

# **VITAMIN D IN DRY EYE AND MYOPIA**

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Submitted in fulfilment of the requirements for the degree of  
Doctor of Philosophy

School of Optometry and Vision Science

Faculty of Health

Queensland University of Technology

2017



# Keywords

Accommodation response  
Amplitude of accommodation  
Axial length  
Dopamine  
Dry eye  
Form deprivation myopia  
Inflammation  
Interleukin-6  
Keratograph  
Lenstar  
Myopia  
Ocular surface  
Saliva  
Tonic accommodation  
Vitamin D  
Vitamin D binding protein

# Abstract

Vitamin D has long been regarded as an essential daily nutrient of benefit to human health, particularly bone health. Studies have shown that low vitamin D levels may be related to ocular dryness. Vitamin D levels also impact dopamine controlled behaviours in animals. Dopamine, a neurotransmitter, may impact eye health and human eye accommodation. It has also been suggested that increased dopamine release can inhibit myopia development.

This study aimed to investigate whether there was an association between vitamin D levels and two eye conditions, dry eye and myopia, and whether an oral vitamin D supplement taken for 60 days could affect ocular dryness and accommodation/refraction in humans.

In Experiment 1, dry eye symptoms were assessed objectively using the keratograph 5 and subjectively using surveys, in 58 older adults aged between 43 and 69 years. Objective measurements included tear meniscus height, non-invasive tear break up time, ocular redness, oxford corneal staining grading, Schirmer's test, and Phenol red thread tests of tear quantity. Subjective assessment included the dry eye symptoms survey and the Ocular Surface Disease Index (OSDI). There were two participant groups, one was recruited from AusSun study, and the other was from Optometry clinic of Queensland University of Technology. Blood samples were taken from each participant of Optometry clinic group for analysis of vitamin D levels and interleukin-6 analysis. The results showed dry eye was correlated with insufficient serum vitamin D levels ( $< 75$  nmol/l).

In Experiment 2, 32 participants with insufficient vitamin D and/or dry eye from the participants of Experiment 1 were included. They were provided with 1000 IU vitamin D supplements to take daily for 60 days. The assessments were identical as Experiment 1. The vitamin D supplement increased the vitamin D levels by 29.08 nmol/l after 60-day of treatment. The main finding was that OSDI significantly reduced in association with the rise of vitamin D levels. Also, Oxford grading score showed a significant reduction. Interleukin-6 levels did not show significant difference before and after the vitamin D treatment.

In Experiment 3, 55 young adults aged between 18 and 25 were recruited from QUT and UQ student populations. Saliva samples were collected from each participant for the analysis of dopamine and vitamin D levels, levels of vitamin D binding protein were measured in 40 participants. Ocular biometry including axial length, anterior chamber, lens thickness, anterior chamber depth, central cornea thickness, corneal astigmatism, and pupil diameter were measured using the Lenstar. Subchoroidal thickness was measured using Optical Coherence Tomography. Accommodation and binocular vision assessments included accommodation accuracy to near targets, tonic accommodation (TA), distance and near phoria, negative relative accommodation (NRA), positive relative accommodation, and near work induced transient myopia, and near work induced effect on TA. Main findings showed NRA was positively correlated with dopamine levels; people with poorer accommodation had lower dopamine levels. Pre-task TA was higher in high myopes. TA was quite stable when comparing pre-task and post-task in each refractive group. The near work induced TA shift in myopes was myopic whereas it was hyperopic in emmetropes. The levels of vitamin D binding protein were higher in emmetropes compared with myopes. People with lighter iris colour had higher levels of vitamin D binding protein.

In Experiment 4, 33 people with myopia/poor accommodation identified in Experiment 3 were recruited and provided with 1000IU vitamin D supplement to take daily for 60 days. Both the shifts in TA and accommodation accuracy at 25 cm were relatively stable after the vitamin D treatment. Choroidal thickness was greater following the vitamin D treatment. Early onset myopes showed higher central corneal thickness than late onset myopes.

In conclusion, dry eye score using OSDI was inversely correlated with serum vitamin D levels in people with vitamin D insufficiency. A 60 day 1000 IU vitamin D supplement was able to show improvement in dry eye symptoms assessed by OSDI and increases in tear meniscus height. Higher dopamine levels had effects on assisting relaxation of accommodation. A 60-day 1000IU vitamin D supplement in young adults stabilised TA and improved accommodation accuracy for higher accommodation stimulus. The results are suggestive of vitamin D being involved in the ocular surface and human accommodation.

# Table of Contents

Keywords .....	i
Abstract .....	ii
Table of Contents .....	iv
List of Figures .....	vi
List of Tables .....	viii
List of Abbreviations .....	i
Statement of Original Authorship .....	ii
Acknowledgements .....	iii
<b>CHAPTER 1: INTRODUCTION .....</b>	<b>1</b>
1.1 Background .....	1
1.2 Purpose .....	1
1.3 Significance and Scope .....	2
1.4 Thesis Outline .....	2
<b>CHAPTER 2: LITERATURE REVIEW .....</b>	<b>4</b>
2.1 What is Vitamin D .....	4
2.1.1 Absorption and transport of vitamin D .....	4
2.1.2 Vitamin D synthesis .....	5
2.1.3 Risk factors for vitamin D insufficiency or deficiency .....	5
2.1.4 The importance of vitamin D to the human body .....	7
2.2 Does higher vitamin D mean more sunlight exposure .....	9
2.2.1 Vitamin D binding protein .....	9
2.2.2 Vitamin D and interleukin-6 .....	10
2.3 Vitamin D and ocular health .....	11
2.4 What is dry eye .....	25
2.4.1 Inflammation in general health .....	25
2.4.2 Inflammation and dry eye and the commonly used diagnostic techniques .....	25
2.4.3 Seasonal variation and dry eye .....	26
2.4.4 Dry eye in animals .....	26
2.5 Group specific component of serum .....	27
2.6 Vitamin D receptor .....	27
2.7 Myopia .....	28
2.8 Myopia in animals .....	30
2.8.1 Activity/exercise/time outdoors and myopia .....	33
2.8.2 Artificial light and sunlight .....	40
2.8.3 Choroidal thickness and myopia .....	41
2.9 Dopamine and experimental myopia .....	41
2.10 Light and dopamine synthesis in the retina .....	42
2.11 Tyrosine hydroxylase and dopaminergic activity .....	43
2.12 Dopamine and horizontal cells .....	44
2.13 Dopamine and Dopamine receptors .....	44
2.14 Insulin like growth factor-1 .....	48

2.15	Horizontal cells, bipolar cells, and amacrine cells in the retina .....	49
2.16	Defocused image, Phoria, accommodation and myopia .....	50
2.17	Accommodation and ciliary smooth muscles.....	50
2.18	accommodation/Tonic accommodation and refractive errors .....	51
2.19	Summary and Implications .....	54
2.20	Hypothesis .....	54
2.21	Implications .....	56
	<b>CHAPTER 3: RESEARCH DESIGN .....</b>	<b>57</b>
3.1	Ethics .....	57
3.2	Methodology .....	57
3.3	Inclusion and exclusion criteria .....	58
3.4	Instruments.....	59
3.4.1	Dry eye questionnaire .....	61
3.4.2	Time spent outdoor survey .....	61
3.4.3	Actiwatch (BMedical) measurement .....	61
3.4.4	Dry eye assessment.....	62
3.4.5	Ocular biometry measurement.....	67
3.4.6	Blood collection:.....	71
3.4.7	The enzyme-linked immunosorbent assay analysis (ELISA) .....	73
3.4.8	Salivary vitamin D analysis .....	77
3.5	Limitations .....	82
3.6	Definitions of technical terms .....	85
	<b>CHAPTER 4: EXPERIMENT 1: VITAMIN D AND DRY EYE.....</b>	<b>86</b>
	<b>CHAPTER 5: EXPERIMENT 2: VITAMIN D SUPPLEMENT AND DRY EYE/LOW VITAMIN D 104</b>	
5.1	Dry eye measurements before and after the vitamin D treatment .....	110
	<b>CHAPTER 6: EXPERIMENT 3: THE ASSOCIATION BETWEEN SUNLIGHT EXPOSURE/VITAMIN D AND MYOPIA/ACCOMMODATION IN YOUNG ADULTS .....</b>	<b>115</b>
	<b>CHAPTER 7: EXPERIMENT 4: VITAMIN D SUPPLEMENT AND REFRACTION/ACCOMMODATION IN YOUNG ADULTS .....</b>	<b>140</b>
	<b>CHAPTER 8: DISCUSSION AND CONCLUSIONS .....</b>	<b>156</b>
	<b>BIBLIOGRAPHY .....</b>	<b>169</b>
	<b>APPENDICES .....</b>	<b>219</b>
	Appendix A Ethics Approval Forms.....	219
	Appendix B Sunlight Exposure Questionnaire .....	237

# List of Figures

Figure 2-1 The flow diagram of vitamin D treatment hypothesis in dry eye .....	55
Figure 2-2 The flow diagram of vitamin D treatment hypothesis in myopia/accommodation .....	55
Figure 3-1 The K5 White-illumination Mode for Tear Meniscus Height.....	63
Figure 3-2 The K5 IR-illumination Mode for Tear Meniscus Height.....	63
Figure 3-3 NIKTBUT of the K5 Display .....	64
Figure 3-4 The R-Scan of the K5 .....	65
Figure 3-5 The image of Corneal Staining of the K5 .....	66
Figure 3-6 Sub-foveal choroidal thickness measurement with OCT.....	67
Figure 3-7: Derivatisation of 25(OH)D <sub>3</sub> with PTAD.....	78
Figure 3-8: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of ultra pure water infusion.....	79
Figure 3-9: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of PTAD derivatised testosterone infusion.....	80
Figure 3-10: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of purified vitamin D <sub>3</sub> standard.....	80
Figure 3-11: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of saliva sample with potential PTAD Vitamin D <sub>3</sub> .....	81
Figure 3-12: QTRAP 4500 ESI-MS/MS total ion chromatogram (TIC) (top) and ESI-MS mass spectrum (bottom) of saliva sample.....	82
Figure 3-13: ESI-MS/MS spectrum of 25(OH)D <sub>3</sub> -PTAD, with methylamine (5 mM) added to the mobile phase when the spectrum was measured, in Higashi et al.'s study (2008).....	82
Figure 4-1 Proportion of showing OSDI greater than 12 in participants with optimal serum vitamin D levels (> 75nmol/l).....	96
Figure 4-2 Proportion of showing OSDI greater than 12 in participants with insufficient serum vitamin D levels (< 75 nmol/l).....	97
Figure 4-3 The OSDI levels in the two groups.....	97
Figure 4-4 The 25(OH) D levels in the two groups .....	97
Figure 4-5 The correlations between OSDI and 25(OH) D in all participants (n = 58) .....	98
Figure 4-6 The correlations between OSDI and 25(OHD) in the participants of Optometry Clinic group (n = 29) .....	98
Figure 4-7 The correlations between OSDI and 25(OH) D in the participants of AusSun group (n = 29) .....	98
Figure 4-8 The correlation between OSDI and dry eye score (n = 58) .....	99
Figure 4-9 OSDI and 25(OH)D levels in different severity of dry eye.....	99
Figure 5-1 Vitamin D levels in serum before and after the vitamin D supplement.....	110
Figure 5-2 Oxford grading scale before and after vitamin D treatment .....	110
Figure 5-3 OSDI before and after the vitamin D treatment .....	111



Figure 5-4 The correlation between the index of bulbar conjunctiva and limbal conjunctiva redness .....	111
Figure 6-1 The correlation between spherical equivalent refraction and axial length.....	124
Figure 6-2 The correlation between spherical equivalent refraction and choroidal thickness.....	125
Figure 6-3 The correlation between axial length and choroidal thickness.....	125
Figure 6-4 The correlation between axial length and anterior chamber depth .....	126
Figure 6-5 The correlation between aqueous depth and lens thickness.....	126
Figure 6-6 The correlation between negative relative accommodation and dopamine.....	126
Figure 6-7 Dopamine levels in young people with normal and poor accommodation .....	127
Figure 6-8 First tonic accommodation (pre-near task) in emmetropes, low myopes, and high myopes. ....	128
Figure 6-9 Second tonic accommodation (post-near task) in emmetropes, low myopes, and high myopes .....	128
Figure 6-10 Pre-and post-near task TA measurements in emmetropes, low myope, and high myopes. ....	129
Figure 6-11 Tonic accommodation shift in emmetrope, low myope, and high myope groups.....	129
Figure 6-12 The proportions of myopes and emmetropes in normal accommodation group .....	130
Figure 6-13 The proportions of myopes and emmetropes in poor accommodation group.....	131
Figure 6-14 Choroidal thickness in emmetrope, low myope, and high myope groups.....	131
Figure 6-15 Choroidal thickness in emmetropes, stable myopes, and progressing myopes.....	132
Figure 6-16 Vitamin D binding protein in myopes and emmetropes.....	132
Figure 6-17 The levels of vitamin D binding protein in emmetropes, low myopes, and high myopes.....	133
Figure 6-18 The levels of vitamin D binding protein in emmetropes, stable myopes, and progressing myopes.....	133
Figure 6-19 The levels of vitamin D binding protein in individuals with dark and light coloured iris. ....	134
Figure 6-20 The levels of vitamin D binding protein in dark and light coloured individuals with similar refractive error.....	134
Figure 6-21 Time spent outdoors during a typical week.....	135
Figure 6-22 Choroidal thickness between eyes with better and poor accommodation accuracy at (25 cm) after near task.....	136
Figure 6-23 The levels of vitamin D binding protein in eyes with normal and poor accommodation accuracy (at 25cm) after near task.....	136
Figure 7-1 Tonic accommodation before and after near task at the first visit.....	147
Figure 7-2 Tonic accommodation before and after near task at the second visit. ....	148
Figure 7-3 The measurements for the second tonic accommodation in the two visits.....	148
Figure 7-4 Tonic accommodation shift before and after the vitamin D treatment.....	149
Figure 7-5 Accommodation accuracy at 25cm before and after near task at the first visit .....	149
Figure 7-6 Accommodation accuracy of the 1 <sup>st</sup> and 2 <sup>nd</sup> measurements at the visit after the vitamin D treatment. ....	150
Figure 7-7 Choroidal thickness before and after vitamin D treatment .....	150

<b>Figure 7-8 Central corneal thickness in emmetropes, early onset myopes, and late onset myopes.....</b>	<b>151</b>
<b>Figure 7-9 Salivary dopamine levels before and after the vitamin D treatment.....</b>	<b>152</b>
<b>Figure 7-10 Dopamine levels before and after the vitamin D treatment in myopes. ....</b>	<b>152</b>
<b>Figure 7-11 Dopamine levels before and after the vitamin D treatment in emmetropes .....</b>	<b>153</b>

## List of Tables

<b>Table 2-1 Studies involving sunlight, vitamin D and dry eye, age-related macular degeneration, intraocular pressure.....</b>	<b>12</b>
<b>Table 2-2 Findings on vitamin D and the eye from animal models .....</b>	<b>12</b>
<b>Table 2-3 Studies of myopia with relation to time spent outdoors/seasons .....</b>	<b>16</b>
<b>Table 2-4 Studies of the association between myopia and accommodation .....</b>	<b>19</b>
<b>Table 3-1 Characteristics of two groups of participants in dry eye study.....</b>	<b>58</b>
<b>Table 3-2 Characteristics of two groups of participants in myopia/accommodation study.....</b>	<b>58</b>
<b>Table 4-1 Vitamin D and Dry Eye Data in the two groups.....</b>	<b>93</b>
<b>Table 4-2 Vitamin D and dry eye data in all the participants .....</b>	<b>94</b>
<b>Table 4-3 Dry eye signs and symptoms from the questionnaire.....</b>	<b>95</b>
<b>Table 5-1 Dry eye measurements before and after the vitamin D treatment.....</b>	<b>108</b>
<b>Table 6-1 Summary of measurements of ocular biometry .....</b>	<b>122</b>
<b>Table 6-2 Summary of measurements in binocular vision and accommodative functions .....</b>	<b>123</b>
<b>Table 6-3 Measurements of salivary DBP, dopamine, and vitamin D levels.....</b>	<b>123</b>
<b>Table 7-1 Summary of measurements of ocular biometry before and after vitamin D treatment.....</b>	<b>145</b>
<b>Table 7-2 Summary of measurements of binocular vision and accommodative functions before and after vitamin D treatment.....</b>	<b>146</b>
<b>Table 7-3 Measurements of salivary dopamine and vitamin D levels.....</b>	<b>151</b>

# List of Abbreviations

AE - Accommodative Error

CCT - Central Corneal Thickness

DOPAC - 3,4-dihydroxyphenylacetic acid

DBP - Vitamin D Binding Protein

FDM - Form Deprivation Myopia

IL-6 - Interleukin-6

LIM - Lens Induced Myopia

NITM - Nearwork Induced Transient Myopia

NRA - Negative Relative Accommodation

OSDI - Ocular Surface Disease Index

PRA – Positive Relative Accommodation

TA - Tonic Accommodation

# Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: QUT Verified Signature

Date: October 2017

# Acknowledgements

This thesis would not have been possible without support from many people. I would particularly like to acknowledge my supervisor Associate Professor Katrina Schmid for her inspiration, advice, and encouragement over the past 3 years.

I would also like to thank The School of Optometry and Vision Science of Queensland University of technology for providing me with a scholarship and access to laboratory space; both of which have supported me throughout the journey.

My associate supervisor Professor Damien Harkin and the Queensland Eye Institute, who kindly provided invaluable advice, professional and technical support and equipment for the analysis of human enzyme-linked immunosorbent assay.

I appreciate associate Professor Jonathan Harris, who kindly provided professional and technical support, and always patiently solved my questions.

My thanks to Professor Michael Kimlin, who provided his research participants for a good start during my PhD career, also the phlebotomist Sam Vaartjes's assistance on blood collection of the participants. I also appreciate the kind participants who gave me their time and travelled a long way to be involved in my study.

I also appreciate Ray Duplock and Xi Zhang who provided statistical advice and support to me. This thesis would not be completed without Sandra Chou's assistance in checking the writing for me. I also wish to acknowledge the staff and other post-graduate students in the School of Optometry and Vision Science, without their assistance this work would not have been completed.

Finally, I thank my parents and my family for their support during this period.



# Chapter 1: Introduction

---

## 1.1 BACKGROUND

Vitamin D is a fat-soluble nutrient that is necessary for human health (DeLuca & Zierold, 1998; Magnuson, 2004), this includes bone health (Dawson-Hughes et al., 2005), neuromuscular function (Dhesi et al., 2004; Flicker et al., 2003), the immune system (Baeke et al., 2010; Cantorna et al., 2004), endothelial cells (Sugden et al., 2008), and reduction of inflammation (Froicu et al., 2003; Hughes & Norton, 2009). The human body makes vitamin D when the skin is exposed to the sun (Cranney et al., 2007; Holick, 2004a). Limited sun exposure from indoor lifestyles has led to large numbers of people with insufficient levels of vitamin D (Jääskeläinen et al., 2013; Tolppanen et al., 2012); few foods naturally contain vitamin D (Holick et al., 2011; Lamberg-Allardt, 2006).

There is limited knowledge on vitamin D and eye health. There is some evidence that vitamin D is involved in corneal epithelial cells ability to help regulate the tear film (Yin et al., 2011). A lack of vitamin D may be a risk factor for myopia (Mutti & Marks, 2011). High sunlight exposure (which enables the human body to produce higher level of vitamin D) has a protective association against age related macular degeneration (Darzins et al., 1997). A correlation between myopia development and vitamin D may exist, however it is not established whether low vitamin D levels directly result in myopia. This project involved two investigations of the impact of vitamin D on the eye. (i) An association and vitamin D supplement study of the ocular surface, and (ii) an association and vitamin D supplement study of accommodation accuracy/ refraction. This research has contributed to the understanding of the role of vitamin D in two aspects of ocular function i) dry eye and ii) accommodation accuracy and refraction.

## 1.2 PURPOSE

The purpose of these studies was firstly to investigate whether there was an association between serum vitamin D levels and dry eye symptoms in humans, and subsequently whether a period of vitamin D treatment produced an observable

improvement in dry eye. Secondly to investigate whether the vitamin D levels would be different between myopes and emmetropes; subsequently to study the effects of a period of vitamin D supplement on human accommodation and the ocular parameters.

### **1.3 SIGNIFICANCE AND SCOPE**

The findings have contributed to current knowledge in the fields of ocular dryness and myopia progression/development. In recent years, there have been many researchers studying vitamin D levels in the human body and dry eye, however there are few studies of the effects of oral vitamin D supplements on ocular dryness.

Previous studies have shown that myopic individuals appear to have lower vitamin D levels. To date there has not been any studies examining the effect of vitamin D supplements on myopia/accommodation. There might be combined effects of vitamin D supplements and vitamin D synthesised from sunlight exposure. A short period (8.6 weeks) of vitamin D supplement was provided to participants to take orally for observing the effects in eye dryness and refraction/accommodation.

### **1.4 THESIS OUTLINE**

The following chapters include:

- **Chapter 2:** provides a comprehensive literature review of the topics related to the projects.
- **Chapter 3:** describes research design and methods.
- **Chapter 4: Chapter 5: Chapter 6: and Chapter 7:** are the chapters describing the experiments.
- **Chapter 4:** is an association experiment, in which dry eye measurements were assessed objectively and subjectively using the Keratograph 5 and the Ocular Surface Disease Index. Criteria of dry eye severity are addressed in this chapter.



- **Chapter 5:** investigates whether a period of vitamin D supplement could affect the symptoms experienced in people with dry eye/low vitamin D levels.
- **Chapter 6:** is an association experiment, in which ocular parameters and accommodation function were assessed. Criteria of myopia severity and onset are addressed in this chapter.
- **Chapter 7:** investigates whether a period of vitamin D supplementation could affect the accommodative functions in people with myopia/poor accommodation.
- **Chapter 8:** Final discussion

# Chapter 2: Literature Review

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## 2.1 WHAT IS VITAMIN D

The chemical structure of vitamin D is similar to that of the steroids (Pérez-López, 2007), it therefore is regarded as a secosteroid hormone to the human body (Guillot et al., 2010). This hormone is primarily produced in the skin after exposure to ultraviolet B radiation (UVB), though some specific foods (i.e. fatty fish such as herring and mackerel) (Lips, 2006) and/or supplements are also sources of this hormone. Lower sunlight exposure was first recognised to be associated with general health problem such as rickets in the early 20<sup>th</sup> century (Holick, 1994), suggesting the significance of vitamin D to the human body.

### 2.1.1 Absorption and transport of vitamin D

The UVB from sunlight is essential for human physiology and is responsible for producing vitamin D. Sunlight over-exposure does not produce greater amounts of vitamin D<sub>3</sub>; instead, the excessive UV radiation is detrimental to the production of vitamin D (Holick, 1994). It is supposed that wavelengths between 295 and 297 nm are the most important for the formation of vitamin D (Hajrasouliha & Kaplan, 2012). Sun protection behaviours also impact vitamin D levels; for example, it has been reported that concealing clothing style can lead to vitamin D deficiency (Hatun et al., 2005) and vitamin D levels can be low at the end of winter even in areas with a sunny climate (Kimlin et al., 2007; Van der Mei et al., 2007).

Vitamin D has long been regarded as one of the indispensable daily nutrients. There are two main forms of vitamin D, ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>). Ergocalciferol can be acquired via specific food intake or irradiation of plants, whereas cholecalciferol is mainly synthesised in the skin after exposure to ultraviolet light (Lips, 2006). As early as a century ago it was observed that low sunlight exposure was associated with physical conditions such as rickets (Holick, 1994). Thus, vitamin D<sub>3</sub> might be more essential than vitamin D<sub>2</sub> for maintaining human health. After vitamin D<sub>3</sub> is transported to the liver, it undergoes a hydroxylation process to form 25-hydroxyvitamin D<sub>3</sub> (25(OH)D) and following that it is hydroxylated into 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D) in the kidney. The

human body needs the active metabolite, 1,25-dihydroxyvitamin D, to stimulate the absorption of calcium from the gut. Sufficiency of 1,25-dihydroxyvitamin D<sub>3</sub> is also required to form 24,25-dihydroxyvitamin D (Dusso et al., 2005) - a vitamin D metabolite essential for bone formation (Ornoy et al., 1978).

### **2.1.2 Vitamin D synthesis**

To produce the active form of vitamin D, 1,25(OH)<sub>2</sub>D<sub>3</sub>, 25(OH)<sub>2</sub>D<sub>3</sub> needs to be catalysed by 1-alpha-hydroxylase. A number of studies have confirmed the expression of vitamin D in the brain of developing adult rats (Burkert et al., 2003; Prüfer et al., 1999; Veenstra et al., 1998). Eyles et al. (2005) have shown that both the vitamin D receptor and 1-alpha-hydroxylase are present in the human brain. The importance of vitamin D for brain development has been demonstrated (Brown et al., 2003; Eyles et al., 2003; Mackay-Sim et al., 2004), and a number of regions have been shown to contain receptors for the vitamin D hormone (Prüfer et al., 1999; Stumpf & O'Brien, 1987; Veenstra et al., 1998). This could indicate that the human brain is capable of synthesising 1,25(OH)<sub>2</sub>D<sub>3</sub> through catalysing 25(OH)<sub>2</sub>D<sub>3</sub>, and does not have to produce 1,25(OH)<sub>2</sub>D<sub>3</sub> via the nephro-pathway. It is hypothesised that insufficient vitamin D during early life might be associated with diseases such as schizophrenia (Bland et al., 2000; McGrath, 2001). Vitamin D levels in the brain may impact the brain-dominant conditions including psychiatric and vision-related disorders. For example, lower vitamin D levels have been shown to have increased risk of schizophrenia (McGrath, 2001) and have potential to inhibit retinoblastoma in mice (Shokravi et al., 1995).

### **2.1.3 Risk factors for vitamin D insufficiency or deficiency**

A 25(OH)D serum level of 75 nmol/L or higher is regarded as optimal, vitamin D deficiency is defined by a serum level lower than 25 nmol/L and vitamin D insufficiency occurs between 25 and 50 nmol/L (Heaney et al., 2003; Nowson & Margerison, 2002; Vieth et al., 2001). A number of factors may contribute to vitamin D deficiency (Holick, 2007). External individual based factors include less time spent outdoors, reduced sunlight intensity, shade seeking behaviour, high skin pigmentation and extensive dense clothing. Other factors associated with deficiency include obesity, older age, and abnormal absorption. The following provides an

overview of the underlying causes of vitamin D insufficiency/deficiency.

**Ageing:** Advanced age can cause vitamin D deficiency resulting from 7-dehydrocholesterol reduction in the skin (Holick, 2006a; Holick & Garabedian, 2006).

**Darker skin pigment:** UVB radiation absorption is affected by melanin in the skin, and individuals with a fair skin colour seem to have better vitamin D absorption efficiency (Holick, 2004b, 2006b). The comparison of 25(OH)D between dark and light skinned individuals revealed that those with darker skins had lower concentrations of 25(OH)D, however, the estimated bioavailability of 25 (OH)D was similar in the two groups (Powe et al., 2013). Also, higher prevalence of vitamin D deficiency in non-Western immigrants (compared to local Caucasians) in Western countries (Lips, 2010) may be suggesting that the importance of skin types/colours overrides residing areas.

**Clothing/shade seeking:** The photosynthesis of vitamin D can be affected by the variety of sunlight exposure behaviours such as clothing or shade seeking. Recently, it has been suggested people wearing protective clothing/ seeking shade have higher odds ratios of having deficient vitamin D (Hansen et al., 2016).

**Weak sunlight season and higher latitude:** Both of these two factors are thought to contribute to vitamin D insufficiency. During the seasons with weaker sunlight (i.e. winter) people living in high latitudes have little vitamin D production (Bouillon et al., 2001; Holich et al., 1995; Van der Mei et al., 2007).

