112-342

Table 3. Estimates of time outdoors by each method.

Table 3 shows the calculation of average minutes of time spent outdoors by each method of measurement for the 20 subjects in the study. There was a large range among all three methods indicating that there was variability from subject to subject. The amount of time that was spent outside may have been affected by the lifestyle of the individual subject or even by the season that the subject participated in the study. The results show a much closer agreement between the survey and Daysimeter mean results and survey and UV badge median results. Each difference was significant by repeated measures ANOVA with $p<0.030$ after Bonferroni correction.

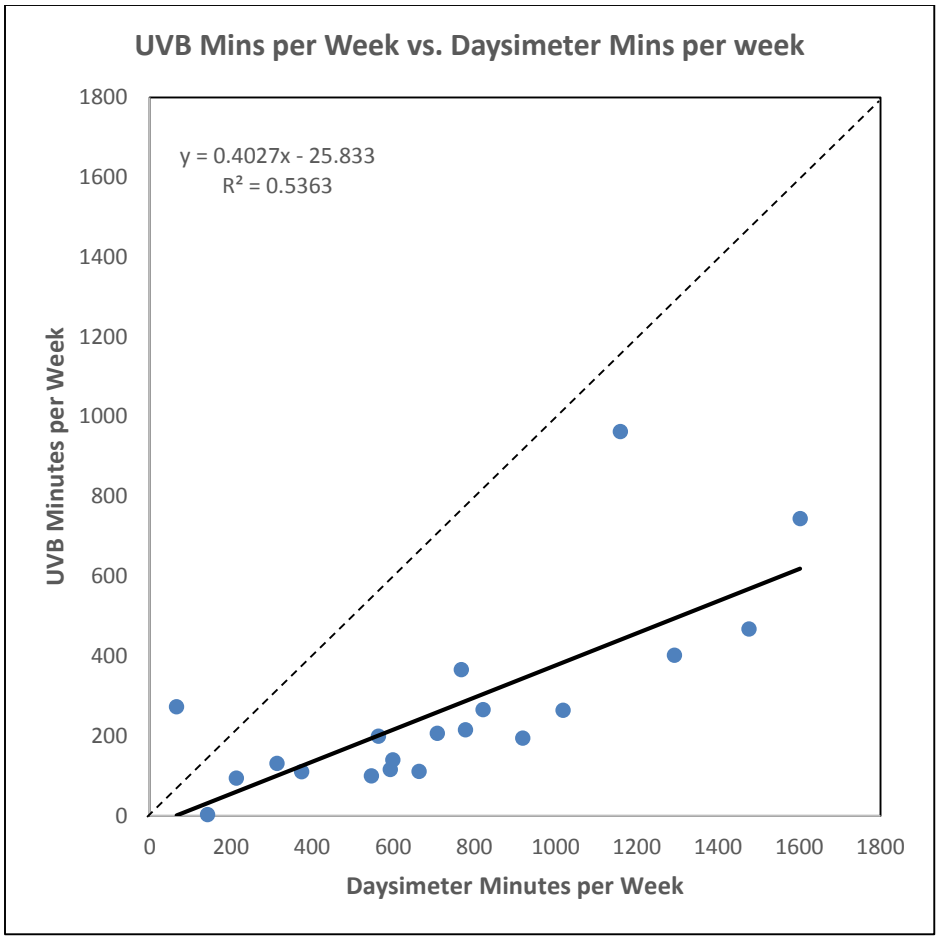

Figure 14. Measurement of minutes per week spent outdoors UVB vs. Daysimeter (the dotted line represents a 1:1 line).

Figure 14 depicts the correlation in the subject population between the minutes of time spent outdoors as calculated by the ultra-violet badge and by the Daysimeter. The dotted one to one line demonstrates an ideal 1:1 correlation. There was a positive correlation between the measurement of time by the two badges. The slope of the linear regression line of 0.40, however, indicates that the ultra-violet badge tended to underestimate the number of minutes spent outdoors vs. the Daysimeter. Only one subject

fell above the one to one line meaning in only one instance did the amount of time measured by the ultra-violet badge exceed the Daysimeter.

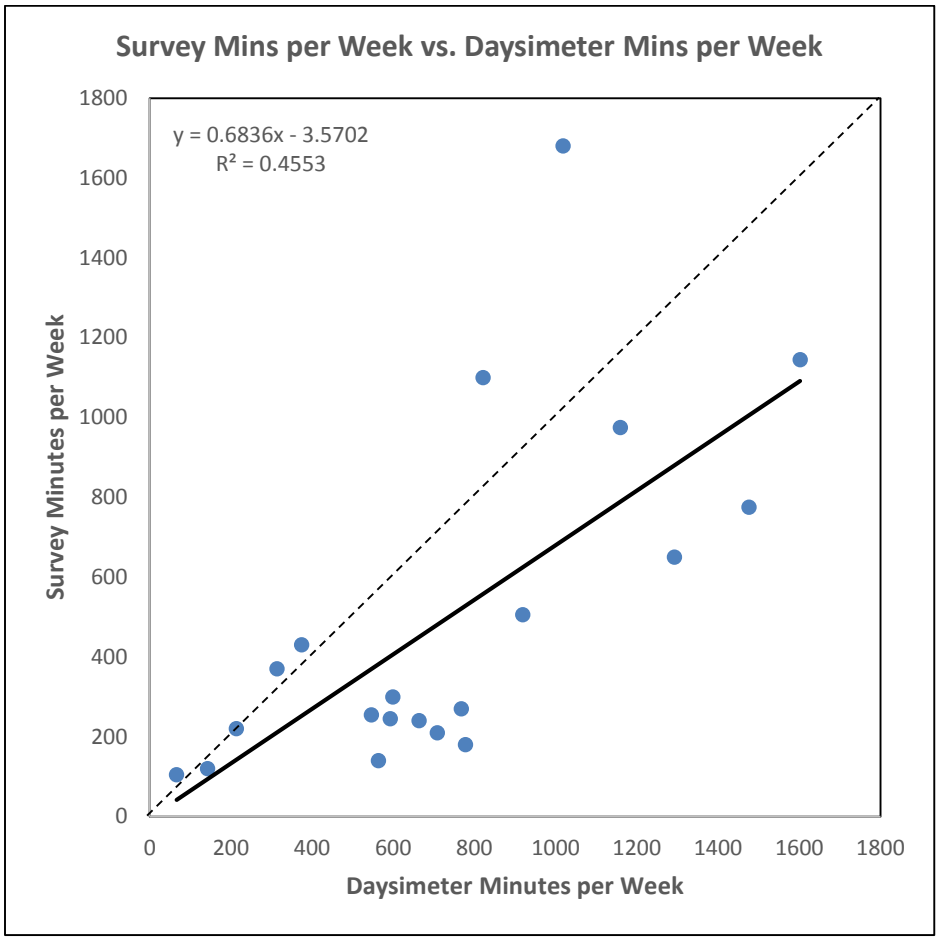

Figure 15. Measurement of minutes per week spent outdoors Survey vs. Daysimeter (one to one line dotted).

Figure 15 depicts the correlation in the subject population between the minutes of time spent outdoors as calculated by the activity survey and by the Daysimeter. The dotted one to one line demonstrates an ideal correlation. There was a positive correlation between the results of the survey and the Daysimeter. The slope of the linear regression

line of 0.68, however, indicates that the survey tended to underestimate the number of minutes spent outdoors vs. the Daysimeter. Though it still underestimated the number of minutes spent outdoors, there was a much closer relationship (slope closer to 1) between the Daysimeter and the activity survey than there was between Daysimeter and the ultra-violet badge.

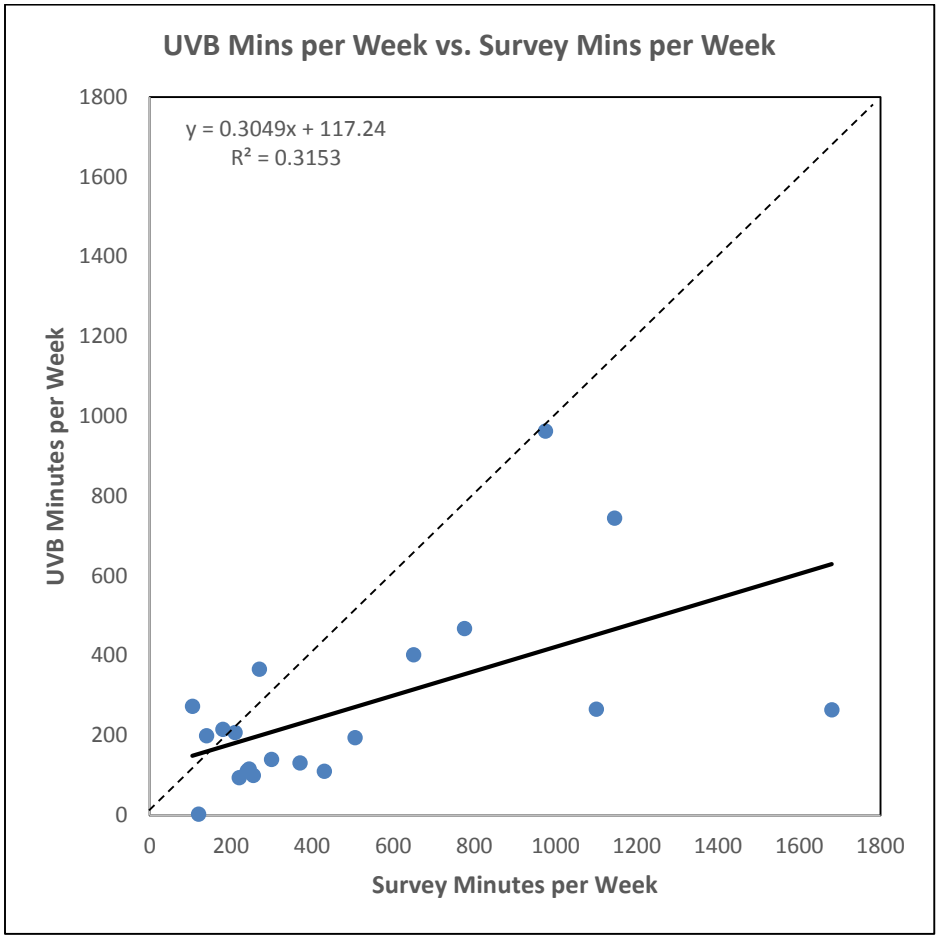

Figure 16. Measurement of minutes per week spent outdoors UVB vs. Survey (one to one line dotted).

Figure 16 depicts the correlation in the subject population between the minutes of time spent outdoors as calculated by the ultra-violet badge and by the activity survey. The dotted one to one line demonstrates an ideal correlation. There was a positive correlation between the UVB badge and the survey results but with a low R^2 of 0.32. The slope of the linear regression line of 0.30, however, indicates that the ultra-violet badge tended to underestimate the number of minutes spent outdoors vs. the activity survey.

