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RESEARCH

Conjunctival ultraviolet autofluorescence (CUVAF) area, but not intensity, is associated with myopia in UK adults.

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Running title: Myopia, conjunctival ultraviolet autofluoresence (CUVAF) and myopia.

Background:

Conjunctival ultraviolet autofluorescence (CUVAF) has been used as a biomarker of time spent outdoors and smaller CUVAF area is associated with myopia in Southern Hemisphere cohorts. Further research is to determine if this association is replicated in northern latitudes and whether average CUVAF intensity is a valuable metric. This prospective study explored the association between myopia, CUVAF (area and intensity) and additional indicators of sun exposure (vitamin D_3 and self-reported sun exposure preferences) across seasons at a location 55°North.

Methods:

Young adults (18-20 years) provided blood samples biannually (Mar/Apr and Sept/Oct) over an 18-month period (four phases) for the assessment of 25-hydroxyvitamin D ($25(OH)D_3$) concentrations (liquid chromatography-tandem mass spectrometry). CUVAF (total area, average intensity) and self-reported sun exposure preferences were recorded at each phase. Axial length and corneal radius were measured. Refractive error was measured by autorefractor and spherical equivalent refraction (SER) used to classify participants into refractive groups: myopic (SER ≤-0.50DS) or non-myopic.

Results:

Fifty-four participants (24 myopes, 30 non-myopes) participated. CUVAF area was negatively associated with the presence of myopia (OR=0.94, 95% CI=0.90-0.98, p=0.002. Myopes=4.5mm^2 (Interquartile range (IQR) 0.95-6.4mm^2), non-myopes=7.0 mm^2 (IQR=2.0 mm^2-10.7 mm^2)). No significant association was found between CUVAF intensity and refractive group (p=0.17). There was no significant association between sun exposure preferences or serum concentration of $25(OH)D_3$ and refractive status (all p≥0.21). CUVAF measures were not associated with ocular biometry measures (all p≥0.084). CUVAF area was unaffected by season (all p≥0.45) and variations in CUVAF

area over the study period did not exceed the repeatability of the measurement technique.

Conclusion:

Myopia was associated with smaller areas of conjunctival ultraviolet autofluorescence indicative of less cumulative UVB exposure. These findings suggest that CUVAF measures are a useful, non-invasive biomarker of the time spent outdoors in adults in Northern Hemisphere populations.

The increase in the prevalence of myopia worldwide[1] is a public health concern owing to the associated risk of visual impairment arising from related ocular pathologies such as glaucoma, retinal detachment and cataract[2,3]. There is an increasing amount of literature reporting that greater time spent outdoors is protective against myopia in children of various ethnicities, from different countries and across various latitudes[4-6].

In myopia research to date, time spent outdoors by study participants has been largely determined using self-reported questionnaires; a methodology which may introduce recall bias. Light exposure dosimeters provide an alternative means of objectively determining the time spent outdoors and have been employed in myopia research in a variety of forms; incorporated into smart watches with a corresponding smart phone app[7], in a badge attached to the child's collar[8], and as a spectacle attachment[9]. Dosimeter outputs and self-reported time spent outdoors measures have been shown to only weakly correlate with each other, undermining confidence in both metrics[10-12].

An alternative objective measure which may be used as a biomarker of time spent outdoors is circulating vitamin D_3 concentrations in the blood[13]. Vitamin D_3 can be obtained in small amounts from diet but it is predominantly synthesised within the skin following ultraviolet-B (UVB) light exposure, before being hydroxylated to 25-hydroxyvitamin D_3 ($25(OH)D_3$): the metabolite quantified to determine an individual's vitamin D status. Outcomes from recent studies exploring the association between vitamin D status and myopia fail to support an active, independent role for vitamin D in myopic eye growth regulation[13,14]. While vitamin D status may be considered a useful objective biomarker of time spent outdoors, concentrations of 25(OH)D are influenced by a wide range of other factors, and sampling and measurement involves invasive procedures which are not routinely used in ophthalmic practice.

