Sun damage to the eyes of young adults in Western Australia: an assessment of the extent and distribution of conjunctival ultraviolet autofluorescence, and other ultraviolet-associated ocular conditions, at the 20-year follow-up of a longitudinal cohort study.

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Abstract

Sunlight is fundamental to life on Earth. Ancient civilisations across the globe and throughout history have recognised the crucial importance of sunlight, with sun deities in various forms featuring prominently in many ancient religions and traditions (Australian Aboriginal Gnowee, Wala and others, Arabian Malakbel, Chinese Xihe, Egyptian Ra, Mayan Ah Kin and many others). A modern form of sun worshipping can also be recognised, with sun, summer and beach culture figuring prominently (for many people) in the Australian national identity.

Inadequate sunlight exposure has many known and postulated detrimental health effects, in part through its role in vitamin D metabolism. Conversely sunlight, particularly wavelengths in the ultraviolet A and B range, is well known to be dangerous in excess. This is particularly significant in regions, such as Australia, with high levels of sunlight and environmental ultraviolet radiation.

The balance between beneficial and detrimental effects of sunlight exposure may also be important for ocular health. This thesis investigates the use of an objective method of quantifying ocular sun exposure, conjunctival ultraviolet autofluorescence photography, in a large cohort of young adults (the Western Australian Pregnancy Cohort (Raine) Study). The associations between ocular sun exposure and established eye diseases in the cohort are reported and discussed.

As detailed in the text, median area of conjunctival ultraviolet autofluorescence was 44.2mm$^2$ (range 0.0mm$^2$ – 180.3mm$^2$). Pterygium was present in 2.0% of male participants and 0.3% of female participants. Median total ultraviolet autofluorescence was higher in participants who had pterygium than those who did not, 73.4mm$^2$ vs 44.0mm$^2$, p=0.001.

23.7% of participants were myopic. Median conjunctival ultraviolet autofluorescence was lower in myopes than non-myopes, 31.9mm$^2$ vs 47.9mm$^2$, p<0.001. Participants in the lowest quartile of conjunctival ultraviolet autofluorescence (least sun damage) had more than two and a half times the odds of myopia than participants in the highest quartile (OR 2.67, 95%CI 1.83-3.89, p<0.001). Possible mechanisms underlying these associations, and the public health implications, are discussed.
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Statement of candidate contribution

This thesis is my own composition, all sources have been acknowledged and my contribution is clearly identified. For any work in the thesis that has been co-published with other authors, I have the permission of all co-authors to include this work in my thesis. The bibliographic details of the work, where it appears in the thesis and my contribution are outlined below.

  • Chapter 1
  • First author on paper. Major contribution including data collection, data analysis and writing the manuscript.

  • Chapter 3
  • Second author on paper. Significant contribution including design of the study, data analysis and revision of the manuscript for publication.
  • Complex statistical analysis of reliability and validity were performed by Dr Sherwin.

  • Chapter 4 and chapter 5
  • First author on paper. Major contribution including data collection, data analysis and writing the manuscript.

  • Chapter 6
  • First author on paper. Major contribution including data collection, data analysis and writing the manuscript.
List of abbreviations

Note: all abbreviations are also defined within the text as they arise.

KEMH: King Edward Memorial Hospital
RCT: Randomised controlled trial
BMI: Body mass index
DOHaD: Developmental origins of health and disease
NHMRC: National Health and Medical Research Council
TICHR: Telethon Institute for Child Health Research
TKI: Telethon Kids Institute
UWA: University of Western Australia
HPA: Hypothalamic pituitary adrenal
NIES: Norfolk Island Eye Study
SD: Standard deviation
IQR: Interquartile range
OR: Odds ratio
CI: Confidence interval
CCC: Concordance correlation coefficient
LOA: Limits of agreement
Introduction: Sunlight and the eye

Ophthalmohelioses and conjunctival ultraviolet autofluorescence photography

Ophthalmohelioses describe a group of ophthalmic conditions in which sunlight is implicated in the pathogenesis. Sunlight has been associated with several different ophthalmic conditions affecting every major anatomical component of the eye, as well as systemic conditions with the potential for ocular involvement.

Excessive ultraviolet radiation exposure plays a pathogenic role in a number of ophthalmic diseases, including pterygium, cortical cataract, squamous cell carcinoma of the ocular surface, acute photokeratitis, solar retinopathy, and malignant skin lesions of the eyelids. In contrast, inadequate ultraviolet exposure is associated with deleterious health effects including osteomalacia, osteoporosis and rickets. Ultraviolet light is not thought to have any direct ocular health benefits, but there is evidence that low levels of outdoor sunlight exposure might have a role in myopia pathogenesis, as outlined below.

To understand the factors driving the development of pterygium and other ophthalmohelioses, research groups must be able to accurately assess individual ultraviolet exposure. This is not straightforward; sun exposure history by questionnaire is inherently subjective and prone to recall bias. Ultraviolet exposure meters are being tested in research settings, but are only able to provide short-term information about current ultraviolet exposure patterns, not the cumulative lifetime exposure that is believed to be of more relevance in development of pterygium, ocular surface neoplasia, cataract and eyelid malignancy.

Conjunctival ultraviolet autofluorescence photography provides an objective, quantifiable measure of ocular ultraviolet exposure. This method avoids the inherent limitations, including subjectivity and recall bias, of measuring outdoor activity using participant recall via questionnaire. The principle is the same as the Wood’s lamp used in dermatology whereby areas of actinic damage on the skin are observed to fluoresce under ultraviolet light. The same phenomenon occurs on the conjunctiva (figure 1), and this conjunctival fluorescence can be captured with a fixed focal-length camera system and digitally analysed to determine the extent of pre-clinical, ocular surface sun damage.

As the technique of conjunctival ultraviolet autofluorescence photography is relatively new, its reliability, reproducibility and validity has not previously been determined. In chapter 3, the
results of an inter- and intra-observer reliability study, and the validity of conjunctival autofluorescence in relation to outdoor activity level, are reported.

The initial studies using conjunctival ultraviolet autofluorescence photography suggested that areas of fluorescence represented precursors to ophthalmohelioses. In people with established ophthalmohelioses, in particular pterygium, it was hypothesised that ultraviolet autofluorescence represented areas of cellular activity within pterygia. These initial studies were limited by small sample sizes and lack of quantitative assessment of the area of ultraviolet autofluorescence.

Little is known about the characteristics or distribution of conjunctival autofluorescence in the population. Distribution of conjunctival autofluorescence has been reported in school-aged children, in the general adult population of sub-tropical Norfolk Island, in a small population of patients with pterygium and in a study of European eye care practitioners. No study has looked exclusively at young adults. This is perhaps the most important age group to target in sun protection research – pterygium has begun to develop in those individuals most at risk, but there remains scope for intervention to prevent development of ultraviolet-related disease later in life. This study looks at the distribution of conjunctival autofluorescence and associated ophthalmic conditions in a large cohort of young Australian adults, with distribution reported in chapter 4 and associations with pterygium and myopia reported in chapters 5 and 6, respectively. The history of the cohort itself is reviewed in chapter 1, to provide background and context for the ophthalmic investigations.

**Pterygium: the archetypal ophthalmoheliosis**

Pterygium is a wing-shaped, fibrovascular lesion extending from the bulbar conjunctiva onto the cornea (figure 2). The first known descriptions of pterygium date from the 4th - 6th century BC, in the Indian surgical text Susruta Samhita and in the Hippocratic scripts. Susruta’s description of removing the pterygium by scratching with a sharp, round-topped instrument before excision is not dissimilar to the procedure performed today. In modern times, Australia is a world leader in pterygium research with many important clinical, epidemiological and pathophysiological studies performed by Australian researchers.

Despite these research efforts, and sun protection campaigns from government and non-government organisations, pterygium remains a significant issue in Australia and surrounding territories.
Visual impairment can occur through several mechanisms\textsuperscript{29}, including directly from obscuration of the visual axis or from induced astigmatism\textsuperscript{30}. With rates of ocular surface neoplasia in excised pterygium around 10-12\%\textsuperscript{15,16}, pterygium should not be universally considered a benign condition. Overall, the disease burden associated with pterygium and its treatment is high\textsuperscript{28,4}. There are strong environmental associations with pterygium, including outdoor activity, occupation, latitude and rural lifestyle, providing clear evidence for the role of lifetime ultraviolet light exposure in pterygium pathogenesis\textsuperscript{17,29}. The study reported in chapter 5 investigates the prevalence of pterygium in the cohort, and describes the associated ophthalmic features and population characteristics.

**Myopia: sunlight may have protective effects**

Myopia is a major health issue worldwide\textsuperscript{31}. It contributes to the burden of uncorrected refractive error, which is a leading cause of visual impairment\textsuperscript{32} and a significant economic issue with billions of dollars of lost productivity globally\textsuperscript{33}. Low levels of myopia may be corrected with glasses, contact lenses or refractive surgery; however, high myopia is associated with a substantial risk of potentially blinding ocular pathologies including myopic maculopathy\textsuperscript{34,35}, retinal detachment\textsuperscript{36}, glaucoma\textsuperscript{37} and cataract\textsuperscript{38,39}.

The prevalence of myopia is increasing worldwide in many populations, with particularly steeper trajectories in east and southeast Asia\textsuperscript{40,41}. This rise in myopia, with corresponding rise in high myopia, is concurrent with myopigenic activities on a background of increasing urbanization of these populations\textsuperscript{40}. One activity underlying this association may be a reduction in time spent outdoors, with a recent meta-analysis revealing a reduction in odds of myopia of 2\% for every additional hour spent outdoors per week\textsuperscript{5}. The protective effect of outdoor activity has also been demonstrated in prospective studies\textsuperscript{42,43} and a randomized clinical trial\textsuperscript{44}.