Development of RAPDx Outcome

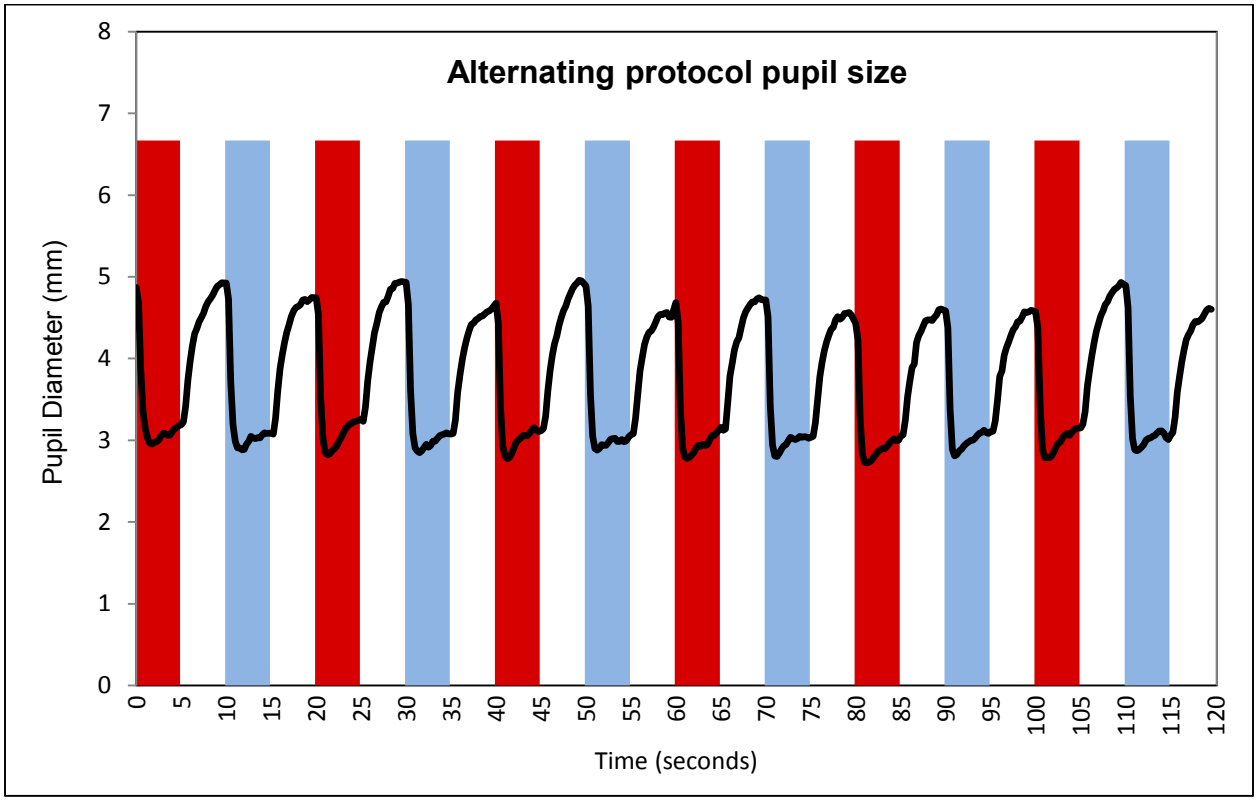

Figure 17. Average pupil diameter from all subjects during alternating protocol.

Figure 17 displays the raw data output from the RAPDx instrument for the alternating protocol. This test was a two-color presentation that lasts for two minutes. Pupil sizes were measured continuously as the oscillating lights were presented. The data showed the expected response of constriction when light was presented and dilation when light was turned off. Redilation was often less prominent following the presentation of blue light compared to red as indicated by a higher peak after exposure to red than compared to the next peak after exposure to blue (for example comparing the peak at 50 seconds after red and the peak at 60 seconds following blue).

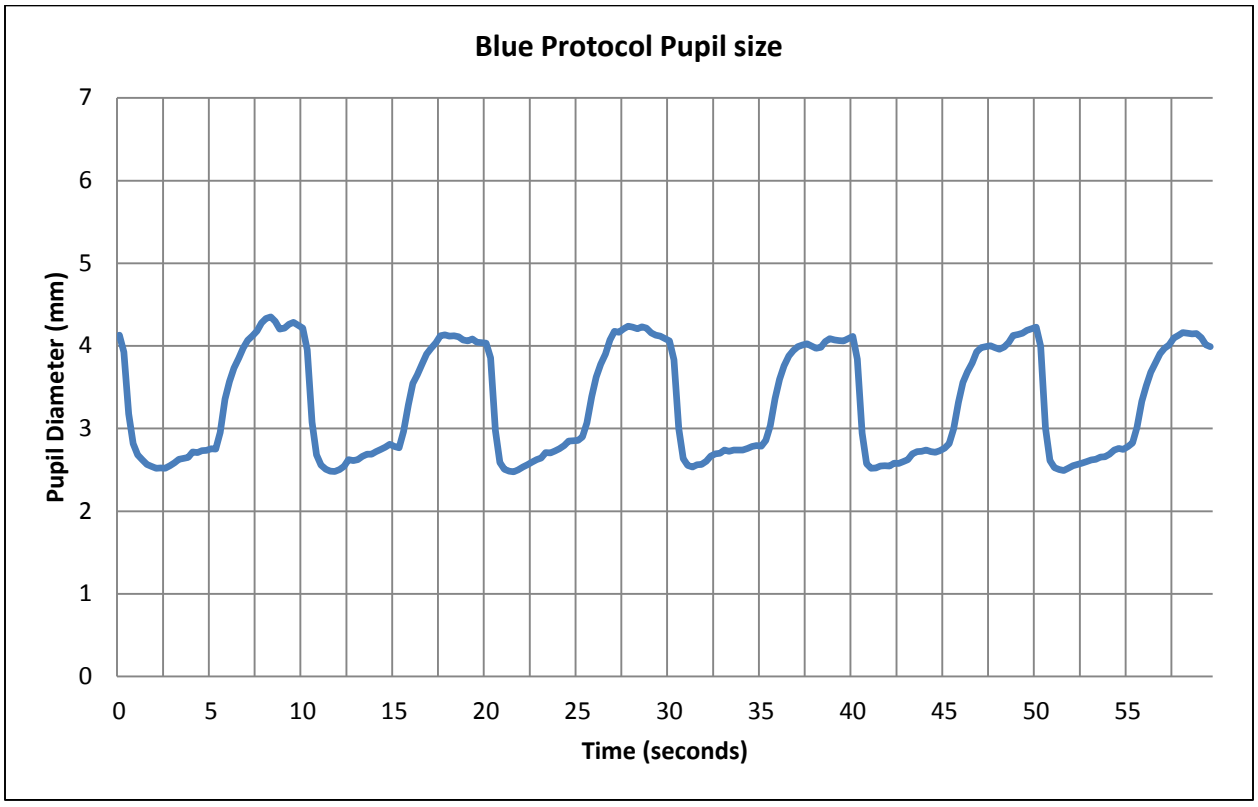

Figure 18. Average pupil diameter from all subjects during blue only protocol.

Figure 18 displays the raw data output from the RAPDx instrument for the single-color blue protocol for one minute. Pupil sizes were measured continuously as the oscillating lights were presented. The data showed an expected response of constriction when light was presented and dilation when light was turned off. The level of constriction and dilation remained relatively consistent, on average, throughout the test.

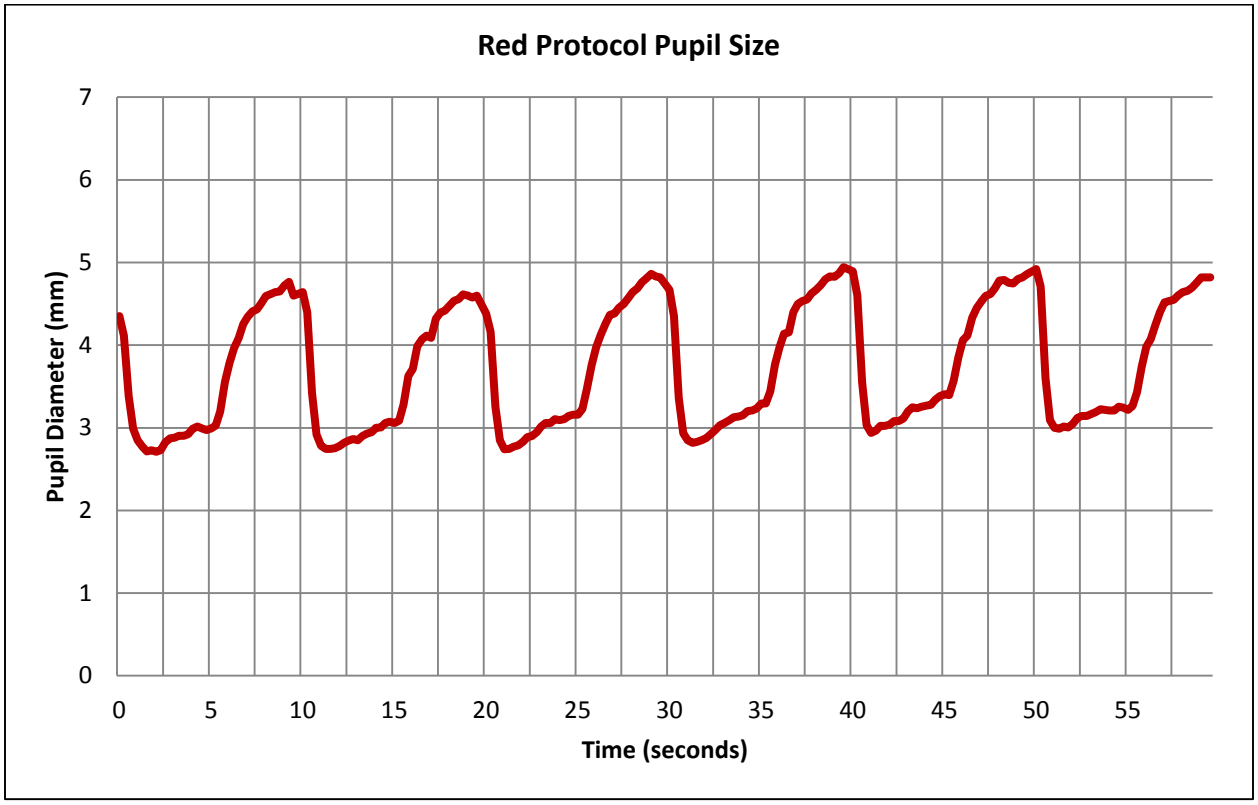

Figure 19. Average pupil diameter from all subjects during red only protocol.

Figure 19 displays the raw data output from the RAPDx instrument for the single-color red protocol for one minute. Pupil sizes were measured continuously as the oscillating lights were presented. The data showed an expected response of constriction

when light was presented and dilation when light was turned off. Again, the level of constriction and dilation remained relatively consistent, on average, throughout the test.

A variety of different measures were considered for the analysis of the data. Fourier analysis was considered which would have yielded an outcome measure of amplitude. A measure that captured the change in constrictions and dilations over time was desired. To achieve this measurement outcome, the data from each subject was normalized on a scale of 0% to 100% with 0% being the minimum amount of constriction (maximum dilation) of that subject's pupil over the course of all tests. 100% represented the maximum amount of constriction of that subject's pupil over the course of all tests. The difference between values was taken at each time point to show whether or not the values were becoming more similar over time. Then the average difference over the final 3 seconds of each pulse was calculated. Lastly the results for the first pulse were subtracted from the last pulse to give the change in time over the course of the test. An example of this analysis is given in Figure 21.

Pupil Data Analysis

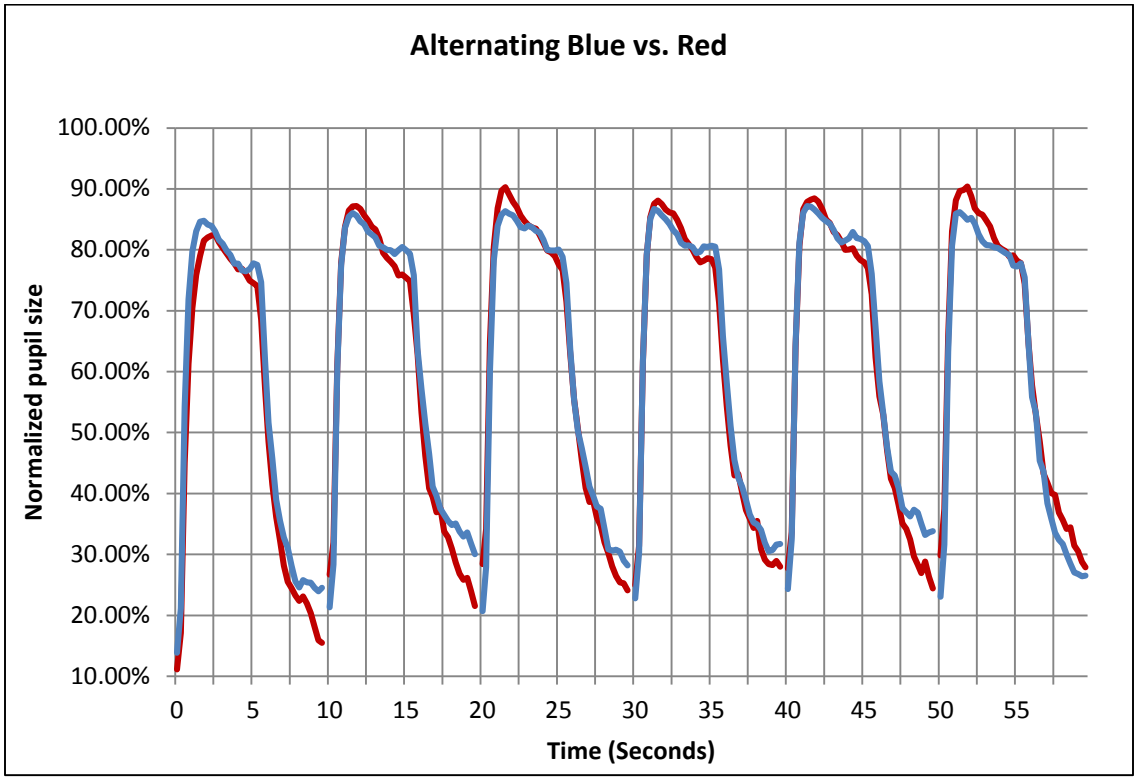

Figure 20. Normalized pupil size from all subjects during alternating protocol.