Conjunctival ultraviolet autofluorescence (CUVAF) photography provides an alternative, less invasive measure of time spent outdoors[15,16]. The technique

derives from Wood's lamp which was originally used in dermatological investigations[17]. UV damaged elastin and collagen, present in conjunctival tissue, emit fluorescence upon exposure to an excitatory light source and this autofluorescence can be captured with relatively simple photographic techniques and measured to determine the extent of UV-related damage[17,18]. A change in the intracellular content of proteins including cytokines and matrix metalloproteinases may also contribute to the CUVAF observed[19].

It is unclear from current literature if CUVAF is indicative of accumulative UV damage. CUVAF has been positively associated with age in some, but not all studies[15,20-21,22] suggesting that repair to conjunctival tissue following UV damage is possible over time. Although a genetic predisposition to CUVAF may exist within the population, environmental exposure to UV light has the greatest influence on the pathogenesis of CUVAF[23]. Expectedly, an outdoor occupation has been associated with greater amounts of CUVAF[24]. Male gender was associated with greater amounts of CUVAF in adults on Norfolk island[21] as the authors postulate that males are more likely to have an outdoor occupation than females in this population.

Research exploring the effects of seasonal variation on CUVAF area is limited. Greater amounts of CUVAF were measured in participants living in Ohio during the winter months[24]. However, the authors report that participants worked indoors for greater hours during the spring which may have confounded this association. Literature also exploring this association is limited to the Southern Hemisphere where a study group did not report an association between season and CUVAF area in young adults living in Western Australia[25,26]. However, there is minimal seasonal variation in the hours of sunshine at this latitude with the average daily hours of sunshine varying from five to nine hours in the winter months and from seven to ten hours in the summer months[27]. In comparison, Northern Ireland experiences a greater seasonal variation in sunshine hours varying from one to two hours in the winter months to five to six hours in the summer months[28]. Therefore, this finding may not be comparable to more

northern latitudes with colder climates and less hours of sunshine such as Northern Ireland. Higher temperatures in the spring and summer time may also influence the amount of CUVAF due to higher intensity UV light but this association has not yet been explored.

Smaller areas of CUVAF have been associated with myopia in Australia[26] and in Norfolk Island[29]. Furthermore, the Australian study by McKnight et al[26] reported that smaller areas of CUVAF was more strongly associated with myopia than self-reported time spent outdoors. However, the association between CUVAF and myopia has not yet been explored at a latitude similar to Northern Ireland.

Research is required to establish the association between CUVAF and myopia at different seasons at more northern latitudes where climatic differences may influence the relationship. Moreover, data are lacking on the association between myopia and CUVAF intensity.

Aims

To explore the association between myopia, CUVAF (area and intensity) and additional indicators of sun exposure including self-reported sun exposure and vitamin D status in a Northern Hemisphere cohort across winter and summer seasons.

METHODS

Study design and subjects

The study methods have been described elsewhere[30]. To summarise, measures were completed biannually, in March/April and September/October, over an 18-month period (commencing September/October 2014) at a latitude of 55° North. This resulted in data being collected at four-phases at the equinoxes for UV exposure; two corresponding to the end of winter (March/April) and two at the end of summer (September/October) [31,32]. The end of summer measurements allowed for an

accumulative measure of UV exposure over the summer months (May to August). Young adults aged 18 to 20 years were recruited.

The cohort was predominantly Caucasian (98.1%). Participants were first or second year undergraduate students enrolled in a variety of subject areas. Ethical approval was granted from the Ulster University Research Ethics Committee (REC/14/0003). Written informed consent was obtained after explanation of the nature and possible consequences of the study and prior to commencing the study protocol. Research adhered to the tenets of the Declaration of Helsinki. Blood samples were processed and stored in accordance with the Human Tissue Act 2004. The data collected from participants in the current study comprised part of a larger study described elsewhere[30] where the second data collection point in the previous study corresponds with phase 4 in the current study.