There are currently no widely utilised interventions to prevent myopia, and interventions proposed to reduce progression of myopia are limited by small effect sizes, rebound myopia and side effects\textsuperscript{45-47}. If the protective association of increased outdoor time and reduced rates of myopia can be confirmed using objective measures of outdoor exposure, it will provide clear evidence of the need for further interventional trials in this area.

Using ultraviolet autofluorescence photography, an inverse association between conjunctival ultraviolet autofluorescence and myopia was demonstrated in a population cohort from Norfolk Island\textsuperscript{48}, although findings were limited by a small sample size, wide age range of participants and
incomplete data on confounding variables. The study reported in chapter 6 was designed to further investigate the association between conjunctival ultraviolet autofluorescence and myopia in a larger population, and determine whether the association could be explained by confounding factors or the ultraviolet-blocking effect of corrective lenses.
Figure 1.

Photographs of the nasal conjunctiva
a) colour image with no visible sun damage
b) ultraviolet image showing area of autofluorescence
Figure 2.

A participant with bilateral nasal pterygia.

Colour photographs in primary gaze (a, b), right gaze (c) and left gaze (d).
Chapter 1. History of the Raine Study

Cohort studies are an effective medical research tool, with Australia having several well-established research cohorts\(^49\). The increasingly valuable Western Australian Pregnancy Cohort (Raine) Study recently celebrated its 21st birthday, with over $20 million in competitive grant funding and its 150th paper published in mid 2011. This chapter outlines where and why the cohort began, and what factors have contributed to the study’s ongoing success.

In the beginning there was an idea... and funding

If not for a chance meeting between an accountant for the Raine Foundation and a young obstetrician newly returned from an overseas fellowship, the Western Australian Pregnancy Cohort (Raine) Study might never have begun. The Raine Foundation had been established nearly 30 years earlier at the bequest of Mary Raine, a successful businesswoman who left her property empire to The University of Western Australia (UWA) for medical research. The accountant mentioned that the Raine Foundation had decided to award a large sum of money to one big, visionary project, and the very next day the grant application was underway.

After a competitive application process, the Western Australian Pregnancy Cohort was funded. The objectives were two-fold: to investigate the hypothesis that preterm birth and other complications of pregnancy might be prevented by a protocol of multiple ultrasound scans, and subsequently to develop a long-term cohort to study the role that events in early life might have on later health.

From 1989 to 1991, 2900 pregnant women were recruited through King Edward Memorial Hospital (KEMH) or nearby private practices and randomised to either routine obstetric ultrasound or intensive multiple ultrasound scans\(^50\). Ninety percent of eligible women agreed to participate\(^51\). Extensive data were collected during pregnancy, the children were assessed at birth and then reviewed at 1, 2, 3, 5, 8, 10, 14, 17, 18 and 20 years (figure 3). Questionnaire data, physical measurements and biological samples have been collected with a focus on growth, cardiovascular, respiratory, immunological, musculoskeletal, nutritional, psychiatric, neurocognitive and ophthalmic health. The current phenotype dataset contains more than 85,000 measures on each cohort participant as well as 2.5 million genetic variants.
Two decades of scientific discovery

Cohort research can contribute to scientific knowledge in many different ways. As well as leading to novel discoveries, cohorts can be used to assess interventions, describe normal ranges and disease prevalence, provide insights into research methodology, identify new epidemiological associations and be used as a healthy control group. Selected findings from the Raine Study are presented below to illustrate each of these approaches.

Assessment of interventions
The Raine Study is the only large, randomised controlled trial (RCT) to have investigated whether multiple ultrasound scans improve pregnancy outcome. Rates of intrauterine growth restriction in the intensive group were significantly higher than the regular group\(^{50}\). The result was both unexpected and controversial, and is likely to have been a chance finding. Importantly, the study clearly demonstrated that additional routine scanning in the second and third trimesters did not improve outcomes. This translated to widespread practice and remains the standard for obstetric ultrasound. Childhood assessments up to 8 years showed no difference in physical characteristics, behaviour or neurocognitive development between the two groups\(^{52,53}\), and the Raine Study remains the only randomised trial in the world providing evidence into childhood of the relative safety of repeated scans in pregnancy.

New discoveries
Research using the Raine cohort has led to novel discoveries across a range of disciplines. Longitudinal data from the Raine Study on metabolic risk factors in childhood and adolescence demonstrated a U-shaped relationship between birth weight and risk\(^{54}\), indicating that preventative interventions should be targeted to infants in the highest, as well as lowest, birth weights. This U-shaped relationship had been observed in the developing world, but the Raine Study findings demonstrated applicability to children in westernised and developed environments. Maternal smoking during pregnancy, shorter duration of breastfeeding and rapid childhood weight gain were also associated with metabolic risk\(^{54}\), providing potential targets for public health intervention.

Raine is the largest study measuring lung function and bronchial responsiveness in preschool-aged children\(^{55}\), providing the first demonstration that ‘pseudo-allergic’ immune responses to respiratory bacteria are associated with protection against asthma\(^{56}\). In conjunction with other cohorts, Raine contributed to the identification of new genetic loci influencing lung function\(^{57,58}\), birth weight\(^ {59}\) and age at menarche\(^ {60}\). Targeted studies of otitis media showed that the FBXO11
gene was associated with severe and recurrent disease\textsuperscript{61}. Genes underlying neurodevelopmental disorders\textsuperscript{62,63} and metabolic risk\textsuperscript{64,65} have also been investigated, with many more genetic studies underway.

**Normal ranges, disease prevalence and longitudinal data**

Population-based cohorts allow tracking of changes over time. The Raine cohort has provided normal ranges for biometric measurements such as height, weight\textsuperscript{66} and blood pressure\textsuperscript{67} throughout childhood and adolescence, as well as biochemical measurements including serum glucose, lipid levels\textsuperscript{68} and inflammatory markers\textsuperscript{69}. Patterns of dietary intake\textsuperscript{68} and activity\textsuperscript{70,71} in this ‘Generation Y’ group have been identified, as have prevalence of otitis media, asthma\textsuperscript{72}, non-alcoholic fatty liver disease\textsuperscript{73}, polycystic ovarian syndrome\textsuperscript{74,75}, attention deficit-hyperactivity disorder\textsuperscript{76}, eating disorders\textsuperscript{77}, spinal pain\textsuperscript{78,79}, visual impairment\textsuperscript{80} and many other diseases.

Longitudinal monitoring of disease phenotypes is also valuable, with Raine cohort data demonstrating that preterm babies have persistently impaired lung function at age 6 years\textsuperscript{55}. Predictors of disease can change over time; the Raine Study identified that sedentary behaviours such as screen use were predictive of obesity in early childhood, whereas physical activity was a better predictor in adolescence\textsuperscript{71}. Body mass index (BMI) tracking showed that children destined to be obese in adolescence had an earlier adiposity rebound than normal or overweight children\textsuperscript{66}.

**Insights into research methodology**

Cohort studies can be used to evaluate research tools or methods. Research using the Raine cohort highlighted the value of continuous, rather than binary, measures of atopy in assessing asthma risk\textsuperscript{69,81}. Cluster analysis for features of the metabolic syndrome\textsuperscript{54,69}, factor analysis of dietary patterns\textsuperscript{68} and linear mixed modelling for BMI trajectories\textsuperscript{66} have been used to help unravel the interplay of factors contributing to cardiovascular risk. The reliability of tools to assess dietary intake\textsuperscript{82} and adiposity\textsuperscript{83} have been investigated, and new techniques for posture assessment developed\textsuperscript{84}.

**Epidemiological associations**

Identifying links between population characteristics and disease is integral to cohort research. The Raine Study has confirmed many suspected risk factors, and identified new associations not previously reported. Prenatal and early life exposures have been assessed, including influence of maternal smoking on birth weight\textsuperscript{85,86}, behaviour\textsuperscript{87} and blood pressure\textsuperscript{86}. Maternal factors such as stress, hypertension and weight were found to influence childhood behavioural and emotional development\textsuperscript{77,88,89}. Birth weight was associated with other variables including blood pressure\textsuperscript{90} and menarche\textsuperscript{91}. The value of breastfeeding was emphasised, as it was associated with better
respiratory health\textsuperscript{92} and language development\textsuperscript{93}, more optimal body weight\textsuperscript{94} and better behavioural outcomes\textsuperscript{95}. Of course the interplay of multiple risk factors and outcomes is complex. The longitudinal design of the Raine Study may help to unravel causal pathways in conditions that present a large burden but are clearly of multifactorial aetiology, such as cardiovascular, respiratory and psychiatric diseases.

\textit{Use as healthy controls}

There are clear benefits to using established cohorts as comparison groups in terms of cost saving and recruitment; in many cases the data required for comparison have already been collected. The Raine cohort has been used to compare outcomes with a group of very preterm infants\textsuperscript{96,97} and with children exposed to antenatal corticosteroids\textsuperscript{98}. A large study looking at children conceived through in-vitro fertilisation is proposed.

\textit{The value of a cohort increases with age}

Publication output from the Raine Study has increased exponentially over time (figure 4).

\textbf{Lessons learned: ingredients for a successful cohort study}

\textit{Start with a good idea}

A good idea does not have to be controversial or all-encompassing. It does, however, need to have scientific merit and the potential to inspire researchers long after the cohort is established. The original ‘good idea’ for this study began with Mary Raine, when she bequeathed her estate to “investigate the origins of disease in humans”. The concept of the developmental origins of health and disease (DOHaD) whereby the interplay of genetic predisposition and environmental effects on the fetus produce disease characteristics in the adult is now entrenched in our understanding of disease causation. The original grant application to establish the cohort contains many references to fetal origins of adult disease, although it preceded the DOHaD theory by several years.