**Obesity and high BMI:** Individuals with high body weight and high body mass index (BMI) have lower blood concentrations of vitamin D (Wortsman et al., 2000). Related to this, a study has shown that the individuals with higher BMI have lower dopamine D<sub>2</sub> (Wang et al., 2001a).

**Liver failure:** Depending on the severity of liver failure, an inability to produce sufficient 25-hydroxyvitamin D may occur (Holick, 2006a).

**Renal or kidney diseases:** Such conditions can affect vitamin D binding protein or result in an inability to produce sufficient 1,25 dihydroxy vitamin D (Dusso et al., 2006; Ritter et al., 2006; Shimada et al., 2004).

**Living in urban areas:** In modern life, people tend to live in places that are closer to cities and the indoor urban lifestyle may contribute to less sunlight exposure (McGrath et al., 2001).

**Air quality:** Although it was shown in vitro that air quality was not associated with vitamin D production (McKinley et al., 2011), it has been suggested that people living in more polluted area had lower vitamin D while air pollution is believed to partially block UV and therefore vitamin D production (Hosseinpanah et al., 2010).

#### **2.1.4 The importance of vitamin D to the human body**

An insufficient or deficient vitamin D level has been linked to a variety of systemic diseases, including cardiovascular disease, osteoporosis, autoimmune disease, multiple sclerosis and diabetes mellitus (Holick, 2004b, 2007; Salzer et al., 2012).

**Osteoporosis:** Osteoporosis is defined as ‘a systemic skeletal disease characterised by low bone mass and deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture’ (Peck et al., 1993). It is estimated that 1.2 million Australians have osteoporosis that is mostly undiagnosed (Ebeling et al., 2013). Vitamin D deficiency has been reported to be associated with lower bone mineral density in elderly women (Rosen et al., 1994). Calcium is essential to prevent fractures, and vitamin D enhances calcium absorption (Tang et al., 2007). Avenell et al. (2009) found that the combination of vitamin D and calcium reduced the incidence of hip fractures.

**Cardiovascular disease:** Although the aetiology of cardiovascular disease remains unclear (Yancy et al., 2003), a number of studies implicate vitamin D deficiency in cardiovascular disease (Holick, 2004a; Wang et al., 2008; Zittermann, 2006). It has been suggested that better muscular performance in elderly required at least 30 ng/ml (Bischoff-Ferrari et al., 2004). Age related high systolic blood

pressure can be reduced by adequate vitamin D levels. Serum levels of vitamin D higher than 32 ng/ml reduced systolic blood pressure by one-fifth compared to blood pressure measured when the vitamin D levels were lower than 20 ng/ml (Judd et al., 2008; Zittermann et al., 2005).

**Multiple sclerosis:** A lower incidence of multiple sclerosis is associated with higher 25(OH)D in Caucasian populations (Kragt et al., 2009; Munger et al., 2006). Moreover, Van der Mei et al. (2003) reported an inverse association between early life sunlight exposure and the risk of multiple sclerosis, i.e. lower sunlight exposure led to increased risk. Latitudinal variation, which alters both vitamin D level and sunlight exposure, is an important environmental factor influencing multiple sclerosis (Simpson et al., 2011).

**Diabetes mellitus:** Diabetes mellitus is defined as a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Alberti & Zimmet, 1998; Association, 2006). It had been estimated the global population of adults with diabetes mellitus was 285 million in 2010 and that this will rise to 439 million by the year 2030 (Shaw et al., 2010). Defects in insulin secretion are also associated with inadequate vitamin D levels (Hyppönen et al., 2001), that is, the lower vitamin D level in serum, the higher the glucose in the blood, and greatest serum glucose increase was observed in individuals with vitamin D levels below 40 nmol/l (Need et al., 2005). Further supporting evidence for the role of vitamin D in diabetes is that the incidence of type 1 diabetes was found to be inversely associated with latitude (Mohr et al., 2008).

**Asthma:** It is estimated that ~300 million people worldwide have asthma, and numbers are projected to increase (Masoli et al., 2004). Asthma is caused by inflammation of the airways; established pathophysiology includes activated eosinophils, mast cells, and T lymphocytes (Louis et al., 2000). Vitamin D's immunomodulatory mechanisms (Ginde & Sutherland, 2010) may be important in limiting asthma development, particularly given the association between vitamin D deficiency and asthma (Celedón et al., 2004; Dixon et al., 2010; Manicourt & Devogelaer, 2008; Masoli et al., 2004; Rajakumar et al., 2005; Wortsman et al., 2000).

## **2.2 DOES HIGHER VITAMIN D MEAN MORE SUNLIGHT EXPOSURE**

Among different ethnic groups, individuals with higher pigmented skins tend to less efficiently produce vitamin. Also, people with high body weight and/or high body mass index tend to have relatively lower vitamin D, suggesting that high body weight/BMI impacts vitamin D production even with the same amount of sunlight exposure. This factor is more significant in systemic diseases and outside the scope of this study. Clothing behaviour was noted as this factor directly affects the amount and intensity of sunlight exposure an individual receives.

### **2.2.1 Vitamin D binding protein**

The primary vitamin D carrier protein that can potentially affect vitamin D concentration in the human body is vitamin D binding protein (DBP), which is synthesised in the liver when stimulated by estrogen (Speeckaert et al., 2006). DBP is responsible for binding up to 90% of total circulating 25(OH)D (Bikle et al., 1986), a 58 kDa glycoprotein that is encoded by the GC gene and previously known as group-specific component (gc-globulin) (Mikkelsen et al., 1977; Sollid et al., 2016). A number of conditions are known to be associated with low level of vitamin D binding protein, for example, nephritic syndrome (Speeckaert et al., 2006), cirrhosis (Lai et al., 2015), and estrogen therapy (Møller et al., 2013). There are more than 120 DBP variations in the human body while only three DBP polymorphic alleles are dominant – GC1F, GC1S, and GC2 (Cleve & Constans, 1988). When bound to the carrier protein, vitamin D is believed to have limited actions on target cells due to its lower bio-availability. Ethnicity, for example, is one of the factors that influence the bio-availability of vitamin D. Better bone health and lower incidence of fractures in African Americans by comparison to European Americans despite the fact they had lower serum 25(OH)D concentration (O'Connor et al., 2013). It may not necessarily directly reflect the difference of biological activity when taking into account the difference in the level of bioavailable 25(OH)D [free 25(OH)D, defined as the 25(OH)D unbound to vitamin D binding protein (Powe et al., 2013)] between the two populations, though there was difference in total serum 25(OH)D level (Powe et al., 2013). This may be indicating that direct measurement of bio-available 25(OH)D could truly compare the different level of response in biological activity between individuals.

### 2.2.2 Vitamin D and interleukin-6

Interleukin-6 (IL-6) is known as a circulating cytokine that is secreted from a number of different cells such as activated lymphocytes and macrophages, and it is thought as a potent regulator of inflammation and immune responses (Kishimoto, 2005; Scheller et al., 2011).

The biological activities are initiated when IL-6 binds to a high-affinity receptor complex (Yudkin et al., 2000).

Fattori et al. (1994) suggested that interleukin-6 is a key mediator of the inflammatory response to localised inflammation. Also, a study has demonstrated that inhibiting the activity of IL-6 leads to the reduction of a number of systemic reactions to inflammation (Oldenburg et al., 1993). It is also observed in mice that by comparison to IL-6 deficient animals, the control showed higher expression of serum amyloid P mRNA (one of the acute phase reactants that can cause amyloid deposits) (Fattori et al., 1994). Noma and the colleagues (2008) investigated both vitreous and aqueous samples in the human with branch retinal vein occlusion and found that there was a significant correlation between vitreous levels of vascular endothelial growth factor and IL-6, the correlation between the suavity of macular oedema and IL-6 was also noticed.

A number of systemic response pathways such as metabolic process, cell growth, and acute phase responses, involve insulin like growth factor-6 (IL-6) (Neurath & Finotto, 2011). The pro-inflammatory effects of IL-6 have also been demonstrated in many systemic diseases such as arthritis, colitis, and encephalitis (Fujimoto et al., 2008; Serada et al., 2008; Yamamoto et al., 2000). Due to the inflammatory characteristics of ocular surface diseases, IL-6 may be a significant regulator of immune responses and inflammation (Kishimoto, 2005; Scheller et al., 2011). Fernandez-Real et al. reported that the levels of IL-6 in the plasma of healthy human was between 1 and 14 pg/mL (Fernandez-Real et al., 2001). Zhang and colleagues (2013) investigated the expression of inflammatory factors in the conjunctival epithelium of dry eye people and found that IL-6 was highly correlated with dry eye symptom scores (using OSDI) and higher in the more severe symptom group by comparison to the less severe symptom groups. If IL-6 plays an important role in ocular surface dryness, people with worse dry eye symptoms should in theory, have higher IL-6 levels.



Topical use of MC903 (a synthetic vitamin D<sub>3</sub> analogue) on human's skin has been demonstrated to reduce the inflammatory effects of interleukin-6 (IL-6) (Oxholm et al., 1988). Considering dry eye is a chronic inflammatory eye disease, vitamin D may have the potential of relieving ocular dryness via the inhibition of IL-6. In human corneal epithelial cells, vitamin D appears to reduce the inflammatory response to infection. Downregulation of the expression of IL-6 using vitamin D treatment has been demonstrated in the infection induced by *Pseudomonas aeruginosa* (Xue et al., 2002). In addition, enhanced corneal epithelial barrier function has also been reported with the treatments of both vitamin D and its active metabolite (1,25-dihydroxvitamin D<sub>3</sub>) (Yin et al., 2011). The results of these studies indicate the potential of vitamin D to reverse the negative effects during ocular infection and inflammatory conditions.

In healthy middle aged individuals, it has been suggested the usual value of IL-6 is  $0.25 \pm 0.31$  pg/ml (Hager et al., 1994), while in younger healthy female it ranged between 0.35 and 1.87 pg/ml (Lutgendorf et al., 1999).

### **2.3 VITAMIN D AND OCULAR HEALTH**

Insufficient or deficient vitamin D level has been linked to myopia (Choi et al., 2014; Mutti & Marks, 2011), dry eye disease (Galor et al., 2014) and age related macular degeneration (Millen et al., 2011). A study of the impact of vitamin D supplements on the eye's intraocular pressure (IOP) reported no differences before and after treatment (Krefting et al., 2013). Other key studies discussing the association between vitamin D and ocular health in both humans and animal models are summarised in Table 2-1 and 2-2.

**Table 2-1 Studies involving sunlight, vitamin D and dry eye, age-related macular degeneration, intraocular pressure**

Research Study	Condition	Participants Age (Year)	Sample Size (n)	Location	Results/Findings	P value
Galor et al. 2014	Dry eye	55 to 95 (mean 69±8.9 years old)	247	Miami, USA	Higher levels of vitamin D were associated with decreased dry eye syndrome symptoms, with a -1.24 decrease in median Dry Eye Questionnaire 5 score for every 10-U increase in Vitamin D levels	P=0.01
Krefting et al. 2013	Intraocular pressure	30 to 75 (received 20,000IU twice weekly for 6 months)	86	Norway	IOP was not different between people with low and high serum 25(OH)D levels	P=0.92
Millen et al. 2011	Age-related macular degeneration	50 to 79 years	1313 (female)	Madison, Iowa City, Oregon; the US	Serum 25(OH)D was associated with decreased odds of early stage AMD in women	P=0.02
Seddon et al. 2011	Age-related macular degeneration	Born between 1917 and 1927	840	USA	A diet higher in vitamin D was consumed by the twin with the less severe AMD	P=0.01

**Table 2-2 Findings on vitamin D and the eye from animal models**

Research Study	Animals	Sample Size	Treatment Duration	Supplements/ Visual Treatments	Results/findings	P value
Lee et al. 2012	Mice	14	6 weeks	Subcutaneous injection (0.9µg in 0.1 mL)	The number of macrophages reduced significantly in subretinal area in the vitamin D <sub>3</sub> treated group	0.008
Lin et al. 2012	Rabbits	12	8 weeks	Fed orally (1.1IU/g to control and 7IU/g to treated group)	Consumption of vitamin D supplements altered vitamin D metabolite concentrations in the anterior segment of the eye	
Dong et al. 2011	Guinea pigs	140	11 days	Subconjunctival injection	Dopamine and DOPAC levels were reduced in the deprived eyes but did not change significantly in the	

				of 100 µl APO of different concentrations	defocused eyes	
Kesby et al. 2009	Rat	15 (control = 7, treated = 8)	Vitamin D deprived in diet for 6 weeks	Vitamin D deficient diet in first generation	Developmental vitamin D deficiency is associated with altered dopaminergic turnover in the neonatal rat	
McBrien et al. 2001	Tree shrews	20	12 days, and 3–20 months	Diffuser wearing (for 12 days and 3 month groups, eyelid closure for groups treated for more than 6 months)	Short term deprived eyes had thinner scleral thickness by 21% as compared to the control eyes; while significant differences (23%) in scleral thickness was observed between myopic and control eyes in long-term-deprived animals	P=0.001 (short-term) P<0.05 (long term)



## **Myopia**

Research have shown that environmental factors, such as leading an outdoor lifestyle, can have a strong impact on myopia development (Dirani et al., 2009; Ip et al., 2008a; Jones et al., 2007; Rose et al., 2008a). The Sydney Myopia Study (Ip et al., 2008a) found that 6 to 12 years old children, who reported to spend more than 2.8 hour/day performing outdoor activities, were on average more hyperopic than children reported to perform less outdoor activity. A large body of evidence now is supportive of the protective role of increasing time spent outdoors both against the development and progression of myopia (French et al., 2013a; Jacobsen et al., 2008; Onal et al., 2007; Pärssinen & Lyyra, 1993; Saw et al., 2002; Wu et al., 2013).

There are studies suggesting an association between myopia prevention and the amount of sunlight exposure, for example, six month follow up found that both eye elongation and myopia progression were slower in longer days as compared to shorter days in young children between the age of 8 and 14 years (Cui et al., 2013). Similarly, Guo et al. (2013b) surveyed grade 1 and 4 students attending schools in rural regions and urban areas in Beijing and found that the students in rural regions spent twice as long being outdoors than those in urban areas (2.2 hours vs 1.1 hours). The prevalence of myopia in grade 1 for boys in urban areas was almost 4 times than those living in rural regions (29.9% vs 7.9%), while the figures for girls were 26.6% in urban areas and 2.7% in rural regions. A relatively higher prevalence was noticed in grade 4 children, the figures for urban and rural boys were 53.2% and 18.8%, while they were 76.2% and 15.6% respectively for girls. Wu et al. (2013) observed the children (aged between 7 and 11 years) in two adjacent schools in Taiwan for a year, a recess outside the classroom was intervened in the children of one school while the other one was control. It was found that the incidence of new onset myopia was only half in the recess outside the classroom group when compared to the control group (8.41% vs 17.65%). Myopia progression was also significantly slower in the recess intervene group (-0.25 D vs -0.38 D).

In Australian urban areas, myopia affects one in every nine 12-year-old children (Ip et al., 2008a) and at least one in every three 15- to 18-year-old teenagers (Rose et al., 2003). Even higher myopia prevalence has been reported in East and Southeast Asian countries, for example, 85% of 17 years old Taiwanese require an optical correction for myopia (Hung, 2001). The possible cause(s) of this large geographical variation in myopia prevalence is of much interest; sunlight varies in intensity and

spectrum around the world and this may be a key factor. Studies focusing on the myopia prevalence with relation to sunlight exposure in Asian countries are discussed and summarised in the following table.

**Table 2-3 Studies of myopia with relation to time spent outdoors/seasons**

Research Study	Condition	Age (Year)	Sample Size (n)	Location	Results/Findings	P value
Dirani et al., 2009	Myopia	11 to 20 (mean 13.7)	1249 (614 boys and 635 girls)	Singapore	Total time spent outdoors was associated with a lower myopic refraction and shorter axial length	<0.001
Guo et al., 2013	Myopia	56.1% were 6.3±0.5, 43.9% were 9.4±0.7	681	Beijing, China	The presence of myopia was associated with less time spent outdoors	<0.001
Donovan et al. 2012	Myopia	6 to 12	85	Guangzhou, China	Myopia progression rates were higher in winter months when compared to summer months	
Fujiwara et al. 2012	Myopia	6 to 12 (11.4± 1.7)	92	Okayama, Japan	Myopia progression was similar across the year (seasons), however axial elongation was slower in the summer	P=0.08 (axial elongation)

A lack of time spent performing outdoor activity is associated with myopia; conversely shorter axial lengths and lower myopic refractions have been demonstrated in teenage children who spend long hours performing outdoor activity (Dirani et al., 2009). Younger children (5 to 8 years old) residing in urban areas where less time was spent outdoors were more susceptible to myopia (Guo et al., 2013b). The mechanism for outdoor activities protective effect remain unclear, a number of possibilities have been raised. The outdoor distant viewing condition may naturally relax the accommodation system, whereas near work and accommodation errors during reading or writing are risk factors for the development of myopia (Gong et al., 2014). Bright light levels may produce reduced image blur on the retina as the pupil constriction minimises the impact of defocus (Ashby et al., 2009a; Ashby & Schaeffel, 2010a), however Ashby and the colleagues (2009a) using artificial pupils and lenses in the chick model found no evidence for this hypothesis. A further possible mechanism relates to the high light levels stimulating retinal dopamine

release (Cohen et al., 2012) and/or vitamin D (Mutti & Marks, 2011). Vitamin D insufficiency is reported to be very high in China; during the winter season, the prevalence of vitamin D insufficiency (<30 ng/mL) was 84% in males and 89% in females (Lu et al., 2012). A positive association between low vitamin D level and myopia has also been reported (Choi et al., 2014), implying the association between the sunlight synthesised vitamin D. It would therefore be rational to speculate that there might be an association between decreased sunlight exposure (and decreased vitamin D) and the progression and development of myopia.

On average, the supply of 25(OH)D is able to remain in the human body for 60 days (Richer & Pizzimenti, 2013), which is shorter than the length of a single season. If the assumption that more sunlight exposure inhibits myopia progression is true, then seasonal changes should impact refractive error; in other words, refractive error variation may be observed in an individual if measured in the end of summer and in the end of winter, respectively. A study investigating Chinese school aged children (Donovan et al., 2012) revealed both slower myopia progression and axial elongation in the summer. In contrast to this, Fujiwara et al. (2012) reported a marked axial length increase in the winter, although the overall seasonal influence on myopia progression was not significant. The fact that they measured noncycloplegic refractions rather than cycloplegic ones, which are known to be required for accurate refraction measurement in children, is a potential reason for their lack of significant findings. Due to potential confounding factors such as geographic location, lifestyle, diet, and ethnicity; to date it could only be presumed that myopia is a multifactorial condition and therefore the conclusion that intensive sunlight exposure plays a sole role in myopia prevention should not be formed.

### **Ciliary muscle, accommodation (tonic accommodation and accommodation accuracy) and pupils**

Annamaneni et al (2011) proposed that the vitamin D<sub>3</sub> receptor (VDR; an intracellular hormone receptor) might contribute indirectly to myopia through regulation of ciliary muscle function (VDR is known to play significant role in the alteration of intracellular calcium levels which might result in impaired contraction and relaxation of the ciliary muscle) but this has not been tested. The prediction would be that low dopamine and/or low vitamin D<sub>3</sub> levels would reduce focusing accuracy. Myopic children with high accommodation lags (accommodation response

less than the demand) have fast myopia progression (Gwiazda et al., 2004).

Accommodation is under autonomic control, with both parasympathetic [muscarinic receptors (Gilmartin & Hogan, 1985)] and sympathetic innervations [beta receptors (Gilmartin et al., 1984)] (Van Alphen, 1976; Wax & Molinoff, 1987). It has been suggested that the increase of accommodation results from raised parasympathetic stimulation (Yörnqvist, 1967). High lags of accommodation during near work (Gwiazda et al., 2004) and longer time to relax accommodation following near work (Vera-Díaz et al., 2002) are characteristic of progressing myopia (Arunthavaraja et al., 2010). Children with higher lags of accommodation show the most rapid myopia progression (Gwiazda et al., 2004). Myopes also tend to have low positive relative accommodation (Goss, 1991), i.e. poor ability to clear negative lenses. Low tonic accommodation is also proposed as a risk factor in the development of myopia (Ebenholtz, 1983; McBrien & Millodot, 1987).

Tonic accommodation (TA) is the resting point of the accommodation system measured when there is no feedback provided, i.e. when there is no visual stimulus (Leibowitz & Owens, 1975). It is believed that eye's refractive state under darkness is the status of being equalised between the sympathetic and parasympathetic tone to the ciliary muscle (Gilmartin & Hogan, 1985; Rosenfield et al., 1993). Given the equilibrium when the accommodative system is at resting position, the status may be the physiological reference point for the neural and motor control (McBrien & Millodot, 1988). Average tonic accommodation values in adults of 1.52 D have been reported, however it has been suggested inter-individual variation of TA is considerable, showing a range between 0 to 4 D (Leibowitz & Owens, 1978; Miller, 1978b), the remarkable difference may be due to varied onset of myopia or difference in refractive properties (McBrien & Millodot, 1987). Maddock et al. (1981) compared adults with high myopia and emmetropes and found that high myopes had lower values of tonic accommodation. Another study (McBrien & Millodot, 1987) further categorised myopic participants into juvenile onset and adult onset myopes; higher tonic accommodation levels occurred in hyperopes compared to both emmetropes and juvenile onset myopes (Maddock et al., 1981; Simonelli, 1979; Smith, 1983). In children, it was discovered that the mean lag of accommodation was higher in those with corrected myopia compared to those with emmetropia (Nakatsuka et al., 2005) Similarly, a higher lag of accommodation was found in young subjects who had developed myopia, i.e. it occurred after rather than before



myopia onset (Mutti et al., 2006). The studies concerning the possible correlations between myopia and accommodative functions are summarised in the following table.

**Table 2-4 Studies of the association between myopia and accommodation**

Research Study	Condition	Age (Year)	Sample Size (n)	Location	Results/Findings	P value
Choi et al. 2014	Refraction	13 to 18	2038	South Korea	Refraction was significantly associated with low serum 25(OH)D levels	P=0.047
Mutti et al. 2006	Myopia/ Tonic accommodation	6 to 15	1107 (568 becoming myopic, 539 remained emmetropic)	California, USA	High accommodation lags occurred after the onset of myopia rather than before the onset of myopia	After the onset (P<0.004); before the onset (P<0.8)
Gwiazda et al. 2004	Myopia/ Accommodation	6 to 11	469	Alabama, Massachusetts, Texas, Pennsylvania; USA	The fastest myopia progression occurred in children with larger accommodative lags wearing single vision lens	
Vera-Díaz et al. 2002	Myopia/ Accommodation	18 to 27	41	Bradford, UK	Progressive myopes had higher post-task accommodation shifts compared to both stable myopes and emmetropes	
Goss et al. 1991	Myopia/ Accommodation	6 to 15	75	Illinois, Iowa, Oklahoma, Oregon; USA	Lower positive relative accommodation was found in subjects becoming myopic as compare to those who remained emmetropic	
Maddock et al. 1981	Refraction/ Accommodation	All under 25 years but one	63	Cardiff, Wales; UK	High myopes had lower values of tonic accommodation	

Kesby et al. (2009) have demonstrated that the rate of dopamine turnover was reduced when vitamin D deficiency occurred in rats. The link between vitamin D and dopaminergic neurotransmission has been demonstrated in research, for example, Puchacz and colleagues (1996) observed in adrenal medulla and showed the potential of vitamin D in regulating tyrosine hydroxylase (the essential enzyme toward conversion of dopamine). In addition, the substantia nigra, a region containing abundant dopaminergic neurones, has the highest expression of vitamin D receptor; experimental vitamin D deficiency during developing period changes dopaminergic behaviours (Cui et al., 2015). Eyes of chicks, guinea pigs, and tree shrews (Dong et al., 2011a; McBrien et al., 2001) with form-deprivation myopia have decreased retinal dopamine. Given dopamine can be affected by vitamin D status and the association between refraction and dopamine, vitamin D has the potential to affect the development of human's refractive error via alterations to the accommodation function.

### **Dry eye disease**

Dry eye disease is a common [affects ~30% of the adult Caucasian population (Viso et al., 2012) and ~60% of the adult Asian population (Siak et al., 2012)] and severe ocular condition that results in ocular discomfort, visual disturbance and damage to the ocular surface (Pinho Tavares et al., 2010). It has been estimated that the average healthcare cost of 1,000 patients with dry eye in developed countries varies between US\$ 0.27 million to US \$1.10 million per year (Clegg et al., 2006); this is likely an underestimation as some patients self-manage their conditions.

Dry eye disease is accompanied by increased tear film osmolality (Sullivan et al., 2010; Zhou & Beuerman, 2012), and desiccation and inflammation of the ocular surface (Stevenson et al., 2012b). Ocular surface inflammation can lead to epithelial squamous metaplasia and apoptosis (Argüeso et al., 2002; De Paiva et al., 2007). Goblet cell (numbers reduce) viability is severely impacted by chronic ocular surface inflammation (Kunert et al., 2002; Pflugfelder et al., 2008). As a result of finding T cells and increased inflammatory cytokines in the conjunctiva and tears of dry eye patients (Massingale et al., 2009; Stern et al., 2002b), inflammatory mechanisms are believed to play a crucial role in this ocular condition. Experimental dry eye (for example induced by high levels of the preservative benzalkonium chloride used in

eye drops) results in pathological changes including epithelial apoptosis, squamous metaplasia and inflammation (Lin et al., 2011). Gradual lacrimal glands dysfunction can result from long term ocular surface dryness (Xiong et al., 2008). Some types of lacrimal glands dysfunction and/or tear production insufficiency may be prevented at the early stage of these conditions via the anti-inflammatory mechanisms of vitamin D.

Although the impact of low vitamin D on general health is not fully clear, associations between low vitamin D and systemic conditions such as Alzheimer disease, depression and peripheral vascular disease have been demonstrated (Afzal et al., 2014; Berk et al., 2007; Cherniack et al., 2009; Holick, 2004a). The level of an inflammatory marker, the receptor of interleukin-2, has also been reported to be higher in low 25(OH)D individuals (Bang, 1999), complying with the general recognition vitamin D does play a modulatory role in inflammatory condition such as arthritis (Patel et al., 2007). While a number of cancers are associated with inflammatory responses (Arthur et al., 2012; Bishayee, 2014; Elinav et al., 2013; Gomes et al., 2014; Sfanos et al., 2014), the preventive characteristics of vitamin D have been tested in cancers and it has been shown that vitamin D deficiency is a risk factor (Krishnan & Feldman, 2011). Similarly, inflammation component appears to play a key role in the etiopathogenesis of ocular surface dryness (Wei & Asbell, 2014; Yagci & Gurdal, 2014). Galor and colleagues (2014) surveyed 247 elder males and investigated the effects of diet and vitamin D levels on dry eye. It was found that for every 10-U increase in vitamin D in the serum the dry eye syndrome symptoms decreased by -1.24, suggesting the concentration of vitamin D in the blood may be associated with the severity of dry eye.

### **Ocular Inflammation**

Increasing evidence has shown that vitamin D<sub>3</sub> is able to influence inflammation (Baeke et al., 2010; Kamen & Tangpricha, 2010). In systematic conditions, for example, Alzheimer disease, the levels of amyloid beta [a protein associated with Alzheimer brain plaques (Glennner & Wong, 1984) that contributes to a chronic inflammatory response and ageing induced extracellular material deposition in Bruch's membrane (Anderson et al., 2002)], were greatly reduced after vitamin D<sub>3</sub> treatment. Inflammation in the retina increases as human age (Lee et al., 2012; Yang et al., 2007). A mice model with a short period of vitamin D<sub>3</sub> administration

demonstrated that not only did the numbers of macrophage cells reduce but also the size of cells increased in the retina (Lee et al., 2012). Another eye condition, transgenic murine retinoblastoma, the angiogenesis could be successfully inhibited through intraperitoneal administration of vitamin D<sub>3</sub> (Shokravi et al., 1995). In the study, the mice groups receiving 0.05 µg and 0.025 µg of vitamin D for 5 weeks had average vessel counts of 8.5 and 10.1 respectively, markedly less than that of the control group (14.1). Likewise, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and a synthetic vitamin D<sub>3</sub> analogue (22-oxa-1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>) also have demonstrated dose-dependent anti-angiogenesis in the chick model (Oikawa et al., 1990).