Based on published data from Sherwin et al[21], sample size calculations (power of 90%, significance 5%) indicated that 13 myopes and 13 non-myopes would be sufficient to detect a significant difference in CUVAF area of approximately 10 mm[216]. Recruitment targets were inflated to allow for a dropout rate of approximately 50% in both the myopic and non-myopic groups over the 18-month study period[33].

Measures

Autorefraction

Non-cycloplegic autorefraction while the participant viewed a distance target was completed at each phase using the Shin-Nippon SRW-5000 binocular open field autorefractor (Shin-Nippon, Tokyo, Japan). The representative value from each eye was determined by the instrument and the average of the right spherical equivalent refraction (SER) and left SER used in analysis. Participants were defined as myopic if the mean SER equated to less than or equivalent to -0.50 dioptre sphere (DS)[34]. One participant had anisometropia of greater than 1.50D (SER: Right eye: +0.25DS, left eye:+2.50DS) and was classified as non-myopic.

Ocular biometry:

Axial length (AL) and corneal radii (CR) were measured using the IOL Master. A total of five AL measures with a signal-to-noise ratio of greater than two were measured from each eye. A total of three CR measures were also recorded from each eye. The AL to CR ratio (AL/CR) was determined from these measures. An average value was derived from both eyes for each participant.

CUVAF photography

A previously validated photography system and novel analysis method (using MATLAB, The MathWorks Inc, Natick, MA, 2000) were used to quantify the area and intensity of CUVAF[15]. The method has been described elsewhere[15].

A specially adapted Sony Nex 6 (Sony, Tokyo, Japan) digital camera with a 50mm f/22 lens and a macro extension tube was used alongside a specially adapted unidirectional Xenon flash (Centon, FG30D) (Fig 1)[15]. A camera setting of f22 3200 ISO sensitivity was used and images were captured in a dark room to ensure ambient visible light did not interfere with imaging.

At least three images of both the temporal and nasal conjunctiva of the right and left eye were captured. The highest quality photograph from each position was chosen for analysis. Images were rejected if the visibility of CUVAF was hindered by lid position or defocus.

CUVAF Image analysis

The red and blue channels were removed from the RGB image producing a green only image which was converted to a greyscale image allowing the contrast of the image to be further enhanced using an automated MATLAB function which applied the same contrast settings to every image. This function made it much quicker and easier to subjectively differentiate CUVAF from non-CUVAF.

An area encompassing the fluorescence was subjectively outlined (Fig 2). An algorithm was created with MATLAB to determine a pixel threshold that provided an automated means of differentiating fluorescence from non-fluorescence within the outlined area. The area of fluorescence in pixels was converted to mm^2 using an algorithm that accounted for camera magnification.

Temporal and nasal conjunctival images were captured from the right and left eye at each phase. Total CUVAF area (mm^2) for an individual was calculated by summing the temporal and nasal areas of the right and left eye. To explore CUVAF intensity the average CUVAF pixel intensity per mm^2 ($x10^3$) was calculated. The values from both nasal and temporal images were used to determine an individual's average CUVAF pixel intensity per mm^2.

Additional sun exposure measures:

Questionnaires

A validated sun exposure questionnaire was completed by the participant at each phase[31]. Sun exposure habits were categorised as 'avoids the sun, sometimes stays in the sun' or 'often stays in the sun'. Participants reported sunglasses and hat use separately using the categories: 'Never', 'Rarely', 'Sometimes', 'Usually' or 'Always'. =

Vitamin D$_3$

A 4ml fasting blood sample (Vacuette; Greiner Bio One, Ltd, Stonehouse, UK) was collected into serum tubes from the antecubital vein between 8.30am and 10am at each phase. Samples were centrifuged at 2200g for 15 minutes at 4^0 C (Harrier 18/80 Refrigerated Centrifuge; MSE, London, UK) and aliquots of serum were stored at -80^0 C prior to analysis.