Figure 20 shows the change in normalized pupil size over the course of the alternating test. Over the course of the test there was a general upward shift in the normalized graph for both the peaks and troughs for red and an upward shift in the troughs only for blue, indicating a decrease in the amount of redilation following both red and blue pulses and an increase in constriction with each subsequent pulse of red light. Figure 21 and Figure 22 show two examples of computations of two of the outcome variables.

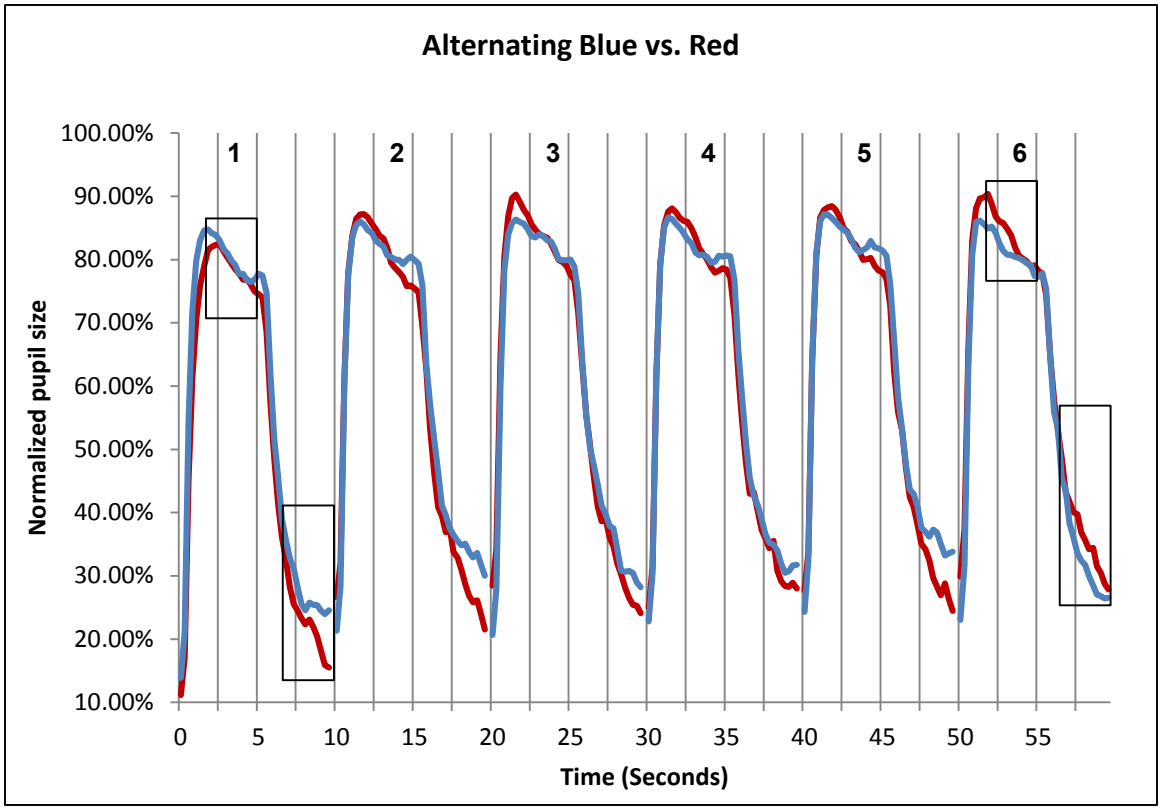

Figure 21. An example of the calculation to get the variable Alt Blue-Alt Red Pulse 6-Pulse 1. The red values in the last 3 seconds of both constrictions and dilations (rectangles) are subtracted from the blue values in the last 3 seconds of both constrictions and dilations for both pulse 1 and pulse 6. Then the average difference of the 6 seconds of interest in pulse 6 is subtracted from the average difference of the 6 seconds of interest in pulse 6. This yields the change in the difference between blue and red over the course of the test.

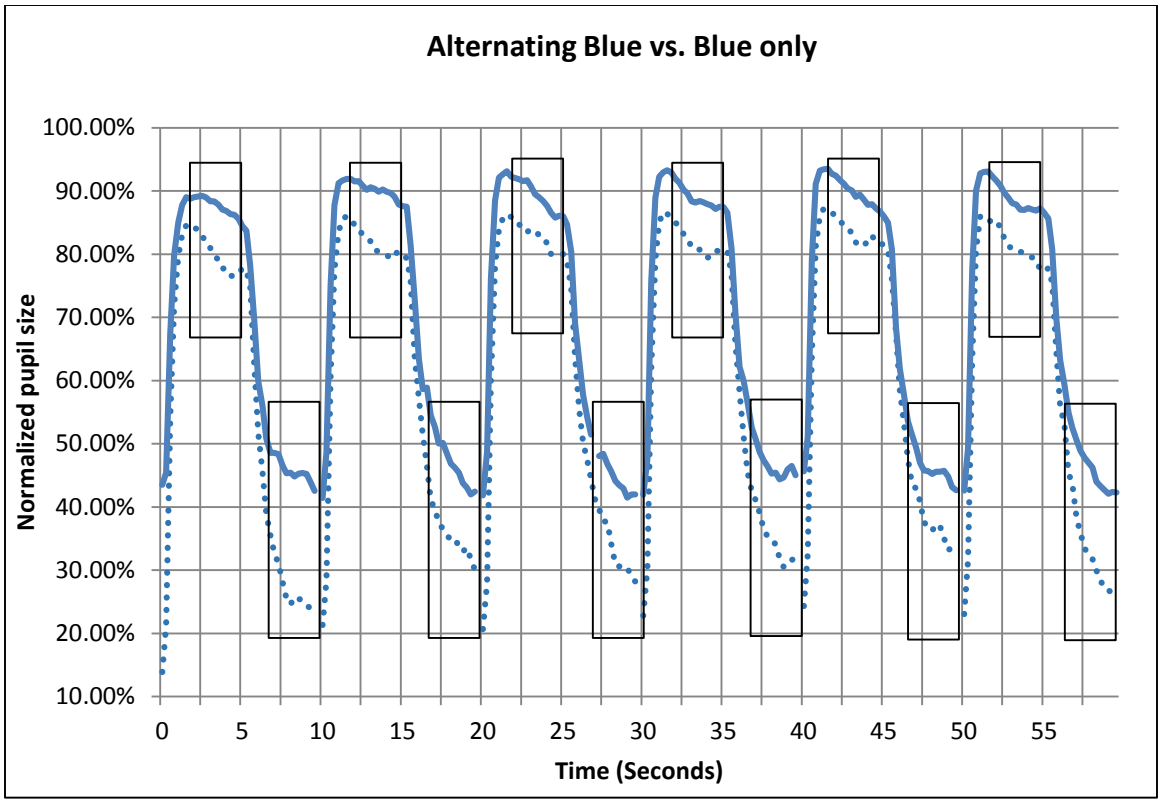

Figure 22. An example of the calculation to get the variable Mono Blue-Alt Blue All Pulses. The Alt values in the last 3 seconds of both constrictions and dilations is subtracted from the mono values in the last 3 seconds of both constrictions and dilations for all pulses. Then the average difference between mono and alt was determined for each subject.

Table 4 shows descriptive statistics of log total lux data over different intervals of time. Table 5 shows descriptive statistics of the potential outcome measures from the RAPDx.

	Mean	Standard deviation
60 minutes	4.50	0.872
180 minutes	5.06	0.612
720 minutes	5.37	0.570
1440 minutes	6.00	0.438
4230 minutes	6.45	0.463
7200 minutes	6.70	0.445

Table 4. Mean and standard deviation of Log Total Lux data over different intervals of time.

	Mean	Standard Deviation
Mono Blue – Mono Red Pulse 6 – Pulse 1	0.081	0.071
Alt Blue – Alt Red Pulse 6 – Pulse 1	-0.051	0.066
Mono Blue – Alt Blue Pulse 6 – Pulse 1	-0.011	0.062
Mono Red – Alt Red Pulse 6 – Pulse 1	-0.14	0.11
Blue – Red Alt Con Pulse 6 – Pulse 1	-0.015	0.069
Mono Blue – Alt Blue All pulses	0.091	0.075
Alt Red – Mono Red All pulses	0.015	0.079

Table 5. Mean and Standard deviation of potential outcome measure from RAPDx.

Table 6 shows possible outcome measures that were explored using the RAPDx pupil data and the lux data. Mono Blue – Mono Red Pulse 6 – Pulse 1 is the difference between pulse 1 and pulse 6 for the single color presentation of both blue and red. Alt Blue – Alt Red Pulse 6 – Pulse 1 is the difference between pulse 1 and pulse 6 for the alternating presentation of both blue and red. Mono Blue – Alt Blue Pulse 6 – Pulse 1 is the difference between pulse 1 and pulse 6 for the single color presentation and

alternating presentation of blue. Mono Red – Alt Red Pulse 6 – Pulse 1 is the difference between pulse 1 and pulse 6 for the single color presentation and alternating presentation of red. Blue – Red Alt Con Pulse 6 – Pulse 1 is the difference in the constrictions between pulse 1 and pulse 6 for blue and red during the alternating protocol.

	60 minutes	180 minutes	720 minutes	1440 minutes	4230 minutes	7200 minutes
Mono Blue – Mono Red Pulse 6 – Pulse 1	0.22 0.36	0.18 0.46	0.25 0.28	-0.13 0.60	0.11 0.65	0.05 0.85
Alt Blue – Alt Red Pulse 6 – Pulse 1	-0.046 0.85	0.11 0.64	-0.040 0.87	-0.074 0.76	-0.31 0.19	-0.31 0.18
Mono Blue – Alt Blue Pulse 6 – Pulse 1	-0.18 0.45	-0.22 0.36	-0.22 0.35	-0.050 0.83	-0.0050 0.98	-0.025 0.92
Mono Red – Alt Red Pulse 6 – Pulse 1	-0.27 0.25	-0.17 0.48	-0.32 0.17	0.0080 0.97	-0.25 0.28	-0.23 0.34
Blue – Red Alt Con Pulse 6 – Pulse 1	0.032 0.89	0.063 0.79	-0.084 0.72	-0.27 0.25	**-0.50** **0.025**	**-0.52** **0.019**
Mono Blue – Alt Blue All pulses	0.43 0.056	0.046 0.85	-0.072 0.76	0.011 0.96	-0.051 0.83	-0.19 0.43
Alt Red – Mono Red All pulses	-0.27 0.25	0.18 0.44	0.13 0.60	-0.056 0.81	0.017 0.94	0.096 0.69

Table 6. Correlation coefficients (and p-values) between potential outcome measures from RAPDx and Log Total Lux data over different intervals of time. The two significant correlations are marked with a larger font in **bold**.

Table 6 shows there was a statistically significant correlation between the difference and the Log total lux values for exposures of 4230 minutes (3 days) and 7200 minutes (5 days) prior to the test. The correlation builds over time and becomes significant in the two most long-term periods of time (-0.50, p = 0.025 and -0.52, p = 0.019). Mono Blue – Alt Blue Last 3 seconds of all pulses is the difference in normalized pupil size between the single color presentation of blue and the alternating presentation of blue. Alt Red – Mono Red Last 3 seconds of all pulses is the difference in normalized pupil size between the alternating presentation of red and the single color presentation of red.