### **Age related macular degeneration (AMD)**

Age related macular degeneration (AMD) is the leading cause of blindness amongst people over 50 in Western countries (Bressler, 2004; Resnikoff et al., 2004). According to the Blue Mountains Eye Study (Mitchell et al., 1995), the prevalence of end stage AMD in Australians aged over 85 years is nearly 20%. There is no effective way of preventing this ocular condition and it cannot be cured (Jager et al., 2008). The pathogenesis of AMD is complex and it is thought to involve inflammation (Hageman et al., 2001), light damage and oxidative stress (Sparrow et al., 2002), a characteristic feature is amyloid deposition (Dentchev et al., 2003).

Although to date there is no effective treatment for AMD, evidence suggests that vitamin D<sub>3</sub> supplementation might assist in preventing further deterioration if used in the early stages of this condition (Millen et al., 2011). Further evidence for the protective functions of vitamin D, it has also been suggested that there was a negative association between the levels of serum vitamin D and early stage AMD (Parekh et al., 2007). An association between activated macrophage cells and chronic inflammation in the retina has been proposed (Grossniklaus et al., 2002; Penfold et al., 2001). AMD is recognised that its inflammatory response is characterised by an infiltration with macrophages and lymphocytes (human body defence and immune cells) in the blood-retina barrier including retinal pigment epithelium layer (Reddy et al., 1995; Seregard et al., 1994). The evaluation of monozygotic twin pairs found that the twin with less severe AMD had the higher dietary vitamin D consumption and thus concluded that behavioural and nutritional factors were crucial in the etiological involvement of AMD (Seddon et al., 2011).

A large proportion of AMD variation seems to be associated with the

inflammatory response (Seddon et al., 2009; Thornton et al., 2005). Of relevance to AMD, Mora et al. (2008) suggested that vitamin D modifies the immune system and can alter the inflammatory response. Furthermore, the risk of a number of age related chronic diseases is showed to be associated with vitamin D insufficiency and/or deficiency (Holick, 2007). There is also evidence that sunlight exposure can lower the risk of developing AMD (Darzins et al., 1997; Delcourt et al., 2001). Given the inflammation inhibitory properties of vitamin D (Mullin & Dobs, 2007), Vitamin D might be capable of impacting the underlying inflammation pathogenesis associated with AMD.

Other possible actions for the beneficial effects of Vitamin D, such as modulating neuronal excitability, have been proposed (Rupprecht & Holsboer, 1999; Zakon, 1998). The neuro-protective action of Vitamin D has been observed in animal models, for example, it has been suggested that over-activation of the glutamate receptor can cause a form of neuronal injury, known as excitotoxicity (Rothman & Olney, 1986). Brewer (2001) demonstrated that the Vitamin D hormone presented a direct neuroprotective action against this excitotoxicity. Also, in a model of brain inflammation (Garcion et al., 1998) it was showed that the number of apoptotic cells was reduced by the addition of 1,25(OH)<sub>2</sub>D<sub>3</sub>. Experimental neurotoxicity in animals was decreased by the administration of vitamin D<sub>3</sub> (Wang et al., 2001b).

### **Intraocular pressure (IOP)**

There is one reported human study on the impact of vitamin D supplements on the eye's IOP, stating that vitamin D had no significant effects (Krefting et al., 2013). In contrast, in animal models (adult cynomolgus monkeys), it has been demonstrated that intraocular pressure was reduced following vitamin D topical ocular application directly to the cornea (Kutuzova et al., 2012). The different findings may be due to the differing routes of administration, the animal study using topical application which has good ocular absorption (Dusso et al., 2005) and the human study using an oral route (Krefting et al., 2013). The recruiting period of the human IOP study was longer than 15 months, during this time there would be seasonal variation in vitamin D levels. The inclusion criterion was a vitamin D serum level of < 42 nmol/L, which is only considered to be an insufficiency (Nowson & Margerison, 2002; Vieth et al., 2001) and might not have a large effect on IOP. The potential of IOP and vitamin D association requires further investigation.

### **Vitamin D within the Eye (animal work)**

The human eye can only perceive light that has wavelengths between 390 to 750 nm; violet for the shortest and red for the longest wavelengths. Ultraviolet light ranges between 10 nm and 400 nm and sunlight consists of different bands of UV wavelength: UVA ranges between 400 and 315 nm; UVB ranges between 315 and 280 nm; UVC ranging between 280 and 100 nm (Hajrasouliha & Kaplan, 2012)

Both vitamin D<sub>3</sub> metabolite and vitamin D receptor mRNA have been measured in rabbit aqueous humour (Yin et al., 2011). Serum levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> were 4 times greater in free range rabbits compared to hutch reared rabbits. Serum level of 1,25(OH)<sub>2</sub>D<sub>3</sub> of free range rabbits were lower in the spring than the winter – highlighting the seasonal variations in vitamin D (Fairham & Harcourt-Brown, 1999). In Yin's study (2011), rabbits supplemented with higher vitamin D<sub>3</sub> were found to have 4 to 5 times elevation of 25(OH)D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> level was eightfold greater; while it was observed significant 24,25(OH)<sub>2</sub>D<sub>3</sub> increases in both control and supplementation group. The level of vitamin D metabolites in tear and aqueous humour was also evaluated and it showed both 25(OH)D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> were significantly elevated. This indicates that vitamin D<sub>3</sub> does enter the ocular circulation, i.e. it is present in aqueous humour and tears.

In animal models, myopia and excessive axial length are inhibited by high light levels both in chicks (Ashby et al., 2009a; Ashby & Schaeffel, 2010a; Cohen et al., 2011) and mammals (Norton et al., 2006; Smith et al., 2012a). To investigate the impact of artificial lighting and sunlight on axial length and refraction, Ashby (2009a) raised chicks under a range of lighting conditions. They reported that the brighter the light during a short period of diffuser removal the greater the myopia retardation, for example, 15 minute sunlight exposure resulted in a refraction of  $-1.10 \pm 0.45$  D and axial length of  $8.81 \pm 0.05$  mm, whereas the 15 minute intense indoor lighting exposure group had a refraction of  $-3.39 \pm 0.56$  D and axial length of  $8.88 \pm 0.04$  mm. A similar greater effect of high light levels retarding form deprivation myopia has also been demonstrated in non-human primates (Smith et al., 2012a). Smith et al. (2012a) showed in the monkey model that the eyes with diffusers in the high-light-reared group were less myopic than the form-deprived eyes of monkeys in the normal-light-reared group. Further, both eyes in the high-light reared monkeys were found to be more hyperopic than those of normal-light-reared-monkeys.

## **2.4 WHAT IS DRY EYE**

The International Dry Eye Workshop group defines dry eye as a “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear-film instability with potential damage to the ocular surface” (Nelson et al., 2011). Inflammation of ocular surface results in discomfort such as visual disturbance, irritation, light sensitivity, and itching, leading to poorer quality of life.

### **2.4.1 Inflammation in general health**

Inflammatory responses in the human body are triggered by the recruitment of inflammatory cells. Studies have demonstrated that inflammation is associated with systemic diseases such as atherogenesis and rheumatoid arthritis (Libby et al., 2011; Pope, 2002). While in atherogenesis, the cells produce pro-inflammatory mediators that would further promote the inflammatory response; in arthritis, macrophages are activated by monocyte, which activates the production of tumour necrosis factor (TNF) and IL-1. The mediator TNF then attracts more cells to the arthritic sites.

### **2.4.2 Inflammation and dry eye and the commonly used diagnostic techniques**

Similarly to the mechanisms involved in systemic diseases, cells including the epithelial cells of cornea and conjunctiva, and mediators are thought to be involved in the inflammatory component of dry eye disease (Wei & Asbell, 2014).

A range of objective techniques are used to examine the external ocular surface and assist in the diagnosis of dry eye disease. One of the most commonly used techniques is measurement of the tear break up time (TBUT). A good quality tear film should persist for at least 10 seconds (Abelson et al., 2002). The traditional technique of assessing the TBUT requires instillation of fluorescein, which may potentially affect the results (Johnson & Murphy, 2005). The non-invasive TBUT involves the corneal tear film stability and it is measured the time taken in seconds between the last complete blink and the presence of the first disturbance of the tear film (Mengher et al., 1985). Other techniques for assessing aqueous tear production include assessment of conjunctival staining, corneal staining, Schirmer's test, and the

Phenol Red Thread test (Bron et al., 2003; Saleh et al., 2006). The height of the tear meniscus, a convex tear component lying between the eye surface and the lower eyelid, can also be measured as an indication of tear volume. A tear meniscus height shorter than 0.3 mm is abnormal (Mainstone et al., 1996; Oguz et al., 2000). Technology to assist in this assessment have been developed, for example, keratograph (Best et al., 2012; Hong et al., 2013).

### **2.4.3 Seasonal variation and dry eye**

Longitudinal study on the symptoms of dry eye across a year showed that greater symptoms of eye dryness occur in spring and winter (Kumar et al.), compared to summer and fall. Excluding allergic factors (which can exacerbate or present as dryness of the ocular surface) that can result from metropolitan environments (Hikichi et al., 1995), Kumar and colleagues have suggested that seasonality is the strongest influencing factor; the changing prevalence of dry eye with season highlights its impact. While the shortest day in a year only has 10 hours and 16 minutes sunlight, the longest day is 13 hours and 43 minutes long, having a difference of 3.45 hours between winter and summer season (Sunlight hours over a year in Brisbane city <http://www.brisbane.climatemps.com/sunlight.php> ). The higher prevalence of dry eye, therefore, might be associated with varied vitamin D status resulted from different intensity of sunlight exposure in different seasons. Humidity, temperature, and the use of air conditioning may have to be considered due to the difference climate brought by seasons over a year.

### **2.4.4 Dry eye in animals**

Short term (2 months) external application of active type vitamin D on the eyelids in mice has also been shown to have effect on relieving Meibomian gland dysfunction (Jin et al., 2014). In rabbits, it has also been demonstrated that vitamin D<sub>2</sub> predominately presents in tear fluids and it was suggested that the source of vitamin D might be from lacrimal and Harderian glands (Lu et al., 2015).



## **2.5 GROUP SPECIFIC COMPONENT OF SERUM**

Gc-globulin (group specific component of serum), now known as vitamin D binding protein (DBP) as it is capable of binding 85 to 90% of total circulating 25(OH)D (Bikle et al., 1986). The bioavailability of vitamin D may be limited when bound with DBP (Safadi et al., 1999), appearing to suppress some actions of vitamin D. It has also been postulated that only “unbound” hormones are capable of entering cells and inducing biological actions (Mendel, 1989). This may be an explanation toward the relatively lower total 25(OH)D in the black as compared to that of the white, however had similar level of estimated bioavailable 25(OH)D (Powe et al., 2013). Also, Powe and colleagues (2011) found that bone density is not correlated with total 25(OH)D, but highly with the level of free 25(OH)D. The findings of these studies may be suggesting that the level of unbound vitamin D in the human body is associated with the biological actions of targeted cells and organs, meaning indirect effects can be produced by the carrier (binding) proteins.

## **2.6 VITAMIN D RECEPTOR**

Spending longer hours outdoor has emerged and been regarded as possessing protective effect toward developing myopia in recent years. The underlying mechanisms of this effect however remain unclear and some claim it is exercise resulting in the cease of myopia development rather than being exposed to sunlight (Deere et al., 2009; Jacobsen et al., 2008). However, studies in children conducted in different areas suggested that spending longer time outdoors is a greater protective factor as compared to physical activity (Dirani et al., 2009; Rose et al., 2008b). There has been the comparison of myopia progression between the seasons that have higher and lower hours of sunlight, demonstrating a slower progression of myopia in the sunnier seasons (Fulk et al., 2002). Also, dietary source of vitamin D has also been examined; for example, Taiwanese adult males have a daily intake of 3.39 µg vitamin D, while boys between the age of 6 and 12 only have 1.74 µg (Lee et al., 2008). Furthermore, myopes (as compared to non-myopes) having lower serum vitamin D has also been reported (Mutti & Marks, 2011). It therefore comes to the speculation that raised vitamin D level derived by cutaneous production might be one of the protective factors. Vitamin D receptor (VDR) can affect the concentration of

vitamin D in the human body, which therefore should be examined. In the Collaborative Longitudinal Evaluation of Ethnicity, it is reported that the single nucleotide polymorphisms rs1635529 is significantly transmitted to myopes (Mutti et al., 2007a). The chromosome that carries the SNP, is located closely to the gene VDR, making it a potential gene that can have effect on myopia (Wojciechowski, 2011).

## **2.7 MYOPIA**

Myopia, also known as short-sightedness, is the most common eye condition in the world, and is regarded as a complicated ocular diseases affected by both environmental and genetic factors (Hornbeak & Young, 2009; Tang et al., 2008). Studies have indicated that the prevalence of myopia is increasing in east and South-East Asian countries such as China, Hong Kong, Singapore, and Taiwan (Morgan & Rose, 2005), as well as Western communities such as the United States (Vitale et al., 2009), Australia (Morgan et al., 2012), and Europe (Williams et al., 2015). A potential consequence of myopia is reduced vision, and it is estimated that a quarter of the cases of reduced vision in people living in Western countries is due to myopia. In some Asian countries (e.g. Korea, Singapore, and Taiwan), myopia accounts for 80% of cases of reduced vision in younger people (Hosaka, 1988).

It has been suggested that the development of myopia occurs between the age of 5 and 15 (Saw et al., 2005a), and the progression ceases between the age 14.44 and 16.66 (Goss & Winkler, 1983) though some evidences have indicated that myopia can develop or progress significantly after adolescence (Bullimore et al., 2002; Mutti & Zadnik, 2000). It is also recognised that the earlier the onset of myopia, the more prominent it will progress (Pcirsinen & Lyyraf, 1993; Saw et al., 1996). Considering the severity and progression rate of myopia, it is crucial to intervene its development at the early stage.

A number of vision-threatening ocular conditions are also associated with myopia, for example:

### **Glaucoma:**

Glaucoma, an optic neuropathy characterised by the progressive degeneration of the optic nerve (Weinreb et al., 2016), is one of the leading causes of eye

conditions that can result in irreversible blindness (Malihi et al., 2014). The most common type in Western society, such as Europe and the United States (Kapetanakis et al., 2015; Vajaranant et al., 2012), is primary open-angle glaucoma (POAG); whereas in Asian countries, angle closure glaucoma is relatively more prevalent (Li et al., 2014a). It was estimated worldwide that the population with glaucoma in the year 2013 was 64.3 million and will increase to 76.0 million in the year of 2020 (Tham et al., 2014). Qie et al. (2013) have reported that myopic eyes have higher possibility of developing visual defects by comparison to emmetropic eyes; as compared to non-myopes, the incidence of developing glaucoma in people with myopia was higher by nearly three times (Mitchell et al., 1999). Furthermore, a recent study found that when comparing to non-myopes and low myopes, the incidence for high myopes to have POAG could go up to two times (Tham et al., 2016), the results of these studies indicate that myopia is at higher risk of acquiring glaucoma.

In high axial myopia, changes in optic nerve head are commonly observed, it has been suggested that the intrapapillary and parapapillary changes of optic nerve head in highly myopic eyes might result in increased susceptibility for glaucomatous optic nerve damage (Vajaranant et al., 2012).

#### **Retinal changes and degeneration:**

Not only does myopia have optical involvement, but also has retinal structure changes. For example, the most frequently observed retinal changes are optic nerve crescents, white-without-pressure, lattice degeneration, microcystoid degeneration, and pigmentary degeneration (Cheng et al., 2013; Takahashi et al., 2013). As compared to non-myopes, deformed, larger disc area, and longer disc-foveola distances have been reported (Hwang et al., 2012; Takahashi et al., 2013). In addition, thinner central retinal thickness (outward to 80°) has also been reported (Cheng et al., 2010), while there is an inverted correlation between the level of myopia and the thickness of peripapillary retinal nerve fibre layer (Choi & Lee, 2006).

#### **Cataracts**

A cataract is an opaque area over the crystalline lens of the eye resulting in decreased vision. Cataracts account for half of blindness and 1/3 of visual

impairment worldwide (WHO, 2012). Although the development of cataracts is gradual and slow, quality of visual acuity and vision related life are significantly affected and have been the concern while being assessed objectively and subjectively (Pokharel et al., 1998; Steinberg et al., 1994). It has been suggested that being myopic was an independent risk factor for cataract (McCarty et al., 1999).

## **2.8 MYOPIA IN ANIMALS**

To date, different animal models have been used in the observation of refractive development, including primate animals such as tree shrews (McBrien et al., 2000; Siegwart & Norton, 1998), monkeys (Smith et al., 2005; Smith III & Hung, 1999), and vertebrate animals such as guinea pigs (Liu et al., 2011; McFadden et al., 2004), chicks (Cohen et al., 2014; Cohen et al., 2012), and cats (Konrade et al., 2012; Rose et al., 1974).

Developing animals have been used to observe the effects of optical component on the refractive development of the human eye. For example, Schmid and Wildsoet (1996) found that chicks wearing high positive powered lens developed hyperopia, and a short period of normal vision intervention helped to prevent myopia development. Chick model also showed that the defocus induced by negative and positive lenses can be compensated, implying the absence of accommodation cues does not impede the blur signal being recognised by retina (Diether & Schaeffel, 1997). The development of refraction in the models of monkey (Hung et al., 1995) and tree shrew (McBrien et al., 1999), can be altered by wearing by positive or negative lenses. Animal models have shown that the development of refraction and axial length during early life can be manipulated by lens wear: a hyperopic defocused image forms behind the retina (using negative lens) causes axial elongation and myopic shift in refraction, whereas a myopic defocused image forms in front of the retina (using positive lens) develops hyperopia (Edwards, 1996; Schmid & Wildsoet, 1996).

However, using animals such as chick in experiments has some limitations. For instance, Infrared Photoretinoscopy has demonstrated that the range of accommodation is approximately 17 diopters, and may be due to the structure of head, each eye's accommodation of a chick acts independently (Schaeffel et al.,

1986), by comparison to the mere 10 diopters (Sun et al., 1988), binocular vision and concurrent accommodation in human. Other disadvantages such as different mechanism of accommodation, scleral composition, and lack of fovea need to be taken into consideration (Glasser & Howland, 1995; Glasser et al., 1995; Schaeffel & Howland, 1987).

In chicks, it has been demonstrated that the refractive development toward myopia is light intensity dependent using chicks reared in different light levels (50 lux, 500 lux, and 10,000 lux) (Cohen et al., 2011). In addition, under light-dark cycle condition, the speed of emmetropisation is light intensity dependent, higher ambient light slows the progress of emmetropisation, making axial length shorter as compared to low ambient light reared chicks (Cohen et al., 2014). However, rapid eye growth in chick model provided researchers to observe essential information on the mechanisms of emmetropisation. It has been reported that lens induced blurred image on the retina can be compensated by adjusting the rate of axial elongation in chicks (Schaeffel et al., 1988). In addition, FDM can be “partially” induced in the retinal areas that were restricted (Fugate-Wentzeak, 1987). Removal of accommodation using different means (Schaeffel et al., 1990; Schmid & Wildsoet, 1996) has excluded emmetropisation is guided by the level of accommodation. In addition, choroidal thickening by strong light illumination has also been observed in chicken (Lan et al., 2013). It is worth noting that in smaller eye model (chick) the moving forward/backward of choroid makes changes of refraction up to 7 D.

Thickness changes in choroid have raised attention of myopia research as the changes have tendency of bringing focal plane of optical system in the eye closer to the retina (Wallman et al., 1995b). In this experiment, one of the chick eyes was covered with a white, translucent diffuser to produce myopia via the mechanism of visual deprivation. Myopic blur was experienced which resulted in thickening of the choroid. Similarly, a plus lens creates myopic defocus and the choroid becomes thicker within hours. In contrast, a minus lens placed in front of the eye creates hyperopic defocus and the choroid thins. Thickening and thinning of choroidal thickness indicate that the defocus signals have to be recognised by the retina to modulate the rate of axial elongation. Recognition of the blur takes only two minutes (Zhu et al., 2005).

Dopamine reduction and myopia inhibitory effects of dopamine agonist during the development of FDM were firstly reported by Stone and the colleagues (1989a). Although the underlying mechanism remains unclear, now it is known that retinal dopamine release can be altered by retinal image quality, either manipulated by the contrast or illumination received on the retina (Feldkaemper et al., 1999). Reduced the contrast received by retina using myopic or hyperopic defocus leads to lower dopamine release (Feldkaemper & Schaeffel, 2013). Although the extents of myopia inhibitory effects using bright light are different in FDM and LIM (Ashby et al., 2009b; Ashby & Schaeffel, 2010b), myopia inhibitory effects induced by high intensity of light can be suppressed by intravitreal injection of dopamine antagonist (Cohen et al., 2012). The relationships between retinal dopamine release, bright light, and use of dopamine antagonist, again raised the interest of myopia research.

Tree shrew is one of the major animal models used in myopia research (Walker et al., 1978). Although tree shrews have poorer visual acuity (2 cycles/degree) than chickens, the scleral structure (one layer) is more closely to human (Schaeffel & Feldkaemper, 2015). Partial FDM on retinal area observed in chicks has also been shown in tree shrews (Norton & Siegwart, 1991). Myopia inhibitory effects of bright light have been demonstrated in both FDM and LIM and the myopia inhibitory effects of positive lens could be enhanced by bright light exposure (Norton & Siegwart, 2013; Siegwart Jr et al., 2012). Pharmacological studies have been focusing to investigate the mechanisms of muscarinic antagonists on anti-myopia effects [see review by Bererman et al. (2010)].

Another animal model relatively newer used in myopia research is guinea pig, initially introduced 2 decades ago (McFadden & Wallman, 1995). The model of guinea pig has been used to investigate dopamine release in non-experimental myopia, however the complexity of myopia development in guinea pigs appears to be higher as different doses of non-selective dopamine agonist (apomorphine) had opposite effects on myopia development (Jiang et al., 2014). A separate issue but worth noting is that LIM in both chicken and guinea pig showed retinal and choroidal retinoic acids can be regulated by the induced refractive errors (McFadden et al., 2004; Mertz & Wallman, 2000).

Marmosets have better visual acuity (30 cycles/degree) than tree shrew (Troilo & Judge, 1993). The thinning and thickening of the choroid may be a compensation

response to the lens induced defocus. This compensation response has also been observed in primates, for example, in infant monkeys it has been demonstrated that the eye's refractive state plays an important role in the increase of choroidal thickness during early stage of development (Hung et al., 2000a). Similarly, a marmoset model also showed that eyes with more myopic refraction had higher axial length and thinner choroids as compared to control eyes, axial elongation however continued upon recovery from FDM induced by lid suture (Troilo et al., 2000). Not only does the blurred image induced on the retina change choroidal thickness, temporary increase of choroidal thickness has also been reported when recovering from experimental myopia (form deprived) (Wallman et al., 1995a; Wallman et al., 1992). Choroidal thickness may be one of the factors for predicting visual acuity in high myopes; choroidal thinning was associated with poorer vision (Nishida et al., 2012). Similar as tree shrew model, the rate of retinoic acid synthesis changes during the development of FDM has also been reported (Troilo et al., 2006).

The animal model most closely to human is rhesus monkey. Myopia cannot be induced in dark-rearing monkey's eyes after lid suture, suggesting visual stimulation is required for experimental myopia (Raviola & Wiesel, 1978). Later it was reported apomorphine reduced FDM in monkey (Iuvone et al., 1991). As the findings in chick model, lens compensatory effects was also observed in monkey eyes (Hung et al., 1995). Choroidal changes were also found by lens induced defocus in monkeys (Hung et al., 2000b). Not only in infant monkeys, FDM can also be induced in adolescent monkeys (Smith III et al., 1999). In additional, the degree of FDM depends on the quality of retinal image - the poorer the quality, the higher the induced myopia (Smith & Hung, 2000).

All the evidence from animal studies to date suggests that light exposure is an essential process, implying the intensity of light, as well as the retinal areas that "perceive" light, could be associated with axial elongation, choroidal thickening, and the development of refraction.

### **2.8.1 Activity/exercise/time outdoors and myopia**

Lower myopic refractions and slower rates of myopia progression were associated with greater time spent outdoors and sport activities, though the

association was only significant in the school boys (Pärssinen & Lyyra, 1993). Mutti and colleagues (2002) reported that myopic children spent less time doing sport activities than emmetropic children. Subsequently it was proposed emmetropic children spent more time in sport with less near work and thus remained emmetropic. Similarly, Jones and the colleagues (2007) found that the children who developed myopia had significantly lower participation rate in sports and outdoor activities. In the longitudinal study, they concluded that children spending less time outdoors and on sport would have greater odds of developing myopia. More interestingly, this trend was observed regardless of parental myopia history. A study observing first year medical students suggested that more physical activity was associated with less change in refraction toward myopia (Dirani et al., 2009; Jacobsen et al., 2008). Similarly, Read and Collins (2011) also demonstrated that a 10 minute physical exercise decreased axial length, indicating the potential preventative effect of physical exercise for axial elongation. However, when analysing the two factors, physical activity and time spent outdoors separately, the latter has been shown to be more prominent in the incidence of myopia (Guggenheim et al., 2012a). Less time spent outdoors and in sport was also observed in children who developed myopia as compared to those remained emmetropic (Jones-Jordan et al., 2011). Likewise, Battersby and the colleagues (2015) compared whether there was difference between emmetropes, low myopes, and high myopes in young using pedometer to record and quantify the number of steps being completed in both semester and holiday periods, which they found that low myopes spent much less time on physical activities and being outdoor during the holiday period, with larger discrepancy in time spent outdoors. In addition, all the groups in the study showed similar low intensity of physical activity, suggesting the amount of physical exercise was not the dominant factor. Rose et al (2008b) further proposed that indoor sport activities did not protect from myopia development, whereas outdoor sports did. The findings of these studies might be indicating that less amount of time spent outdoors is a contributing factor to myopia development. While cross sectional design might not truly reflect the total time spent outdoors, as the data collected was based on self-reporting at a single point in time. Longitudinal studies have shown that time spending outdoors was a significant factor in myopia progression and time outdoors could be used to predict whether myopia would develop in young children (aged between 8 and 9 years) (Guggenheim et al., 2012b).



There are some studies which have indicated no association between time spent outdoors and myopia development, while the results of most studies have reported the protective effects of spending more time outdoors. Earlier studies conducted in Singapore showed time spent outdoors was not associated with myopia (Saw et al., 2006; Saw et al., 2001). Since then Saw and her colleagues have reported, in Loughheed's study (2014), a significant association between myopia and outdoor exposures when they adopted a modified version of the Sydney Myopia Study questionnaire. Lu and the colleagues (2009) examined 1232 school children aged between 13 and 16 years and reported no association between time outdoors and myopia development. However, the time spent outdoors reported by the children was between 4.8 and 7.6 (mean 6.1) hours. The low amount of variation in time spent outdoors could be the main obstacle to producing a statistically significant association. Young children (n = 3009, preschool age, between 6 months and 6 years) have also been assessed to determine if there were association between time spent outdoors and myopia (Low et al., 2010). No association was observed in this study, it might be the lower proportion of myopes in the sample, the sample size for emmetropes was nearly 8 times than myopes. In addition, eyes of children in this age are still under the process of emmetropisation (Siegwart Jr & Norton, 2011), genetic factors would be more prominent in the eyes that present refractive error.