Concentrations of 25(OH)D$_3$ (nmol/L) were quantified using liquid chromatography-tandem mass spectrometry (LC/MS-MS) by the Biochemistry Department of St James's Hospital, Dublin which is accredited to ISO 15189. The quality and accuracy of the method was monitored by the use of internal quality controls, participation in the Vitamin D External Quality

Assessment Scheme (DEQAS) and the use of the National Institute of
Standards and Technology (NIST) 972 vitamin D standard reference material.
The respective inter- and intra-assay CVs were 6.5% and 7.5%.

A validated Food Frequency Questionnaire (FFQ) was used to estimate the
habitual dietary intake of vitamin D once a year[35]. The questionnaire comprised
of 17 questions pertaining to 13 food groups which contribute to dietary intake
of vitamin D including milk and dairy products, fish and dietary supplements.
The results from the FFQ were entered into a custom-built spreadsheet which
calculated the average dietary intake of vitamin D in micrograms (μg/d) as a
continuous variable.

Parental myopia was determined at Phase one using a validated refractive
status questionnaire[36] and responses categorised as either
'Neither parent myopic', '1 parent myopic' or 'Both parents myopic'.

Statistical analyses:
Statistical tests were performed using a statistical significance of 5% using
Stata 13.1 (StataCorp, College Station, TX, USA). CUVAF data were not
normally distributed and could not be adequately transformed.

The association between continuous variables (AL, CR, AL/CR) and average
summer CUVAF measures (average of phase 1 and phase 3) and average
winter CUVAF measures (average of phase 2 and phase 5) was explored using
Spearman's correlation. Kruskal Wallis test was used to explore the presence of
seasonal variation in the myopic and non-myopic groups separately. Season
was categorised as summer=1 (phase 1 and phase 3) and winter=0 (phase 2
and phase 4).

Multiple imputation analyses

A single MI repeated logistic regression model was used to explore the
association between the outcome variable (the presence of myopia (yes/no))

and measures of CUVAF, serum $25(OH)D_3$ and sun exposure preferences. This multivariate analysis allowed for the inclusion of confounding factors such as parental myopia and season of measurement. Other confounding factors known to influence CUVAF and serum $25(OH)D_3$ including sunglasses use, wearing a hat and dietary intake of vitamin D were included in the analysis. The odds ratio (OR) and corresponding 95% confidence interval (CI) were reported.

Multiple-imputation (MI)[37] was used to account for missing data in longitudinal analyses. Data were missing at random. A total of 34 imputed datasets were generated for CUVAF measures.

RESULTS

Participant characteristics

A total of 24 myopes and 30 non-myopes were recruited at phase 1 (March/April 2014). The study protocol and the participant characteristics at each phase are outlined in Figure 3 and Table 1.

There was no significant difference between the participants who re-attended and those who dropped out with regards to sex ($X^2 = 0.58$, p=0.45), SER (degrees of freedom (d.f)=52, p=0.47) or parental myopia ($X^2=0.31$, p=0.079). Participants initially classified as myopic or non-myopic remained within their respective refractive status category for the duration of the study. Refractive error over the study period was relatively stable (mean change in SER (±standard deviation (SD)): myopes: -0.24±0.27DS, non-myopes: +0.01±0.42DS). No significant changes in refractive error were recorded over the study period in either the myopic or non-myopic groups (all p≥0.078).

The association between CUVAF, season, measures of sun exposure and myopia

Table 2 summarises CUVAF measures obtained from myopic and non-myopic participants at each phase. There was no significant seasonal variation in average CUVAF measures in either refractive group (all p≥0.45) (Fig 4).

When comparing CUVAF measures at phase 1 (winter) to CUVAF measures at phase 4 (winter), the non-myopic group demonstrated no significant change in total CUVAF area (p=0.45) whereas myopes demonstrated a decrease in this metric (p=0.044) over the study period. Conversely, myopes demonstrated no significant change in average CUVAF intensity (p=0.13) whilst a statistically significant increase was measured in the non-myopic group (p=0.002).