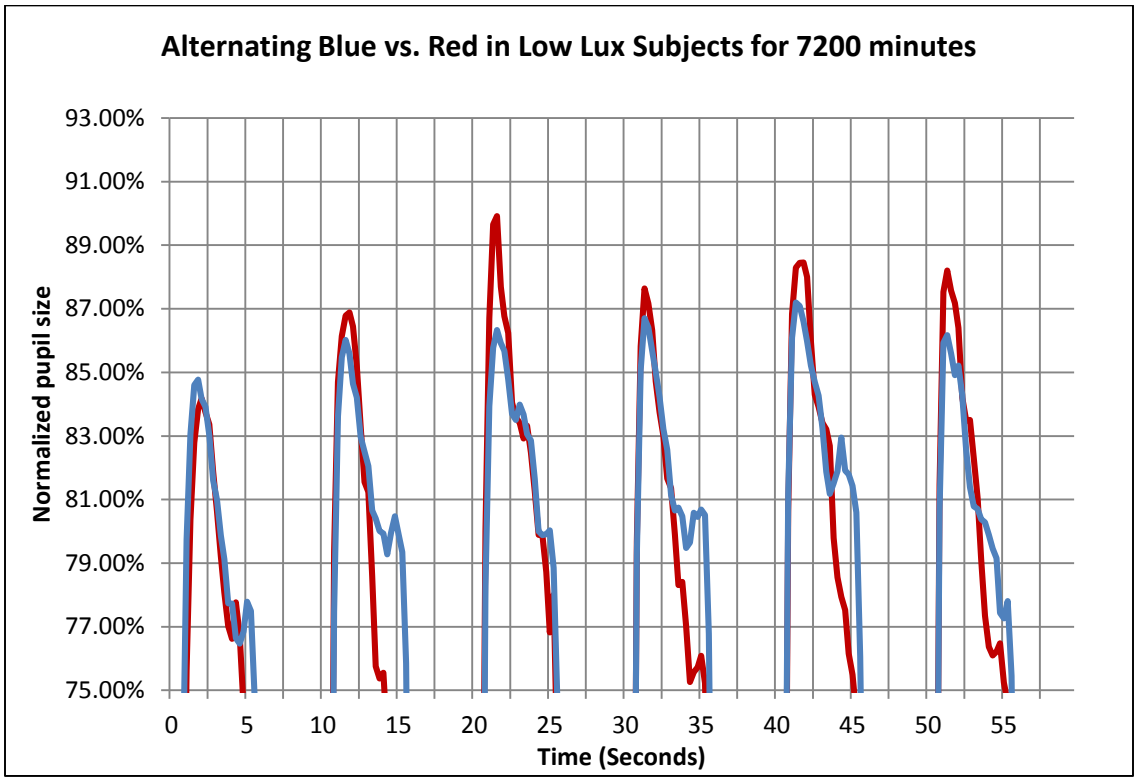

Figure 23. Normalized pupil size during alternating protocol from low lux case over 7200 minutes prior to test.

The relationship between light exposure history and constrictions to red and to blue during the alternating protocol was explored in more detail. Figure 23 shows the change in normalized pupil size over the course of the alternating test as a function of light exposure. The truncated y-axis is designed to highlight the constriction period for each of the six pulses of red and blue light, as suggested by the results in Table 6. In subjects with a low log lux value (defined as below a median split of 6.65 log lux-minutes) over the 7200 minutes (5 days) prior to the test, the increase in constriction with each subsequent pulse of red light was relatively low (from 84% to 88%). The constrictions to red closely match the constrictions with blue.

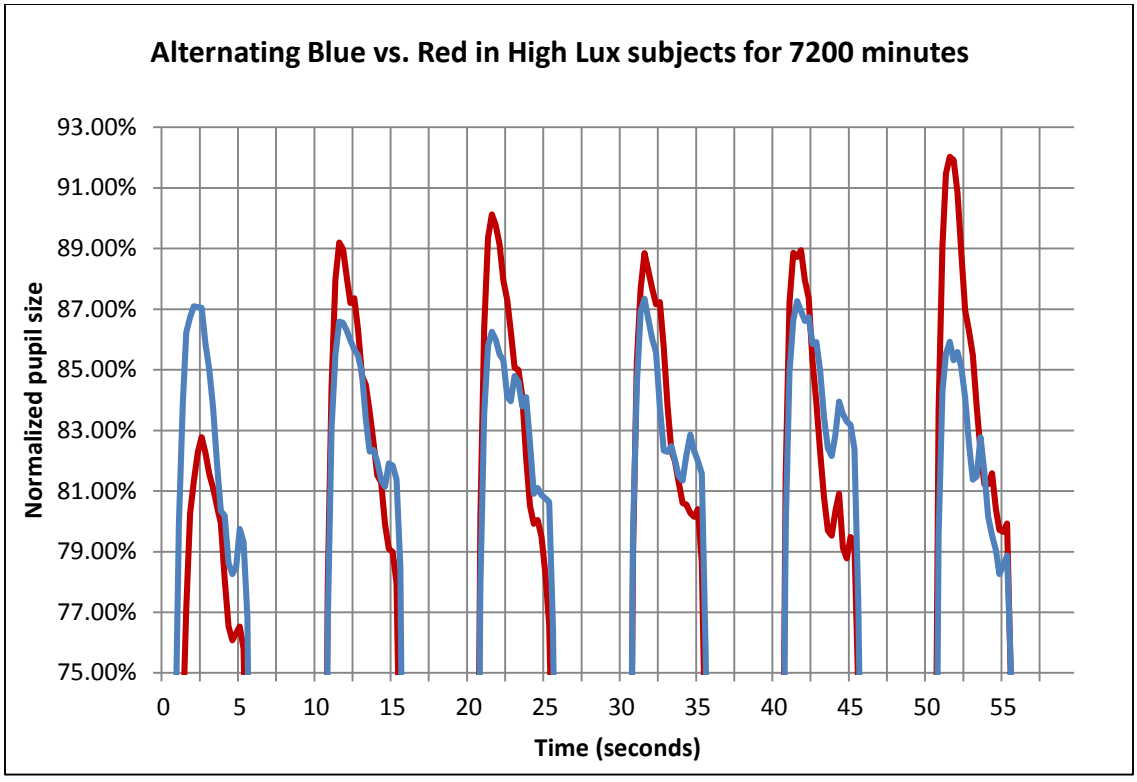

Figure 24. Normalized pupil size during alternating protocol from high lux case over 7200 minutes prior to test.

This pattern differed in subjects with a 5-day light exposure history greater than the median. Figure 24 shows the change in normalized pupil size over the course of the alternating test. The truncated y-axis is designed to highlight the constriction period for each of the six pulses of red and blue light. In subjects with a high lux value (defined as above a median split of 6.65 log lux-minutes) over the 7200 minutes (5 days) prior to the test, the increase in constriction with each subsequent pulse of red light was greater. The constrictions following blue pulses remain relatively constant throughout the test. The constrictions following red pulses increased from the first pulse to the last pulse (from 83% to 92%).

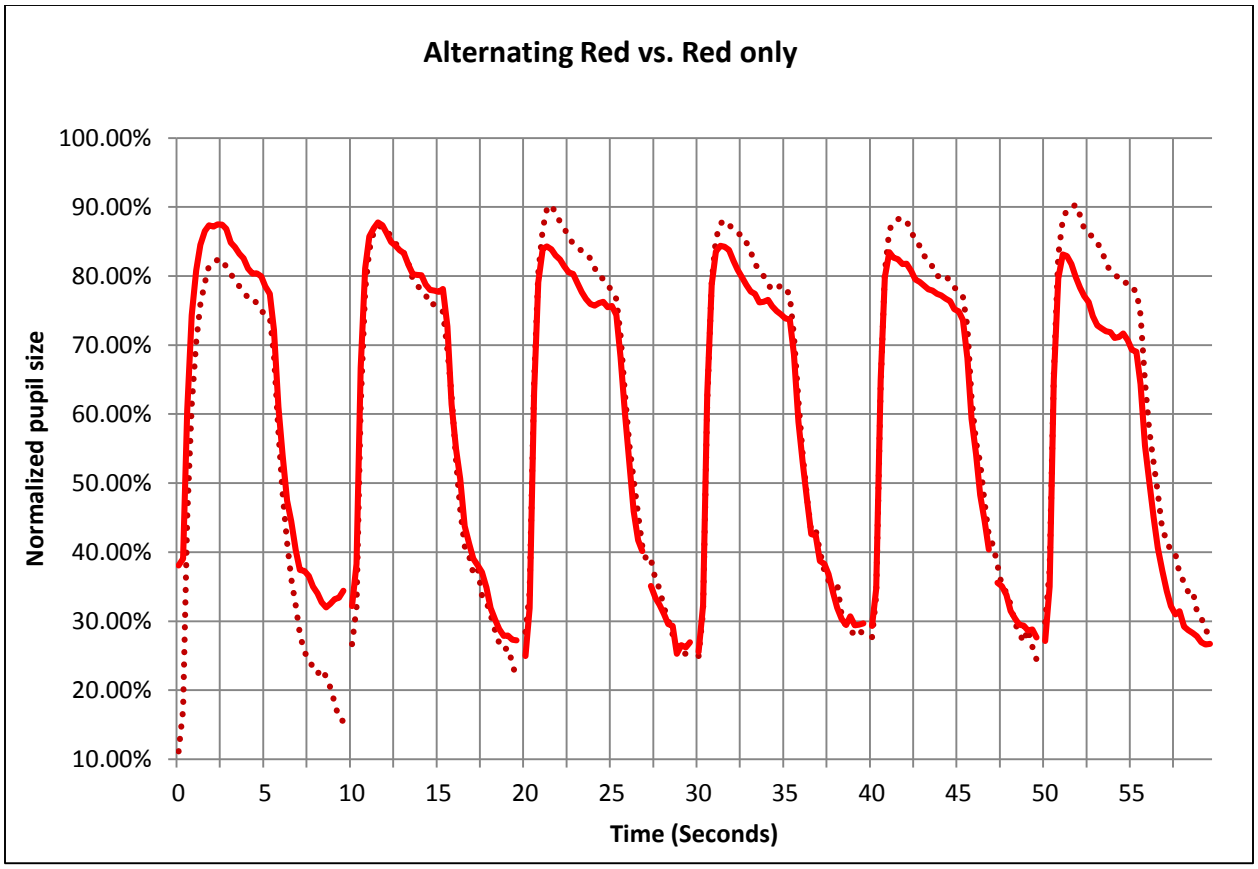

Figure 25. Normalized pupil size from all subjects for red pulses during alternating protocol vs. red only protocol. The dotted line indicates the responses during the alternating protocol.

Figure 25 shows the increase in constriction over time to red in the alternating protocol vs. the red only protocol. The red only responses showed a slight decrease in constriction throughout the course of the test while the constriction in response to alternating red showed a slight increase. Except for the first pulse, the dilations were consistent over the course of the test.