The most significant difference between spending time outdoors and indoors is the light intensity is much higher, the average illuminance indoor is lower than 1000 lux (Read et al., 2014), while outdoor illuminance can reach 90000 lux (Li & Lam, 2000), and on a sunny day up to 120,000 lux (Lan et al., 2016). Evidence suggested by Rose et al (2008b) showed that the amount and intensity of light exposure, rather than activity changes, influenced myopia development. Although time and intensity of sunlight exposure can be measured by device such as Actiwatch (Ostrin, 2016), times spent outdoors in individuals could be largely varied depending on location, ethnicity, or age. For example, it was found that children in Australia who are of East Asian ancestry spent less time outdoors (up to 4 hours/daily), compared to longer hours in children of European Caucasian ancestry (6 hours/daily) (French et al., 2013b). Different region is also a determinant for time spending outdoors, school children in Taiwan spend less than half an hour outdoors per day (Wu et al., 2010), while a study in Beijing compared children living in rural and urban areas reported

the outdoors time were 2 hours/daily and 1 hour/daily, respectively (Guo et al., 2013b).

Initially, it was suggested that there was an inverse correlation between outdoor activities and myopia development (Dirani et al., 2009; Guo et al., 2013b; Jones-Jordan et al., 2011; Jones et al., 2007; Lee et al., 2013; Wu et al., 2010). In subsequent studies, some studies reported that the essential factor associated with myopia is likely bright outdoor light exposure rather than greater physical activity (Hua et al., 2015; Karouta & Ashby, 2015; Read et al., 2014). The current direction now might have a trend moving to investigating whether it is the high intensity of the natural light, or the violet spectrum contained in sunlight that would potentially inhibit myopia development (Torii et al., 2016).

Although to date there has been a conclusion that high illumination can prevent myopia development, a meta-analysis on 7 published works conducted during past 2 decades has indicated the consistency of these studies that greater time spent outdoors during a week reduced the risk of developing myopia (Sherwin et al., 2012c). While most of these studies suggested the protective effects of more weekly bright light exposure against myopia development, greater benefits of additional daily bright light exposure has also been raised (French et al., 2013b; Guggenheim et al., 2012a), implying frequency of bright light exposure might be of more importance when comparing to total weekly bright light exposure.

### **Sunlight**

It could be suggested that lower sunlight exposure might be the result from being myopic, rather than the cause of myopia, considering that blurred vision or spectacle wear may be obstacles for sport/exercise participation. A multiple-site study in the U.S. reported that myopic children spent fewer hours outdoors (by 1.1 to 1.8 hours) as compared to emmetropic children before and after the onset of myopia (Jones-Jordan et al., 2011), suggesting that long period spent indoors plays an important role in myopia development. Some studies have suggested an association between light and myopia (Chapell et al., 2001; Czepita et al., 2003; Saw et al., 2002; Vannas et al., 2003), more importantly and recently, it has been demonstrated that myopes exposed to shorter sunlight hours (by 2.5 hours to 2.8 hours) as compared to

non-myopes in the week in the young children (Dharani et al., 2012), though there was some degree of disagreement between different measure methods for sunlight exposure hours. However, other studies in earlier years did not report this association (Guggenheim et al., 2003; Saw et al., 2002; Zadnik et al., 2000). Although the survey in the study conducted by Vannas and colleagues (2003) was limited in conscript population, it still implied that a possible role of natural light in the pathogenesis of myopia. The findings of the study might also be suggesting that the development of refractive error in the human can be influenced via a biological signal generated by the amount and/or intensity of light exposure.

Whether the protective effect is principally caused by outdoor light exposure or physical activities has been debated. Not only have studies suggested that physical activities conducted indoors do not have inhibitory effect on myopia (Dirani et al., 2009; Rose et al., 2008b), it has also been suggested that both intensity (Rose et al., 2008b) and duration of light exposure (Guggenheim et al., 2012a) are key factors in myopia development. More recently, the intervention of daily 40 minute outdoor activity from a longitudinal study has been found to have potential to reduce the incidence of myopia in young school children (He et al., 2015a). Earlier intervention of being outdoor between classes during the day also showed protective effect from developing myopia, particularly in those children who had not developed myopia (Wu et al., 2013). This may be implying that the intensity and frequency of exposing to natural light are principle factors in myopia development.

### **Animal studies on light levels and myopia**

Induced myopia has been used in animal models to study the mechanism and gene expression of myopia development (Chakraborty et al., 2015; Chen et al., 2013; He et al., 2013; Smith et al., 2013; Ward et al., 2016; Zuo et al., 2015). Two of the commonly used methods for inducing myopia are form deprivation myopia (FDM) and lens induced myopia (LIM). Morgan and the colleagues (2013) have suggested that in FDM there is no defined endpoint and it is an open loop condition; while LIM is a closed loop condition, its lens induced hyperopic defocus can be reduced by axial elongation, and the elongation discontinues upon the neutralisation of the growth stimulus. Evidence for this is that a sectioned optic nerve cannot cease the development of FDM (Troilo et al., 1987), while an optic nerve section in LIM has a

mismatch with the power of the negated lenses that are applied to impose myopia (Wildsoet & Wallman, 1995).

During the past century, myopia has been extensively induced in experiments with FDM, given the term is it is through suturing the eyelids or blocking the vision with a translucent goggle. A number of animal species have demonstrated that the disruption of emmetropisation occurs leading to myopic-shifted refraction and axial elongation when there is FDM in the deprived eye; for example, chicks (Beresford et al., 2001; Feldkaemper et al., 1999; Schaeffel & Howland, 1991; Wildsoet & Wallman, 1995), and mammals (including monkeys, cats, tree shrews, and squirrels) (Funata & Tokoro, 1990; Hendrickson & Rosenblum, 1985; Jinren & Smith, 1989; Marsh-Tootle & Norton, 1989; McBrien et al., 1993; McBrien & Norton, 1992; Nathan et al., 1984; Norton & McBrien, 1992; Siegwart & Norton, 2001; Smith & Hung, 2000; Smith et al., 1987; Yinon, 1984).

FDM in chickens could be suppressed by constant light (Bartmann et al., 1994) and a similar inhibitory effect on myopia has been demonstrated in rhesus monkeys exposed to high intensity of light (range between 18000 and 28000 Lux) (Smith et al., 2012a). However, Lauber (1987) raised that constant light could induce glaucoma in the chicks about three decades earlier. Furthermore, chickens reared in dim environment have been shown to have larger eye size (Lauber & Kinnear, 1979). The progress of recovery from FDM under dim light was found to be much slower than that occurred under bright light (Wallman et al., 1995a), showing light also plays a crucial role in the recovery from induced myopia.

On the other hand, LIM given the term is to induce myopia by placing a negative lens in front of the eye. It has been suggested that visual feedback plays a role in LIM during the emmetropisation process (Schaeffel et al., 1988).

Similar as observed in FDM, animal studies using rhesus monkeys have also demonstrated that LIM can be impeded by sunlight exposure (Wang et al., 2015). The results of these studies thus indicate that light is a crucial factor that can affect refraction/refractive development. It is therefore suggested that cone activity is involved in both the development of refraction and emmetropisation (Crewther, 2000). However, Lan et al. (2014b) used 15000 lux to irradiate chickens in a constant mode and a minute cycle mode, which they discovered that the inhibitory effect of the bright light against myopia development is frequency dependant in light-dark

cycle in the animal, rendering the underlying mechanism may principally dependant on how often the eyes expose to intensive light.

The underlying mechanism signalled by FDM and LIM may be different, though the induced myopia in two experimental myopia causes axial elongation and thinner choroid in the eye. Past studies have shown dopamine is more effective to FDM than LIM [for review, see (Chen et al., 2003)]. However, the visual resolution blocked by the two means is essentially different. The underlying mechanism may be related to accommodation, the visual clue is mostly removed in FDM, while accommodation can compensate the defocused image in LIM. Reductions of dopamine and its metabolites were observed with 13-day constant light, accompanying myopia inhibition; whereas eye growth still continued in LIM (Bartmann et al., 1994). This may be indicating LIM is not dopamine rhythm dependant as observed in FDM. Further studies are required to confirm the difference of the two forms of experimental myopia. Schmid and Wildsoet (2004) reported myopia inhibitory effects of apomorphine (non-selective dopamine agonist) in both LIM and FDM in chicks, however animal model using guinea pig demonstrated only the inhibitory effects in FDM and both the levels of dopamine and dopamine metabolites were lower in FDM but such reduction was not observed in LIM (Dong et al., 2011b), indicating dopaminergic pathway is of higher relevance to FDM. In addition, less myopic shifts were observed with normal vision intervention in both FDM and LIM of chicks (Napper et al., 1995; Schmid & Wildsoet, 1996). Spiperone, a D2 dopamine antagonist, should therefore subdue myopia inhibitory effects of normal vision intervention. However, the subdued effects of spiperone were only observed in FDM but not LIM (Nickla & Totonelly, 2011). The results again suggest that dopamine mediates myopia development of the two types of experimental myopia differently.

Animals with better night vision, for example, cats, are found to develop toward more myopic shift if they are caged, without significant axial length change as compared to non-caged cats (Belkin et al., 1977). In chicks, Ashby and colleagues (2009a) placed translucent diffusers in front of the animals' eyes for 5 days, and during the period the diffusers were removed for 15 minutes each day under intense laboratory lighting (15,000 lux), the removal of diffusers resulted in significantly less myopic refraction than control group. In guinea pigs reared in high intensity of both

single spectrum light and broad band light showed significantly lower myopic shift by comparison to low intensity reared groups (Li et al., 2014b). The cat model might be indicating, firstly, that axial elongation resulting in myopia is only observable in cone predominant retina and cone cells have a mandatory role in myopia development; secondly, accommodation plays a relatively more important role in myopia caused by near adaptive vision. Lastly, myopia inhibitory effect of light may be more prominent in animals with cone predominant retina, indicating the “trigger” may primarily be activated via exposing to light.

### **2.8.2 Artificial light and sunlight**

Lighting source of light bulbs used in indoor environments such as classroom and/or office usually contains the visual spectrum between 400 and 780 nm, ultraviolet (UV) light (with wavelength from 10 nm to 400 nm) is not included. It is thus plausible that the protective effect of being outdoors is the fact that sunlight contains a broader spectrum including UV; A recent study showed that school children that spent longer hours outdoor have slower myopia progression (Jin et al., 2015), while a study conducted in the same area by Hua et al. (2015) suggested that higher indoor light intensity also helped cease further myopia development and axial elongation. .

To quantify the amount of UV light the eye is exposed to, a UV recording technique, conjunctival UV autofluorescence (UVAF) has been used. Compared to non-myopic, myopic eyes exposed to longer and stronger sunlight show decreased axial elongation and slower progression toward myopic shift (Cui et al., 2013); the prevalence of myopia was inversely associated with exposed UV intensity (Sherwin et al., 2012a), indicating the protective effect might be from UV light exposure.

Also worth noting is, Schmid and colleague (2013) applied different measuring methods with HOBO logger and UV dosimeter to assess daily illuminance and total dose of UV exposure over 3 separate days, they further categorised myopes into stable myopes and progressing myopes and found that individuals with progressing myopia had significant lower UV exposure. The results might be indicating longer time spending outdoor has protective effect against myopia development.

It is reasonable, on the other hand, to question the other difference between artificial light and sunlight, the intensity; while the usual illuminance indoor is lower than 1000 lux (Read et al., 2014), the outdoor illuminance can reach 90000 lux (Li & Lam, 2000), and on a sunny day up to 120,000 lux (Lan et al., 2016). The significant difference between the two lighting sources might be another potential influencing factor in the development of refractive error. While the protective mechanism for myopia development is not clear yet, Mori and the colleagues (2016) used a wavelength of 305 nm irradiation (short wavelength UV) to increase the vitamin D serum level by more than two times of the control group, and found that the eyes of chicks exposed to UV had significantly shorter axial length and vitreous chamber depth, indicating vitamin D level synthesised in the body might be associated with very early stage of myopia. However, Schaeffel and Smith also commented that light of this wavelength does not reach the retina (Mori et al., 2016)

### **2.8.3 Choroidal thickness and myopia**

A number of studies have suggested that eyes with myopia have relatively thinner posterior layers (choroid, retina, and sclera) as compared to those with emmetropia, or hyperopia (Curtin et al., 1979; Esmaeelpour et al., 2010; Ikuno & Tano, 2009; Kremser et al., 1999; Lam et al., 2007). Not only are thinner choroids observed in myopic eyes, eyes developing myopia were also found to have thinner choroids, whereas higher choroidal thickness was observed in non-myopic eyes (Troilo et al., 2000). The exact mechanism of choroidal thinning in myopic eyes is unknown, however it has been demonstrated in animal models that physiological factors such as creep, mechanical stretching, active matrix remodelling and/or outflow of layer fluid result in the thinning of these posterior segment layers (McBrien & Gentle, 2003; Nickla & Wallman, 2010; Phillips et al., 2000; Siegwart & Norton, 1999; Wallman et al., 1995a; Yinon et al., 1982).

## **2.9 DOPAMINE AND EXPERIMENTAL MYOPIA**

In experimental myopia using LIM and FDM, it has been suggested that both the metabolism and release of dopamine are reduced when placing diffusers or negative lens (Feldkaemper & Schaeffel, 2013). Significant and immediate reduction

in dopamine release has been demonstrated in eyes with developing FDM (Megaw et al., 1997b). To date it is unclear whether there is association between the reduction of dopamine release and the rate of axial elongation. Although dopamine agonists have been shown the capability of preventing further axial elongation in both FDM and LIM, while a model using guinea pigs has suggested that the potency of ceasing axial elongation was only observed in FDM (Dong et al., 2011b).

Earlier works have demonstrated that myopia and significant axial elongation can be induced when animals are reared under constant light and darkness, indicating a light/dark cycle is essential for emmetropisation. A FDM shares partially common property as constant darkness and both lead to the same consequences.

A rabbit model has shown that a dose of 1  $\mu\text{g}/\mu\text{l}$  (20  $\mu\text{l}$ ) by direct intravitreal injection was capable of preventing further myopic shift (Gao et al., 2006).

## **2.10 LIGHT AND DOPAMINE SYNTHESIS IN THE RETINA**

As early as three decades earlier, deprived vision in chicks' eye showed a reduction in retinal dopamine content (Stone et al., 1989a). Iuvone et al. (1989) occluded one of the eyes of rhesus monkeys and found that in the occluded eyes, not only the dopamine metabolite - 3,4-dihydroxyphenylacetic acid (DOPAC) showed decreased levels, but also lower level of tyrosine hydroxylase (the rate limiting enzyme required to synthesise dopamine) activity was observed, as compared to the un-occluded eyes. Similarly, other animal models using chicks, guinea pigs, and tree shrews also demonstrated reduced level of retinal dopamine in the deprived-vision eyes (Dong et al., 2011a; McBrien et al., 2001; Papastergiou et al., 1998).

It has been demonstrated that dopamine is contained in amacrine cells and interplexiform cells of a number of mammal species (Ehinger & Falck, 1969; Haggendal & Malmfors, 1965; Malmfors, 1963), this neurotransmitter/neuromodulator may play an important role in processing visual information. For example, changes in the signal transmitting to the human brain. Also, visual cortex signal can be controlled via dopamine alteration (with dopamine D1 manipulation) in macaques has been demonstrated (Noudoost & Moore, 2011).



Although immediate light exposure to dark adapted eyes of chicks does not significantly increase dopamine and DOPAC in the retina, the increase of dopamine and DOPAC in vitreous is almost two times; over the following 6 hours, DOPAC levels continue to rise, though the rate is slower as compared to the first hours (Megaw et al., 2001). The rise pattern of vitreal DOPAC may be indicating that the first hour is critical toward dopamine release, also implying the frequency of light exposure to the eyes is relatively more significant as compared to the total hours of light exposure.

The required enzyme for producing dopamine– tyrosine hydroxylase, has been found to be affected by surrounding lighting conditions (Iuvone et al., 1978b). Iuvone et.al (1978a) showed that the activity of tyrosine hydroxylase increased rapidly after exposing to light and it decreased following the removal of light in rats. In the study, the rate of dopamine formation by amacrine cells was also found to be raised by four times. The value of investigating the association concerning dopamine formation regulated by tyrosine hydroxylase activation therefore may be significant. If the higher dopamine formation being capable of inhibiting myopia development is accurate, constant light should cease further increase of refractive error in myope. However, Bartmann et al. (1994) found that constant light only had inhibitory effect on myopia development in chickens when the eyes were form deprivation myope (FDM), with no significant changes in eye with lens induced myopia (LIM), implying that the two types of myopia have different triggers; while normal diurnal dopamine rhythms is required in developing FDM, LIM may not be developed via dopaminergic pathways.

## **2.11 TYROSINE HYDROXYLASE AND DOPAMINERGIC ACTIVITY**

To form L-DOPA (the precursor of dopamine) in the human body, tyrosine, one of the types of amino acids in the human body, has to be hydroxylated by the rate limiting enzyme, tyrosine hydroxylase (TH) (Molinoff & Axelrod, 1971).

A number of studies have demonstrated that the turnover rate of dopamine and tyrosine hydroxylase in striatum of rats can be increased via administration of neuroleptic drugs (Andén et al., 1970; Carlsson & Lindqvist, 1963; Lloyd & Bartholini, 1974; Nyback & Sedvall, 1968). Electrical stimulation, meanwhile, is

also capable of raising tyrosine hydroxylase in rats (Murrin et al., 1976). Iuvone et al. (1978a) observed similar tyrosine hydroxylase increase in rats being exposed to a short period of light. In their studies, the light exposure was both applied to the whole animals and isolated eyes and tyrosine hydroxylase increased in both conditions, demonstrating that the behaviour of this enzyme is affected intrinsically through the tissue that perceives the light – retina. In the study, the increase of tyrosine hydroxylase was observed both in a short period of light exposure and under lighting condition of a normal 12 hour dark-light cycle; also, tyrosine hydroxylase activity is also reduced upon light removal. The effect toward activating dopamine in the retina might be due to the depolarization of amacrine cells (Werblin & Dowling, 1969).

## **2.12 DOPAMINE AND HORIZONTAL CELLS**

One of the retinal neurones, horizontal cells, helps regulate and integrate visual input from photoreceptor cells by providing inhibitory feedback to rod and cone photoreceptors (Demb & Singer, 2015; Masland, 2012). It has been suggested that horizontal cells could be depolarised by the application of dopamine and the amplitude of responses to light was reduced by the depolarisation (Dowling & Ehinger, 1978). This might be indicating that dopamine to some extent has the capability of triggering the responses of horizontal cells as light does. Although how the effect of dopamine to horizontal cells is produced is not clear yet, two activity pathways have been suggested, being via diffuse release, or via G-protein/second messenger (Dowling, 1991). While it is known that there are four types of horizontal cells, cone driven horizontal cells (3 types) are predominately in the retina, suggesting the generation of retinal depolarisation by dopamine could be via these cells. Neither membrane potential nor membrane resistance could be altered by a dopamine concentration of 300 $\mu$ M (Lasater & Dowling, 1982), implying the effects on horizontal cells from dopamine were via a second messenger.

## **2.13 DOPAMINE AND DOPAMINE RECEPTORS**

A number of studies have investigated the dopamine level in plasma (Pieske, 2012), urine (El-Beqqali et al., 2007; Kajiwarra et al., 2016), and serums (Xue et al., 2013). Measurement on dopamine levels in plasma using radioimmunoassay has a

value between 0 and 11470 ng/mL (TriCat RIA; DRG International, Marburg, Germany). Being one of the secretory fluids derived from blood (Sreebny et al., 1992) and the relatively more convenient and faster access, saliva can be used for measuring biometry in the human.

Vitamin D not only involves calcium homeostasis, but is also regarded as a neuroprotective nutrient in the human body (Garcion et al., 2002; Pérez-López, 2007). Dopamine, is found in amacrine cells in most vertebrates (Kramer, 1971; Witkovsky & Dearry, 1991) and it acts as a neurotransmitter. The level and behaviour of dopamine in the central nervous system of adult animal models have been investigated via the manipulation of vitamin D (Tekes et al., 2009; Tenenhouse et al., 1991). The forebrain of the neonatal rats investigated in a study (Kesby et al., 2009) revealed that dopaminergic turnover was altered in association with developmental vitamin D deficiency; in the study, the vitamin D deficient group appeared to have nearly half as much catechol-O-methyl transferase (COMT) expression as the control group. Although studies have suggested that COMT in post-synaptic neurons and glial cells (Kaakkola & Wurtman, 1992; Karhunen et al., 1995), it has been recognised that dopamine transport into these cells involves the pathway of uptake<sub>2</sub> (Trendelenburg, 1990), implying the dopaminergic behaviours may be affected through the change of COMT. Not only can prenatal experimental vitamin D deficiency affect the level of dopamine, but also post-natal vitamin D deficiency in the animal model showed association with higher levels of both dopamine and dopamine metabolites in the cortex and hypothalamus (Baksi & Hughes, 1982). In contrast Tenenhouse (1991) found increased dopamine but unchanged dopamine metabolites in the adult rats with vitamin D deficiency.

Past studies, primarily involving the chick myopia model, have shown that the development of form-deprivation myopia is associated with retinal dopamine metabolism (Stone et al., 1989b; Weiss & Schaeffel, 1993). Lower concentrations of dopamine induced by form-deprivation myopia lead to both longer axial length and larger equatorial diameter. Similarly, lens induced refractive errors in chicks have confirmed there was increased retinal dopamine level and 3,4-dihydroxyphenylacetic acid (DOPAC, the metabolite of dopamine) in hyperopia as compared to control eyes, while both retinal dopamine and DOPAC were found relatively lower in myopic eyes (Guo et al., 1995). The lower concentration of dopamine is thought to

be a consequence of decreased tyrosine hydroxylation during light adaptation (Stone et al., 1989b). In contrast, the concentration of dopamine is higher in eyes recovering from form-deprivation myopia (Pendrak et al., 1997). These studies have been confirmed using guinea pigs (Dong et al., 2011a) in form-deprivation myopia, reductions in both dopamine and its metabolites were observed. Other studies observing chickens also showed similar results (Bartmann et al., 1994; Megaw et al., 2006; Stone et al., 1989b).

In an animal model using rabbits, Mancino and colleagues found that the ciliary bodies contain dopamine receptors (Mancino et al., 1992). In humans, the smooth muscles of the iris are under autonomic control. Constriction of the iris sphincter via stimulation of the parasympathetic system results in pupil constriction, whereas constriction of the iris dilation via stimulation of the sympathetic system results in pupil dilation. Dopamine and its precursor tyrosine hydroxylase are involved in sympathetic transmission. Sonnenberg et al. (1986) found that in rat brain nuclei, the elevated choline acetyltransferase activity could be induced by  $1,25(\text{OH})_2\text{D}_3$  treatment, suggesting vitamin D has potential to influence certain aspects of function in the brain. Acetylcholine has been used in constricting pupil size following cataract surgery (Elliott & Carter, 1989). Clinically and physiologically, the amplitude of pupillary constriction can be reduced by the administration of an acetylcholine antagonist (Heller et al., 1990), while the sphincter muscle of iris can be stimulated by the administration of an acetylcholine agonist to induce higher pupillary constriction (Drummond, 1991). Thus, vitamin D may alter pupil size (this has never been assessed). In humans dopamine induced mydriasis should, theoretically, reduce the depth of field, transmitting higher demand of accommodation to visual system in order to compensate the relatively blur image of retina (Crawford et al., 1990; Hennessy et al., 1976).

Recently and more interestingly, Wu and colleague (2016) injected different doses of 6-hydroxydopamine in the vitreous of mice and found that dopamine levels were reduced by 20% to 60% demonstrating that experimental decreased retinal dopamine resulted in myopic shifts in the refraction of mice, the results indicate ocular growth might be regulated by dopamine levels.

### **The action of dopamine and the eye**

To act and produce effects in the human body, targeted cells (or organs) would

require dopamine receptors. There are already 2 groups of dopamine receptors identified, D<sub>1</sub>-like (D<sub>1</sub> and D<sub>5</sub>) family and D<sub>2</sub>-like (D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>) family; D<sub>1</sub>-like family stimulates adenylate cyclase and cyclic adenosine monophosphate (cAMP), while D<sub>2</sub>-like receptors inhibit adenylate cyclase and cAMP (Witkovsky, 2004). Animal models using rats (Giorgi et al., 1992) and chicks (Zawilska et al., 2004) have revealed that D<sub>1</sub>-like dopamine receptors present in the retina.

Agents that mimic dopamine such as apomorphine (non-selective agonist) and quinpirole (D<sub>2</sub> receptor agonist) on ocular growth have been examined (Nickla et al., 2010), and both appear to be significantly effective in inhibiting the development of myopia; the inhibitory effect of the two dopaminergic agonists is thought to result from the inhibition of axial length by their pharmacological potency. Furthermore, their study found that the thickness of choroid of lens induced myopic eyes showed transient increase following intravitreal injection of both quinpirole and apomorphine, while D<sub>1</sub> agonist (SKF-38393) had no effect in inhibiting ocular growth and choroid thickening.

Animal models using chicks, tree shrews, and guinea pigs have demonstrated that eyes with fully or partially deprived vision have reduced level of retinal dopamine (Dong et al., 2011b; McBrien et al., 2001; Stone et al., 1989b). In monkeys, the enzyme required to synthesise dopamine and tyrosine hydroxylase also showed a reduction (Stone et al., 1989b). Due to the stability of dopamine and its metabolites in human's vitreous (Cohen et al., 2012), the concentration of dopamine metabolite - 3,4-Dihydroxyphenylacetic acid (DOPAC) in vitreous has been thought to be a reliable reflex of retinal dopamine (Megaw et al., 1997a; Megaw et al., 2001); DOPAC was found to reduce in negative lens wearing chicks (Ohngemach et al., 1997). More interestingly, studies using chicks also found that there was only reduction of DOPAC in the section of the deprived area in the retina and also only the deprived area grew further (Ohngemach et al., 1997; Stone et al., 2006). The results of the studies might be indicating that there is a negative association between retinal dopamine release and ocular growth, also the refraction can be affected via alteration of dopamine level results from deprived vision.

Dopamine requires receptors to be utilised in the human body. There are two types of dopamine receptors in the human body, D<sub>1</sub> like and D<sub>2</sub> like receptors (Self et al., 1996; Stoof & Keibian, 1981). A number of studies using vertebrates have

shown that there are both D1 and D2 like dopamine receptors in the retina (Cahill & Besharse, 1991; Lankford et al., 1988; Nguyen-Legros et al., 1999; Schorderet & Nowak, 1990; Wagner et al., 1993). Dopamine D1 and D5 receptors are categorised as the D<sub>1</sub> like receptors, while D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> are of the D<sub>2</sub> like receptor family. When being activated, D<sub>1</sub> like receptors are stimulated to increase cyclic adenosine monophosphate (cAMP), while D<sub>2</sub> like receptors act to decrease cAMP. All types of retinal neurones contain dopamine receptors, and it is believed that biological activities such as retinal development and light adaptation are regulated by dopamine receptors (Nguyen-Legros et al., 1999; Nir et al., 2002). Dopamine D<sub>1</sub> receptors, for example, regulate connections of gap junctions between horizontal cells and amacrine cells (Kothmann et al., 2009; Urschel et al., 2006; Zhang et al., 2011). Acetylcholine release in amacrine cells (Hensler et al., 1987), neurite outgrowth (Lankford et al., 1987), and adaptation and sensitivity to light sensed in retinal ganglion cells (Van Hook et al., 2012), are all regulated by dopamine D<sub>1</sub> receptors. While dopamine D<sub>2</sub> receptors are expressed by amacrine cells (Nguyen-Legros et al., 1999; Weber et al., 2001), retinal ganglion cells (Mills et al., 2007), and photoreceptors (Ribelayga et al., 2008). Recently and more importantly, electroretinography has demonstrated that dopamine D<sub>2</sub> receptors more dominantly regulate the development of light responses of the inner retina (Tian et al., 2015).