\textit{Initial funding}

Long-term projects requiring ongoing financial support, and for which the most important research outcomes may not be seen for decades, do not intrinsically appeal to funding bodies. How then does a research group fund the establishment of a cohort?

The approach used by Raine researchers was to structure the study, initially, as a RCT. This has the potential for novel findings in a defined period of time, and allows recruitment and collection of baseline cohort data while simultaneously evaluating the chosen intervention.
Planning for future work

The initial RCT was well funded and well designed; however, the practicalities of following the cohort through childhood and into adulthood were not clearly defined. In the early years dedicated volunteer staff maintained the cohort on a shoestring budget until ongoing funding could be established. Planning for the future should engage not only the investigators and institutions involved in recruitment and the initial phase of the study, but also those who will be involved later as the cohort become older and the research focus changes.

Ongoing funding

Over the first 20 years the Raine Study has received 53 competitive national and international research grants; however, a cohort cannot survive on short-term project grants. Ongoing funding is necessary for staff and infrastructure. For many years core funding of the Raine cohort was through consecutive National Health and Medical Research Council (NHMRC) program grants, awarded to the Telethon Institute for Child Health Research (TICHR), now Telethon Kids Institute (TKI).

More recently core funding has been through ‘institutional buy-in’, whereby organisations associated with the study (universities, research foundations and institutes) each contribute a set amount of funds, at least $50,000 per annum, over 5 years. Beyond the financial benefit to the study, ‘investment’ in the cohort encourages research commitment from these organisations. Furthermore, while they all have a sense of ownership, multiple sources of funding ensure that no single organisation ‘owns’ the cohort or has exclusive access. An additional funding opportunity for established cohorts is to charge collaborating researchers for data access.

Given the significant contributions made by population-based studies, additional government funding is required to specifically address the longitudinal needs of cohort research.

Executive governance

The Raine Executive is chaired by the Dean of the UWA Faculty of Medicine, Dentistry and Health Sciences and consists of the original and more recent investigators, a representative of the Raine Foundation, a senior Emeritus Professor of Medicine and a scientific director. The Executive approves requests for access to data and proposed research projects, manuscripts and collaborations. There are many benefits to a transparent, centralised approval process, led by an independent body prioritising the interests of the cohort. It ensures that research is not duplicated, that all projects undertaken are of scientific merit, that authorship issues are addressed early, and that cohort data are protected. The Executive also has the important functions of determining
which data will be collected at each follow-up and of developing a long-term plan for the future of the cohort.

The Executive formed when the cohort was aged 15, but clearly the lesson from the Raine Study experience is to establish a governance structure from the outset. The Executive should include not only leaders from the major research disciplines, but also representatives from organisations with an independent interest in the future of the cohort.

Avoiding participant loss to follow-up

Initial data collection. As much demographic data as possible should be recorded. Many families will move during the early years of a child’s life, so it is imperative to collect grandparents’ names and addresses. A process of checking and updating information, including email, mobile phone and even Facebook contact details, should be in place as the cohort ages.

Engagement. The families need to feel they are ‘part of something’. The Raine Study management maintains contact with the cohort beyond requests for participation in research projects, with regular newsletters, birthday cards, a kids’ club, and engagement through social networking websites. Large functions were organised for the cohort’s 10th and 21st birthdays. Engagement is age-specific and relies heavily on energetic staff involvement.

Cohort overload. The extent to which the cohort is ‘burdened’ with research activity is carefully controlled. This includes restricting length of questionnaires, limited use of invasive procedures, frequency of assessments and even how many times participants are contacted to rebook appointments. These decisions are not made arbitrarily but are informed by research evidence. Investigators must be aware of what else is likely to be happening in participants’ lives; an extensive follow-up in the year of the cohort’s final high school examinations may not be advisable.

Consultation. A formal feedback and consultation process ensures research remains relevant and acceptable to participants, and as such Raine cohort representatives work closely Raine Study management. Participant workshops are used to plan new research, gauge what the cohort would (or would not) be prepared to do, and what information they would like returned to them.

Record linkage. Linking the cohort, with their permission, to total population data sets available in Western Australia is an effective and very cheap way to increase the information on those lost to follow-up, as well as to get more complete information on those who remain in the study. These data include complete hospitalisation, disability and mental health contacts and specific
disease registers. By linking back into total birth information, it is possible to compare how well the Raine follow-up population reflects the total population from which they were sampled, i.e. how generalisable the data are to all children and youth. As the Western Australian linkage capacity now includes data from educational, child protection and justice agencies, the cohort can be tracked in to all contacts with these services. With linkage to Commonwealth data sets such as the Pharmaceutical Benefits Scheme, their prescription drugs can be tracked as a check on morbidity.

**Impeccable ethics**

The very strictest confidentiality must be maintained, and only de-identified data distributed to researchers and collaborators. An auditing process should be established to ensure database integrity. When findings are potentially controversial they must be managed carefully; the facts do not change, but presentation might. Every question must have a valid reason for being asked, with prioritisation of scientific rigour and research output.

**What does the future hold? The next 20 years of the Raine Study.**

Twenty-three research groups have worked with the Raine cohort to date, and local, national and international collaborations are expanding (figure 5). The value of the Raine cohort will increase over time, particularly in later adult life when the major chronic diseases have developed, allowing assessment of genetic and environmental influences across the lifespan. At that point we will have truly fulfilled Mary Raine’s request in “seeking, diagnosing and investigating the nature, origin and cause of disease in human beings... and the prevention, cure, alleviation and combating of such diseases”.
Figure 3.

Graph of participant attendance at each follow up assessment.
*18-year follow-up: volunteers for Trier stress test only, no cohort assessment.
Figure 4.

Graph of publication output over time, showing exponential increase in number of publications in later years. This pattern is typical for longitudinal cohort studies, highlighting the need for identification of cohorts with sound methodology and provision of funding during the first decade.
Figure 5.

Twenty-three research groups currently work with the Raine Study. Collaboration occurs between the groups, and with other cohorts internationally. DOHaD: Developmental Origins of Health and Disease, HPA: Hypothalamic pituitary adrenal.
Chapter 2. Methods

Participants

This thesis comprises a cross-sectional analysis of 1344 participants in the Western Australian Pregnancy Cohort (Raine) Study, a population-based longitudinal birth cohort established in 1989\textsuperscript{50,99}. At the 20-year follow-up, participants underwent a comprehensive ophthalmic assessment\textsuperscript{80}.

The cohort participants were predominantly of Caucasian ethnicity; 90.3% had a Caucasian mother, 90.0% had a Caucasian father, and for 85.5% both parents were Caucasian. Chinese and Indian were the most common non-Caucasian ethnicities, with small numbers of participants having Indigenous Australian, Torres Strait Islander, Polynesian or Vietnamese ethnicities. The recruitment and follow-up were performed in Perth, Western Australia, which has a subtropical climate with a latitude of 31°60 south.

Inclusion and exclusion criteria for the initial recruitment of the Raine cohort have been previously published\textsuperscript{50}. Pregnant women of gestational age 16 to 20 weeks were enrolled from public and private antenatal clinics in Western Australia between May 1989 and November 1991. There were no specific exclusion criteria for the 20-year follow-up of their children (the Raine cohort). All 2135 active members of the Raine cohort were invited to participate, and of these 1743 (81.6%) agreed to take part. During the 24-month period from March 2010 to March 2012, 1344 (77.1%) underwent the ophthalmic examination. There was no significant difference in age or gender between those who attended the eye examination and those who did not. Those who attended the eye examination were more likely to be of Caucasian ancestry (85.4% vs 80.1%, \( p<0.001 \)).

For the reliability and validity study (chapter 3), a population-based cohort from Norfolk Island was used rather than the Raine cohort. The Norfolk Island cohort was selected for this study as it has a much wider age range (16 to 85 years) than the Raine cohort. This allowed determination of the reliability of conjunctival autofluorescence measurement in populations of all ages, thereby validating it for use in future research cohorts. Norfolk Island is an Australian territory with a subtropical climate. It is located in the Pacific Ocean at latitude 29°02 south. The Norfolk Island Eye Study (NIES) has been described in detail elsewhere\textsuperscript{100}.
**Ethics**

Ethics approval was obtained from the Human Research Ethics Committee at the University of Western Australia. For the reliability and validity study (chapter 3), ethics approval was obtained from the human research and ethics committees of Griffith University and the Royal Victorian Eye and Ear Hospital in Melbourne.

The study was conducted in accordance with the tenets of the Declaration of Helsinki and informed consent was obtained from all participants.

**Measurement of conjunctival ultraviolet autofluorescence**

High resolution ultraviolet photographs of the nasal and temporal conjunctiva were taken using a specially designed camera system\(^\text{10}\). A Nikon D100 (Nikon, Melville, New York USA) digital camera with 105mm 147 f/2.8 Micro Nikkor (Nikon, Melville, New York, USA) macro lens was used with an external electronic flash system including ultraviolet transmission filters (transmittance range 300-400nm, peak 365nm). The camera, lens and flash system were mounted on a table with an adjustable subject headrest and joystick-controlled camera positioning assembly. Photographs were taken in a dark room so only ultraviolet fluorescence was recorded by the camera.

The quality of the images was assessed on the camera digital display at the time of photography. If the initial images were decentred, defocused or had eyelid artefact, further photographs were taken as needed. The highest quality photographs for each participant were used for analysis.