Myopes demonstrated a significantly smaller CUVAF area than non-myopes (OR=0.94, 95% CI=0.90-0.98, p=0.002) (Myopes= 4.5mm^2, interquartile range (IQR)=0.95-6.4mm^2. Non-myopes=7.0 mm^2, IQR=2.0 mm^2-10.7 mm^2).

Average CUVAF intensity was not significantly different between myopes and non-myopes (OR=0.97, 95% CI=0.92-1.0, p=0.17) (Myopes=86, IQR=81-9. Non-myopes=88, IQR=82-94). Myopia was not associated with sun exposure preferences (p=0.38) or with serum 25(OH)D$_3$ (p=0.25) (Table 3).

Table 4 summarises the results pertaining to the association between CUVAF measures and ocular biometry. CUVAF measures were not associated with AL or AL/CR. Although a larger CUVAF area measured in the winter was associated with a flatter cornea, the p value pertaining to this association (p=0.041) was greater than the Bonferroni corrected p value (p=0.017) indicating this association is not significant.

Mean serum 25(OH)D$_3$ equated to 41.9±17.6nmol/L in the myopic group and 40.8±19.8nmol/L in the non-myopic group. In the group as a whole, the mean seasonal change in serum 25(OH)D$_3$ equated to 22±16nmol/L. There was no significant difference in vitamin D intake from diet between refractive groups (p=0.14. Myopes=3.1µ/d (SD=3.5µ/d). Non-myopes=2.3µ/d (SD=3.0µ/d)).

DISCUSSION

This study illustrates that myopia is associated with significantly smaller areas of CUVAF in a cohort of young myopes in the UK. This finding provides empirical evidence for an association between less UVB exposure and adult myopia in the UK supporting results from a larger cross-sectional study in adults from multiple European countries[14]. While the area of CUVAF was inversely associated with myopia, average CUVAF intensity was not. Our findings suggest that the measurement of CUVAF intensity is a less useful measure for myopia researchers than measures of CUVAF area. CUVAF area and intensity were not associated with AL or AL/CR, likely due to the small number of high myopes represented in the study.

Myopes demonstrated smaller areas of CUVAF at all phases except at phase 1. The outlying point at phase 1 reflects the variability of CUVAF measures and demonstrates the value of multiple sampling points at this latitude to ensure fluctuations in measures are accounted for. The difference in CUVAF area between myopes and non-myopes in the current study is modest compared with that previously reported in a similar aged Australian cohort[26]. This differential is most likely attributable to the distinct climatic differences between the UK and Western Australia. Measures more comparable to those from the present study were obtained by Sherwin et al[29] from an older cohort of adults (mean age 54.1 ± 16.2 years) living on Norfolk Island in the Pacific Ocean between Australia and New Zealand. The use of an additional objective measure of time spent outdoors such as a wearable dosimeter in future studies may further strengthen exploration of inter-group differences.

CUVAF metrics did not vary systematically by season. However, it is not possible to conclude from this that there is no annual variation in CUVAF as sampling at only two time points may have missed peaks and troughs which may have occurred throughout the year. Although there was some variability in both CUVAF area and intensity measures between visits in the two refractive

groups, these differences, while statistically significant, failed to exceed the limits of repeatability of the technique[38] and are unlikely to be meaningful. As CUVAF is an indicator of accumulative UV damage and has been associated with chronic indicators of UV exposure such as pterygium[18,24], previous sunlight exposure during childhood may also have masked any small changes in CUVAF that occurred over the study period at this northerly latitude.

Data from the present study support previous UK reports which identify that while vitamin D status may be a useful biomarker for time spent outdoors, it is not significantly associated with myopic status in adults. Vitamin D_3 synthesis is triggered by recent exposure to UVB and the measurement of vitamin D_3 concentrations in this 18-month prospective study provides a current 'snapshot' of vitamin D status in our adult participants. In contrast, CUVAF measures provide an indication of accumulative UVB exposure during the period when myopic eye growth was active[15,20].