Interestingly, Wu and the colleague (2016) injected different doses of 6-hydroxydopamine in the vitreous of mice and found that dopamine levels were reduced by approximately 20% to 60% (dose dependent). The results demonstrated that experimental decreased retinal dopamine resulted in myopic shifts in the refraction of mice through the steepening cornea. The myopic shift induced by reduced retinal dopamine may support the hypothesis that the protective effect against myopia development can be achieved by enhancing retinal dopamine level through outdoor activity and high ambient illumination (Hua et al., 2015; Lan et al., 2014a; Norton & Siegwart, 2013; Siegwart Jr et al., 2012).

## **2.14 INSULIN LIKE GROWTH FACTOR-1**

Insulin, synthesised in the pancreas, plays an important role in absorbing glucose from the blood into fat, liver and skeletal muscle cells (Stryer, 1995). The production of glucose by the liver is regulated by the concentrations of insulin in the

blood (White, 2003). The insulin like growth factors (IGFs), synthesised by the liver, are structurally similar to insulin but have an extended carboxy terminus (Humbel, 1990). The two types of IGFs – IGF- I and IGF- II, are essential in functioning most organs in the body (LeRoith et al., 1991). Although removal of both IGF- I and IGF- II in mice has shown effects of growth deficiency (Baker et al., 1993), IGF-I is believed to predominantly regulate postnatal growth (Le Roith, 1997). Both insulin and insulin like growth factor -1 (IGF-1) have the effect of promoting the rate of ocular elongation in the eyes without optical interference (Zhu & Wallman, 2009). Also, it has been demonstrated that the mice of higher expressed IGF-1 showed an overgrowth of the brain (D’Ercole et al., 1996).

Feldkaemper et al (2009) have shown in chicks that negative lens wearing eyes can have further increase in axial length with insulin injection, while the development of hyperopia is blocked by insulin. Similarly, it is also observed that in eyes without optical interference, the growth rate of axial length increase is also induced by insulin like growth factor -1 (IGF-1) (Zhu & Wallman, 2009). In addition, it has been demonstrated that high grade myopia is genetically associated with IGF-1 in human (Metlapally et al., 2010). The results of these studies may be indicating the ocular growth can be promoted by IGF-1. IGF-1 can be measured in serum, urine, and saliva (Antonelli et al.; Parkinson et al., 2001). In consideration of both the perspectives of convenience and non-invasion, saliva sampling procedure would be feasible and applied in this experiment. The participants were asked to give some saliva. An immunoenzymatic method (ELISA) was used for salivary analysis.

## **2.15 HORIZONTAL CELLS, BIPOLAR CELLS, AND AMACRINE CELLS IN THE RETINA**

The application of dopamine in the retina of fish has shown to be capable of depolarising horizontal cells (Hedden & Dowling, 1978).

Two main types of bipolar cells, cone bipolar cells and rod bipolar cells, have been discovered in the retinae of mammals (Sterling et al., 1995). Animal model using monkey has provided evidence that signal is transmitted from the cone bipolar cells in the outer plexiform layer to the dopaminergic amacrine cells in the inner plexiform layer via a light mediated pathway (Hokoc & Mariani, 1987).

Different responses to light further divide bipolar cells into two types (Kolb & Nelson, 1995), ON-bipolar cells and OFF-bipolar cells. The former have axons terminating in the inner half of the inner plexiform layer (IPL) and depolarising in response to light, while the latter show hyperpolarising light response and have axons which terminate in the outer half of the IPL (Euler et al., 1996; Hartveit, 1997; Wassle, 2004). As the different responses to light between the two types of bipolar cells, it may be possible that one is responsible for triggering light response while the others are functioning as inhibition in order not to over respond.

## **2.16 DEFOCUSED IMAGE, PHORIA, ACCOMMODATION AND MYOPIA**

In most viewing conditions, the human eyes tend to accommodate less than the demand, resulting in under-accommodation and exhibiting a lag of accommodation. In this situation, the image is formed slightly behind the retina which leads to hyperopic defocus. A hyperopic defocused image has been suggested as one of the contributing factors toward axial elongation and myopia progression (Gwiazda et al., 1993).

The onset of myopia has also been indicated as one of the influencing factors to accommodation accuracy (Gwiazda et al., 2005). Early onset myopia tends to have greater refraction as compared to myopia developed at a later onset (Grosenor & Scott, 1993). However, Seidel and the colleagues (2005) demonstrated no significant difference in accommodation accuracy among early onset myopia, late onset myopia and emmetropia under free space viewing conditions.

Type of phoria in myopic individuals may also be related to the progression of myopia as phoria reflects the status of oculomotor system. For example, near addition using bifocal lenses has been shown to be more beneficial in reducing the progression rate of myopia in children with esophoria than those with exophoria (Fulk et al., 2000).

## **2.17 ACCOMMODATION AND CILIARY SMOOTH MUSCLES**

To understand the action of human accommodation, one must have knowledge of neural control and receptors distribution in the ciliary muscle.



Parasympathetic system dominantly innervates in the ciliary muscle and the action of ciliary muscle is controlled by the transmit of acetylcholine on muscarinic receptors (Lograno & Reibaldi, 1986; Pang et al., 1994), while the sympathetic innervation is mediated by noradrenaline on post-synaptic receptors, generating an inhibitory effect on the input of parasympathetic system. Two types of post-synaptic receptors –  $\alpha_1$  and  $\beta_2$ -adrenoceptors, have been identified in the ciliary muscle (Wax & Molinoff, 1987; Wikberg-Matsson et al., 2000; Zetterström & Hahnenberger, 1988). Action of accommodation is controlled by the interaction between sympathetic and parasympathetic inputs. It has been demonstrated that phenylephrine (alpha-agonist) induced accommodation reduction while thymoxamine (alpha-antagonist) increased accommodation (Culhane et al., 1999; Garner, 1983). Instillation of isoprenaline (nonselective beta-agonist) would induce the resting accommodation (tonic accommodation) toward hyperopic shift, while it has been found that the use of timolol (beta-antagonist) and betaxolol (beta<sub>1</sub>-antagonist) resulted in myopic shift in accommodation (Gilmartin, 1986; Gilmartin & Hogan, 1985; Gilmartin et al., 1984). Due to the sustained accommodation required for near tasks and the characteristics of sympathetic control, it is believed that sympathetic is dominant when performing continuous near work (Gilmartin & Bullimore, 1987; YöRnqvist, 1967).

## **2.18 ACCOMMODATION/TONIC ACCOMMODATION AND REFRACTIVE ERRORS**

The association between performing near tasks and myopia development has been reported in a number of studies (Hepsen et al., 2001; Li et al., 2015b; Saw et al., 2002; Tan et al., 2000). Accommodative functions should differ between myopes and emmetropes if myopia is to be triggered via performing near works. Differences in the autonomic control to the ciliary muscles might be the underlying mechanism associated with refraction developing toward myopic shift (Chen et al., 2003). Pharmacological effects using dopaminergic and cholinergic agents have been examined in experimental myopia (Dong et al., 2011b; Nickla et al., 2010; Schmid & Wildsoet, 2004; Schwahn et al., 2000).

Clinically, the measure of an eye's amplitude of accommodation (AA) is an indication of the maximum accommodative ability (Rosenfield & Cohen, 1996). AA

is frequently measured for assessing the efficacy of topically pharmaceuticals, such as cycloplegic and mydriatic agents. AA can also be significantly altered by a number of systemic medications (Sears, 1989; Sloan, 1962). When the AA of an eye is minimal, more accommodation reserve is used for near work, and a myopic shift could occur for lower accommodation demand (Chen et al., 2003). The association between AA and refractive error has been repeatedly investigated. However, the inconsistency of the findings in studies investigating AA and refractive error leaves an indefinite conclusion. For example, higher AA in myopes has been suggested in a number of studies (Fledelius, 1981; Maddock et al., 1981; McBrien & Millodot, 1986), whereas Fong (Fong, 1997) found myopes had lower AA, and other studies found no association (Fisher et al., 1987; Gawron, 1981). Possible increasing lag of accommodation may result from a mismatch between accommodation accuracy (under-accommodate) and accommodation demand, leading to hyperopic defocus on retina and promote axial elongation (Gwiazda et al., 1993; Schmid & Strang, 2015). Two different methods haven been frequently used in measuring AA: one is to change the dioptric power of the lens measuring the amplitude, whereas the other is to change the distance of observing target in front of the eye. Myope is believed to have higher lag of accommodation though measuring methodology varied in the studies examining eye's accommodative response. However, ambiguous findings from previous study leave a conclusion suspended. Whether a lag of accommodation is the result of being myopic or the cause toward myopia development requires more suspend studies. Higher lag of accommodation was found in young myopes (between 5 and 17 years of age) than in emmetropes (Gwiazda et al., 2003). However, increased lag of accommodation was observed in myopic children but not in those emmetropes who became myopic (Mutti et al., 2006), indicating lags of accommodation might occur after the onset of myopia. Measuring method might also affect the results. For example, when comparing accommodative lags between the use of negative lens and changing distance in emmetropic children, it has been reported that the measurements of negative lens series showed larger lags of accommodation (Chen & O'Leary, 2002; Gwiazda et al., 2003).

Human's (young) accommodation remains in the resting position when there is no visual stimulation, presenting as intermediate dioptric power between far and near points. This dioptric power is known as tonic accommodation (TA) (Gwiazda et al.,

1995). While it is believed that the underlying cause for adult onset myopia being environment, juvenile onset myopia occurs due to genetic characteristics. The association between refractive errors and TA has been investigated. A number of studies have indicated that emmetropes have higher TA (Fisher et al., 1987; Jiang, 1995; Maddock et al., 1981), while some have reported conflicting results (Gawron, 1981; Tokoro, 1988). Although TA has been shown to be stable in an individual (Johnson et al., 1984; Mershon & Amerson, 1980; Miller, 1978b), there are varied influencing factors that can contribute to inconsistent finding from previous studies, for example, variability between human subjects (Schaeffel et al., 1993), set up of instruments and the criteria of refractive error (Bullimore et al., 1986; Rosenfield et al., 1993), and varied measuring conditions (Gray et al., 1998). Onset of myopia may be another influencing factor in measuring TA (McBrien & Millodot, 1988). However, the results from previous have not been able to reach a consensus. Studies comparing TA between emmetropes and late onset myopes have suggested that adult onset myopia appears to have lower TA (Bullimore & Gilmartin, 1987; Gilmartin & Bullimore, 1991; Jiang, 1995; McBrien & Millodot, 1987), while some have indicated that early onset myopes have lower TA than emmetropes (Gwiazda et al., 1995; Rosner & Rosner, 1989; Woung et al., 1998; Zadnik et al., 1999). In spite of varied onset of myopia, the results of these studies suggested TA is lower in myopes. Interestingly, most results of the studies showing early onset myopes with lower TA were measured in young children and teenagers. However, other studies found TA in young adults was higher in myopes than in emmetropes (Gawron, 1981; Maddock et al., 1981; Tokoro, 1988). In addition, it is observed that a sustained near task of 15 minutes had an increase in TA more significantly in adult onset myopia (McBrien & Millodot, 1988), indicating this equilibrium can temporarily be altered.

A sustained near work can temporarily change accommodation response toward myopic direction (Ebenholtz, 1983; Rosenfield & Gilmartin, 1999). This myopic shift occurred in TA and accommodation is known as accommodative hysteresis or adaption (Hung, 1992; Rosenfield et al., 1993). Depending on types of refractive errors, the recovery time from the status of accommodative hysteresis to the original level of TA ranges from few seconds to hours (Baker et al., 1983; Ebenholtz, 1983), which the large variation between individuals might be related to the onset and development of myopia.

## **2.19 SUMMARY AND IMPLICATIONS**

Limited sun exposure from indoor lifestyles has led to large numbers of people with insufficient levels of vitamin D (Jääskeläinen et al., 2013; Tolppanen et al., 2012). Few foods naturally containing vitamin D can be another contributing factor (Holick et al., 2011; Lamberg-Allardt, 2006). Insufficient or deficient vitamin D level has been linked to myopia (Choi et al., 2014; Mutti & Marks, 2011), dry eye disease (Galor et al., 2014) and age related macular degeneration (Millen et al., 2011). Furthermore, Yeung et al. (2001) has demonstrated that accommodation accuracy can be affected by dopamine antagonists, implicating dopaminergic activity plays a role on accommodative facility. To date, there is limited knowledge on vitamin D and eye health.

### **Aims**

This project investigated in humans whether there was an association between

- (i) serum vitamin D levels, ocular surface inflammation and dry eye in older adults, and
- (ii) vitamin D levels and accommodation functions/refraction in young adults.

This project investigated whether vitamin D supplements could

- (iii) reduce ocular dryness in older adults with low vitamin D levels and/or
- (iv) improve accommodation facility in younger adults with low vitamin D levels.

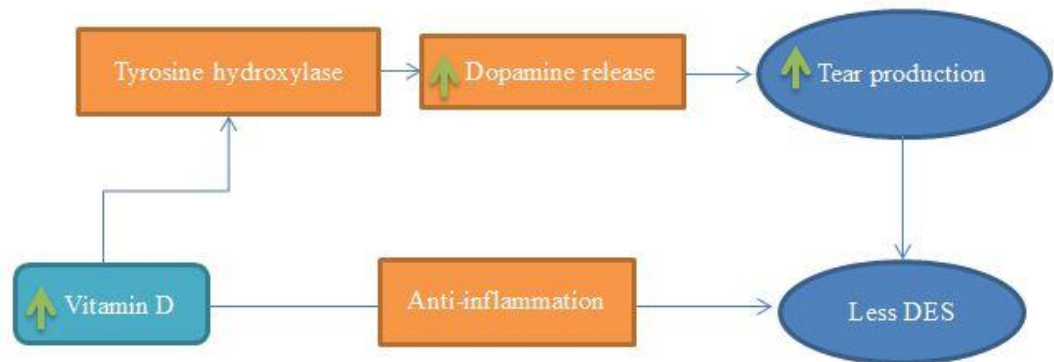
The main hypotheses were:

- (i) In the human eye, vitamin D would produce anti-inflammatory effects on the ocular surface thus improving ocular surface health and decrease dryness.
- (ii) In the human eye, vitamin D would improve the function of the accommodation system, resulting in reduced lags of accommodation and greater accommodative tone and decreased myopia risk.

## **2.20 HYPOTHESIS**

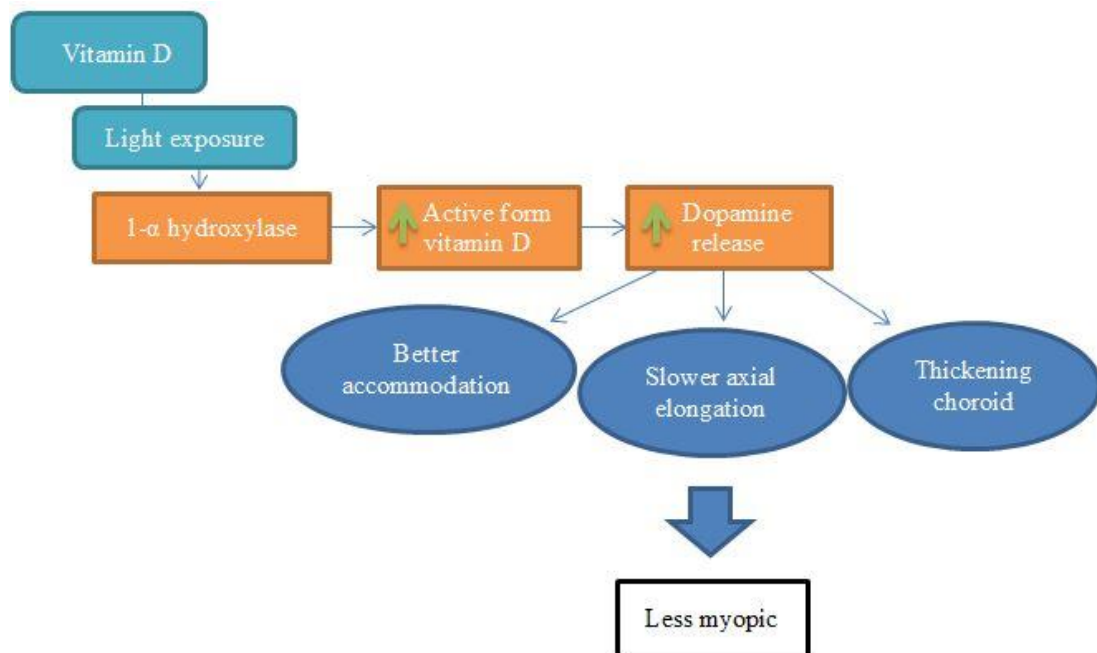
The hypothesis is the increase of vitamin D levels will enhance the activity of tyrosine hydroxylase, resulting in higher dopamine release to stimulate more tear

production; the anti-inflammatory property of vitamin D also leads to less dry eye syndromes (see **Figure 2-1**).



**Figure 2-1** The flow diagram of vitamin D treatment hypothesis in dry eye

Increased vitamin D or higher light intensity will produce more 1-alpha hydroxylase, assisting more active form of vitamin D production and therefore higher dopamine release. The produced higher levels of dopamine will lead to better accommodation, slower axial elongation, and thicker choroid, resulting in less myopia (see **Figure 2-2**).



**Figure 2-2** The flow diagram of vitamin D treatment hypothesis in myopia/accommodation

## 2.21 IMPLICATIONS

Dry eye affects about 1/3 of adult population in the world. People with dry eye often report dryness, irritation, fatigue, and fluctuating visual disturbances (Miljanović et al., 2007a). The discomforts and symptoms caused by dry eye considerably impact dry eye patients' quality of life, in particular in carrying out daily tasks including reading, computer use, driving, and watching television (Miljanović et al., 2007b).

To date, there has been more natural treatment developed, for example, an anti-bacterial eye drop that contains medical honey. However, the adverse effects such as ocular redness and stinging have been reported (Albietz & Lenton, 2006). If vitamin D levels in the human body are associated with ocular surface dryness, this study may be able to bring a new perspective toward dry eye management in the future.

The tendency of an earlier onset of myopia leading to a longer duration of myopia progression and higher myopic refractive error, by comparison to a late onset myopia, is generally accepted (Thorn et al., 2005). Although the rates of myopia progression have been shown to be relatively lower in Western countries (-0.10 to -0.30 D) (Hirsch, 1952, 1962; Zadnik et al., 1993) as compared to Asian countries such as Singapore (-0.56 D to -2.00D) (Saw et al., 2000) and Hong Kong (-0.63 D) (Fan et al., 2004). A preventive treatment should be applied on the younger generation in order to avoid future ocular complications and higher health expenditure.

# Chapter 3: Research Design

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This chapter includes the information concerning ethics approval, inclusion/exclusion criteria of the participants, statistics analysis, preliminary experiments and various instruments/techniques used for measuring ocular biometry.

## 3.1 ETHICS

This research involved “low risk” experiments. According to the Queensland University of Technology Ethical Conduct in Human Research guidelines, research is “low risk” where the only foreseeable risk is discomfort, which can involve body and/or mind. All instruments except for the handheld tonometer used in the Experiment 3 and 4, were non-contact. Appropriate training, assessment, and inductions were undertaken at the Optometry clinic of Queensland University of Technology and the Institute of Health and Biomedical Innovation. A health and safety research risk assessment was carried out as part of the ethics application. The experiments of the study followed the tenets of the declaration of Helsinki and were approved by the Human Research Ethics Committee of the Queensland University of Technology (Dry Eye: #1400000555) (Myopia: #1500000591). The nature of experimental procedures was explained to participants and written informed consent was obtained before undertaking measurements.

## 3.2 METHODOLOGY

There were two main stages in the targeted groups. Stage 1 was recruiting either older (Dry Eye study: aged between 43 and 69 years) or younger (Myopia/accommodation study: aged between 18 and 25 years) people. Stage 2 was to identify older people with dry eye/low vitamin D or young people with poor accommodation/myopia for the Vitamin D supplement study. For characteristics of participants, see summary information in

**Table 3-1 and Table 3-2.**

**Table 3-1 Characteristics of two groups of participants in dry eye study**

	Experiment 1		Experiment 2	
Group	AusSun	Optometry Clinic	AusSun	Optometry Clinic
Age	54.2 ± 7.8	56.10 ± 6.7	56.04 ± 7.07	56.00 ± 8.47
Source of recruiting	Recruited from a previous study investigating seasonal variation of	Recruited from the Optometry clinic of QUT, all had dry eye history	Recruited from Experiment 1 showing lower vitamin D	Invited from Experiment 1 from those who were willing to participate
Vitamin D status	Lower mean serum vitamin D levels	Higher mean serum vitamin D levels		
Dry eye status			Six (86%) had dry eye	Seventeen (68%) showed dry eye in the testing of Experiment 1

**Table 3-2 Characteristics of two groups of participants in myopia/accommodation study**

	Experiment 3	Experiment 4
Age	22.2 ± 2.22	22.79 ± 2.27
Gender	Male = 19 (34.55%) Female = 36 (65.45%)	Male = 10 (30.3%) Female = 23 (69.7%)
Refractive status	Myopes = 35 (63.6%) Emmetropes = 20 (36.4%)	Myopes = 4 (12%) Emmetropes = 29 (88%)



### 3.3 INCLUSION AND EXCLUSION CRITERIA

Criteria for the assessment of participant eligibility were the same, excluding age, for all main experiments, and were assessed using routine clinical tests. Tests included visual acuity measurement were conducted under normal lab room illumination (68-84 lux; measured with Watt Watcher FX-200 Light Meter illuminometer). Bailey-Lovie LogMAR visual acuity chart was adopted as it provides an accurate and rapid measurement (Lovie-Kitchin, 1988), refraction (to ensure there was no high myopia, hyperopia, and astigmatism), tonometry (to excluding those who were potentially at risk of glaucoma which might influence eye sight) and natural pupil ophthalmoscopic examination (to confirm there were no retinal/lenticular diseases). In the dry eye study, ages were limited to the range between 40 and 70 years because the incidence of dry eye is higher in older population. In the myopia/accommodation study, ages were limited to between 18 and 25 years because the refractive status would be relatively stable and the accommodative function would still have sufficient flexibility. Participants had best corrected visual acuity better than or equal to 6/7.5 with astigmatism within -0.50 D for myopia study group. Only participants with no ocular conditions were included, therefore intraocular pressure during selection screening tests was limited to  $\leq 21$  mmHg. Individuals with any evidence or previous history of any ocular disease or surgery were excluded from participation.

### 3.4 INSTRUMENTS

The following tests were performed on all participants (in all experiments) to determine suitability. Inclusion criteria were: no ocular pathology aside from ocular surface disease, strabismus, amblyopia or other vision problems aside from refractive ametropia.

**Spectacle/sunglasses use history:** A biomarker of outdoor sunlight exposure, conjunctival ultraviolet autofluorescence (UVAf), has been suggested to be associated with myopia (Sherwin et al., 2012a). Thus, variations in the time length of sunglasses/glasses use, which can directly influence the amount or intensity of

sunlight reaching the eye, was considered.

**Medications (systemic/ocular) history:** An implication that specific medication use may interfere the metabolism/absorption of vitamin D was suggested (Gröber & Kisters, 2012). In addition, a number of medications such as antiretroviral, antiestrogens, antiepileptic agents, and glucocorticoids have been reported to have an association with bone damage (Gröber et al., 2011). Therefore, medication use history was inquired and noted.

**Refraction and Visual acuity:** Distance visual acuity was measured binocularly with and without correction by the Natural Vision Auto Refkeratometer (Nvision-K 5001 Shin-Nippon) (to minimise instrument myopia) for all participants. The visual target was the NVRI 3 meter LogMAR chart (Bailey-Lovie). During testing, the optical correction was worn in accordance with auto-refraction examination followed by subjective refraction using the maximum plus for best visual acuity principle (Carlson & Daniel, 2003), corrected visual acuity in each eye was at least 6/7.5.

**Slit lamp biomicroscopy:** This was performed using a slit lamp microscopes (TAKAGI SM-70N) to evaluate the health of the ocular surface and anterior segment to ensure there was no ocular disease (other than dry eye).

**Ophthalmoscopy:** Ophthalmoscopy (Ophthalmo-Retinoscope Set BX $\alpha$ -13-RX) was performed to ensure the health of the ocular posterior segment and exclude conditions such as macular degeneration and glaucoma.

**Intraocular pressure:** For the dry eye studies, intraocular pressures were measured using a non-contact tonometer (TX-20P, Canon) to avoid disturbing the tear film assessment and ocular surface. During measurements, participants were asked to look at the red light in the tonometer and an air puff was generated. An intraocular pressure was generated once the air puff was in contact with central cornea, and the average of three consecutive readings was recorded.

In the accommodation/myopia study, intraocular pressure of each eye was measured by hand-held tonometer (Icare® TA01) without anaesthetic. Participants

were asked to relax and look straight ahead on distant visual target. The tip of the probe from the cornea was approximately 4-8 mm. The tip then gently contacted central cornea after pressing the measurement button lightly. The average intraocular pressure was shown on the screen after six consecutive readings in the tonometer and was recorded.

### **3.4.1 Dry eye questionnaire**

A questionnaire regarding the symptoms of dry eye disease and their impact on daily living activities was conducted. The Ocular Surface Disease Index (OSDI), a validated dry eye questionnaire (Schiffman et al., 2000) was used. It was suggested that dry eye can be diagnosed by the presence of one or more symptoms of ocular surface irritation (“dry”, “gritty”, “stingy”, “tired”, “painful”, “itching”) occurring at least half of the time (Johnson & Murphy, 2007). Fifteen commonly experienced symptoms for dry eye (burn, blurriness, discomfort, itch, soreness, redness, irritation, grittiness, scratchiness, tired eyes, foreign body sensation, dryness, filmy vision, pain, and crustiness/stickiness) and the frequency (lowest to highest, 1 for some of the time, 2 for half of the time, 3 for most of the time, and 4 for all the time) were also recorded.

### **3.4.2 Time spent outdoor survey**

Participants self-reported the time spent outdoors (in minutes) for week days and non-weekdays by indicating 1 of 5 categories (0, <15, 15–29, 30–44 or 45–60 minutes). Total daily time spent outdoors was calculated by summing midpoint values (0, 7.5, 22.5, 37.5 and 52.5 minutes, respectively) of each of the 5 time categories for the 13 hourly intervals. This was performed with respect to the fact that during the study period the sunrise and sunset occurred at 5am to 6pm (Australian Eastern Standard Time) respectively. However, the quantification of vitamin D levels relying on questionnaire data might be considered inaccurate, it has been suggested that a more objective method should be applied when estimating vitamin D status in conducting research (McCarty, 2008).

### 3.4.3 Actiwatch (BMedical) measurement

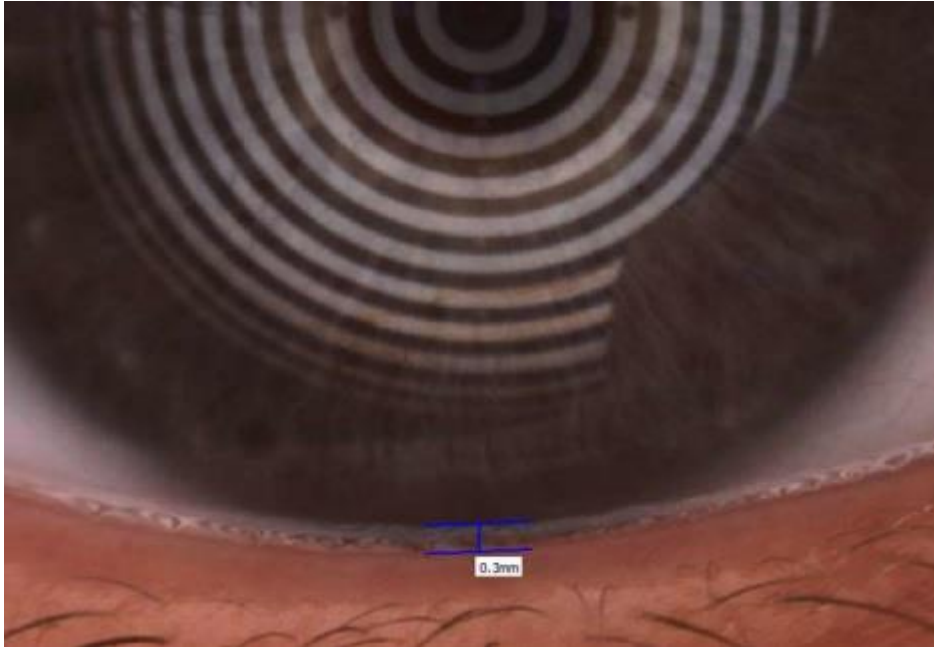
This light exposure measurement was completed by in 10 participants (5 from the myopic group, and 5 from the emmetropic group) in Experiment 3 to examine whether there was difference in bright light exposure between young myopes and emmetropes. The measuring period was from 08<sup>th</sup>/Oct/2015 to 10<sup>th</sup>/Nov/2015. Ten participants were instructed to wear an actiwatch on their non-dominant hands continuously for 7 days. The actiwatch was portable, waterproof, and had an inbuilt light sensor that records the intensity and duration of light exposure to illuminance in lux every 30 seconds from 6 am on the first day to 5:59am on the last day. The participants were instructed to wear the actiwatch all the time with the light sensor facing upward and not to cover it.