Area measurement was performed using Adobe Photoshop CS4 Extend (Adobe Systems Inc, San Jose, California, USA). Four photographs were analysed for each participant (left and right eyes, nasal and temporal conjunctiva) by one investigator. The area of fluorescence in mm\(^2\) for each photograph was determined using the Lasso tool and Analysis function on the Photoshop toolbar (pixel length = 3008, logical length = 2.4), and added together to produce a total area of fluorescence in mm\(^2\) for each participant.
Pterygium assessment

High resolution colour photographs of nasal and temporal conjunctiva were taken using a Nikon D100 (Nikon, Melville, New York USA) digital camera with inbuilt flash and 105mm f/2.8 Micro Nikkor (Nikon, Melville, New York, USA) lens. The camera was mounted on a table with an adjustable subject headrest and joystick-controlled camera positioning assembly. Four photographs for each participant (left and right eyes, nasal and temporal conjunctiva) were assessed for presence or absence of pterygium (figure 6). Pterygium was considered to be present in a photograph if a wing-shaped fibrovascular abnormality was evident extending from the conjunctiva onto or beyond the corneal limbus.

Myopia and hypermetropia

Cycloplegic autorefraction was performed using the Nidek ARK-510A (Nidek Co Ltd, Japan) autorefractor. Tropicamide 1% and phenylephrine 10% eye drops were administered for mydriasis. Myopia was defined as spherical equivalent (sum of spherical error and half cylindrical error) less than -0.50 diopters. High myopia was defined as spherical equivalent less than -6.00 diopters. Hypermetropia was defined as spherical equivalent greater than +0.50 diopters. As the correlation between right and left eye spherical equivalent was high (R=0.955), only right eye spherical equivalent was used in statistical analysis.

Sun exposure and protection behaviour

Time spent outdoors in summer (none, less than ¼ of the day, ½ of the day, greater than ¾ of the day), location of leisure time in winter (mostly indoors, ½ and ½, mostly outdoors), and frequency of use of hats and sunglasses when outdoors in the sun (never, seldom, ½ of the time, usually, always), were determined by questionnaire completed at the time of the eye examination.

Other variables

Level of education (university studies started/completed), parental history of myopia, and use of glasses and contact lenses were determined by questionnaire. BMI was calculated from height (cm) and weight (kg) measured at the time of the eye examination.
Statistical analysis

Total conjunctival ultraviolet autofluorescence was analysed in quartiles and in increments of 10mm$^2$. Continuous variables were assessed for normality and summarized using mean (standard deviation [SD]) or median (interquartile range [IQR]) as appropriate. Differences between categorical variables were assessed with the chi-squared test. Differences between continuous variables were assessed with the Mann-Whitney U Test. Trends across categories were assessed using Cuzick’s non-parametric test for trend.

Logistic regression was utilised to estimate the odds ratio (OR) and 95% confidence interval (95% CI) of myopia. Covariates that were statistically significant (p<0.05) in univariable analyses were included in the multivariable models, in addition to age and sex. Separate multivariable models were constructed containing either total conjunctival ultraviolet autofluorescence or time spent outdoors, due to the expected collinearity between the two covariates. Interaction was evaluated with the likelihood ratio test.

Statistical significance was set at p<0.05. Statistical analyses were undertaken in Stata 10.1 for Macintosh (Stata-Corp, College Station, 2009).

For inter-observer reliability testing, two assessors calculated the area of ultraviolet autofluorescence in 196 photographs (49 people) and were masked to each other’s results. For the intra-observer component, one assessor determined the area of ultraviolet autofluorescence twice with the same photographs (60 photographs; 15 people). Using ultraviolet autofluorescence as a continuous variable, concordance correlation coefficients (CCC) were determined. The CCC calculates measures of both precision and accuracy to determine whether the observed data significantly deviate from the line of perfect concordance plotted at 45°. Mean difference (and SD) between the two graders (inter-observer) or measurements (intra-observer) was reported. Ninety-five per cent limits of agreement (LOA) between the two different graders or measurements were calculated by the method outlined by Bland and Altman.$^{101}$

In the reliability analysis, measurements of total ultraviolet autofluorescence were categorised into increasing increments of 5mm$^2$ or 10mm$^2$. Ultraviolet autofluorescence was also grouped into either ‘none’ (0mm$^2$) or ‘any’ ultraviolet autofluorescence. For this approach, proportion of agreement and the Kappa statistic (k) were used to measure agreement between graders. Interpretation of k was performed according to the guidelines provided by Altman.$^{102}$
For the validity study, ultraviolet autofluorescence photographs were used to validate degree of ultraviolet autofluorescence against a questionnaire-based assessment of outdoor activity. Trends across categorical variables were assessed using Cuzick’s nonparametric test for trend or Kendall’s rank correlation. The OR of having an ultraviolet autofluorescence area in the first quartile compared with the other three quartiles, according to outdoor activity level, was performed using logistic regression.
Figure 6.

Ultraviolet autofluorescence of established pterygia
a-d) Colour photographs of a participant with bilateral nasal pterygia
e-h) Ultraviolet photographs of the same participant
Chapter 3. Reliability and validity of conjunctival ultraviolet autofluorescence measurement

**Inter-observer reliability assessment**

196 conjunctival ultraviolet autofluorescence photographs of 49 people were evaluated. Mean age was 53.3 +/- 14.2 years (range 16-85 years) and 28 (57.1%) were women. A histogram of inter-observer differences revealed that differences in measurements between the two assessors approximated a normal distribution (not shown).

CCCs were performed for all conjunctival ultraviolet autofluorescence photograph combinations (table 1). The highest CCC corresponded to the total conjunctival ultraviolet autofluorescence category. Difference in mean area between graders, and 95% LOA increased with increasing numbers of photos contributing to the autofluorescence measurement.

Inter-observer reliability was also assessed using normal (Q-Q) and repeatability plots of total conjunctival ultraviolet autofluorescence (figure 7). The linearity of the Q-Q plot demonstrated a normal distribution. Variation between assessors was mildly dependent on the magnitude of measurements. When the area of autofluorescence was much greater, the variation between the two observers increased slightly. There was no evidence of a major systematic bias. The null hypothesis of equal variance between the two measurements was not rejected for total conjunctival ultraviolet autofluorescence (Pitman’s test of difference in variance, r=0.099, p=0.500).

For total conjunctival ultraviolet autofluorescence categories delineated by 10mm², agreement was good: agreement = 79.6%, k statistic (k) = 0.71, p<0.001. Reliability was reduced, but still good, with 5mm² categories: agreement = 73.5%, k = 0.61, p<0.001. Reliability of presence or absence of autofluorescence revealed: agreement = 98.0%, k = 0.79, p<0.001.

**Intra-observer reliability assessment**

The total conjunctival ultraviolet autofluorescence category was associated with a substantial strength of agreement, greater than that of the inter-observer analysis. Mean differences between the two measurements, and in the 95% LOA, were considerably less compared with the results of the inter-observer analysis. A histogram of differences between the two measurements approximated a normal distribution, as supported by the normal (Q-Q) plot. There was no
evidence of systematic bias. Pitman’s test for total conjunctival ultraviolet autofluorescence revealed $r=0.033$, $p=0.801$. For both 5mm$^2$ and 10mm$^2$ categories, reliability was very high: agreement = 86.7%, $k = 0.81$, $p<0.001$ for both categories.

**Association between conjunctival ultraviolet autofluorescence and outdoor activity**

Total conjunctival ultraviolet autofluorescence increased with increasing time outdoors in both genders. For men, median autofluorescence increased across the three groups of outdoor activity (p trend <0.001): <1/4 day outside 28.3 mm$^2$ (IQR 11.4-37.0); ~1/2 day outside 32.8 mm$^2$ (IQR 17.6-55.7); >3/4 day outside 45.1 mm$^2$ (IQR 26.6-66.1). Similarly, for women, median total ultraviolet autofluorescence increased from 20.0 mm$^2$ (IQR 9.1-34.1) to 25.5 mm$^2$ (IQR 15.2-45.5) to 35.0 mm$^2$ (IQR 17.8-63.9) across the three categories (p trend<0.001). Median conjunctival ultraviolet autofluorescence increased across the three categories when both sexes were analysed together.

There was also a significant trend of increasing median conjunctival ultraviolet autofluorescence with increasing time spent outdoors in winter (p trend<0.001): mostly indoors 23.3 mm$^2$ (IQR 9.7-35.2); half indoors/outdoors 28.7 mm$^2$ (13.8-49.0); mostly outdoors 33.9 mm$^2$ (IQR 21.6-58.1).

**Discussion**

These findings demonstrate that conjunctival ultraviolet autofluorescence is a highly reliable measurement, and differences between intra- and inter-observer reliability measurements were minor. Conjunctival ultraviolet autofluorescence correlated very well with questionnaire-based outdoor activity level.

The highest CCCs were for total conjunctival ultraviolet autofluorescence measurements, representing sum of area in four measurements in both eyes; k statistics for total autofluorescence in 5mm$^2$ or 10mm$^2$ increments indicated good and very good agreement, respectively. Possibly of more importance is the ability to discriminate between individuals with and without any discernible conjunctival ultraviolet autofluorescence, and our inter-observer agreement was nearly perfect at 98%. The inter-observer CCC figure revealed that the variation between the measurements increased as the degree of conjunctival ultraviolet autofluorescence increased, as displayed by increased numbers of outlier values. This may be expected, as delineation between
an area of autofluorescence and no autofluorescence is often equivocal, especially when the quality of the image is reduced.