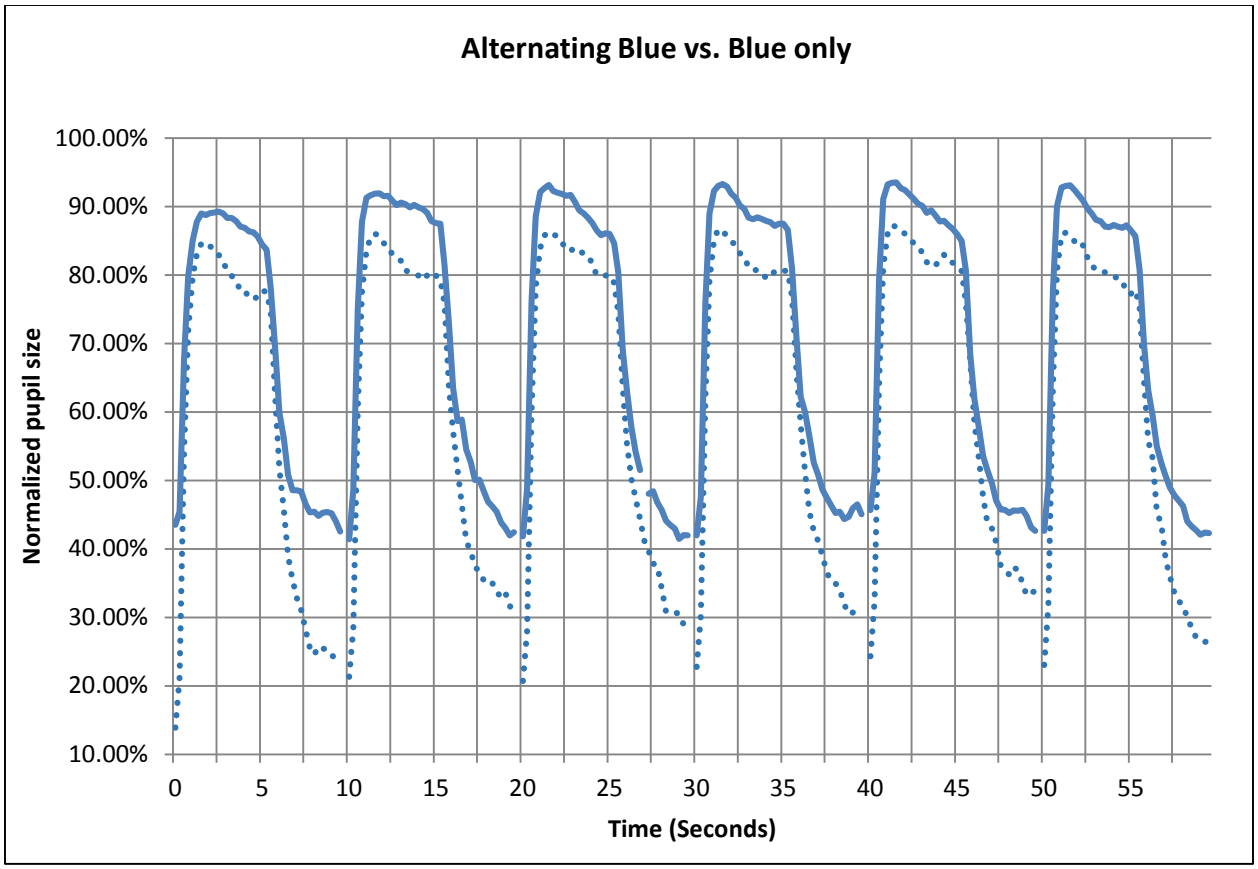

Figure 26. Normalized pupil size from all subjects for blue pulses during alternating protocol vs. blue only protocol. The dotted line indicates the responses during the alternating protocol.

Figure 26 shows a relatively consistent response to blue over the course of the test within each of the alternating and the single color protocols. Both constrictions and dilations remained relatively constant during the duration of each individual test. However, there was a marked separation between the two with the single color blue only presentation yielding more robust constriction and less redilation compared to the responses to blue when alternated with red stimuli.

	SEQ
Mono Blue – Mono Red Pulse 6 – Pulse 1	-0.14 0.54
Alt Blue – Alt Red Pulse 6 – Pulse 1	-0.25 0.29
Mono Blue – Alt Blue Pulse 6 – Pulse 1	-0.033 0.89
Mono Red – Alt Red Pulse 6 – Pulse 1	-0.075 0.76
Blue – Red Alt Con Pulse 6 – Pulse 1	-0.16 0.51
Mono Blue – Alt Blue Last 3 sec all pulses	**0.58** **0.0080**
Alt Red – Mono Red Last 3 sec all pulses	**-0.57** **0.0090**

Table 7. Correlation coefficients (and p-values) between potential outcome measures and to spherical equivalent refractive error values. The two significant correlations are marked with a larger font in **bold.**

Because of the interest in the effects of time outdoors on refractive error, all outcome measures were checked for any correlations with spherical equivalent. Table 7 depicts these correlations between previously defined outcome variables vs. the spherical equivalent refractive errors of the study subjects. A statistically significant positive correlation with refractive error was found for the variable that captures the last 3 seconds of all pulses, constrictions and dilations, in the normalized pupil size for single color

presentation and alternating presentation of blue. An equally strong statistically significant negative correlation with refractive error was found for the variable that captures the last 3 seconds of all pulses, constrictions and dilations, in the normalized pupil size for single color presentation and alternating presentation of red.

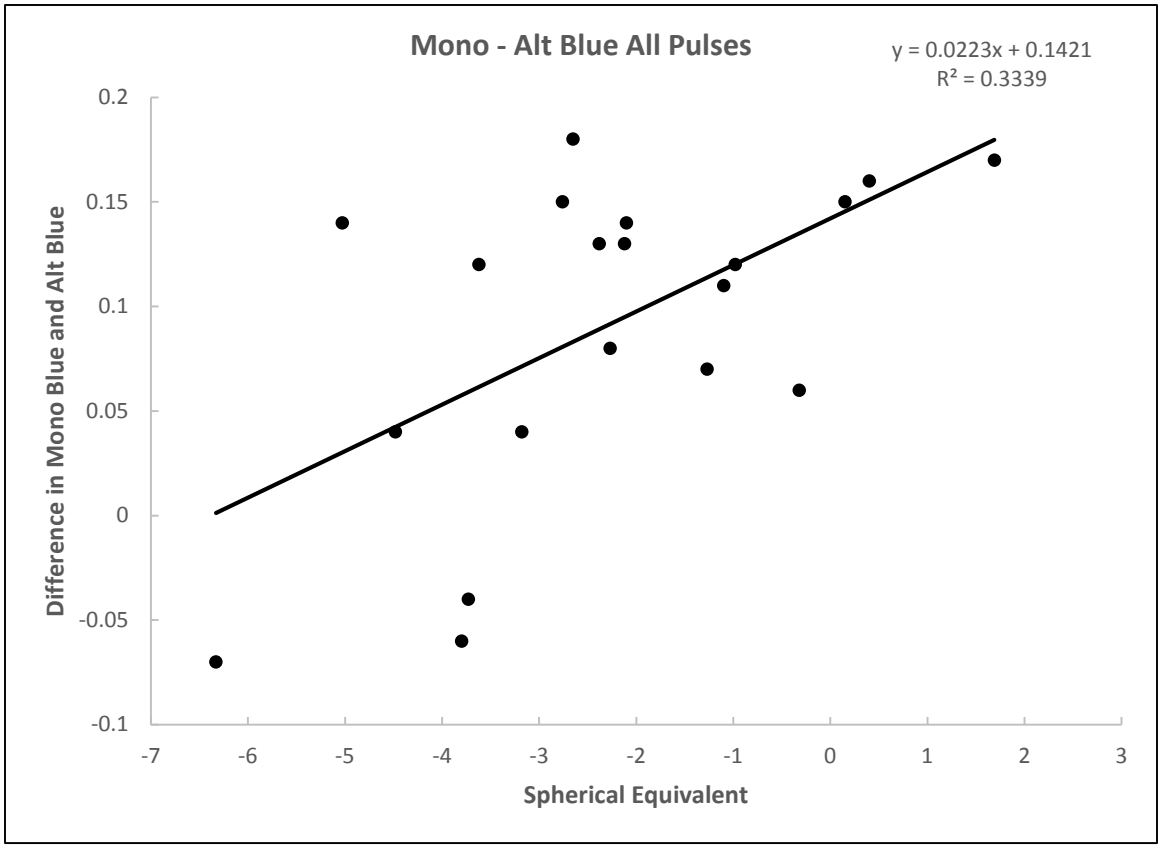

Figure 27. Difference in single color blue and alternating blue vs. spherical equivalent.

Figure 27 shows the positive correlation between subjects' average spherical equivalent and the difference between single color blue and alternating blue. This shows

that as the subject becomes less myopic and more hyperopic, the difference between single color blue and alternating blue is greater.

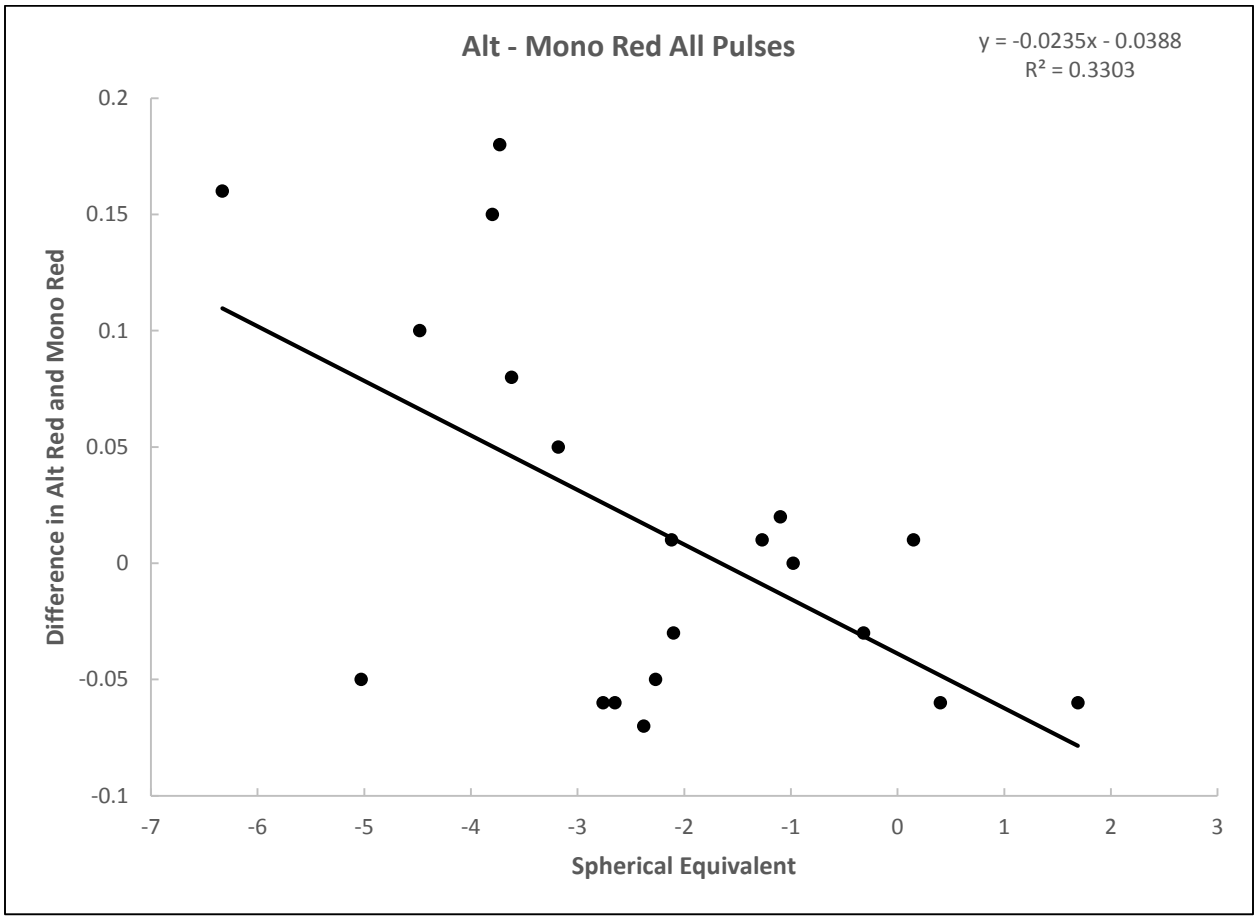

Figure 28. Difference in alternating red and single color red vs. spherical equivalent.

Figure 28 shows a negative correlation between subjects' average spherical equivalent and the difference between alternating red and single color red. For less myopic and more hyperopic subjects, the difference between alternating red and single color red was near zero or slightly negative.