The rationale of the time setting was based on the data acquired from <http://www.timeanddate.com>, where the sunrise and sunset time between the period of 08<sup>th</sup> Oct 2015 and 10<sup>th</sup> Nov 2015 were 04:51am and 6:13pm respectively (average day length over the 34 days was 12.945 hours). Also, an intensity of 10000 lux was used for the indication of being outdoors to ensure the measurements had distinct border. Although the intensity of light levels rarely exceeds 1000 lux, which is the usual indoor illuminance level, spaces close to window may be greatly higher than that (Read, 2016). Furthermore, a study conducted by Cohen and colleagues (2011) found that chicks raised under high light level (10000 lux) have been shown to develop less myopia compared to those raised in low light levels (50 lux).

### 3.4.4 Dry eye assessment

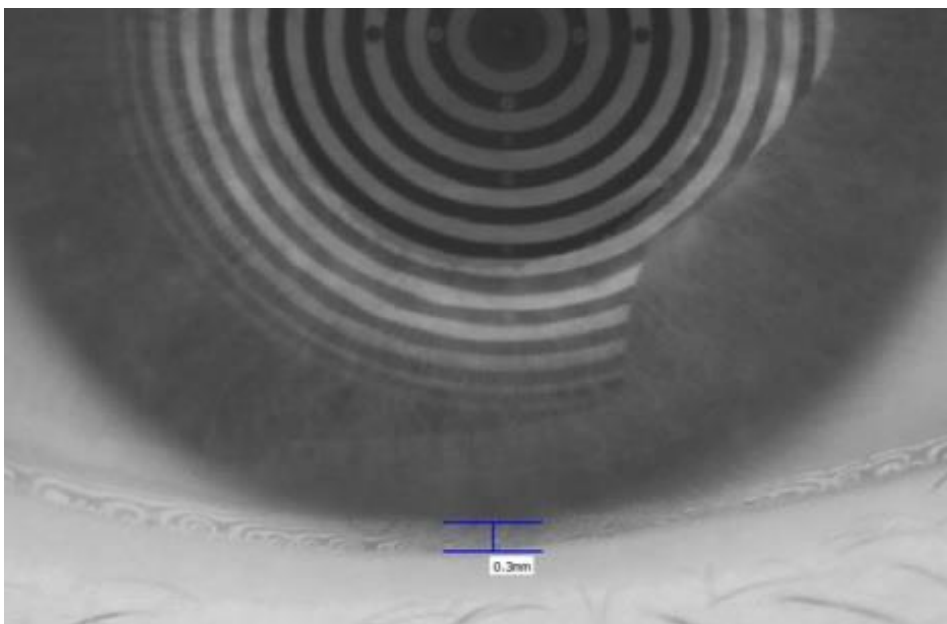
**Tear meniscus height:** The height of the tear meniscus is clinically used as an estimate of tear film quantity. A tear meniscus height of less than 0.2 mm is indicative of a low tear volume and potentially dry eye (Mainstone et al., 1996; Shen et al., 2009). The Keratograph 5 was used to assess the tear meniscus. Mainstone et al. (1996) have demonstrated the height of tear film meniscus to be diagnostically accurate, having the highest reference value on predicting tear film insufficiency. They showed that there were strong correlations between tear meniscus height and other dry eye assessments such as cotton thread test, non-invasive breakup time, and ocular surface staining scores. Tear meniscus evaluation has been stated to have

satisfactory repeatability (Fukuda et al., 2013). The in-built blue integrated ruler was used to measure the tear meniscus height from a position directly below the pupil centre. The measurements were taken with the K5 IR-illumination mode to avoid stimulation of reflex tear secretion (see **Figure 3-2**).



**Figure 3-1 The K5 White-illumination Mode for Tear Meniscus Height**

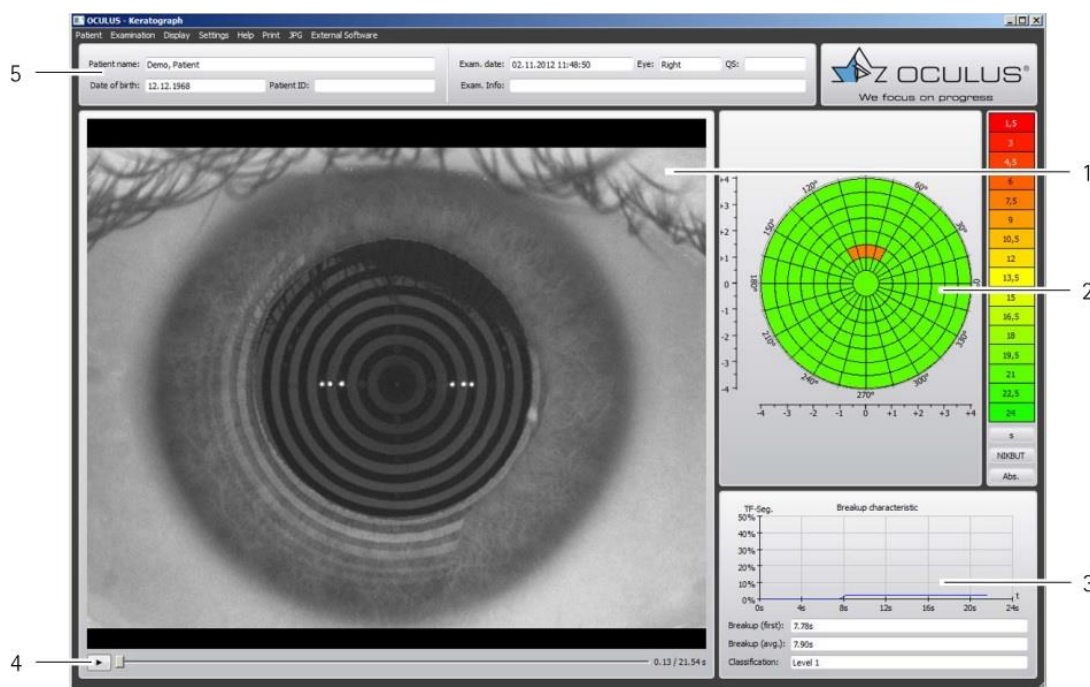
In the white-illumination mode, a nature white light would project from the K5 while the image is taken, which would potentially stimulate tear production and directly affect measurement.



**Figure 3-2 The K5 IR-illumination Mode for Tear Meniscus Height**

In the IR-illumination mode, the image is taken by a projection of infra-red rather than natural white light. The potential stimulation from the white light is therefore absent during measurement.

**Non-invasive tear break-up time (NITBUT):** The time it takes for the tears to disappear (i.e. break-up) on the corneal surface is a measure of tear stability and quality (Norn, 1969). Clinically, tear break-up time (BUT) is measured when lacrimal insufficiency is suspected (Carlson et al., 2003b). Statistical difference was not observed in the outcomes of non-invasive BUT and standard BUT (Amaechi & Osunwoke, 2004; Mohidin et al., 2002). NITBUT was measured using the NIKBUT (Non-invasive Keratograph Break-Up Time) mode of the Keratograph 5. The Keratograph 5 takes the break-up time measurements automatically and without touching the eye. There are also infra-red and white illumination modes available, with the infra-red mode being used to avoid elevated estimated due to stimulation of tear secretion (see **Figure 3-3**). The participants were asked to blink twice and then keep their eyes open. The time was measured automatically by the K5 from the last blink until the tear film break-up was detected.

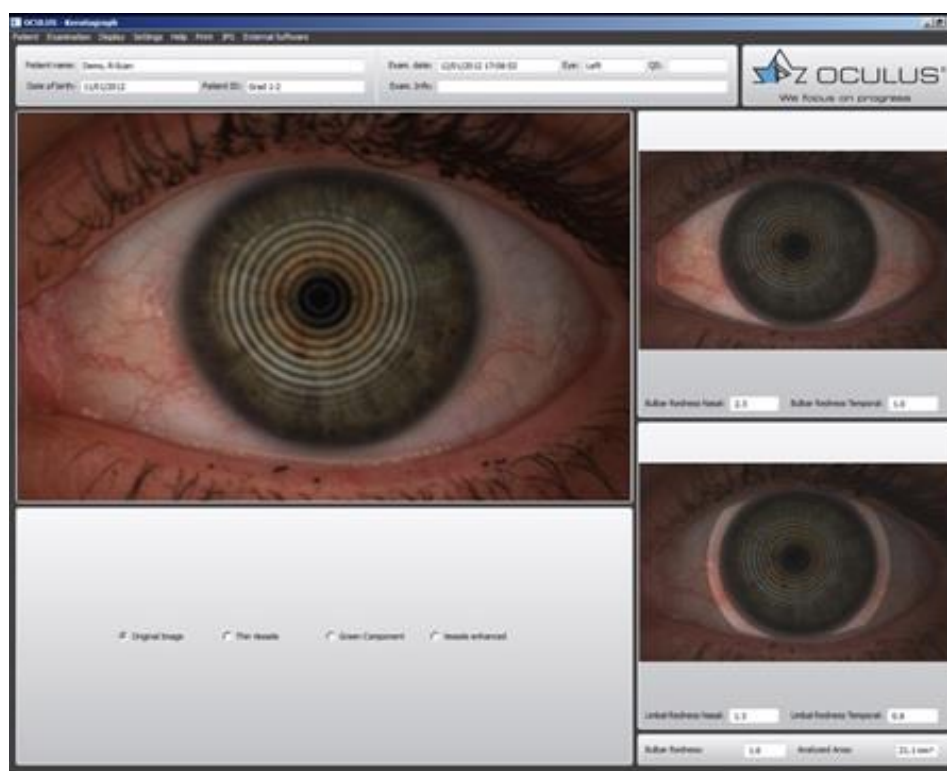


**Figure 3-3 NIKTBUT of the K5 Display**

In the mode of NIKTBUT, the right bar shows tear break-up time by seconds, with the shortest on the top indicating 1.5 seconds and bottom representing 24 seconds. The circle stands for the central ocular section (diameter of 8mm) and shows the tear section that breaks at earliest, as well as the average break-up time for the whole surface.

**Ocular surface redness (bulbar and limbal conjunctival redness):** The presence of ocular redness is considered one of the signs indicating inflammation of the ocular surface due to ocular surface desiccation (Wong & Wood, 2014). The R-Scan function of the K5 records and grades bulbar redness automatically and objectively. The redness scores for temporal and nasal bulbar conjunctiva, as well as to the temporal and nasal sections of the limbal conjunctiva were obtained, with a score of 0 indicating clear conjunctive surface (without redness) while 2.50 being the highest level in redness (see

**Figure 3-4).** The grading with the K5 is objective and stated to detect minor changes in redness (Downie et al., 2016).



**Figure 3-4 The R-Scan of the K5**

The R-scan mode of K5 measures both bulbar and limbal sections nasally and temporally by giving each section a score for the level of redness, with 0 showing the least redness and the number increases corresponding with the colour intensity.

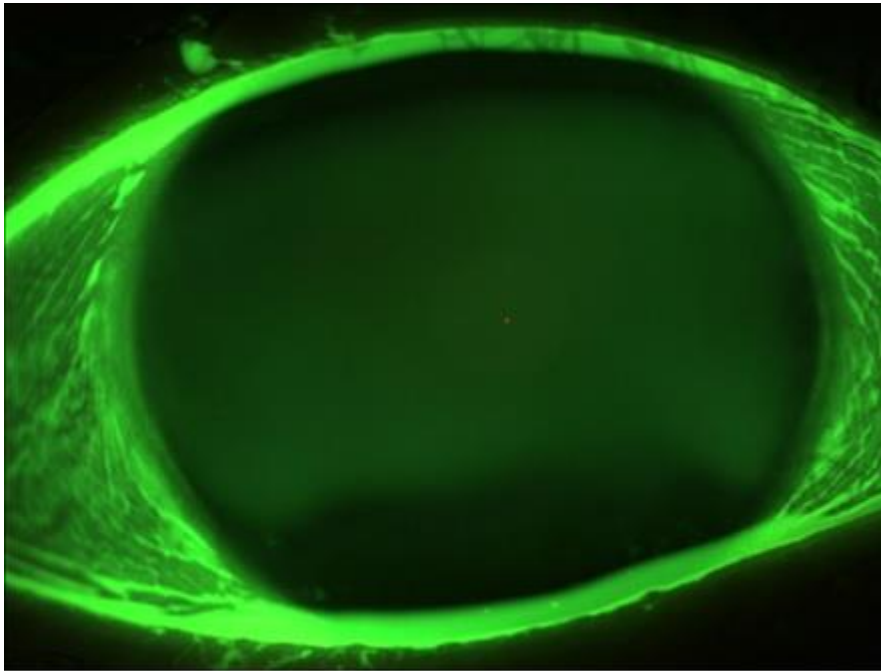
**Schirmer tear test:** This test was performed on the 29 participants recruited from the AusSun Study. Schirmer tear secretion test (Carlson et al., 2003a) was performed to evaluate the tear quantity and thus by inference the integrity of the lacrimal

secretion system (which produces the aqueous component of the tears). Participants were instructed to look up and the lower lid gently pulled down and the Schirmer paper hooked over the lower lid. They were asked to keep the eyes open and to continue to look up to avoid significant reflex tearing. The Schirmer paper was removed after 5 minutes and the reading was noted by the scale reached by of the colour bar on the paper.

**Phenol red thread test:** This test was performed on the 29 participants recruited from the Optometry clinic. A soft thread was placed inside the lower lid, approximately 1/3 of the distance from the temporal canthus for 15 seconds. The participants were instructed to look straight ahead and try not to blink. Following that, the thread was removed with an upward motion to avoid causing discomfort. The part of thread changed to red then was measured by the scale on the back of the pack. The reading was deducted by 3 mm (excluding the length of the angled thread component that sits under the lid). A measure of less than 11 mm indicates aqueous deficient dry eye (Albietz, 2000), whereas a value greater than 20 mm is considered normal. It has been suggested that phenol red thread test is equally sensitive as the Schirmer test in detecting dry eye (Vashisht & Singh, 2011).

**Grading of corneal staining:** Fluorescein sodium was used, due to its nature of preserving the intact of cornea and also as it does not stain vital tissue, to assess corneal epithelial staining (Maurice, 1967). Fluorescein staining was evaluated and graded soon after the fluorescein application in order to avoid the high luminosity that results from the rapid dye diffusion (Bron et al., 2003), which might affect the grading. The staining was evaluated 30 seconds after fluorescein application using the Fluo Imaging of Keratograph 5 (see **Figure 3-5**).



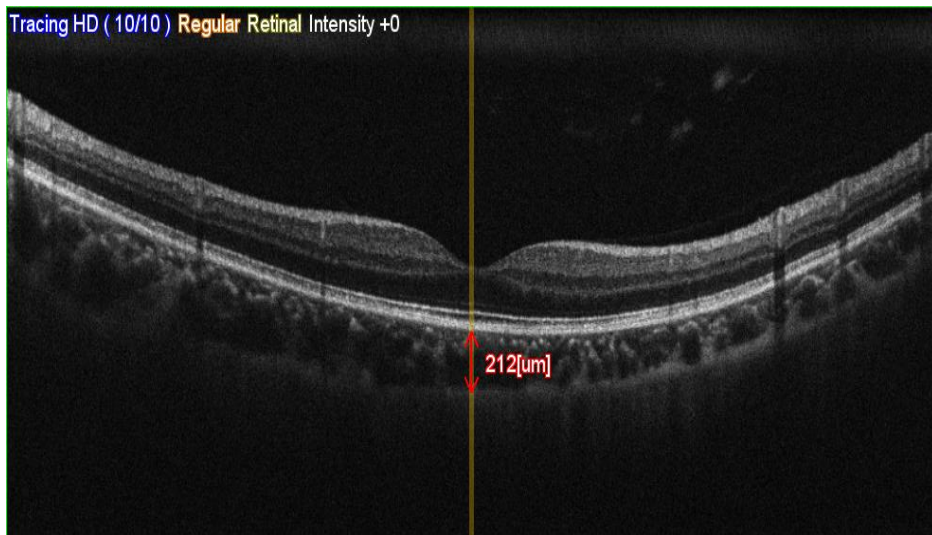


**Figure 3-5 The image of Corneal Staining of the K5**

The fluorescein staining pattern can be photographed by the K5 for documenting and grading.

#### **3.4.5 Ocular biometry measurement**

**Choroidal thickness measurement:** This measurement was conducted on study participants in Experiment 3 and 4. The choroidal thickness was measured according to the manufacturer's instructions (ref) using the cross line mode of an Optical Coherence Tomography (OCT) (NIDEK RS-3000). The participants were instructed to look at the central cross of the OCT. During the measurement, 120 continual scans were conducted and a clear image of cross section of posterior section was recorded. Image was enhanced using the inbuilt function of high contrast of the OCT in order to obtain the measurement accurately. The thickness was determined by measuring sub-foveal choroid (see **Figure 3-6**). An inter-observer variability of 32  $\mu\text{m}$  on young adult has been reported (Rahman et al., 2011). Also, choroidal thickness measurement in humans shows a diurnal variation, being thinnest during the day and thickest at night (Chakraborty et al., 2011).



**Figure 3-6 Sub-foveal choroidal thickness measurement with OCT.**

Choroidal thickness was measured from the section below the fovea, an inbuilt digital scale was used to accurately measure the thickness. In this example, the choroidal thickness on axis is 212  $\mu\text{m}$ .

**Biometry measurements:** All biometry measurements for the eyes were taken by Lenstar (LS-900, HAGG-STREIT, USA) according to the manufacturer's instructions ("Instructions for use biometer lenstar ls900,"). Lenstar is the first optical biometer on the market that is capable of measuring the thickness of the crystalline lens. With one click, it also measures axial length, anterior chamber depth, aqueous depth, central corneal thickness, corneal astigmatism, and pupil diameter. Another commonly used device for ocular biometry measurements is the IOLMaster. The IOLMaster uses lateral-slit illumination at approximately 30 degrees to the optical axis and image analysis, while Lenstar uses Optical Low Coherence Reflectometry (OLCR) to analyse anterior chamber depth (Buckhurst et al., 2009). By comparison to lateral-slit illumination, the OLCR has higher resolution. Also, OCT takes the measurements on the axis while IOLMaster measurements could be off axis (Huang et al., 2012). Huang and colleagues (2012) compared ocular biometry measured by Lenstar and IOLMaster with and without cycloplegia, and found that the measurements of anterior chamber depth with both methods were significantly higher than those without cycloplegia. While some reported non-cycloplegic measurements of anterior chamber depth with Lenstar to be higher than IOLMaster (Buckhurst et al., 2009; Holzer et al., 2009), one study found no difference. Zhao and colleagues (2013) evaluated the repeatability of Lenstar by comparing the measurements (undilated

pupil) taken using the Lenstar, IOL Master and Pentacam-HR. They found that the ocular measurements taken using the Lenstar had good repeatability, with the highest repeatability being axial length, whereas central corneal thickness measures had the lowest repeatability. To assess changes/differences of ocular structure and dimension in myopia studies, it is essential to obtain accurate measures of the ocular parameters (Saw et al., 2005b).

**Contact lens correction:** Individuals with refractive errors have different accommodation/vergence demands when being corrected with spectacle lenses or contact lenses (Alpern, 1949; Robertson & Ogle, 1967). In optical theory, altered magnification caused by wearing contact lens in myopes make the effect similar as the near object fixated moving closer toward the eye, resulting in eyes wearing contact lens requiring higher accommodation effort. Previous studies have shown inconsistent findings in the relationship between accommodative amplitude and myopia, with some indicating lower amplitude of accommodation (Fong, 1997; Zhai & Guan, 1988), while others showing higher (Maddock et al., 1981; McBrien & Millodot, 1986), and some further suggesting no difference (Fisher et al., 1987; Gawron, 1981; Mäntyjärvi, 1987). Refractive errors were corrected by contact lens (QASYS, Base curve 8.4, Diameter 14.0) (to minimise potential varying accommodative demands and vergences by correcting with spectacle lenses) in accordance with participants' spherical equivalence measured by an autorefractometer (Nvision-K 5001, Shin-Nippon).

**Phoria measurement:** Both distant and near phoria were taken with Howell Dywer phoria cards. Phoria at distance was measured prior to near. A prism base down was placed in front of participants' right eye at 33 cm for near and at 3 m for distance to induce diplopia of a numbered scale in Howell Dywer phoria card, adopted from Allen et al.'s study (2013). The participants were asked if two rows of numbers were seen to confirm image dissociation. The participants were instructed to keep the numbers clear at all times and look at the top arrow and report what number it pointed to. The number reported indicated the amount of phoria (numbers on yellow represent esophoria and blue numbers stand for exophoria). Measurements were recorded in prism diopters.

Commonly used phoria testing clinically includes von Graefe test, modified Thorington test (using a tangent scale and a Maddox rod in front of one eye), and Howell Dwyer phoria. It has been suggested that modified Thorington test has higher repeatability when comparing to von Graefe test (Casillas & Rosenfield, 2006; Goss et al., 2010; Rainey et al., 1998; Schroeder et al., 1996; Wong et al., 2002). Participants reported the number the dissociated arrow pointed to and the number was noted as phoria at distance and near, respectively.

**Relative accommodation (NRA/PRA):** The relative accommodation was measured through phoropter at 40 cm. The participants were asked if the letters on the near target were clear at the beginning of the test. Negative relative accommodation (NRA) was performed first by adding lenses binocularly, +0.25 D for each step until the participant reported the first sustained blur. The adding plus power was recorded as NRA. Subsequently, PRA was measured after returning the power of phoropter to zero. Minus lenses were added until the participant reports the first sustained blur. The total adding minus power was recorded as PRA.

**Accommodation accuracy:** Abbott and colleagues (1998) used three different methods to measure accommodative stimulus response curve in myopes and emmetropes, and they found accommodative inaccuracy was the greatest when assessing with negative lens series and the least with positive lens series. Their results showed negative lens series tended to overestimate accommodative inaccuracy (i.e. higher lag or lead) while an underestimation was observed in positive lens series. Decreasing distance series showed relatively more accurate measurements. Gwiazda and colleagues (1993) also suggested negative lens series had lower accuracy in measuring accommodative response. This may be due to the fact that there is no clue of distance away from eye's plane, also vergence is absent when measuring accommodative accuracy with this technique.

A metered rod was fixed on the top of the autorefractometer to avoid varied size of retinal image perceived at different distances, and the size of letters on the near targets was adjusted for three different distances of 25, 33, and 40 cm respectively. Accommodation accuracy at 40 cm was measured first, followed by 33 cm and lastly 25 cm. The participants were asked to keep visual clarity when looking

at the words on the targets. Ten consecutive readings were obtained and the average was used as the measurement. To determine the effects of the near task on the accommodation accuracy, the measurement was taken again following the near task.

**Impact of near task on the measurements:** It has been suggested that both continues reading and close reading distance are associated with myopia (Ip et al., 2008b), indicating refractive status/ accommodative response might temporally be altered by a period of near task. A computer screen was placed 40 cm away from the eye's plane for 5 minutes while they were completing the questionnaire. Accommodation accuracy at 25 cm, near induced transient myopia, and tonic accommodation were assessed as soon following the near task. Gwiazda and colleagues (1995) compared tonic accommodation before and after a 15 minute video game playing (at 25 cm) in children and found that tonic accommodation increased (by 0.24 D to 1.15 D), also that myopes had greater shifts in tonic accommodation after the game playing task. The results might be implying the relatively stable measurement – tonic accommodation, can also be affected by near tasks. It has been reported that myopes had greater transient myopia after near task than emmetropes, and early onset myopes exhibited longer decay of near induced transient myopia (Vasudevan & Ciuffreda, 2008). Abbott and colleagues (1998) measured accommodation using lens induced accommodative demand and found that progressing myopes had lower accommodation response as compared to stable myopes, suggesting that there is a link between myopia progression and accommodation accuracy.

**Near induced transient myopia (NITM):** A five minute computer screen watching with a 40 cm eye-screen distance was used as near task. It has been suggested that both continues reading and close reading distance are associated with myopia (Ip et al., 2008b), indicating refractive status/ accommodative response might temporally be altered by a period of near task. Accommodation accuracy at 25 cm, near induced transient myopia, and tonic accommodation were assessed. Following the near task, accommodation accuracy at 25 cm was measured again; NITM was immediately measured after the accommodation accuracy measurement, 10 consecutive readings were recorded and the average was used as the measurement.

**Tonic accommodation measurement:** Tonic accommodation was measured twice in a completed dark room (Rosenfield et al., 1993) in the beginning after contact lens insertion and after 5 minutes of near viewing, a fixation red light was stuck 6 meters away on the wall at the same level of the participants' eyes, 10 consecutive readings were recorded as soon as the room lighting was switched off and the average of the readings was used as the measurement.

### **3.4.6 Blood collection:**

Ten ml of blood was collected by the phlebotomist from each participant in the Optometry clinic group in the first experiments and those who returned to for the follow up assessments in the second experiment, using vacutainer and 21 gauge needle into a 10 ml serum separator tube (SST). The tubes were centrifuged at a speed of 2400 RPM for 10 minutes. The blood samples were stored at -80°C until later analysis.

Following that, participants' ID numbers were correctly labelled on SST tube and transported SST tubes to the lab. Collected blood samples in the tubes were centrifuged at a speed of 2400RPM for 10mins. Two labelled 1ml Cryo vials with the participants' ID numbers written by a black permanent marker were prepared and put in fume cabinet. When blood was being spun, transfer SST tube to the fume cabinet on a rack. Two ml of serum from the SST tube was pipetted and divided between two Cryo vials, one ml into each vial. SST tubes were then discarded and the caps on the vial were tightened. Transfer Cryo vials to -80°C freezer and stored for further analysis. The equipment was then cleaned with Ethanol.

### **Saliva collection:**

All the participants in the Experiments 3 and 4 were asked to have their saliva samples (4ml at least) collected.

It has been suggested that the salivary concentration of vitamin D would be overestimated when measured with gum chewing (Higashi et al., 2013). Unstimulated drooling was used and the participants were asked to rinse the mouth before saliva sample collection. Fifty ml conical centrifuge tube was used for the participants to spit in 4 mL of saliva and sample collection and storage.

### **Human saliva sampling procedure:**

During saliva collecting sessions the participants were asked to donate saliva into collection tubes (fifty ml conical centrifuge tube) provided by us. Human saliva was collected using the standard “Drool” method. The Drool method is described as follows:

1. The participants were asked to refrain from eating and drinking (except for drinking plain water) for 1 hour prior to the collection of saliva.
2. The mouth was rinsed with water five minutes prior to collection. During saliva collection/expectoration, participants sat comfortably in an upright position with head slightly tilted forward so that saliva pools to the front of the mouth. Collection was completed under assistance of the trained research student.
3. Pool saliva (head tilted slightly down) in the mouth for about 2-5 minutes, and expectorated into the tube (to collect at least 4ml of saliva).
4. Collected human saliva was labelled and immediately frozen on dry ice.