The mean difference between graders (inter-observer) and measurements (intra-observer) was approximately 3 and 1.5mm$^2$, respectively for total conjunctival ultraviolet autofluorescence. The corresponding 95% LOA was between -13.7 and 19.7mm$^2$ and between -5.3 and 2.4mm$^2$, for inter- and intra-reliability measures, respectively.

Median conjunctival ultraviolet autofluorescence correlates well with outdoor activity and can be considered a valid biomarker for usual outdoor exposure in both summer and winter.

There remains some uncertainty about what the area of autofluorescence precisely represents, but there are several possibilities. Conjunctival ultraviolet autofluorescence may represent changes in extracellular matrix that are features of pterygium, or areas of cellular activity, and/or altered stem cells. It has been proposed that ultraviolet-inducible cytokines, growth factors and matrix metalloproteinases contribute to the histological changes in pterygium that are similar to those seen in photo-aged skin. Knowledge of the precise composition of conjunctival ultraviolet autofluorescence is vital as it may pave the way for specific local or systemic therapies to retard formation or progression of ophthalmohelioses.

There were several limitations inherent in this study. The findings were obtained in a small, geographically isolated population. Even so, there may be benefits in performing this analysis in a population with a homogenous environment (including latitude, weather patterns and ultraviolet radiation exposure). The study was also limited by use of a questionnaire-based assessment of outdoor exposure, which was only categorised into three groups. Finally, the numbers of measurements used in the reliability analyses, especially intra-observer reliability, were relatively small.

In conclusion, this study shows that conjunctival ultraviolet autofluorescence is a highly reliable measurement that correlates strongly with the level of outdoor activity in the Norfolk Island cohort.
Table 1.

Inter-observer reliability
Concordance correlation coefficients (CCCs), inter-grader difference and 95% limits of agreement (LOA) for inter-observer reliability conjunctival ultraviolet autofluorescence measurement

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CCC (95% CI)</th>
<th>p Value</th>
<th>Difference between graders (mm&lt;sup&gt;2&lt;/sup&gt;) mean (SD)</th>
<th>95% LOA (mm&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right nasal (1 photo per participant)</td>
<td>0.861 (0.770 to 0.918)</td>
<td>&lt;0.001</td>
<td>-0.01 (3.62)</td>
<td>-7.11 to 7.08</td>
</tr>
<tr>
<td>Right temporal (1 photo per participant)</td>
<td>0.918 (0.868 to 0.948)</td>
<td>&lt;0.001</td>
<td>1.14 (2.49)</td>
<td>-3.74 to 6.01</td>
</tr>
<tr>
<td>Left nasal (1 photo per participant)</td>
<td>0.872 (0.786 to 0.925)</td>
<td>&lt;0.001</td>
<td>0.99 (2.82)</td>
<td>-4.53 to 6.52</td>
</tr>
<tr>
<td>Left temporal (1 photo per participant)</td>
<td>0.827 (0.723 to 0.894)</td>
<td>&lt;0.001</td>
<td>0.89 (3.83)</td>
<td>-6.61 to 8.40</td>
</tr>
<tr>
<td>Total nasal (sum of 2 photos)</td>
<td>0.894 (0.821 to 0.939)</td>
<td>&lt;0.001</td>
<td>0.99 (5.38)</td>
<td>-9.55 to 11.52</td>
</tr>
<tr>
<td>Total temporal (sum of 2 photos)</td>
<td>0.909 (0.851 to 0.954)</td>
<td>&lt;0.001</td>
<td>2.04 (5.24)</td>
<td>-8.23 to 12.31</td>
</tr>
<tr>
<td>Total left (sum of 2 photos)</td>
<td>0.908 (0.828 to 0.944)</td>
<td>&lt;0.001</td>
<td>1.09 (4.77)</td>
<td>-7.45 to 11.23</td>
</tr>
<tr>
<td>Total right (sum of 2 photos)</td>
<td>0.913 (0.852 to 0.950)</td>
<td>&lt;0.001</td>
<td>1.12 (5.27)</td>
<td>-9.21 to 11.46</td>
</tr>
<tr>
<td>Total ultraviolet autofluorescence (sum of 4 photos)</td>
<td>0.924 (0.870 to 0.956)</td>
<td>&lt;0.001</td>
<td>3.02 (8.52)</td>
<td>-13.67 to 19.71</td>
</tr>
</tbody>
</table>
Figure 7.

Q-Q and repeatability plots
a) Normal (Q-Q) plot of inter-reliability measurement of total conjunctival ultraviolet autofluorescence.

b) Repeatability plot of inter-reliability measurement of total conjunctival ultraviolet autofluorescence. The dashed line at 45° equals the line of perfect concordance.
Chapter 4. Extent and distribution of conjunctival ultraviolet autofluorescence

Distribution of conjunctival ultraviolet autofluorescence

Of 1344 participants who attended the eye examination, 16 were excluded due to inadequate or incomplete conjunctival ultraviolet autofluorescence photographs. 1328 participants (98.8%) were included in this analysis. The mean age of participants at the time of the examination was 20.0 years (standard deviation 0.45, range 18.3-22.1 years). 684 participants (51.5%) were male.

When comparing cohort participants who attended the eye examination with those who did not, there was no clinically relevant difference in age (mean age [January 2014] 22.98 years vs 23.03 years, p=0.047) or gender (males 51.5% vs 50.1%, p=0.45). As with other Raine follow-up studies, those who attended the eye examination were more likely to be of Caucasian ancestry (85.4% vs 80.1%, p<0.001).

Total conjunctival ultraviolet autofluorescence was non-normally distributed (skewness = 0.66, kurtosis = 3.17). Median total conjunctival ultraviolet autofluorescence was 44.2mm$^2$ (interquartile range 20.2-69.8mm$^2$). 47 participants (3.5%) had no detectable conjunctival ultraviolet autofluorescence, and the highest total autofluorescence measurement was 180.3mm$^2$.

There was no difference in median conjunctival ultraviolet autofluorescence between right eyes and left eyes (22.3mm$^2$ vs 22.6mm$^2$, p=0.661). Median autofluorescence was higher in nasal quadrants than in temporal quadrants (23.8mm$^2$ vs 18.9mm$^2$, p<0.001). The season in which the eye examination was performed showed no association with measured conjunctival ultraviolet autofluorescence.

Associations with conjunctival ultraviolet autofluorescence

Median total conjunctival ultraviolet autofluorescence was higher in males than females; however, this was not statistically significant (45.6mm$^2$ vs 43.2mm$^2$, p=0.079). There was no association between age and total conjunctival ultraviolet autofluorescence (p=0.170); however, the spread of participant ages was small.

BMI was inversely associated with total conjunctival ultraviolet autofluorescence (-0.39mm$^2$ per unit BMI, 95% CI -0.75 to -0.03, p=0.035). This remained significant after adjustment for age, gender and height (-0.38mm$^2$, 95% CI -0.74 to -0.02, p=0.033). Obese participants (BMI > 30)
had significantly lower median total ultraviolet autofluorescence than non-obese participants (36.5 mm$^2$ [IQR 14.7 – 61.3] vs 45.2 mm$^2$ [IQR 21.0 – 71.2], p=0.004).

Total conjunctival ultraviolet autofluorescence increased with increasing time spent outdoors in summer and winter, p trend <0.001 (figure 8).

There was no association between frequency of wearing sunglasses and total conjunctival ultraviolet autofluorescence. There was a positive association between wearing a hat and total conjunctival ultraviolet autofluorescence, with increasing median autofluorescence as frequency of hat wear increased (p trend = 0.031).

Participants with no detectable conjunctival ultraviolet autofluorescence (n=47) spent less time outdoors than participants with detectable conjunctival ultraviolet autofluorescence. 30.3% of participants with no conjunctival ultraviolet autofluorescence spent half or more of the day outside, compared with 51.6% of the rest of the cohort (p=0.016). There was no difference in gender or obesity between the groups.

**Discussion**

This study describes the population distribution of conjunctival ultraviolet autofluorescence. The NIES$^{10}$, which looked at 641 individuals aged 15 to 89 years, also reported distribution of conjunctival ultraviolet autofluorescence in their cohort. Median total conjunctival ultraviolet autofluorescence in the NIES was 28.2 mm$^2$, much lower than the median total autofluorescence of 44.2 mm$^2$ observed in this study. Geographic differences in ultraviolet exposure are unlikely to explain the discrepancy; Perth and Norfolk Island both have subtropical climates and are located at similar latitudes (31°S and 29°S, respectively).

It is possible that the age range of participants accounts for some or all of the difference in median conjunctival ultraviolet autofluorescence between these studies. In the NIES there was a significant inverse relationship between conjunctival ultraviolet autofluorescence and age, with less autofluorescence observed in older participants. It is not known whether this represents the natural history of the conjunctival autofluorescence phenomenon, or if it is a cohort effect of different ultraviolet exposure patterns between generations. In either case, the Raine Study includes only young adults, which is the age group found in the NIES to have the most conjunctival ultraviolet autofluorescence. The relationship between age and conjunctival ultraviolet autofluorescence is interesting; in a cross-sectional study of children aged 3 to 15
years\textsuperscript{6} conjunctival ultraviolet autofluorescence was found to increase with age, the opposite of the relationship observed in the NIES. It is possible that the young adults in the Raine Study represent the peak of the autofluorescence age curve. Longitudinal data are needed, and to this end a follow-up assessment of the NIES cohort is planned.