Self-report of sun-exposure behaviours across winter and summer seasons were not significantly related to myopic status in our Northern Hemisphere cohort. Similar to the vitamin D measures, these self-reported measures indicate current behaviour rather than reflecting behaviour during an earlier more dynamic period of ocular growth. Such qualitative metrics are likely to be less reliable in reflecting actual exposure to outdoor light compared with objective measures such as CUVAF or vitamin D status[8].

The OR of 0.94 for myopia associated with CUVAF area, indicates that participants with larger areas of CUVAF were slightly (6%) more likely to be myopic than those with less CUVAF. As the participants were young adults with stable refractive errors, previous exposure to sunlight when they were children exhibiting refractive change is likely to have influenced this outcome. As noted previously, CUVAF is thought to represent accumulative exposure and damage to UVB. The wide confidence intervals reported in these analyses may be attributed to the relatively small sample size and to the variation in CUVAF measures illustrated by the standard deviation. The power calculations used to

inform sample sizes were based on a difference in CUVAF area of 10 mm^2 between myopes and non-myopes from Southern Hemisphere data. Our participants demonstrated smaller CUVAF measures and smaller between group differences than these Southern Hemisphere reports, likely attributable to climatic differences between the cohorts. Given the lack of data exploring CUVAF's association with refractive error in the Northern Hemisphere, the current data will be helpful in powering future, larger studies in similar climates.

Although it may be postulated that the protective UV filtering effects of wearing spectacles or contact lenses may also have influenced CUVAF measures and hence outcomes, the literature reports that CUVAF is significantly lower in spectacle wearing myopes than in spectacle wearing hyperopes[26]. Furthermore, no difference in CUVAF area between hyperopes wearing or not wearing corrective lenses or between myopes wearing or not wearing corrective lenses was reported, indicating that refractive correction is unlikely to have confounded the results presented.

Although fairer skin phenotypes and pale coloured irises have been associated with greater CUVAF, the cohort in this study were predominantly Caucasian which will have minimised this potential confounder[39]. As the majority of literature exploring factors influencing CUVAF is largely confined to the Southern Hemisphere, it is unclear if other environmental factors including snow and humidity may also influence the amount of CUVAF measured.

CONCLUSIONS

This study demonstrates that smaller areas of CUVAF, indicative of lower levels of accumulative UVB exposure, are significantly associated with myopia in young adults living in the Northern Hemisphere. Outcomes suggest that CUVAF area, but not intensity, can be used as an objective, non-invasive biomarker of time spent outdoors in Northern Hemisphere cohorts.

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Table 1 Summary of the mean (±SD) spherical equivalent refraction (SER), axial length (AL) and AL to CR ratio (AL/CR) for myopes and non-myopes at each Phase. *Refractive and ocular biometric change over the study period was not significant in either group (all p≥0.078).*

	Average right and left eye (Mean (±SD))

Phase 1		Myopes n=25	Non-myopes n=29
	SER	-2.37±1.27	+0.62±0.89
		(-5.88 to -0.94)	(-0.38 to +4.44)
	AL	24.7±0.90	23.3±0.80
	CR	7.8±0.26	7.9±0.28
	AL/CR	3.2±0.09	2.9±0.09
Phase 2		**Myopes** **n=24**	**Non-myopes** **n=26**
	SER	-2.33 ±1.28	+0.76 ±1.07
		(-5.81 to -0.63)	(-0.31 to +5.31)
	AL	24.8 ±0.84	23.4 ±0.78
	CR	7.9 ±0.27	7.9 ±0.27
	AL/CR	3.2 ±0.09	2.9 ±0.09
Phase 3		**Myopes** **n=21**	**Non-myopes** **n=24**
	SER	-2.73 ±1.39	+0.60 ±1.13
		(-5.69 to -0.69)	(-0.19 to +5.13)
	AL	24.9 ±0.91	23.4 ±0.76
	CR	7.8 ±0.27	7.9 ±0.28
	AL/CR	3.2 ±0.09	3.0 ±0.10
Phase 4		**Myopes** **n=21**	**Non-myopes** **n=24**
	SER	-2.34±1.12	+0.78±1.16
		(-5.44 to -0.88)	(-0.19 to +5.31)
	AL	24.7±0.76	23.4±0.81
	CR	7.8±0.24	8.0±0.26
	AL/CR	3.2±0.09	2.9±0.08