### **3.4.7 The enzyme-linked immunosorbent assay analysis (ELISA)**

#### **Serum concentration of vitamin D:**

Serum vitamin D levels were measured by ELISA (in pg/ml) (Sigma-Aldrich, St. Louis, MO, USA), while the serum concentrations of interleukin-6 were measured by ELISA (Abcam, MA, US). All blood samples of dry eye group were taken in May 2014 for the first visit, and between July and October 2014 after vitamin D supplementation. For the serum samples of participants from the AusSun study, the lab method used was the measurement of 25(OH)D concentration (in nmol/L) using the Liaison semiautomated chemiluminescence assay (DiaSorin, S.p.A., Saluggia, Italy).

#### **25(OH) vitamin D ELISA (Sigma-Aldrich):**

All standards, controls, and samples were run in duplicate. All samples and kit reagents were brought to room temperature (18-26 °C) and gently mixed. Ten µL of 25(OH)D standard, controls, and serum samples were dispensed into the wells. Two hundred µL of 1x working solution of biotinylated 25(OH)D reagent was added to

the wells. The wells were shaken for 20 seconds using a plate shaker at 300 rpm. Following that, the plate was sealed and incubated for 90 minutes at room temperature (18-26 °C). The seal was removed and the contents were aspirated. Dispensed 300 µL wash buffer into each well and aspirated the contents of the wells. Repeat twice for a total of 3 washes and the plate was tapped on an absorbent paper. Dispensed 200 µL of enzyme conjugate (Streptavidin-HRP) into each well and incubate for 30 minutes, at room temperature (18-26 °C). Contents of the wells were discarded. Dispense 300 µL of 1x wash buffer into all the wells, and the contents in the wells were discarded. This was repeated for a total of 3 washes. The plate was tapped on absorbent paper. Dispense 200 µL of TMB substrate into the wells. Incubated for 30 minutes with foil wrapped at room temperature. Dispensed 50 µL of stop solution into each well and carefully mixed plate contents for 20 seconds. Read absorbance on ELISA reader at 450nm immediately. The results were expressed in ng/mL.

#### **Interleukin-6 High Sensitivity Human ELISA kit:**

All reagents were thoroughly mixed. One hundred µL of each standard was added to the first 12 wells, followed by 100 µL of sample, 1X control solution, and 50 µL of 1X biotinylated anti-IL6. The wells were covered and incubated for 3 hours at room temperature (18-26 °C). After incubation, washed the plate in the following procedure and repeated for 3 times: aspirate the liquid from each well, add 300 µL of 1X wash buffer, and aspirate the liquid. After the addition of 100 µL of 1X streptavidin-HRP solution, the wells were covered and incubated at room temperature for 30 minutes. The wells were again washed for three 3 times with 1X wash buffer, followed by adding 100 µL of chromogen TMB substrate solution and incubating in the dark (wrap the plate with aluminium foil) for 15 minutes at room temperature. The results were recorded immediately after the addition of 100 µL of stop solution, with the spectrophotometer using 450 nm as the primary wavelength and 620 nm as reference wavelength.

#### **Dopamine ELISA kit:**



The enzyme plate was prepared with 10  $\mu\text{l}$  standard, 10  $\mu\text{l}$  control, and 100  $\mu\text{l}$  sample solution pipetted into the respective wells. They were then each filled up with distilled water to a final volume of 500  $\mu\text{l}$ . After the addition of 25  $\mu\text{l}$  of TE buffer in each well, the plate was covered with adhesive foil and shaken on a shaker (600rpm) for 60 minutes at room temperature (20-25°C). This was followed by the removal of the foil and inversion of the plate on an absorbent towel. The plate was washed by adding 1ml of wash buffer into each well and shaken for 5 minutes at room temperature on the shaker. The plate was then inverted and tapped on the towel. The washing process was performed again. One hundred and fifty  $\mu\text{l}$  of acylation buffer was added into the wells, along with 25  $\mu\text{l}$  of acylation reagent. After being placed on the shaker at room temperature for 20 minutes, the plate was again inverted on the towel. Repeated the washing process. This process was performed again after shaking the plate for another 5 minutes and inverted on the towel. Subsequently, 100  $\mu\text{l}$  of hydrochloric acid was pipetted into all wells, covered with foil and shook for 10 minutes. Prepared a microtiter plate, transferred 90  $\mu\text{l}$  of the extracted standard, control and sample solution from the original plate into the respective wells and added 25  $\mu\text{l}$  of enzyme solution to all wells. After shaking for 1 minute at room temperature with foil covered, the microtiter plate was incubated for 2 hours at 37°C. Prepared pre-coated dopamine microtiter strips and added 100  $\mu\text{l}$  of standard, control and sample solution respectively from the enzyme plate. It was followed by pipetting 50  $\mu\text{l}$  of the respective dopamine antiserum into all wells. Again, covered the plate with foil and shook for 1 minute at room temperature. After incubating for 20 hours at 2-8°C, removed the foil and aspirated the contents of the wells. They were then washed for 4 times by adding 300  $\mu\text{l}$  of wash buffer. After adding 100  $\mu\text{l}$  of enzyme conjugate into all wells, they were covered with foil and shaken for 30 minutes at room temperature. Removed the foil, aspirated the contents of the wells, followed by 4 times washing with 300  $\mu\text{l}$  of wash buffer. 100  $\mu\text{l}$  of substrate was then added into all wells, shook for 20-30 minutes at room temperature without direct sunlight exposure. After the addition of 100  $\mu\text{l}$  of stop solution into all wells, the absorbance of the solution was recorded in 10 minutes using a microplate reader that was set to 450 nm and reference wavelength at 620 nm.

### **Vitamin D binding protein ELISA kit:**

In each well, 100 µl of assay diluent RD1-19 was added followed by 50 µl of standard, control, and sample. The wells were covered with adhesive strip and incubated for 1 hour at 20-25°C on a shaker (550rpm). After incubation, the wells were each aspirated and washed for four times by filling each well with wash buffer (400 µl). After the last wash, the remaining wash buffer was completely removed. Subsequently, 200 µl of vitamin D BP conjugate was added to each well, covered with adhesive strip and incubated for 2 hours at 20-25°C on a shaker. The wells were again washed and wash buffer being removed the same way in the previous procedure. The processed wells were added 200 µl of substrate solution and incubated for 30 minutes at 20-25°C, with adhesive foil cover to protect from light. After incubation, 50 µl of stop solution was added to each well and the optical density was determined immediately after, using a microplate reader set at 450 nm and wavelength correction set at 535 nm.

#### **Liquid chromatography tandem-mass spectrometry:**

Salivary samples were collected from a 36-year-old healthy male during a week (from 13 Dec 2016 to 19 Dec 2016), 5 samples were collected each day at 9:00am, 11:00am, 01:00pm, 3:00pm, and 5:00pm to test the diurnal variation of vitamin D levels.

#### **Data analysis for each experiment is explained in chapter 4 to chapter 7.**

For Experiment 1 and 2, the severity of participants' ocular dryness was graded and potential associations with sunlight and vitamin D levels analysed. Ocular redness and Oxford staining score were assessed using non-parametric tests and other dry eye measurements were analysed by parametric tests. For Experiment 3 and 4, variables in each severity and onset of myopia were categorised and the interactions between variables were analysed. All data for correlation and association were analysed by parametric tests. Statistical data analysis was conducted using GraphPad Prism (GraphPad Software, Inc., CA, USA). Correlations were examined using Pearson Correlation coefficients and Spearman correlation. Variables between each group were compared using two-way ANOVA for analysis.

Experiment 1: The correlation had a statistical power of 99.85%, the unpaired 2 sample t-test had a statistical power of 83.01% and 2-sample 2-sided equality had a

power of 86.2%. For a significant correlation between OSDI and 25(OH)D to reach a level of 50% and a power of 90%, a sample size of 37590 is required.

Experiment 2: The correlation had a statistical power of 96.33%, the paired sample t-test had a statistical power of 78.23% and 1-sample 2-sided equality had a power of 78.2%. For a significant difference in IL-6 levels to reach an effect size of 0.5 and a power of 90%, a sample size of 35650 is required.

Experiment 3: To reach a power of 90% for the observation based on acti-watch measurements, a sample size of 117 is required. The correlation had a statistical power of 97.8%, and 2-sample 2-sided equality had a power of 97.81%. For a significant difference in dopamine levels between emmetropes and myopes (to reach an effect size of 0.5 and a power of 90%), a sample size of 69200 is required.

Experiment 4: The correlation had a statistical power of 85.5%, and 1-sample 2-sided equality had a power of 79.54%. To observe a significant difference in change of tonic accommodation after vitamin D treatment (to reach an effect size of 0.5 and a power of 90%), a sample size of 35650 is required.

### 3.4.8 Salivary vitamin D analysis

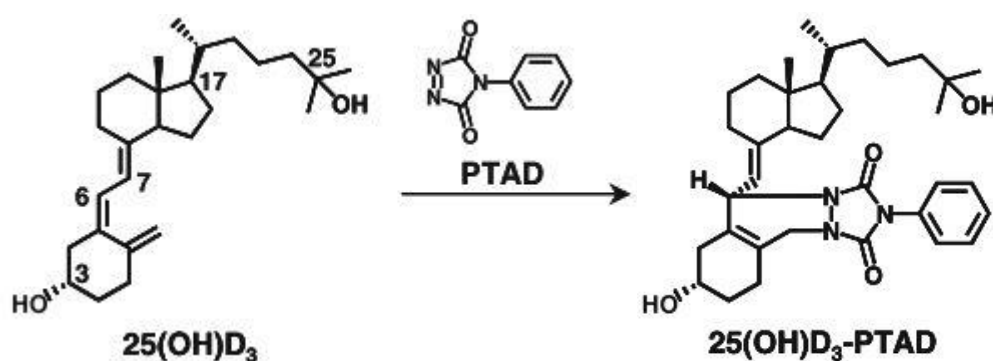
The ELISA plate assay is ideal for quantitating vitamin D<sub>3</sub> in blood, however in saliva it appears to have insufficient sensitivity for detecting and measuring vitamin D<sub>3</sub> levels. The stated limit of detection for the ELISA kit is about 1000 fold above expected level of vitamin D<sub>3</sub> in saliva, according to specifications supplied by the manufacturer.

Compared with the ELISA assay, the electrospray tandem mass spectrometry provides potentially 100 times higher level of sensitivity and was therefore utilised in the study. Nevertheless, the sensitivity was still found insufficient due to the limitation imposed by the ability to ionise vitamin D<sub>3</sub>. To increase ionisation, vitamin D<sub>3</sub> was derivatised with phenyl-1,2,4-triazoline-3,5-dione (PTAD) (see **Figure 3-7**). A Cookson reagent has a chromophore, fluorophore or electrophore at the 4-position, and is often employed for enhancing the sensitivity in the determination of analytics which are difficult to ionise. PTAD derivatisation of vitamin D<sub>3</sub> improves ionisation and was successfully used by Higashi et al (2008) to measure Vitamin D<sub>3</sub> in saliva.

The mass spectrometric analyses were performed utilising an ABSciex QTRAP 4500 triple quadrupole mass spectrometer equipped with an electrospray ionisation interface (ESI). Multiple reaction monitoring was applied to identify the presence of vitamin D<sub>3</sub> in the sample. Quantitation was made possible by the inclusion of an internal standard, a mixture of Testosterone acetate, propionate and butyrate which was intended to be calibrated against standard concentrations of vitamin D<sub>3</sub>.

PTAD derivatisation was carried out according to published protocols (Higashi et al., 2008). An ethanolic stock solution of 25(OH)D<sub>3</sub> was prepared with a concentration of 100 µg/ml. This concentration was confirmed by UV spectroscopy using a molar absorptivity of 18,200 at 265 nm. Subsequent dilutions were carried out with ethanol to prepare 0.2, 0.5, 1.0, 2.0 and 5.0 ng/ml solutions. An ethanolic solution of IS with a concentration of 2.0 ng/ml was also prepared.

The LC-MS/MS was performed using an ABSciex QTRAP 4500 triple stage quadrupole-mass spectrometer connected to a Shimadzu LC-20 AD chromatograph. Following derivatisation, samples were subject to solid phase extraction using C18 Phytips (Phynexus Inc - San Jose California) according to the manufacturer's instructions. HPLC separation was performed at a flow rate of 0.2 ml/min at 40°C with 50% Acetonitrile, 50% ultrapure water, 5 mM ethylenediamine as solvent. Intact 25(OH)D<sub>3</sub> and its PTAD derivative were analysed by ESI-MS in positive-ion mode with the following conditions: declustering potential 50 (intact) or 10 V (PTAD derivative), focusing potential: 220 (intact) or 380 V (PTAD derivative), entrance potential: 10 V, ion spray voltage: 5kV, curtain gas (nitrogen): 45 psi, ion source gas 1 (nitrogen): 80 psi, ion source gas 2 (nitrogen): 80 psi, turbo gas temperature: 350 (intact) or 500°C (PTAD derivative) and interface heater on.



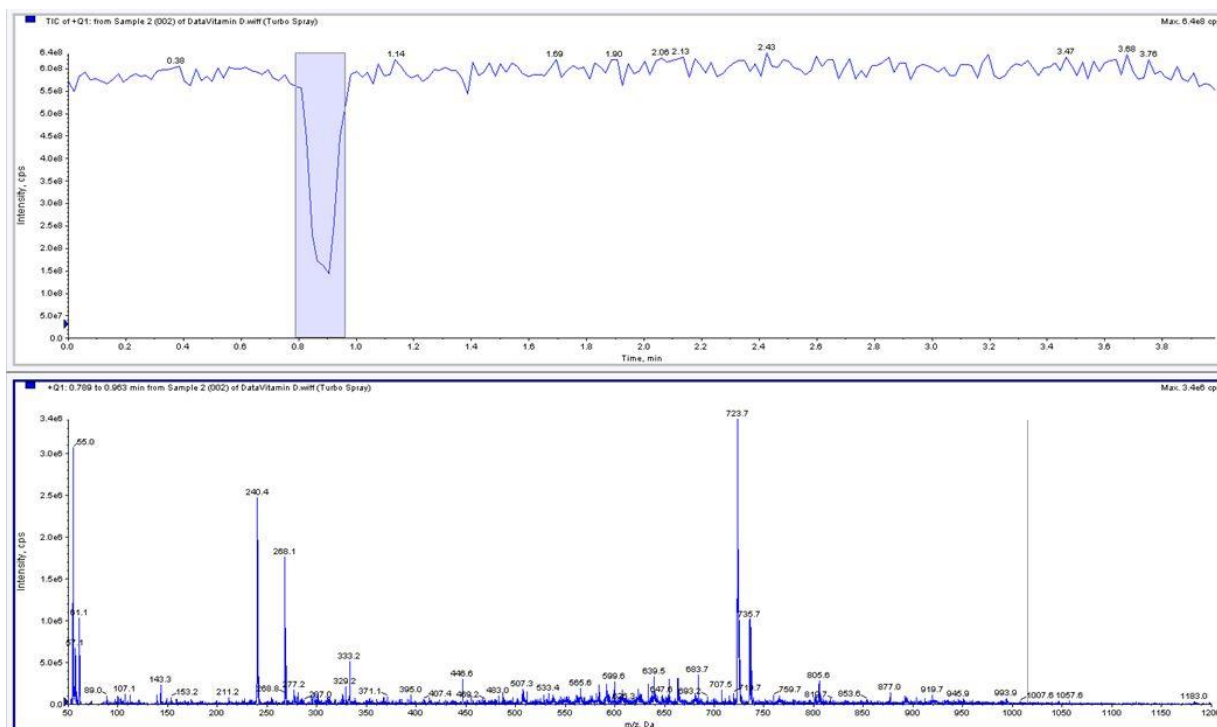
**Figure 3-7: Derivatisation of 25(OH)D<sub>3</sub> with PTAD**

### *Salivary measurements of vitamin D levels*

The internal standard provided a readable signal compared to blank run (see **Figure 3-8** and **Figure 3-9**), indicating PTAD derivatisation was successful. However, standard vitamin D<sub>3</sub> was not detected in this experiment (see **Figure 3-10**). Two potential explanations leading to this result would be the failure of PTAD derivatisation despite the internal standard being detectable at ng levels, or insufficient sensitivity of the instrument.

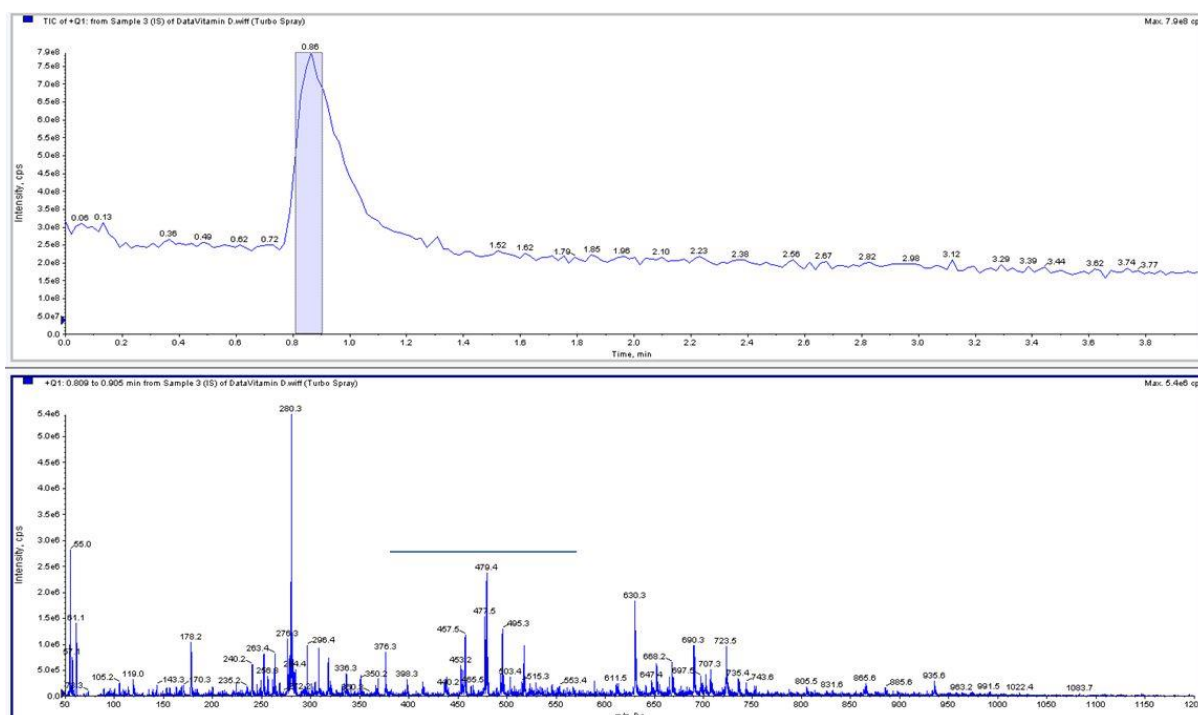
An apparent vitamin D<sub>3</sub> peak was observed in saliva sample (see **Figure 3-11**). However, MS/MS fragmentation pattern appeared inconsistent with vitamin D<sub>3</sub> even with a correct mass (see **Figure 3-12**). Fragmentation suggested it was oxidised glutathione thiolate anion molecular weight 611.63 which split on MS/MS to give two daughter ions of similar molecular mass, rather than the 298, 558 and 607 expected and showed in **Figure 3-13**.

Therefore, although the internal standard was successfully detected, it seems that the ionisation of vitamin D<sub>3</sub> was still poor even in the presence of PTAD.



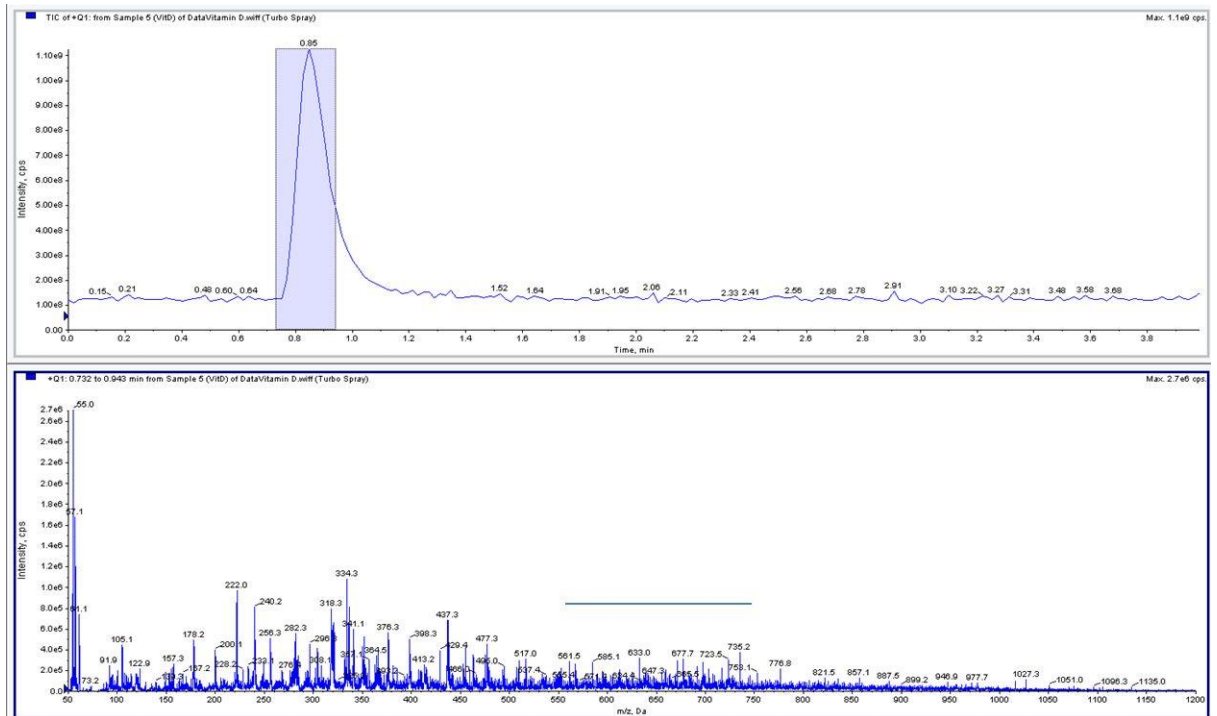
**Figure 3-8: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of ultra pure water infusion.**

The y axis of both plots shows intensity (count per second), the X axis of the top plot represents time (per minute). In the bottom plot, M stands for mass and Z stands for charge number of ions. The top plot shows a negative peak from the signal of pure distilled water.



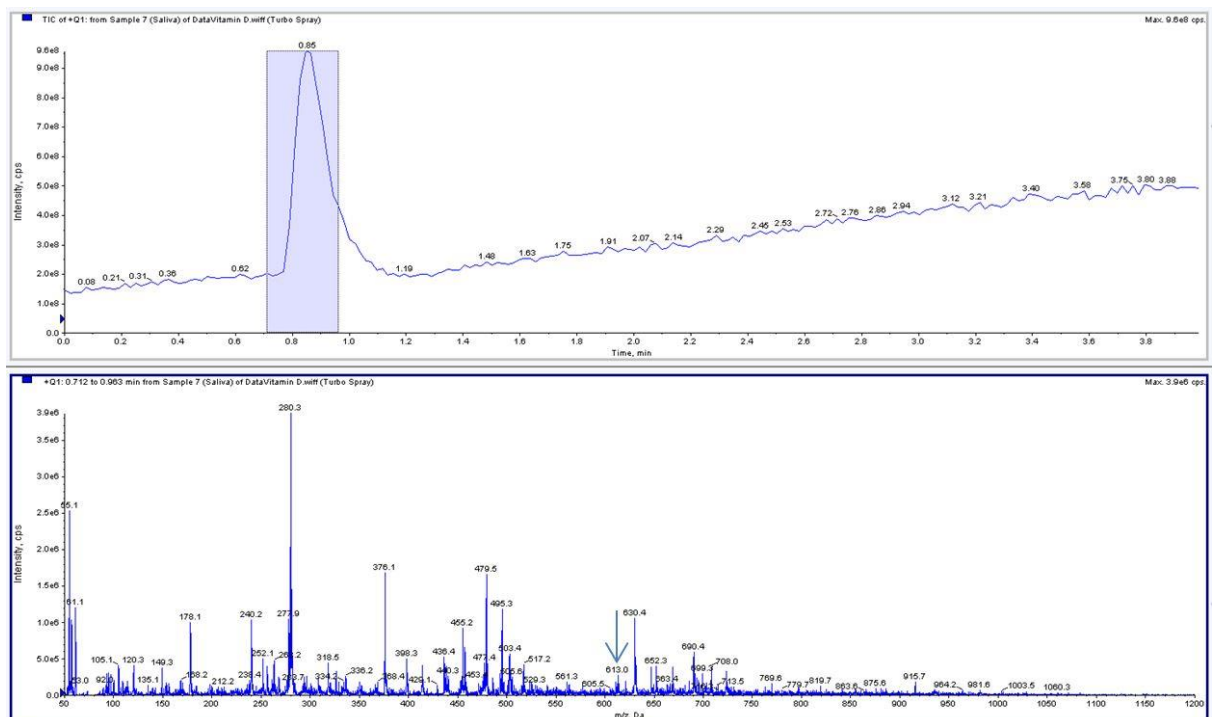
**Figure 3-9: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of PTAD derivatised testosterone infusion.**

The y axis of both plots shows intensity (count per second), the X axis of the top plot represents time (per minute). In the bottom plot, M stands for mass and Z stands for charge number of ions. The sample spiked with 25(OH)D<sub>3</sub> (100 µg/ml) between 0.809 and 0.905 minutes was analysed after the PTAD derivatisation and shown in the bottom plot.



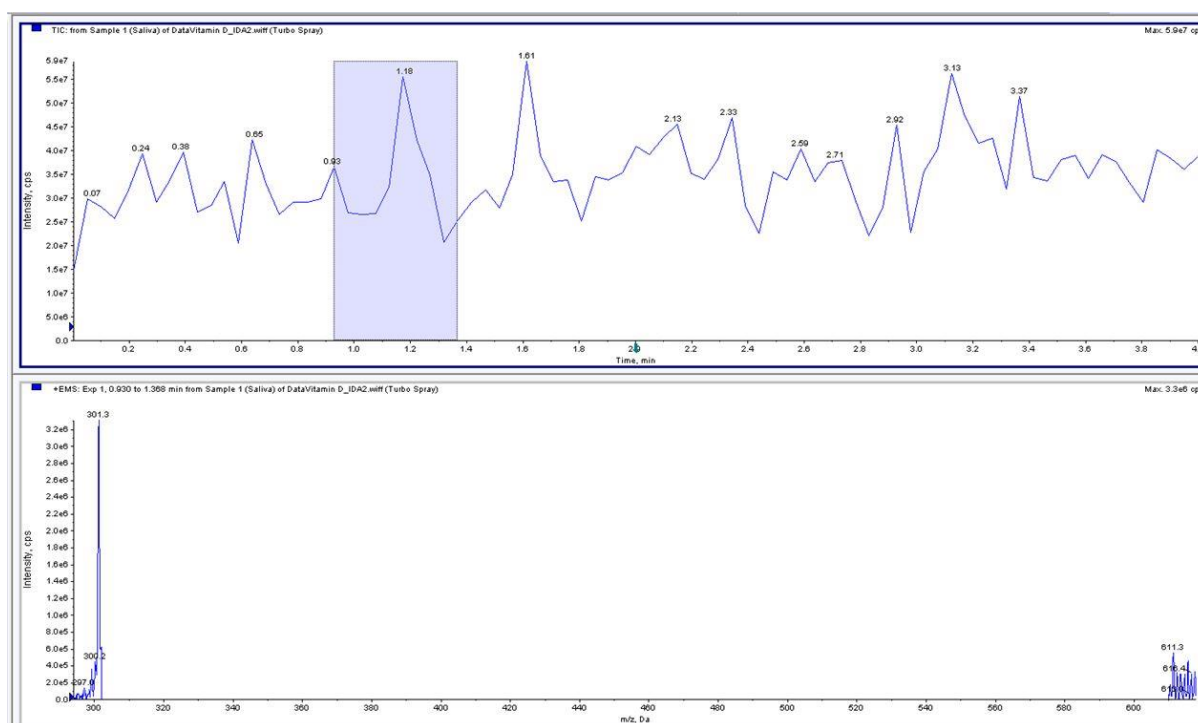
**Figure 3-10: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of purified vitamin D<sub>3</sub> standard.**

The y axis of both plots shows intensity (count per second), the X axis of the top plot represents time (per minute). In the bottom plot, M stands for mass and Z stands for charge number of ions. The sample spiked with purified 25(OH)D<sub>3</sub> between 0.809 and 0.905 minutes was analysed and shown in the bottom plot.