A study of 307 European eye care practitioners aged 19 to 68 years\textsuperscript{11} found even lower levels of conjunctival ultraviolet autofluorescence. Median autofluorescence in the right eye was 2.9mm\textsuperscript{2} nasally and 2.1mm\textsuperscript{2} temporally, compared with 7.1mm\textsuperscript{2} and 6.8mm\textsuperscript{2}, respectively, in NIES, and 12.0mm\textsuperscript{2} and 10.1mm\textsuperscript{2}, respectively, in Raine. The lower conjunctival ultraviolet autofluorescence observed in the European study is likely due to lower levels of environmental ultraviolet light exposure in the home countries of participants. Unfortunately, total conjunctival autofluorescence was not reported, limiting comparison between the studies.

The observation that participants with higher BMI had less conjunctival ultraviolet autofluorescence is interesting and to our knowledge has not previously been reported. On average, obese participants had 20\% less conjunctival ultraviolet autofluorescence than non-obese participants (36.5mm\textsuperscript{2} vs 45.2 mm\textsuperscript{2}). It could be speculated that an active, outdoor lifestyle might result in both lower body weight and more sun exposure, compared with a sedentary, predominantly indoor lifestyle.

Increased frequency of wearing sunglasses did not protect against development of conjunctival ultraviolet autofluorescence, even when those with high levels of outdoor activity were considered alone. This is interesting, as current sun-protection guidelines published in a joint position statement from the Royal Australian and New Zealand College of Ophthalmologists and Cancer Council Australia\textsuperscript{104} recommend wearing close-fitting, wraparound style sunglasses to protect the eyes from ultraviolet radiation. As this study did not differentiate between different styles of sunglasses, no comment can be made about the effect of wraparound sunglasses specifically on conjunctival ultraviolet autofluorescence.

Hats did not confer any protective effect against conjunctival ultraviolet autofluorescence, as has been previously demonstrated for hat wear and pterygium\textsuperscript{105}. Participants who spent more time outdoors had more autofluorescence, and were also more likely to wear a hat (data not shown), explaining the observed positive correlation between hat wear and conjunctival ultraviolet autofluorescence.

The association of conjunctival ultraviolet autofluorescence with pterygium is reported in chapter 5. The relationship of conjunctival ultraviolet autofluorescence to other ophthalmohelioses,
including cataract, ocular surface squamous neoplasia and eyelid malignancy has yet to be
determined. Conjunctival ultraviolet autofluorescence photography may have a role in research
into the pathophysiology of these diseases, and has potential for use in health promotion and
patient education regarding ocular sun protection.
Conjunctival ultraviolet autofluorescence and participant recall of outdoor time measured by questionnaire.
Chapter 5: Pterygium and conjunctival ultraviolet autofluorescence

Prevalence of pterygium

Prevalence of pterygium in either eye was 1.2% (95% CI 0.6-1.8%). Three participants had bilateral pterygium; the other 13 participants had unilateral pterygium. In most cases the pterygium occurred on the nasal conjunctiva, with only two pterygia observed on the temporal conjunctiva.

Pterygium was more common in males than females, 2.0% (95% CI 1.0-3.1) vs 0.3% (95% CI 0.0-0.7), p=0.004. The odds of having pterygium was higher in males than females (OR 6.71 [95% CI 1.51-29.90], p=0.012). Age was not significantly associated with pterygium (OR 1.22 per year [95% CI 0.43-3.50], p=0.706).

Pterygium and conjunctival ultraviolet autofluorescence

Median total conjunctival ultraviolet autofluorescence was higher in participants who had pterygium than those who did not, 73.4mm$^2$ (IQR 48.3-94.7) vs 44.0mm$^2$ (IQR 20.2-69.0), p=0.001. There was no difference in median conjunctival ultraviolet autofluorescence between male and female participants with pterygium (73.4mm$^2$ vs 77.5mm$^2$, p=0.874). For every 10mm$^2$ increase in autofluorescence, odds of pterygium increased by 23% (OR 1.23 [95% CI 1.09-1.39], p=0.001).

When conjunctival ultraviolet autofluorescence was considered in quartiles, each additional quartile was associated with two and a half times the odds of pterygium (OR 2.52, 95% CI 1.39-4.60, p trend = 0.002). In the lowest quartile (least autofluorescence) there were no participants with pterygium. The second and third quartiles each had three participants with pterygium, while the highest quartile (most autofluorescence) had ten participants with pterygium (p trend < 0.001).

Other associations with pterygium

61.5% of participants with pterygium reported spending half the day or more outdoors in summer compared with 50.8% of participants without pterygium, however the difference was not statistically significant (p=0.441).
Height, weight and level of education showed no significant association with pterygium.

When BMI was assessed as a continuous variable, there was no association with pterygium (OR 0.92, 95% CI 0.80-1.04, p=0.189). There was a significant difference in rates of overweight/obesity, with 6.3% of participants with pterygium meeting criteria for overweight/obesity (BMI > 25) compared with 33.8% of participants without pterygium (chi-square p=0.020). When obesity (BMI > 30) was considered alone, the difference was not significant (chi-square p=0.128).

Discussion

In this population of young Australian adults, pterygium was present in 2% of males (1 in 50) and 0.3% of females (1 in 330). Prevalence of pterygium is known to increase with age, and it is likely that rates of pterygium in this cohort will increase as the participants pass into later adulthood. Epidemiological studies of pterygium in Australia have found a wide range of prevalence estimates, related to differences in the age, ethnicity and latitude of the study population. The National Trachoma and Eye Health Program\textsuperscript{18} looked at all ages, and found a pterygium prevalence of 1.1% in the non-Aboriginal population and 3.4% in the Aboriginal population. More recently the Central Australian Ocular Health Study\textsuperscript{19} reported pterygium prevalence of 9.3% in Aboriginal adults. The Melbourne Visual Impairment Project\textsuperscript{20}, Blue Mountains Eye Study\textsuperscript{21} and NIES\textsuperscript{22} reported pterygium prevalence of 2.8%, 7.3% and 10.9%, respectively, reflecting the decreasing latitude of the study population (37°S, 33°S and 29°S). Worldwide, prevalence estimates of pterygium vary according to study location and demographics. A recent meta-analysis looked at data from 20 population-based studies published in English and Chinese and found an overall pooled prevalence of 10.2\textsuperscript{106}.

The finding that participants with pterygium were less likely to be overweight or obese than those without pterygium (6.3% vs 33.8%) is interesting, and supports the association between BMI and conjunctival ultraviolet autofluorescence described in chapter 4. Caution must be used in interpreting these results, particularly as BMI overall did not show an association with pterygium. Clearly more research is needed in this area.

It is well established that pterygium and ocular surface squamous neoplasia are more common in males than females\textsuperscript{106,107}, attributed to differences in occupational and recreational ultraviolet exposure between genders. Certainly in this study males had more than six times the odds of
pterygium than females. It is interesting, then, that although males had slightly more conjunctival ultraviolet autofluorescence than females the difference did not reach statistical significance (see chapter 4). This was also the case with self-reported outdoor activity by questionnaire; males spent more time outdoors in summer than females, but the difference was not significant (data not shown). If both conjunctival ultraviolet autofluorescence and questionnaire data suggest that there was little difference in ocular ultraviolet exposure between the genders in this cohort, why was pterygium so much more common in males? It is possible that early-onset pterygium has a particular predilection for males, or perhaps that males are in some way inherently more sensitive to even moderate increases in ultraviolet light exposure.

The nasal conjunctiva had higher levels of ultraviolet autofluorescence than the temporal conjunctiva, and was more affected by pterygium, likely due to corneal light-focusing across the anterior chamber. Total conjunctival ultraviolet autofluorescence was significantly higher in participants with pterygium compared with the rest of the cohort, presumably due to higher levels of ocular ultraviolet light exposure. Odds of having pterygium increased with increasing quartile of autofluorescence, demonstrating a dose-response relationship. This relationship was also observed in the NIES, and is of particular interest as it demonstrates that the asymptomatic and essentially invisible phenomenon of conjunctival autofluorescence has a direct link to real-world clinical ophthalmic disease.
Chapter 6: Myopia and conjunctival ultraviolet autofluorescence

Prevalence of myopia

Of 1344 participants who attended the ophthalmic examination, 13 were excluded due to absence of cycloplegic autorefraction (refused dilation) and 16 were excluded due to inadequate or incomplete autofluorescence photographs. 1315 participants (97.8%) had complete autorefraction and conjunctival ultraviolet autofluorescence data.

The mean age of participants was 20.1 years (SD 0.46) and 684 (51.5%) were male. 311 participants (23.7%) were myopic (95% CI 21.4-25.9). There was no significant difference between myopia prevalence in males compared with females, 22.7% (95% CI 19.6-25.9) vs 24.6% (95% CI 21.3-28.0), p=0.410.

Median conjunctival ultraviolet autofluorescence was lower in myopes than non-myopes, 31.9mm$^2$ (IQR 10.6-55.9) vs 47.9mm$^2$ (IQR 23.2-72.5), p<0.001.

Associations with myopia

Variables associated with myopia are shown in table 2. Increasing age, parental myopia and tertiary education were associated with increased odds of myopia, although due to the limited age range in the cohort (19 to 22 years) age only reached statistical significance in the adjusted model. Higher level of conjunctival ultraviolet autofluorescence was associated with decreased odds of myopia, as was participant recall of increased time outdoors during summer and winter. Gender, height, weight and BMI were not associated with myopia.

As they were not significant in the univariable analysis height, weight and BMI were not included in the multivariable models (table 3 and table 4). Participant recall of time outdoors was not included in the multivariable analysis due to its collinearity with conjunctival ultraviolet autofluorescence, but when it replaced conjunctival ultraviolet autofluorescence in the multivariable analysis it remained significantly inversely associated with myopia (data not shown). Conjunctival ultraviolet autofluorescence showed a stronger inverse association with myopia than participant recall of time outdoors. There was no interaction between education and
conjunctival ultraviolet autofluorescence (p interaction = 0.482) or between parental myopia and conjunctival ultraviolet autofluorescence (p interaction = 0.141).