Table 2 Table illustrating the difference in total area and average pixel intensity between myopes and non-myopes. Myopia was negatively associated with total CUVAF area (OR=0.94, 95% CI=0.90-0.98, p=0.002) but not with average CUVAF intensity OR=0.97, 95% CI=0.92-1.0, p=0.17)

	Total area (mm^2) (Median (IQR)) (Mean, SD)		Median difference (mm^2)	Average pixel intensity (x10^3/mm^2) (Median (IQR)) (Mean, SD)	
Phase	**Myopes**	**Non-myopes**		**Myopes**	**Non-myopes**
1: Summer	4.9 (0.88-6.7) *(6.0, 5.7)*	4.7 (2.2-10.8) *(6.4, 5.4)*	-0.2	83 (73-86) *(67, 36)*	85 (81-88) *(72, 33)*
2: Winter	4.7 (0.9-5.9) *(5.1, 4.6)*	7.5 (2.3-11) *(7.1, 5.2)*	2.8	85 (81-88) *(75, 29)*	87 (82-92) *(76, 33)*
3: Summer	3.9 (1.2-6.3) *(4.5, 3.5)*	7.7 (1.5-10.2) *(6.8, 6.0)*	3.8	90 (87-93) *(82, 28)*	93 (86-97) *(78, 36)*
4: Winter	4.0 (1.5-7.1) *(4.2, 3.7)*	7.4 (0.9-10.1) *(6.7, 6.1)*	3.4	89 (79-94) *(71, 39)*	92 (76-97) *(73, 40)*

Table 3 Summary of the data pertaining to the difference in serum $25(OH)D_3$ concentrations between myopes and non-myopes. There was no significant difference in serum $25(OH)D_3$ between refractive groups (p=0.25).

Phase	Serum $25(OH)D_3$ (nmol/L)		Mean difference (nmol/L)
	Myopes	**Non-myopes**	
1: Summer	49.8 (36.6-70.7)	53.6 (35.6-72.8)	3.8
2: Winter	24.1 (15.3-37.2)	24.6 (16.5-34.9)	0.5
3: Summer	43.9 (32.6-56.5)	41 (31.9-54.1)	2.9
4: Winter	22.3 (14.7-36.7)	22.2 (17.1-34.3)	-0.1

Table 4 Table summarising the association between season measures of CUVAF and refractive and ocular biometric measures. All analyses were performed using Spearman's correlation. This association did not remain significant after the Bonferroni correction was applied.

	Total CUVAF area				Average CUVAF pixel intensity			
	Summer average		Winter average		Summer average		Winter average	
	ρ	p	ρ	p	ρ	p	ρ	p
AL	-0.10	0.48	-0.10	0.52	-0.10	0.49	-0.05	0.76
CR	0.25	0.084	0.31	0.041	-0.05	0.73	0.04	0.78
AL/CR	-0.14	0.32	-0.19	0.23	0.01	0.96	-0.01	0.96

Figure 1. CUVAF photography system used in methodology.

Figure 2. Sample image used in analysis. An area encompassing the CUVAF has been subjectively outlined prior to MATLAB analysis.

Figure 3 Flow chart illustrating the participant characteristics and participant dropouts at each phase.
Spherical equivalent refraction (SER)
Conjunctival ultraviolet autofluorescence (CUVAF)
Food Frequency Questionnaire (FFQ)

Figure 4 Box plots illustrating CUVAF measures in the myopic and non-myopic groups over the study period. *There was no significant seasonal variation in CUVAF measures (all p≥0.45). Participants with a value of 0 for average CUVAF intensity were removed from the average CUVAF intensity plots to more easily visualise the spread of data.*