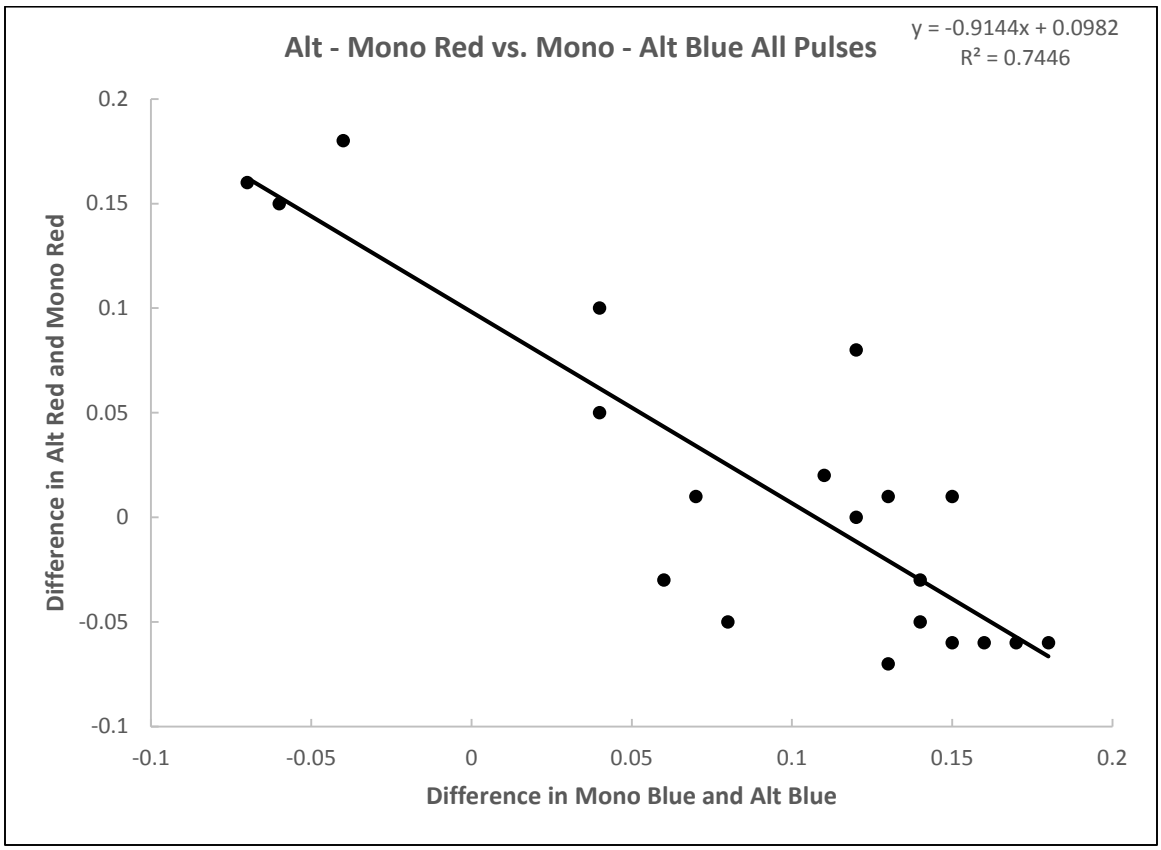

Figure 29. Difference in alternating red and single color red vs. difference in mono blue and alt blue.

Figure 29 shows a negative correlation for the difference between single color blue and alternating blue and the difference between alternating red and mono red. For subjects where the blue response was most different, the difference in red responses was nearly zero to slightly negative. Conversely, in subjects where the difference in red was high, the difference in blue responses was near zero or slightly negative. Interestingly, neither of these two variables (single color blue minus alternating blue or alternating red minus single color red) showed any association with any of the periods of light exposure

history nor were there any interactions between the periods of light exposure history and spherical equivalent refractive error with respect to these two pupil outcomes.

Chapter 4. Discussion

Measurement of time outdoors

The results of this study showed that the Daysimeter badges were the more valid way to measure time outdoors. Both the Daysimeter and the NIWA ultraviolet badges had a positive correlation with the self-reported time spent outdoors from the activity surveys. However, the Daysimeter badge appears to be a superior method of measurement because it correlated more closely with the survey results with a slope of 0.68. This is much closer to 1 than the slopes of UVB vs. Daysimeter (0.40) and UBV vs. survey (0.30). The survey that was used for this study is not well established, but is based upon an activity frequency methodology that has been used previously and successfully studies such as the Framingham Heart and EPIC (European Prospective Investigation into Cancer) studies for several disease outcomes such as cardiovascular disease and stroke research (Kiely, Wolf, Cupples, Belser and Kannel, 1994; Sherman, D'Agostino, Silbershatz and Kannel, 1999). For the purpose of the initial analysis, the activity survey was considered the gold standard of comparison. The Daysimeter became the preferred method of determining time outdoors as supported by the preliminary study in which one subject kept a detailed journal with amount of time spent outdoors and the greater number of hours recorded compared to the survey. Under typical indoor lighting conditions, recordings greater than 1000 lux will be infrequent but not impossible. There are special circumstances such as sitting near a sunny window and driving in a car where, although

the subject is not considered outdoors, the Daysimeter might record greater than 1000 lux. This may be an additional reason why the Daysimeter recorded the largest amount of time outdoors versus the UV badge or the survey.

The variability in the data for measuring time outdoors may be explained by a few simple considerations. In most situations, the ultra-violet badge yielded the lowest amount of minutes spent outdoors. One potential explanation is the amount of ultra-violet light available throughout the day is variable. As Figure 3 shows, the changing cloud cover can greatly affect the amount of available ultra-violet light. This reduction in available UV from cloud cover can cause zero readings while a subject is outdoors. This is demonstrated in Figure 13 when the UV badge counts drop to zero during periods of outdoor exposure. The UV badge appears to need direct sunlight to be most effective. Shaded areas and body positioning may have similar effects to cloud cover and cause further variability in the data.

Although surveys are a well-established as a means to measure time outdoors, they may still be inaccurate. These data are subject to a recall bias. Only activities that subjects recall and self-report will be counted. Subjects in some instances may just not be very good at recalling activities. The survey is also limited by the completeness of the list of activities. There was an opportunity for the subject to list “Other” indoor or outdoor activities, and some would, but it is impossible to list all possible ways to spend time outdoors in one survey.

In future studies the preferred method for measuring time spent outdoors is with the visible light sensitive Daysimeter. In preliminary studies, this was shown to most

closely match the detailed journal, and in the subject population, the Daysimeter showed the best agreement with the survey results. The Daysimeter is safe, reliable, and not subject to any recall bias. This method of personal dosimetry is fast becoming the norm in studies of time outdoors related to refractive error (Dharani et al., 2012; Schmid, Leyden, Chiu, Lind, Vos, Kimlin and Wood, 2013).

Light exposure and pupil response

Not only did the study results suggest a valid method of measuring time outdoors in the Daysimeter, but the results also showed a correlation between light exposure history and pupil response. Due to the emphasis on ipRGC activity in the second part of the study, the primary choice of variable shifted from time spent outdoors to log total lux. The log total lux values correlate highly with time spent outdoors as seen in Figure 30.

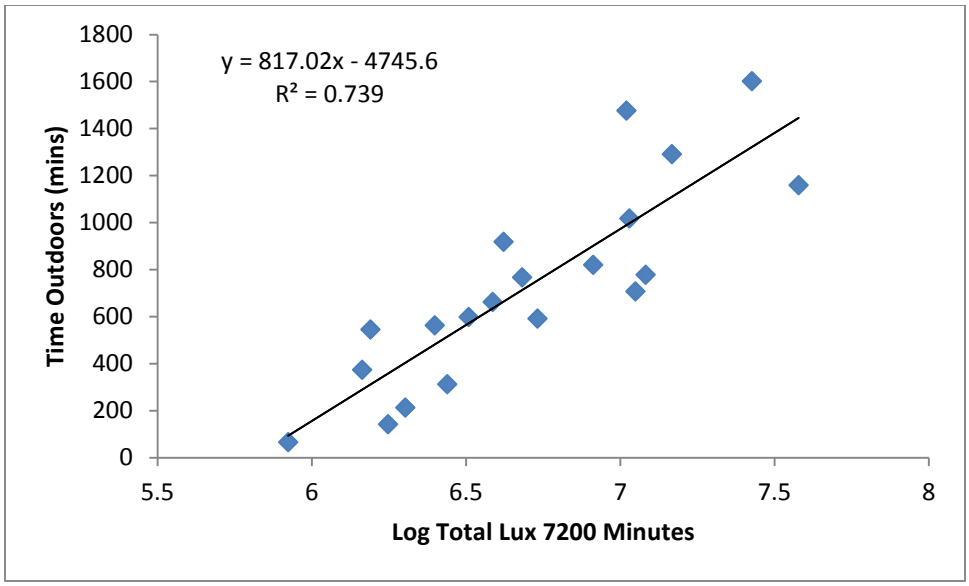

Figure 30. This displays the comparison of log total lux values over 7200 minutes versus the calculation of minutes spent outdoors using the criterion of >1000 lux.

	Time Outdoors Survey	Time Outdoors Lux	Time Outdoors UV
Log Total Lux 7200 Minutes	0.63 0.0030	0.86 <0.001	0.76 <0.001

Table 8. Correlation coefficients (and p-values) between log total lux values over 7200 minutes and three methods of calculating time outdoors.

Lux was chosen because it is widely used and it is an easily understandable measurement. Although a measurement of lux is weighted according to peak cone spectral sensitivity (555 nm) and not peak ipRGC sensitivity (480 nm), the two are closely correlated. The Daysimeter measured values of intensity of a circadian weighted (melanopsin cell stimulating, proprietary Lighting Research Center algorithm) wavelength simultaneously to lux. Example data from the Daysimeter from one subject is seen in Figure 31 and shows that the two variables are nearly identical.

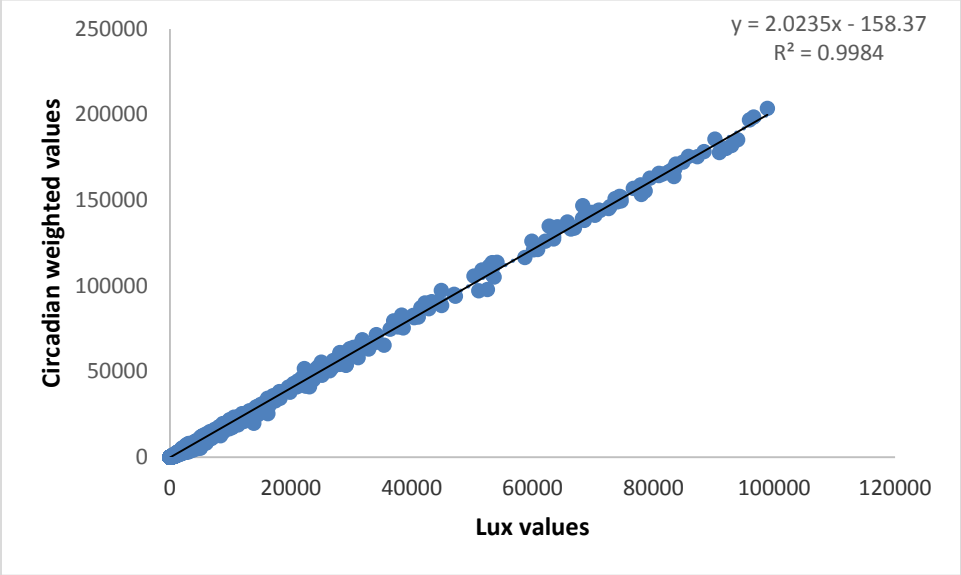

Figure 31. The comparison of lux values to circadian weighted values as measured by the Daysimeter in a study subject over the course of 1 week.

The results in Table 6 showed that there was a statistically significant correlation between the difference in red and blue constrictions over the course of the alternating protocol and increased exposure to light over a longer period of time prior to the test (3 and 5 days). The correlation builds with increased light exposure over time from $p = 0.893$ over the most recent 60 minutes, $p = 0.790$ over the most recent 180 minutes, $p = 0.724$ over the previous 720 minutes, $p = 0.252$ over the previous 1440 minutes until the correlation becomes significant with $p = 0.025$ over the previous 4230 minutes and $p = 0.019$ over the previous 7200 minutes. The increase in difference between these two values is due to the rise in constriction following red pulses, seen in Figures 23 and 24. The constriction following blue pulses remained constant, while the constriction

following red pulses increases to a larger degree in patients with high log total lux values during the 7200 minutes (5 days) prior to the testing.

When thinking of an ipRGC-based response, one does not often think of response to red light because the peak spectral sensitivity for ipRGCs is at about 480 nm (Do et al., 2010). Response to red light seems more consistent with a predominantly long wavelength sensitive cone photoreceptor driven response. If there were little ipRGC input into the red response system, then the response should remain relatively constant across all the tests or fall perhaps due to light adaptation as seen in Figure 25 during the single color red presentation. The response is not therefore strictly a response to exposure to red light. What was found instead is that the intervening blue pulses during the alternating color protocol caused an increase in the red constriction response (Figures 20 and 24). The presence of the blue light, which is associated with greater ipRGC function, perhaps feeds into the photoreceptor-based response in some way so that the ipRGC input to the pupil response amplifies responses during exposure to red. In the average data for the alternating presentation, the pupil response to red tend to track the pupil response for blue; both move upward, particularly the dilations for both colors and the constriction to red (Figure 20). The increase in constrictions to red light over the course of the test was greatest, however, in subjects with the highest amounts of visible light exposure (Figure 24). This is potential evidence of an interaction between ipRGCs and photoreceptors. This increase in constriction based on light exposure history appears to be a form of photopotentiation.