**Analysis by increments of 10mm²**

Prevalence of myopia decreased with increasing conjunctival ultraviolet autofluorescence (figure 9). For every 10mm² reduction in total conjunctival ultraviolet autofluorescence, odds of myopia increased by 15% (OR 1.15, 95%CI 1.10-1.19, p<0.001). This remained significant after adjustment for age, gender, educational level and parental history of myopia (OR 1.12, 95%CI 1.08-1.19, p<0.001).

**Analysis by quartiles**

Prevalence of myopia decreased with increasing quartiles of conjunctival ultraviolet autofluorescence. In the first quartile (least autofluorescence) prevalence of myopia was 33.0%. Prevalence was 27.9% in the second quartile, 18.0% in the third quartile and 15.6% in the fourth quartile (most autofluorescence). This was statistically significant (trend across quartiles p<0.001).

Participants in the first quartile had more than two and a half times the odds of myopia than participants in the fourth quartile (OR 2.67, 95%CI 1.83-3.89, p<0.001). This remained significant after adjustment for age, gender, educational level and parental history of myopia (OR 2.52, 95%CI 1.66-3.81, p<0.001).

For every quartile increase in conjunctival ultraviolet autofluorescence, refraction increased by 0.24 diopters (95%CI 0.16-0.31, p trend<0.001). After adjustment this remained significant, with refraction increasing by 0.21 diopters (95%CI 0.13-0.29, p trend<0.001) for every quartile increase in conjunctival ultraviolet autofluorescence. When myopes were considered alone, the effect of increasing quartiles was higher, with refraction increasing by 0.37 diopters (95%CI 0.17-0.56, p<0.001) per quartile in the unadjusted analysis and 0.33 diopters (95%CI 0.14-0.53, p trend= 0.001) per quartile after adjustment.
Use of glasses and contact lenses

To determine whether the inverse relationship between conjunctival ultraviolet autofluorescence and myopia was due to a protective ultraviolet-blocking effect from corrective lenses (i.e. whether the reduced autofluorescence in myopes was due to lower amounts of ultraviolet light reaching the conjunctiva after being filtered by glasses or contact lenses), conjunctival ultraviolet autofluorescence was compared between myopes who wore corrective lenses and hypermetropes who wore corrective lenses. Conjunctival ultraviolet autofluorescence remained significantly lower in the myopes compared to the hypermetropes (median 31.9mm² vs 43.8mm², p<0.001)

No difference in conjunctival ultraviolet autofluorescence was seen between myopes who wore corrective lenses and those who didn’t (median 31.9mm² vs 31.6mm², p=0.821). Similarly, no difference was seen between hypermetropes who wore corrective lenses and those who didn’t (43.8mm² vs 49.1mm², p=0.144). There remained no significant difference after adjustment for potential confounders.

High myopia and other refractive error

There were 17 participants with high myopia (SE <-6.00 diopters). Median conjunctival ultraviolet autofluorescence in high myopes was 20.7mm² (IQR 2.6-35.8). Eight participants were in the first quartile (least autofluorescence), seven were in the second quartile, two were in the third quartile, and no participants were in the fourth quartile (p trend <0.001). For every 10mm² reduction in total conjunctival ultraviolet autofluorescence, odds of high myopia increased by 8% (OR 1.08, 95%CI 1.01-1.15, p=0.04). Adjusted analyses were not performed due to small numbers in the high myopic group.

There was no difference in median conjunctival ultraviolet autofluorescence between emmetropes and hypermetropes, 47.4mm² (IQR 22.6-72.2) vs 48.2mm² (IQR 23.2-74.9), p=0.572.

Conjunctival ultraviolet autofluorescence and outdoor exposure recall

Conjunctival ultraviolet autofluorescence showed a strong correlation with participant recall of time spent outdoors, even in winter when environmental ultraviolet radiation is relatively reduced
Median conjunctival ultraviolet autofluorescence increased with longer duration of time outdoors in both summer and winter (p trend <0.001).

**Discussion**

The results of this study demonstrate a strong inverse relationship between myopia and conjunctival ultraviolet autofluorescence, an objective biomarker of ocular sun exposure and outdoor time. The relationship remained significant after adjustment for age, gender, educational level, and parental history of myopia. The difference in prevalence of myopia between the groups appears to be clinically significant as well as statistically significant, with more than two and a half times the odds of myopia in the first quartile compared with the fourth.

The relationship between conjunctival ultraviolet autofluorescence and high myopia (SE <-6.00D) was also considered. Median conjunctival ultraviolet autofluorescence in the high myopic group was 20.7mm², significantly lower than in myopes overall (31.9mm²) and non-myopes (47.9mm²). This demonstrates an interesting dose-response effect, with the lowest levels of autofluorescence seen in participants with the most severe myopia. Caution should be exercised in interpreting these results due to small numbers of high myopes in the study population. Further studies looking specifically at conjunctival ultraviolet autofluorescence and high myopia are needed.

An inverse relationship between conjunctival ultraviolet autofluorescence and myopia has been previously reported in a population-based study of 636 Norfolk Island residents aged 15-89 years, although findings were limited by the population sample size and incomplete data on potential confounders. This study includes more than double the number of participants and reports the effect of parental myopia and level of education, important variables to consider when analysing associations with myopia. The narrow age range of this study compared with the Norfolk Island population also reduces confounding by eliminating age cohort differences in outdoor activity patterns or myopia prevalence.

This study addresses the question of whether reduced conjunctival ultraviolet autofluorescence in myopes might be due to an ultraviolet-protective effect from corrective lenses rather than differences in outdoor time between the groups. The significantly lower autofluorescence in myopes was still observed when only participants who wore corrective lenses were included in the analysis, indicating that an ultraviolet-protective effect was unlikely. Further evidence against an ultraviolet-protective effect was the observation that no differences in autofluorescence were observed between myopes who wore corrective lenses and those who did not. This finding is
interesting as most commercially available spectacle lenses have ultraviolet filters. It has been previously shown that light rays entering the eye obliquely are focused most intensely at the corneo-scleral limbus \(^2\) (the site of conjunctival ultraviolet autofluorescence), and it may be that these oblique rays are not blocked by conventional spectacle design. Ultraviolet-filtering contact lenses, however, may provide some protection against oblique corneal rays. Further research into the protective effect of corrective lenses is needed.

Conjunctival ultraviolet autofluorescence was more strongly associated with myopia than participant recall of time outdoors. This may indicate that autofluorescence photography is a more robust and reliable method of determining outdoor exposure than participant questionnaire. Certainly there is inherent imprecision in questionnaire categories (such as when estimating the proportion of the day spent outdoors), with potential for misclassification bias. Further, outdoor time may or may not directly represent sun exposure. It is also possible that ocular sun exposure, which conjunctival photography measures specifically, is more important in the process of emmetropisation than systemic exposure. The potential benefit of objective, rather than subjective, measurement of outdoor time is self-evident. Using wearable light sensor devices is one approach to objectively quantifying outdoor time and light exposure, and these devices are currently being trialled in myopia research. They have the advantage of providing prospectively collected, detailed data on current light exposure, whereas conjunctival ultraviolet autofluorescence photography might better reflect cumulative lifetime light exposure. There may also be a consumer market for these devices, with growing interest generally in gadgets that provide quantified self-data (Tictrac dashboard, fitbit, Jawbone UP, Nike+ FuelBand and others).

Conjunctival ultraviolet autofluorescence photography has been shown to have high reliability, with intra-observer and inter-observer CCCs exceeding 0.900 as detailed in chapter 3. The validity of the measurement as a biomarker of ocular sun exposure and outdoor time is supported by the strong correlation between total conjunctival ultraviolet autofluorescence and outdoor time measured by questionnaire (\(p\) trend across categories <0.001). The reliability and validity of autofluorescence photography is a strength of this study. Other strengths include the large sample size and narrow age range of participants. The predominantly Caucasian ethnicity may reduce confounding by Caucasian differences in patterns of conjunctival ultraviolet autofluorescence or myopia, but does limit application of the results to non-Caucasian populations.

It should be emphasised that the study has a cross-sectional design, and thus does not demonstrate a causal link between myopia and outdoor time, only an association. Any causality (if present) could be working in either direction, and it could be argued that different activity patterns between myopes and non-myopes might result in different levels of conjunctival ultraviolet.
autofluorescence. Clearly prospective data on conjunctival ultraviolet autofluorescence and development or progression of myopia are needed. The longitudinal cohort design of the Raine Study does allow for further ophthalmic examinations to be performed at future follow-up assessments, and this has the potential to provide valuable prospective data.

Serum vitamin D level is known to be associated with sun exposure and outdoor time, and it is possible that it may play a role in myopia development. Serum measurement of vitamin D has been tested in the Raine cohort, and it will be interesting to analyse the association between vitamin D, conjunctival ultraviolet autofluorescence and myopia over time.

Mechanisms underlying the association between outdoor time and myopia have been recently reviewed. Although causality cannot be determined from this cross-sectional study, it confirms a strong inverse relationship between ocular sun exposure and myopia. As increasing outdoor activity has potential as a low-risk intervention for preventing myopia, this study provides further evidence of the need for interventional trials, particularly given the rising burden of myopia worldwide.
### Table 2.