The photopotentiation response seen in the rise in red constrictions was not found to be statistically significant with light exposure with shorter intervals of time preceding the test. This is unexpected because changes in response over the short course of the testing protocol are seen even though those changes do not correlate with the most immediate light exposure history. This may be due to the similarity in the light exposure of the subjects over the shorter periods of time, especially the 60 minute interval prior to testing. All subjects entered the building, walked down the hall to the testing room, were interviewed briefly by the investigator, and then had 5 minutes of dark adaptation before pupillometry. This may also be because the Daysimeter may not have had the ability to track subtle or more rapid changes in lux, but that feature of the Daysimeter was not assessed. The fact that longer-term light exposure was correlated with the response indicates that there may be some sort of priming of the system that happens slowly and makes a subject more likely to respond strongly. This could be a potentially useful feature. A person who spends more time outdoors may benefit from a small pupil size. If a person spends more time outside over a long period of time, the pupillary response reacts with a more robust constriction response in the short term when presented with a longer wavelength red light.

In addition to correlations between pupil response and light exposure history, the results also show that there were correlations between the pupil response and refractive error. Table 7 shows that there was not a significant correlation between refractive error and any of the five outcomes based on differences from first to last pulse. However, there was a significant correlation between refractive error and the two outcomes based on the

differences between single color and alternating color presentations over the last 3 seconds of constrictions and dilations over all pulses. The last 3 seconds of each pulse best encapsulated what the pupil size was following its initial movement to either constrict or dilate. The separation in pupil size can be seen in Figures 25 and 26 for both blue and for red.

The positive correlation between spherical equivalent and the pupil response difference evoked by single color and alternating blue light stimuli is shown in Figure 27. To summarize, subjects with larger differences between their pupil responses to the two blue light presentations (alternating versus single color) tended to have less myopic refractive error. The intervening presentation of single color flashing red stimuli prior to the final trial involving single color flashing blue stimuli appeared to potentiate the blue light-evoked pupil responses, as there was increased pupil constriction in this single color trial as compared to the first trial with alternating stimuli. One might suggest that the difference is simply the result more exposure to blue, but this explanation seems unlikely. The response after the first pulse of single color blue is larger than the last pulse in response to alternating blue and red (Figure 26). The intervening responses to single color red were either stable or declining (Figure 25). The immediate increase in response to the single color blue seems most likely attributable to the effect of the intervening exposure to the single color red presentation.

Figure 28 shows a negative correlation between the spherical equivalent and the difference between response for the alternating color and the single color red pulses. Thus, in the more myopic subjects, there was a greater difference in their pupil responses

to the two types of red light stimuli (monochrome versus alternating). The gradual decrease in the pupil responses to the red single color stimuli may reflect a form of light adaptation that occurs in the absence of significant ipRGC stimulation. Figure 29 shows that if a subject shows robust photopotentiation in their pupil responses to blue light (more constriction and less dilation in the third single color trial versus the first alternating trial), their response to the single color red stimuli holds steady. In other words, high positive values for the differences in the blue light responses are associated with low values for the differences in the red light responses. However, if there is little photopotentiation in the blue light responses, then the response to the single color red dissipates, that is low positive values of the differences for blue are associated with high values for the differences for red. All of these data point to a more robust photopotentiation response in subjects with a less myopic refractive error. This effect can be seen in a short amount of time (throughout the timing of the testing) and is not related to light exposure history.

These results are consistent with current theories regarding dopamine as an inhibitor of growth of the eye. Someone with a robust photopotentiation response is assumed to have active ipRGC input. The ipRGCs have been previously documented to synapse with the dopaminergic amacarine cells (Munch et al., 2013). Therefore, someone who exhibits greater photopotentiation is hypothesized to have more dopamine released locally within the retina upon light stimulation. This dopamine, which has a role in light adaptation mechanisms within the retina, may serve the dual purpose of inhibiting the axial elongation of the eye. This growth inhibition may be the mechanism by which

myopia risk is reduced. Therefore, the data support the idea that the benefit of being outdoors in myopia risk reduction is the presence of visible light driving the production of retinal dopamine, mediated by ipRGCs, to slow eye growth.

Studies have shown that time outdoors reduces the onset of myopia but does not alter the rate of progression (Jones-Jordan et al., 2012). This result raises the question of why effects were seen in this primarily myopic sample. One potential explanation is that although myopes may benefit from time outdoors they do not spend much time outdoors and therefore do not reach a threshold of benefit from time outdoors. Another explanation may be that myopes who have good photopotentation might have delayed the onset of their myopia and are therefore less myopic than they might have been otherwise. Therefore ipRGC activity might still be related to the amount of myopia even though time outdoors is independent of the rate of progression.

While the data are exciting, there may be some study limitations present. Most of the subjects chosen were myopes and were measured with non-cycloplegic autorefraction. Although measurements were non-cycloplegic, a Badal track was used to relax accommodation during testing. Non-cycloplegic measurement compares very well to cycloplegic autorefraction in samples of myopes (Gwiazda, Marsh-Tootle, Hyman, Hussein and Norton, 2002). The correlation is present when myopia is treated as a continuous variable but more non-myope information would be useful to compare responses. This study was done in adults who had already become myopic or remained non-myopic. Pre-adolescence is a common age for the development of myopia so future

studies should be done near the age of onset to determine if the ipRGC response can predict who will become myopic and who will not.

There were some effects of order of testing that could not be avoided. Although some of the order effects mediated the desired responses, a longer washout or dark adaptation period, or randomization of the order of testing may be more desirable to avoid specific order effects. A previous protocol used a 30 second to 2 minute inter-stimulus interval for recovery to baseline (Park et al., 2011). These may be too short as order effects were present in the current study with 5 minutes of dark adaptation between presentations. Future studies may include determining how deliberate short-term exposure to known lighting changes the pupil response of subjects.

There was also a great deal of variability in the data. Figures 27-29 show significant associations but there are a wide range of responses. Though the trend is for myopes to have a weak response, due to the spread of the data, it is still possible for an individual myope to have a robust ipRGC response. There are also non-myopes with a very weak response. This indicates that there are other factors involved in the producing an individual's level of refractive error beyond one's ipRGC response.

Some study strengths include good sampling rate for good temporal resolution of both the RAPDx and the Daysimeter. This provided the most possible data for analysis and gave an accurate picture of responses in real time. This study is also novel because no other research has looked at time outdoors vs. pupil response before.

In summary, the study shows an association between pupil response to red and blue flickering light and refractive error that is consistent with the hypothesis that visible

light through the action of ipRGCs may be the source of the protective effects of time outdoors in myopia. This relationship is promising for future research into the mechanisms of myopia development as well as the potential for the development of future screening mechanisms for children who are at risk for myopia development.

References

Ainsworth, B., Haskell, W., Whitt, M., Irwin, M., Swartz, A., Strath, S., O'Brien, W., Bassett Jr, D., Schmitz, K., Emplaincourt, P., Jacobs Jr, D. & Leon, A. (2000). Compendium of Physical Activities: an update of activity codes and MET intensities. *Medicine & Science in Sports & Exercise,* 498-516.

Anstice, N.S. & Phillips, J.R. (2011). Effect of dual-focus soft contact lens wear on axial myopia progression in children. *Ophthalmology, 118*, 1152-61.

Ashby, R., Ohlendorf, A. & Schaeffel, F. (2009). The effect of ambient illuminance on the development of deprivation myopia in chicks. *Invest Ophthalmol Vis Sci, 50*, 5348-54.

Atchison, D.A., Jones, C.E., Schmid, K.L., Pritchard, N., Pope, J.M., Strugnell, W.E. & Riley, R.A. (2004). Eye shape in emmetropia and myopia. *Invest Ophthalmol Vis Sci, 45*, 3380-6.

Bourne, R.R.A., Stevens, G.A., White, R.A., Smith, J.L., Flaxman, S.R., Price, H., Jonas, J.B., Keeffe, J., Leasher, J., Naidoo, K., Pesudovs, K., Resnikoff, S. & Taylor, H.R. (2013). Causes of vision loss worldwide, 1990–2010: a systematic analysis. *The Lancet Global Health, 1*, e339-e349.

Choi, J.A., Han, K., Park, Y.-M. & La, T.Y. (2014). Low Serum 25-Hydroxyvitamin D Is Associated With Myopia in Korean Adolescents. *Investigative Opthalmology & Visual Science, 55*, 2041.

Dharani, R., Lee, C.F., Theng, Z.X., Drury, V.B., Ngo, C., Sandar, M., Wong, T.Y., Finkelstein, E.A. & Saw, S.M. (2012). Comparison of measurements of time outdoors and light levels as risk factors for myopia in young Singapore children. *Eye (Lond), 26*, 911-8.

Do, M.T. & Yau, K.W. (2010). Intrinsically photosensitive retinal ganglion cells. *Physiol Rev, 90*, 1547-81.

Fulk, G., Cyert, L. & Parker, D. (2002). Seasonal Variation in Myopia Progression and Ocular Elongation. *Optometry and Vision Science, 79*, 46-52.

Gamlin, P.D., McDougal, D.H., Pokorny, J., Smith, V.C., Yau, K.W. & Dacey, D.M. (2007). Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res, 47*, 946-54.

Guggenheim, J.A., Williams, C., Northstone, K., Howe, L.D., Tilling, K., St Pourcain, B., McMahon, G. & Lawlor, D.A. (2014). Does vitamin D mediate the protective effects of time outdoors on myopia? Findings from a prospective birth cohort. *Invest Ophthalmol Vis Sci, 55*, 8550-8.

Guler, A.D., Ecker, J.L., Lall, G.S., Haq, S., Altimus, C.M., Liao, H.W., Barnard, A.R., Cahill, H., Badea, T.C., Zhao, H., Hankins, M.W., Berson, D.M., Lucas, R.J., Yau, K.W. & Hattar, S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature, 453*, 102-5.

Gwiazda, J., Hyman, L., Hussein, M., Everett, D., Norton, T.T., Kurtz, D., Leske, M.C., Manny, R., Marsh-Tootle, W. & Scheiman, M. (2003). A Randomized Clinical Trial of Progressive Addition Lenses versus Single Vision Lenses on the Progression of Myopia in Children. *Investigative Opthalmology & Visual Science, 44*, 1492.

Gwiazda, J., Marsh-Tootle, W., Hyman, L., Hussein, M. & Norton, T.T. (2002). Baseline Refractive and Ocular Component Measures of Children Enrolled in the Correction of Myopia Evaluation Trial (COMET). *Invest Ophthalmol Vis Sci, 43*, 314-321.

Gwiazda, J., Thorn, F., Bauer, J. & Held, R. (1993). Myopic Children Show Insufficient Accommodative Response to Blur. *Invest Ophthalmol Vis Sci, 34*, 690-694.

He, M., Xiang, F., Zeng, Y., Mai, J., Chen, Q., Zhang, J., Smith, W., Rose, K. & Morgan, I.G. (2015). Effect of Time Spent Outdoors at School on the Development of Myopia Among Children in China: A Randomized Clinical Trial. *JAMA, 314*, 1142-8.

Huang, H.M., Chang, D.S. & Wu, P.C. (2015). The Association between Near Work Activities and Myopia in Children-A Systematic Review and Meta-Analysis. *PLoS One, 10*, e0140419.

Jones, L.A., Sinnott, L.T., Mutti, D.O., Mitchell, G.L., Moeschberger, M.L. & Zadnik, K. (2007). Parental history of myopia, sports and outdoor activities, and future myopia. *Invest Ophthalmol Vis Sci, 48*, 3524-32.

Jones-Jordan, L.A., Sinnott, L.T., Cotter, S.A., Kleinstein, R.N., Manny, R.E., Mutti, D.O., Twelker, J.D. & Zadnik, K. (2012). Time Outdoors, Visual Activity, and Myopia Progression in Juvenile-Onset Myopes. *Invest Ophthalmol Vis Sci, 53*, 7169-7175.

Kiefer, A.K., Tung, J.Y., Do, C.B., Hinds, D.A., Mountain, J.L., Francke, U. & Eriksson, N. (2013). Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia. *PLoS Genet, 9*, e1003299.

Kiely, D., Wolf, P., Cupples, L.A., Belser, A. & Kannel, W.B. (1994). Physical Activity and Stroke Risk: The Framingham Study. *American Journal of Epidemiology, 140*, 608-620.

Munch, M. & Kawasaki, A. (2013). Intrinsically photosensitive retinal ganglion cells: classification, function and clinical implications. *Curr Opin Neurol, 26*, 45-51.

Mutti, D.O., Jones, L.A., Moeschberger, M.L. & Zadnik, K. (2000). AC/A Ratio, Age, and Refractive Error in Children. *Invest Ophthalmol Vis Sci, 41*, 2469-2478.

Mutti, D.O., Mitchell, G.L., Hayes, J.R., Jones, L.A., Moeschberger, M.L., Cotter, S.A., Kleinstein, R.N., Manny, R.E., Twelker, J.D., Zadnik, K. & Group, C.S. (2006). Accommodative lag before and after the onset of myopia. *Invest Ophthalmol Vis Sci, 47*, 837-46.

Mutti, D.O., Mitchell, G.L., Moeschberger, M.L., Jones, L.A. & Zadnik, K. (2002). Parental Myopia, Near Work, School Achievement, and Children's Refractive Error. *Invest Ophthalmol Vis Sci, 43*, 3633-3640.

Mutti, D.O., Mitchell, G.L., Sinnott, L.T., Jones-Jordan, L.A., Moeschberger, M.L., Cotter, S.A., Kleinstein, R.N., Manny, R.E., Twelker, J.D. & Zadnik, K. (2012). Corneal and Crystalline Lens Dimensions Before and After Myopia Onset. *Optometry and Vision Science, 89*, 251-262.

Park, J.C., Moura, A.L., Raza, A.S., Rhee, D.W., Kardon, R.H. & Hood, D.C. (2011). Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci, 52*, 6624-35.

Schaeffel, F., Glasser, A. & Howland, H. (1988). Accommodation, refractive error and eye growth in chickens *Vision Res, 28*, 639-657.

Schmid, K.L., Leyden, K., Chiu, Y., Lind, S., Vos, D., Kimlin, M. & Wood, J. (2013). Assessment of Daily Light and Ultraviolet Exposure in Young Adults. *Optometry and Vision Science, 90*, 148-155.

Schroder, A., Colli, E., Maggi, M. & Andersson, K.-E. (2006). Effects of a vitamin D3 analogue in a rat model of bladder outlet obstruction. *BJU International, 98*, 637-642.

Sherman, S.E., D'Agostino, R.B., Silbershatz, H. & Kannel, W.B. (1999). Comparison of past versus recent physical activity in the prevention of premature death and coronary artery disease. *American Heart Journal, 138*, 900-907.

Smith, E.L., 3rd & Hung, L.F. (1999). The role of optical defocus in regulating refractive development in infant monkeys. *Vision Res, 39*, 1415-1435.

Smith, E.L., 3rd, Hung, L.F., Arumugam, B. & Huang, J. (2013). Negative lens-induced myopia in infant monkeys: effects of high ambient lighting. *Invest Ophthalmol Vis Sci, 54*, 2959-69.

Smith, E.L., 3rd, Hung, L.F. & Huang, J. (2009). Relative peripheral hyperopic defocus alters central refractive development in infant monkeys. *Vision Res, 49*, 2386-92.

Swift, N., Hamlin, J.D., Nield, K.M. & McKenzie, R. (2010). Characterisation of AlGaN detectors for UV measurements *Proceedings of the NIWA UV Workshop,*

Verhoeven, V.J., Hysi, P.G., Wojciechowski, R., Fan, Q., Guggenheim, J.A., Hohn, R., MacGregor, S., Hewitt, A.W., Nag, A., Cheng, C.Y., Yonova-Doing, E., Zhou, X., Ikram, M.K., Buitendijk, G.H., McMahon, G., Kemp, J.P., Pourcain, B.S., Simpson, C.L., Makela, K.M., Lehtimaki, T., Kahonen, M., Paterson, A.D., Hosseini, S.M., Wong, H.S., Xu, L., Jonas, J.B., Parssinen, O., Wedenoja, J., Yip, S.P., Ho, D.W., Pang, C.P., Chen, L.J., Burdon, K.P., Craig, J.E., Klein, B.E., Klein, R., Haller, T., Metspalu, A., Khor, C.C., Tai, E.S., Aung, T., Vithana, E., Tay, W.T., Barathi, V.A., Consortium for Refractive, E., Myopia, Chen, P., Li, R., Liao, J., Zheng, Y., Ong, R.T., Doring, A., Diabetes, C., Complications Trial/Epidemiology of Diabetes, I., Complications Research, G., Evans, D.M., Timpson, N.J., Verkerk, A.J., Meitinger, T., Raitakari, O., Hawthorne, F., Spector, T.D., Karssen, L.C., Pirastu, M., Murgia, F., Ang, W., Wellcome Trust Case Control, C., Mishra, A., Montgomery, G.W., Pennell, C.E., Cumberland, P.M., Cotlarciuc, I., Mitchell, P., Wang, J.J., Schache, M., Janmahasatian, S., Igo, R.P., Jr., Lass, J.H., Chew, E., Iyengar, S.K., Fuchs' Genetics Multi-Center Study, G., Gorgels, T.G., Rudan, I., Hayward, C., Wright, A.F., Polasek, O., Vatavuk, Z., Wilson, J.F., Fleck, B., Zeller, T., Mirshahi, A., Muller, C., Uitterlinden, A.G., Rivadeneira, F., Vingerling, J.R., Hofman, A., Oostra, B.A., Amin, N., Bergen, A.A., Teo, Y.Y., et al. (2013). Genome-wide meta-analyses of multiancestry cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nat Genet, 45*, 314-8.

Vitale, S., Cotch, M.F., Sperduto, R. & Ellwein, L. (2006). Costs of refractive correction of distance vision impairment in the United States, 1999-2002. *Ophthalmology, 113*, 2163-70.

Vitale, S., Ellwein, L., Cotch, M.F., Ferris, F.L. & Sperduto, R. (2008). Prevalence of Refractive Error in the United States, 1999-2004. *Arch Ophthalmol, 126*, 1111-1119.

Walline, J.J., Jones, L.A. & Sinnott, L.T. (2009). Corneal reshaping and myopia progression. *Br J Ophthalmol, 93*, 1181-5.

Walline, J.J., Zadnik, K. & Mutti, D.O. (1996). Validity of Surveys Reporting Myopia, Astigmatism, and Presbyopia. *Optometry and Vision Science, 73*, 376-381.

Wu, H.L., Seet, B., Yap, E.P., Saw, S.M., Lim, T. & Chia, K. (2001). Does Education Explain Ethnic Differences in Myopia Prevalence? A Population-Based Study of Young Adult Males in Singapore. *Optometry and Vision Science, 78*, 256-299.

Wu, P.C., Tsai, C.L., Wu, H.L., Yang, Y.H. & Kuo, H.K. (2013). Outdoor activity during class recess reduces myopia onset and progression in school children. *Ophthalmology, 120*, 1080-5.

Yazar, S., Hewitt, A.W., Black, L.J., McKnight, C., Mountain, J., Sherwin, J., Oddy, W., Coroneo, M., Lucas, R. & Mackey, D.A. (2014). Myopia Is Associated With Lower Vitamin D Status in Young Adults. *Invest Ophthalmol Vis Sci, 55*, 4552-4559.

Appendix: Activity Survey

Think about your physical activities, visual activities, and sun exposure over the last 7 days.

How many times in the last week did you participate in each activity listed below and, usually, for how many minutes each time?

Physical activities in the last week	How many times in the last week?	Usually, how many minutes each time?
Basketball (indoors)		
Basketball (outdoors)		
Boating (sail or motor)		
Skating or rollerblading (indoors)		
Skating or rollerblading (outdoors)		
Rowing machine (indoors)		
Rowing (outdoors)		
Fishing		
Riding a horse		
Billiards		
Bowling		
Ping pong (table tennis)		
Indoor racquet sports (squash or racquetball)		
Tennis or other outdoor racquet sports		
Football		
Baseball, softball		
Golf		

Soccer		
Volleyball (indoors)		
Volleyball (outdoors)		
Shopping (indoors)		
Shopping (outdoors)		
Walking at a brisk pace indoors (indoor track or treadmill)		
Running indoors (indoor track, treadmill, or stairclimber)		
Climb a flight of stairs		

Physical activities in the last week	**How many times in the last week?**	**Usually, how many minutes each time?**
Walking at an easy pace outdoors		
Walking at a brisk pace outdoors		
Running outdoors (jogging)		
Running outdoors (running hard, like racing or competitively)		
Spinning or riding a stationary bicycle indoors		
Bicycling outdoors (easy riding for recreation)		
Bicycling outdoors (transportation)		
Bicycling outdoors (riding hard, like racing or competitively)		
Aerobics (low impact) or other low impact indoor workout		
Aerobics (high impact), “boot camp” or other high impact indoor workout		
Weight training		
Yoga or Pilates		
Martial arts		
Swimming indoors (recreation)		

Swimming indoors (swimming hard, like laps or competitively)		
Swimming outdoors (recreation)		
Swimming outdoors (swimming hard, like laps or competitively)		
Dancing		
Skiing		
Backpacking or hiking in hills		
Backpacking or hiking over flatter terrain		
Housework (light cleaning, sweep, mop, vacuum, cook)		
Housework (moderate cleaning, washing windows, scrubbing floors)		
Yardwork (light: power mowing lawn, power clipping hedge, weeding)		
Yardwork (moderate: push mowing lawn, hand clipping hedge, raking)		
Yardwork (heavy: shoveling, digging, using heavy hand tools)		
Do it yourself projects, construction, or other work outdoors		
Do it yourself projects, carpentry, or other work indoors		

Visual or non-physical activities in the last week	**How many times in the last week?**	**Usually, how many minutes each time?**
Texting on a phone		
Watching TV, videos, or DVDs (other than games)		
Playing a hand-held video game		
Playing video games on a TV sitting still (mostly using thumbs and hands)		
Playing video games on a TV not sitting still (mostly using arms and legs)		
Computer for work, school, or gaming		
Reading books or doing paperwork for work or school		

Reading books or magazines for pleasure		
Playing chess, cards, or board games		
Drawing, painting, or writing (other than homework)		
Other hobbies and crafts involving work within arm's length		
Playing a musical instrument (indoors)		
Playing a musical instrument (outdoors)		
Outdoors, but no particular physical activity (sitting at a picnic, park, or pool)		
Other significant activities (list each activity, then give the number of times done this week and, usually, how many minutes spent on it each time)		

Sun exposure in the last week	**How many times in the last week?**	**Usually, how many minutes each time?**
Outside with whole torso in daylight (shirtless or in a bathing suit)		
Outside with just the back of your torso and shoulders in daylight (like when wearing a halter)		
Sun exposure and clothing outdoors	**What percent of the time?**	
What percent of your time **outside** did you wear a hat?	%	
What percent of your time **outside** did you wear **sleeveless** clothing (**not** clothing with short or long sleeves)?	%	**These three answers**

What percent of your time **outside** did you wear **short sleeves** (**not** sleeveless clothing or long sleeves)?	%	**should add up to 100%**
What percent of your time **outside** did you wear **long sleeves** (**not** sleeveless clothing or short sleeves)?	%	
What percent of your time **outside** did you wear short pants instead of long pants?	%	
What percent of your time **outside** did you wear sunglasses?	%	
Sunscreen outdoors	**What percent of the time?**	
When outside without a hat, what percent of the time did you use sunscreen on your head, face, or neck?	%	
When outside and wearing short sleeves or no sleeves, what percent of the time did you use sunscreen on your arms or shoulders?	%	
When outside and wearing short pants, what percent of the time did you use sunscreen on your legs?	%	
When outside and shirtless or in a bathing suit, what percent of the time did you use sunscreen on most exposed skin?	%	
When outside and with just the back of your torso and shoulders in daylight (like when wearing a halter), what percent of the time did you use sunscreen on your back and shoulders?	%	
Sunscreen factor	**What SPF?**	
When you used sunscreen, what factor sunscreen did you usually use?	SPF =	
Sleep and waking hours	**How many hours?**	
How many hours of sleep did you usually get each night?	hours	