Univariable associations with myopia

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>1.20</td>
<td>0.91 – 1.60</td>
<td>0.191</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>1.11</td>
<td>0.86 – 1.44</td>
<td>0.410</td>
</tr>
<tr>
<td>Parental Myopia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No parents (ref)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One parent</td>
<td>2.17</td>
<td>1.57 – 3.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Both parents</td>
<td>3.37</td>
<td>2.01 – 5.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University started/completed</td>
<td>1.81</td>
<td>1.47 – 2.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (per 10 cm)</td>
<td>0.94</td>
<td>0.82 – 1.07</td>
<td>0.343</td>
</tr>
<tr>
<td>Weight (per 10 kg)</td>
<td>1.04</td>
<td>0.97 – 1.11</td>
<td>0.296</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.02</td>
<td>0.99 – 1.04</td>
<td>0.102</td>
</tr>
<tr>
<td>Time spent outdoors in summer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than ½</td>
<td>2.00</td>
<td>1.18 – 3.42</td>
<td>0.010</td>
</tr>
<tr>
<td>~ 1/2</td>
<td>1.37</td>
<td>0.79 – 2.37</td>
<td>0.265</td>
</tr>
<tr>
<td>More than 3/4</td>
<td>1</td>
<td>(ref)</td>
<td></td>
</tr>
<tr>
<td>Time outdoors in winter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mostly indoors</td>
<td>1.85</td>
<td>1.01 – 3.39</td>
<td>0.046</td>
</tr>
<tr>
<td>50/50</td>
<td>1.18</td>
<td>0.63 – 2.21</td>
<td>0.607</td>
</tr>
<tr>
<td>Mostly outdoors (ref)</td>
<td>1</td>
<td>(ref)</td>
<td></td>
</tr>
<tr>
<td>Conjunctival ultraviolet autofluorescence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(per 10mm² decrease)</td>
<td>1.15</td>
<td>1.10 – 1.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3.

Multivariable associations with myopia. Odds of myopia increased with decreasing conjunctival ultraviolet autofluorescence (decreasing ocular sun exposure).

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>1.42</td>
<td>1.03-1.95</td>
<td>0.03</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.87</td>
<td>0.65-1.16</td>
<td>0.34</td>
</tr>
<tr>
<td>Parental Myopia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No parents</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One parent</td>
<td>1.98</td>
<td>1.41-2.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Both parents</td>
<td>2.98</td>
<td>1.80-4.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University started/completed</td>
<td>1.66</td>
<td>1.24-2.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Conjunctival ultraviolet autofluorescence (per 10mm² decrease)</td>
<td>1.12</td>
<td>1.08 – 1.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4.

Multivariable associations with myopia. Conjunctival ultraviolet autofluorescence (ocular sun exposure) measured in quartiles. 1\textsuperscript{st} quartile had the least autofluorescence, 4\textsuperscript{th} quartile had the most autofluorescence.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>1.42</td>
<td>1.03-1.96</td>
<td>0.03</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.89</td>
<td>0.66-1.19</td>
<td>0.43</td>
</tr>
<tr>
<td>Parental Myopia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No parents</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One parent</td>
<td>2.01</td>
<td>1.44-2.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Both parents</td>
<td>2.98</td>
<td>1.80-4.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University started/completed</td>
<td>1.67</td>
<td>1.25-2.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Conjunctival ultraviolet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>autofluorescence 4\textsuperscript{th} quartile (&gt;69.8mm\textsuperscript{2})</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3\textsuperscript{rd} quartile (44.2-69.8mm\textsuperscript{2})</td>
<td>1.07</td>
<td>0.69-1.68</td>
<td>0.760</td>
</tr>
<tr>
<td>2\textsuperscript{nd} quartile (20.2-44.2mm\textsuperscript{2})</td>
<td>1.64</td>
<td>1.08-2.51</td>
<td>0.021</td>
</tr>
<tr>
<td>1\textsuperscript{st} quartile (&gt;20.2mm\textsuperscript{2})</td>
<td>2.52</td>
<td>1.66-3.81</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 9.

Myopia and conjunctival sun exposure. Myopia prevalence decreased with increasing area of conjunctival ultraviolet autofluorescence (participants with more sun exposure had less myopia).
Table 5.

Conjunctival ultraviolet autofluorescence and participant recall of outdoor time measured by questionnaire.

<table>
<thead>
<tr>
<th>Questionnaire response</th>
<th>Median ultraviolet autofluorescence (mm$^2$)</th>
<th>Interquartile range</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>What part of the day do you spend outside in summer?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>24.8</td>
<td>11.0-55.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Less than ¼ of the day</td>
<td>36.6</td>
<td>15.7-61.4</td>
<td></td>
</tr>
<tr>
<td>½ of the day</td>
<td>48.4</td>
<td>27.7-73.7</td>
<td></td>
</tr>
<tr>
<td>Greater than ¾ of the day</td>
<td>56.7</td>
<td>35.0-80.5</td>
<td></td>
</tr>
<tr>
<td>Where has your recreation time usually been spent in winter?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mostly indoors</td>
<td>39.7</td>
<td>17.8-63.6</td>
<td></td>
</tr>
<tr>
<td>½ and ½</td>
<td>47.3</td>
<td>21.7-72.2</td>
<td></td>
</tr>
<tr>
<td>Mostly outdoors</td>
<td>55.3</td>
<td>39.8-86.9</td>
<td></td>
</tr>
</tbody>
</table>
What was previously known

Sunlight and the eye
- Sunlight exposure is required for normal physiological homeostasis.
- Inadequate sunlight exposure has detrimental systemic health effects.
- Excessive sunlight exposure, particularly wavelengths in the ultraviolet range, also has detrimental systemic health effects.
- Ultraviolet light exposure to the eye plays a pathogenic role in pterygium, cortical cataract, squamous cell carcinoma of the ocular surface, acute photokeratitis, solar retinopathy, and malignant skin lesions of the eyelids.

Conjunctival ultraviolet autofluorescence
- Actinic sun damage on the skin fluoresces under ultraviolet light.
- Areas of fluorescence on the bulbar conjunctiva can also be observed under ultraviolet light.
- This fluorescence can be quantified using a fixed-focus camera system and digital analysis.
- The reliability and validity of the photographic technique of measuring autofluorescence must be established if it is going to be widely used as a research tool for quantifying sun exposure.
- There has been little previous research into the phenomenon of conjunctival ultraviolet autofluorescence.
- The normal distribution in the population and its associations with ocular disease are poorly understood.

Pterygium
- Pterygium is associated with environmental ultraviolet light exposure.
- Although generally considered a benign condition, 10-12% contain ocular surface neoplasia.
- Pterygia can be visually significant, and the disease burden associated with the condition and its treatment is high.

Myopia
- Myopia is a major health issue worldwide, with increasing prevalence in many populations.
- Environmental factors contribute to the pathogenesis of myopia.
- There is an inverse correlation between myopia and outdoor activity measured by questionnaire, demonstrated in multiple cross-sectional and prospective studies.
Contribution of this work

Conjunctival ultraviolet autofluorescence
- The photographic analysis technique of measuring conjunctival autofluorescence is highly reliable with intra-observer and inter-observer CCCs exceeding 0.900.
- The highest CCC corresponded to the total conjunctival ultraviolet autofluorescence measurement (sum of four quadrants), suggesting that this measurement should be used for future research in the area.
- Young Australian adults have the most conjunctival autofluorescence of any group previously studied, with a median of 44.2mm$^2$.
- The finding from other studies that nasal quadrants have more autofluorescence than temporal quadrants has been confirmed.
- The finding from other studies that conjunctival autofluorescence is directly associated with time outdoors measured by questionnaire has been confirmed.
- The finding that frequency of wearing sunglasses has no association with conjunctival ultraviolet autofluorescence is new.
- The finding that BMI is inversely associated with conjunctival ultraviolet autofluorescence is new.

Pterygium
- Pterygium was present in 2% of males (1 in 50) and 0.3% of females (1 in 330) in this cohort of young Australian adults.
- As demonstrated in other studies, pterygium was more common in males and more common on the nasal conjunctiva.
- Median total conjunctival ultraviolet autofluorescence was higher in participants with pterygium than those without pterygium (73.4mm$^2$ vs 44.0mm$^2$, p=0.001).

Myopia
- Median conjunctival ultraviolet autofluorescence was lower in myopes than non-myopes (31.9mm$^2$ vs 47.9mm$^2$, p<0.001).
- As demonstrated in other studies increasing age, parental myopia and tertiary education were associated with increased odds of myopia.
- Higher level of conjunctival ultraviolet autofluorescence was associated with decreased odds of myopia, as was participant recall of increased time outdoors during summer and winter.
- The finding that the difference in conjunctival ultraviolet autofluorescence between myopes and non-myopes was not due to an ultraviolet-blocking effect of corrective lenses is new.
Future directions

Conjunctival ultraviolet autofluorescence

- Histological studies to determine the composition of the fluorescing tissue.
- Prospective studies looking at change in conjunctival ultraviolet autofluorescence over time.
- Association of conjunctival ultraviolet autofluorescence with other ultraviolet-related ophthalmic diseases.

Pterygium

- Prospective studies looking at change in ultraviolet autofluorescence characteristics of pterygia over time.
- Prospective studies using the conjunctival ultraviolet autofluorescence camera system to identify features predictive of pterygium progression or recurrence after surgery.
- Further investigation into the aetiology of the significantly higher rate of pterygium in young adult males, when there was little difference in subjective or objective measures of outdoor time between males and females in this cohort.

Myopia

- Prospective studies looking at whether development of myopia, or progression of established myopia, can be predicted with conjunctival ultraviolet autofluorescence photography.
- Randomised interventional studies to determine whether increasing outdoor light exposure can prevent development or progression of myopia.
- Studies designed to investigate conjunctival ultraviolet autofluorescence in high myopes, who are at the greatest risk of blinding complications.
References