**Figure 3-11: QTRAP 4500 ESI-MS total ion chromatogram (TIC) (top) and ESI-MS spectrum (bottom) of saliva sample with potential PTAD Vitamin D<sub>3</sub>.**

The y axis of both plots shows intensity (count per second), the X axis of the top plot represents time (per minute). In the bottom plot, M stands for mass and Z stands for charge number of ions. The sample spiked with potential PTAD 25(OH)D<sub>3</sub> between 0.712 and 0.963 minutes was analysed and shown in the bottom plot.



**Figure 3-12: QTRAP 4500 ESI-MS/MS total ion chromatogram (TIC) (top) and ESI-MS mass spectrum (bottom) of saliva sample.**

The y axis of both plots shows intensity (count per second), the X axis of the top plot represents time (per minute). In the bottom plot, M stands for mass and Z stands for charge number of ions. The sample spiked with 25(OH)D<sub>3</sub> between 1.093 and 1.368 minutes was analysed and shown in the bottom plot.



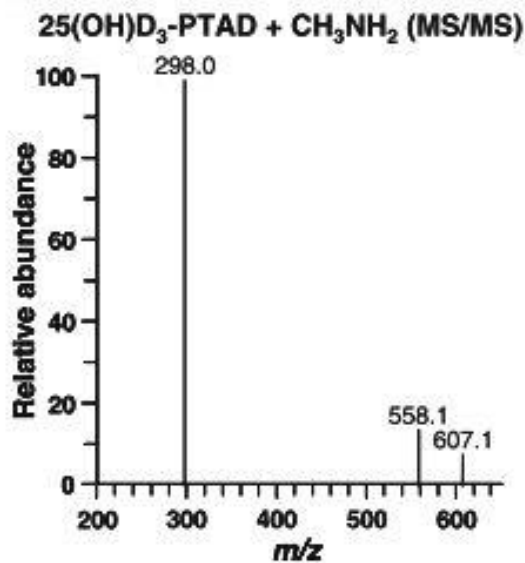


Figure 3-13: ESI-MS/MS spectrum of 25(OH)D<sub>3</sub>-PTAD, with methylamine (5 mM) added to the mobile phase when the spectrum was measured, in Higashi et al.'s study (2008).

### 3.5 LIMITATIONS

The measurements of 25(OH)D<sub>3</sub> levels in serum in the dry eye study used different method. For the participants in Optometry clinic group, the method used was the enzyme-linked immunosorbent assay (ELISA) analysis (Sigma-Aldrich, St. Louis, MO, USA); while it was the Liaison semiautomated chemiluminescence assay (DiaSorin, S.p.A., Saluggia, Italy) for those recruited from AusSun study. This might affect the accuracy of the results.

Seasonal variation could not be controlled while the recruiting period lasted for more than 12 months. This might have affected the results of vitamin D measurements.

OSDI in some participants might have been underestimated while the participants in Experiment 1 and 2 tended to answer all questions.

For tonic accommodation measurements in Experiment 3 and 4, as 10 consecutive readings had to be noted, the screen brightness of Natural Vision Auto Refkeratometer might have given eyes clues of distance, influencing the results measured. In addition, for the measurements of salivary vitamin D levels, a further 10 fold increase of signal to noise ratios in material would be required in order to reach sufficient sensitivity. The minimum required quantity of saliva for current

method is 10ml, however with the availability of the saliva samples in this study, it was not possible to be achieved. Alternatively, a more sensitive instrument, such as an Orbitrap mass spectrometer, could be used.

The onset and progression of myopia in Experiment 3 might not be accurate when they were based on participants' self-report.

In Experiment 2 and 4, compliance on taking vitamin D could not be well controlled though participants were given a weekly text reminder, few participants had more than 10 vitamin D capsules left at returned visits, which might have affected the vitamin D levels in the body.

### 3.6 DEFINITIONS OF TECHNICAL TERMS

Definition of mild dry eye: Ocular Surface Disease Index between 13 and 22

Definition of moderate dry eye: Ocular Surface Disease Index between 23 and 32

Definition of severe dry eye: Ocular Surface Disease Index greater than 33

Definition of spherical equivalent refraction: A spherical refraction that has a focal point coinciding with the circle of least confusion of a spherocylindrical lens. Hence, the spherical equivalent of a prescription is equal to the algebraic sum of the value of the sphere and half the cylindrical value, i.e. sphere + cylinder/2. Example: the spherical equivalent of the prescription -1 D sphere -0.5 D cylinder is equal to -1.25 D)

Definition of emmetropes: spherical equivalent refraction between +0.75 D and -0.75 D

Definition of myopia: Spherical equivalent refraction  $\leq -0.75$  D

Definition of low myopia: Spherical equivalent refraction between -0.75 D and -3.00 D

Definition of high myopia: Spherical equivalent refraction  $\leq -3.00$  D

Definition of poor accommodation: NRA  $\leq +1.50$  D or PRA  $\geq -1.50$  D, or a lag of accommodation  $\geq 1.00$  D at near of 25, 33, or 40 cm.

Definition of dark iris colour: brown colour in iris

Definition of light iris colour: Iris is of hazel, green, or blue

# Chapter 4: Experiment 1: Vitamin D and Dry Eye

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## Abstract

Dry eye affects up to a third of adults. Current treatments (e.g. ocular lubricants, punctal plugs) are not very effective. Dry eye causes or can be caused by ocular surface inflammation. Vitamin D (a steroid like vitamin that can be synthesised by sun exposure) has been shown to have anti-inflammatory properties. In this study, we aimed to investigate whether there was an association between vitamin D levels and dry eye.

**Methods:** Fifty-eight adults aged between 43 and 69 years participated. Half were recruited from the Optometry clinic of the Queensland University of Technology (QUT) with a previous diagnosis of dry eye. The other half were adults randomly selected from those who had participated in the AusSun study. Objective dry eye measurements in this study included tear meniscus height, non-invasive tear break up time, phenol red thread test, Schirmer test, and corneal staining grading. The Ocular Surface Disease Index and the Dry Eye Symptoms survey were used as subjective measures. Vitamin D levels of the participants in the AusSun study had been previously measured, whereas blood samples were collected from other participants for analysis.

**Results:** No correlations were found between vitamin D levels and the dry eye measurements. The dry eye symptoms survey was correlated with the OSDI ( $r = 0.58$ ,  $p < 0.001$ ). The serum vitamin D levels of the Optometry group ( $87.7 \pm 6.0$  nmol/L) were significantly higher than that of the AusSun group ( $61.0 \pm 4.1$  nmol/L) ( $p < 0.001$ ). OSDI was also higher in the Optometry clinic group ( $22.8 \pm 3.0$ ) than the AusSun group ( $11.4 \pm 2.2$ ) ( $p < 0.01$ ). OSDI showed no significant correlation with vitamin D levels of the Optometry clinic group ( $r = -0.23$ ,  $p > 0.05$ ), while it was correlated with vitamin D levels of AusSun group ( $r = -0.56$ ,  $p = 0.0015$ ). Similarly, the correlation between dry eye score and vitamin D levels was not observed ( $r = -0.1$ ,  $p = 0.59$ ) in the Optometry clinic group, while dry eye score was significantly

correlated with vitamin D levels in AusSun group ( $r = -0.51$ ,  $p = 0.0051$ ). Dye eye survey score was significantly correlated with OSDI ( $r = -0.58$ ,  $p < 0.05$ ). When categorising participants as 4 groups (normal, mild dry eye, moderate dry eye, and severe dry eye) in accordance with OSDI, the severity of dry eye was correlated with vitamin D levels ( $r^2 = 0.68$ ,  $p < 0.001$ ).

**Discussion:** An association between the dry eye measurements and serum vitamin D levels was not observed in the study. The dry eye symptoms - OSDI, was only correlated with vitamin D levels when there was a presence of vitamin D insufficiency, however the study lacked power (e.g. correlation analysis). In future research, a larger population that controls confounding factors such as seasonal variation is suggested.

## Introduction

Dry eye is an ocular surface condition defined by a deficiency in tear quality or quantity. Dry eye sufferers often report dryness, irritation, fatigue, and fluctuating visual disturbances (Miljanović et al., 2007a). The discomforts and symptoms caused by dry eye considerably impact sufferers' quality of life, particularly in carrying out daily tasks such as reading, computer use, driving, and watching television (Miljanović et al., 2007b).

Dry eye is an age related condition, it is reported that up to 33% of the adult population suffering from dry eye (Shimmura et al., 1999; Smith, 2007). It has been suggested that people aged above 30 have higher risk of suffering dry eye (Uchino et al., 2013). Between 51 and 70 years of age, the prevalence of dry eye was 13.7%, and the figure rose to almost three times in the people aged between 71 and 90 years (Onwubiko et al., 2014). Age is generally considered a risk factor (Moss et al., 2000; Shimmura et al., 1999) for dry eye, though there have been opposite opinions (Lin et al., 2003; Schein et al., 1997).

Two underlying etiopathologies of dry eye have been identified; these are tear quality deficient (evaporative) dry eye and quantity deficient (aqueous deficient) dry eye. Study has suggested that Meibomian gland dysfunction can contribute to tear deficiency (Bron & Tiffany, 2004). Other causes of dry eye such as reflex block,

impairment of the lacrimal glands, lack of lacrimal gland, and medication effects, have been suggested (Fraunfelder et al., 2012; Tincani et al., 2013).

Vitamin D is a lipophilic prohormone and possesses immunomodulatory properties (Prietl et al., 2013; Smyk et al., 2013). Although the preventive effects of vitamin D in a number of autoimmune diseases and cancer have not been proven, the importance of vitamin D in the development/ prevention of the systematic diseases is recognised (Prietl et al., 2013; Smyk et al., 2013).

Although it has been suggested that the severity of dry eye was not associated with vitamin D levels (Galor et al., 2014), it has been reported that dry eye symptoms (assessed with DEQ5) were reduced when vitamin D levels were higher (Galor et al., 2014). Kurtul and the colleague (2015a) assessed the association between human's serum vitamin D and dry eye measurements using TBUT and Schirmer test, they found the dry eye measurements were reduced in people with vitamin D deficiency. Another recent study also found that there was a negative correlation between vitamin D levels and OSDI, while a positive correlation was found between vitamin D levels and Schirmer test/TBUT scores (Yildirim et al., 2016).

The types of benefits and their magnitude of taking vitamin D supplements are under much investigation. It is thought that vitamin D status is particularly important for regulating bone metabolism (Holick, 1996, 2004a; Rizzoli et al., 2013). There is some evidence relating low serum vitamin D levels to chronic systemic inflammatory diseases, such as asthma, atherosclerosis and autoimmune conditions (Ascherio et al., 2010; Stojanovic et al., 2011; Székely & Pataki, 2012). For example, inflammation associated with asthma results from increased airway smooth muscle mass and is associated with low vitamin D serum levels (Gerber & Sutherland, 2011; Gupta et al., 2011). Emerging evidence is indicating that vitamin D might be involved in the anti-inflammatory response. Also, studies observing individuals have found lower risk in a number of disorders such as mental disorders, infection condition, type 2 diabetes mellitus, cardiovascular disease, autoimmune disorders, and certain types of cancer when the serum 25(OH)D levels are higher than 70 to 80 nmol/L (28 to 32 ng/mL) (Holick, 2007; Holick et al., 2011; Nowson et al., 2012).

Similarly dry eye has an inflammatory component and might be immune mediated (Contreras-Ruiz et al., 2013; Hessen & Akpek, 2014; Pinazo-Durán et al., 2013; Stevenson et al., 2012a). Given the potential anti-inflammatory characteristics

of vitamin D and the inflammation related pathogenesis of dry eye, we sought to investigate whether there was an association between the vitamin D status and dry eye in human.

We targeted older population who are more likely to have dry eye/vitamin D insufficiency/deficiency and sought to determine if there was an association between vitamin D serum levels and severity of dry eye symptoms, investigated by their ocular surface inflammation and tear film.

We aimed to investigate the relationship between vitamin D and ocular surface inflammation and tear film in older adults. It is hypothesised that people with lower vitamin D levels will have more prominent dry eye symptoms.

## **Methods**

Participants (n=58) were aged between 42 and 69 years ( $55.1 \pm 7.3$  years). The recruitment period was from September 2014 to November 2015, AusSun participants had blood samples collected between September 2013 and July 2014.

This project involved two participant groups:

**AusSun Group:** The recruitment emails were sent to 110 participants of the AusSun study; 29 responded and completed the dry eye assessment. Participants (n=29) were aged between 43 and 69 years ( $54.2 \pm 7.8$  years). All the participants had their blood level of vitamin D measured [using the Liaison semiautomated chemiluminescence assay (DiaSorin, S.p.A., Saluggia, Italy)].

**Optometry Clinic Group:** Twenty-nine patients from the Optometry clinic of QUT with a dry eye diagnosis (Schirmer test < 8 mm or tear break up time < 8 seconds recorded at the last visit) (McCarty et al., 1998) were identified and invited for dry eye assessments. Twenty-nine were recruited and had their dry eye assessed and blood collected.

All experiments were conducted with ethics approval in accordance with the Declaration of Helsinki and the requirement of the Queensland University of Technology Human Research Ethics Committee. Written informed consent was obtained from participants.

### **Blood sample collections:**

Ten ml of blood from each participant was collected, using vacutainer and 21 gauge needle into a 10 ml serum separator tube (SST). The tubes were centrifuged at a speed of 2400 RPM for 10 minutes. The blood samples were stored at -80°C until later analysis.

### **Ocular Assessment:**

#### **Acuity and health**

Visual acuity was measured using the Bailey-Lovie 3 meter chart at 3 m; inclusion criteria included best corrected distance acuity of at least 6/7.5 in each eye. All participants reported good general and ocular health, ophthalmoscopy was performed to screen for ocular abnormalities. Individuals who were taking medications/ supplements or had received eye surgery were excluded.

#### **Questionnaire**

Participants completed a 12-item questionnaire - The Ocular Surface Diseases Index (OSDI) concerning their dry eye symptoms, the reliability and validity of OSDI has been tested (Schiffman et al., 2000), dry eye symptoms and their frequency were also recorded (see chapter 3 for detail).

#### **Dry eye assessments**

The Keratograph 5 (Best et al., 2012) was used for a number of ocular surface measurements, including tear meniscus height, non-invasive tear break up time (NITBUT), bulbar conjunctiva redness, and limbal conjunctiva redness (see methods chapter for details).

#### **Tear meniscus height**

The height of the tear meniscus is used as an estimate of tear film quantity. A tear meniscus height of less than 0.2 mm is indicative of a low tear volume and potentially dry eye. The Keratograph 5 was used to assess the tear meniscus (see Chapter 3). The in-built blue integrated ruler was used to measure the tear meniscus height from a position directly below the pupil centre. The measurements were taken with the K5 IR-illumination mode to avoid stimulation of tear secretion.

#### **Non-invasive tear break up time (NITBUT)**



The time it takes the tears to disappear (i.e. break-up) on the corneal surface is a measure of tear stability and quality (Norn, 1969). NITBUT was measured using the NIKBUT (Non-invasive Keratograph Break-Up Time) mode of the Keratograph 5. The Keratograph 5 takes the break-up time measurements automatically and without touching the eye. There are also infra-red and white illumination modes available; the infra-red mode was used to avoid elevated estimates due to stimulation of tear secretion. The participants were asked to blink twice and then were required to keep their eyes open. The time was measured automatically by the K5 from the last blink until the tear film break up was detected.

### **Ocular surface redness (bulbar and limbal conjunctival redness)**

The presence of ocular redness is considered one of the signs indicating inflammation of the ocular surface due to ocular surface desiccation (Wong & Wood, 2014). The R-Scan function of the K5 records and grading bulbar redness automatically and objectively; redness scores for temporal and nasal bulbar conjunctiva, as well as to the temporal and nasal sections of the limbal conjunctiva were obtained.

### **Schirmer tear test**

This test was performed only on the 29 participants recruited from the AusSun study. Schirmer tear secretion test (Carlson et al., 2003a) was performed to evaluate the tear quantity and the integrity of the lacrimal secretion system. Participants were instructed to look up and the lower lid pulled down and the Schirmer paper hooked over the lower lid. They were asked to keep the eyes open and to continue to look up to avoid significant reflex tearing. The Schirmer paper was removed after 5 minutes and the reading was noted by the scale reached by of the colour bar on the paper.

### **Phenol red thread test**

This test was performed on the 29 participants recruited from the Optometry clinic. A soft thread was placed inside the lower lid at approximately 1/3 of the eye from the temporal canthus for 15 seconds. The participants were instructed to look straight and try not to blink. Following that, the thread was removed with an upward motion to avoid causing discomfort. The part of thread changed to red then was measured by the scale on the back of the pack. The reading was deducted by 3 mm (excluding the length of the angle). A less than 11mm measurement indicates aqueous deficient dry eye (Albietz, 2000), while greater than 20mm is considered

normal. It has been suggested that phenol red thread test is equally sensitive as Schirmer test in detecting dry eye (Vashisht & Singh, 2011).

### **Grading of corneal staining**

Fluorescein sodium was used due to its nature of preserving the intact of cornea and also does not stain vital tissue (Maurice, 1967). Fluorescein staining was evaluated and graded soon after the fluorescein application in order to avoid the high luminosity that results from the rapid dye diffusion (Bron et al., 2003), which might affect the grading. The staining was photographed 30 seconds after fluorescein application using the Fluo Imaging of Keratograph 5 (see chapter 3 for details).

### **Blood sample collection procedures**

Ten ml of blood from each participant was collected, using vacutainer and 21 gauge needle into a 10 ml serum separator tube (SST). The tubes were centrifuged at a speed of 2400 RPM for 10 minutes (see chapter 3 for details).

### **Vitamin D and interleukin- 6 analysis**

Serum vitamin D levels were measured by ELISA (in pg/ml) (Sigma-Aldrich, St. Louis, MO, USA), while the serum concentrations of interleukin-6 were measured by ELISA (Abcam, MA, US). All blood samples of dry eye group were taken in May 2014 for the first visit. For the serum samples of participants from the AusSun study, the lab method used was the measurement of 25(OH)D concentration (in nmol/L) using the Liaison semiautomated chemiluminescence assay (DiaSorin, S.p.A., Saluggia, Italy).

### **Data analysis and statistics:**

Data analyses were performed using Pearson correlation to evaluate the relationship between the 25(OH)D and the other variables, and two sample unpaired t-test was conducted to compare the variables between the two groups. For categorical variable, Mann-Whitney U-test was used for statistical evaluations. A two-tailed p-value < 0.05 was considered as significant.

## Results

There were more female (65.52%) than male participants. There were 24% aged between 40 and 49 years, 47% aged between 50 and 59 years, and 29% aged between 50 and 59 years. Of the 29 participants from AusSun study, 24 showed vitamin D insufficiency ( $< 75\text{nmol/L}$ ), while 12 of 29 dry eye participants in the Optometry Clinic group showed vitamin D insufficiency.

**The correlation between vitamin D levels and dry eye symptoms analysed by various measurements.** There were no significant correlations observed between serum vitamin D levels and any of the dry eye measurements. An analysis of the data of the 58 participants tested across the two groups is included in the **Table 4-1**, **Table 4-2** and **Table 4-3**. The vitamin D levels were not correlated with tear meniscus height ( $r = -0.22$ ,  $p = 0.09$ ), NITBUT ( $r = -0.06$ ,  $p = 0.67$ ), limbal conjunctiva redness ( $r = -0.11$ ,  $p = 0.41$ ), phenol red thread test ( $r = -0.07$ ,  $p = 0.72$ ), OSDI score ( $r = -0.11$ ,  $p = 0.40$ ), bulbar conjunctiva redness ( $r = 0.0002$ ,  $p = 0.99$ ), and dry eye score ( $r = 0.03$ ,  $p = 0.84$ ). Also, no significant difference was observed in Oxford grading scores between Optometry clinic and AusSun groups ( $p > 0.05$ ). Based on the questionnaire the participants completed, the mean amount of time they spent outdoors across one week was  $942.6 \pm 723.9$  minutes. There was no association between 25 (OH) D levels and time spent outdoors ( $r = -0.04$ ,  $p = 0.84$ ). Dry eye score did not correlate with serum vitamin D levels in the Optometry Clinic group ( $r = -0.1$ ,  $p = 0.59$ ), while a significant correlation was observed in between dry eye score and serum vitamin D levels in the AusSun group ( $r = -0.51$ ,  $p = 0.0051$ ).

**Table 4-1 Vitamin D and Dry Eye Data in the two groups**

	Aus Sun		Optometry Clinic	
	Mean $\pm$ SD	Range (Min - Max)	Mean $\pm$ SD	Range (Min - Max)
Age (year)	$54.2 \pm 7.8$	43 - 69	$56.1 \pm 6.7$	43 - 69
Serum 25 (OH) D (nmol/l)	$61.08 \pm 22.09$	29.7 - 136	$87.66 \pm 32.48$	33.8 - 171.2

OSDI	11.37± 11.59	0.00 - 39.58	22.84± 15.97	2.08 - 57.5
Tear Meniscus Height (mm)	0.27 ± 0.05	0.16- 0.4	0.28 ± 0.10	0.14 - 0.57
NITBUT (s)	13.7 ± 6.6	3.6 - 24.2	11.8 ± 6.6	2.1 - 24.5
Bulbar Conjunctiva Redness	1.19 ± 0.55	0.35 - 2.65	1.25 ± 0.33	0.65 – 1.8
Limbal Conjunctiva Redness	0.83 ± 0.48	0.00 – 2.5	0.85 ± 0.33	0.35 – 1.40
Schirmer Test (mm)	11.37 ± 11.6	0.00 – 39.58	-	-
Phenol Red Thread Test	-	-	7.47 ± 5.76	-1 - 21
Oxford Grading	0.38 ± 0.62	0 - 2	0.38 ± 0.62	0 - 2
Optometry Clinic IL-6 (pg/ml)			2.26 ± 2.32	0.13 - 10.41

**Table 4-2 Vitamin D and dry eye data in all the participants**

	Mean ± SD	Range (Min - Max)
Genders Male = 20 (24.48%) Female = 38 (65.52%)		
Age (year)	55.14 ± 7.29	43 - 69
Serum 25 (OH) D (nmol/L)	74.37 ± 30.62	29.7 - 171.2
OSDI	17.11 ± 14.99	0 - 57.5
Tear Meniscus Height (mm)	0.27 ± 0.07	0.14 - 0.57
NITBUT (s)	12.88 ± 6.52	2.13 - 24.46

Bulbar Conjunctiva Redness	1.22 ± 0.45	0.35 - 2.65
Limbal Conjunctiva Redness	0.84 ± 0.41	0 - 2.5
Oxford Grading	0.38 ± 0.62	0 - 2
IL-6 (pg/ml)	2.26 ± 2.32	0.13 - 10.41

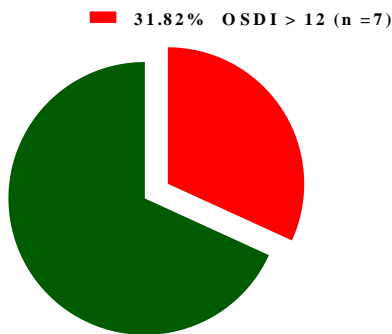
**Table 4-3 Dry eye signs and symptoms from the questionnaire**

Symptoms	Mean ± SD	Range (Min - Max)
Burn	0.5 ± 0.91	0 - 4
Blurriness	0.92 ± 0.86	0 - 4
Discomfort	0.69 ± 0.88	0 - 4
Itch	0.82 ± 0.87	0 - 4
Soreness	0.36 ± 0.76	0 - 4
Redness	0.79 ± 1.00	0 - 4
Irritation	0.74 ± 0.90	0 - 4
Grittiness	0.62 ± 0.77	0 - 4
Scratchiness	0.45 ± 0.89	0 - 4
Tired eyes	1.06 ± 0.89	0 - 4
Feeling of something in the eyes	0.60 ± 0.73	0 - 3
Dryness	1.08 ± 1.18	0 - 4
Filmy vision	0.63 ± 0.93	0 - 3
Pain	0.24 ± 0.61	0 - 3
Crustiness	0.44 ± 0.74	0 - 4

**The association of Interleukin-6 (IL-6) level with vitamin D levels and dry eye symptoms.** The IL-6 level of Optometry clinic participants was 2.26 ± 2.32 pg/ml, there was no correlation between the vitamin D levels and IL-6 ( $r = 0.28$ ,  $p = 0.141$ ). However, Interleukin-6 was found to be correlated with the following dry eye symptoms: burn ( $r = 0.87$ ,  $p = 0.01$ ), discomfort ( $r = 0.53$ ,  $p = 0.04$ ) soreness ( $r = 0.98$ ,  $p < 0.001$ ), irritation ( $r = 0.67$ ,  $p = 0.009$ ), grittiness ( $r = 0.66$ ,  $p = 0.003$ ),

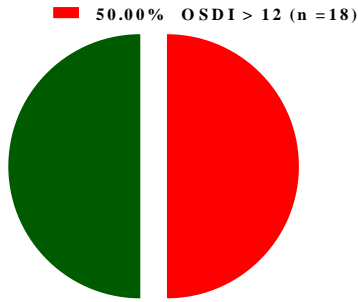
feeling of something in the eye ( $r = 0.67$ ,  $p=0.009$ ), pain ( $r = 0.99$ ,  $p = 0.009$ ), crustiness ( $r = 0.95$ ,  $p < 0.001$ ).

**The correlation between vitamin D levels, OSDI and dry eye condition.** Of the participants with optimal vitamin D levels ( $> 75$  nmol/l), there were nearly 1/3 showing OSDI greater than 12, while there were half with OSDI greater than 12 in the participants with vitamin D insufficiency ( $< 75$  nmol/l) (see **Figure 4-1** and **Figure 4-2**). The OSDI for the Optometry Clinic group was  $22.8 \pm 16.0$ , while it was  $11.4 \pm 11.6$  ( $p = 0.0028$ ) (see **Figure 4-3**); the serum vitamin D levels differed by  $-26.6 \pm 7.3$ ,  $87.7 \pm 32.5$  nmol/ml and  $61.1 \pm 22.1$  nmol/ml for the Optometry Clinic and AusSun groups, respectively ( $p < 0.001$ ) (see **Figure 4-4**). No significant correlation was observed between OSDI and serum vitamin D levels in all participants ( $r = -0.11$ ,  $p = 0.40$ ) (see **Figure 4-5**). The OSDI in the Optometry Clinic group did not show significant correlation with serum vitamin D levels ( $r = -0.23$ ,  $p = 0.23$ ) (see **Error! Reference source not found.**). However, the OSDI in the AusSun group was significantly correlated with serum vitamin D levels ( $r = -0.56$ ,  $p = 0.0015$ ) (see **Figure 4-7**). Also, dry eye survey score was significantly correlated to OSDI ( $r = 0.58$ ,  $p < 0.05$ ) (see **Figure 4-8**).



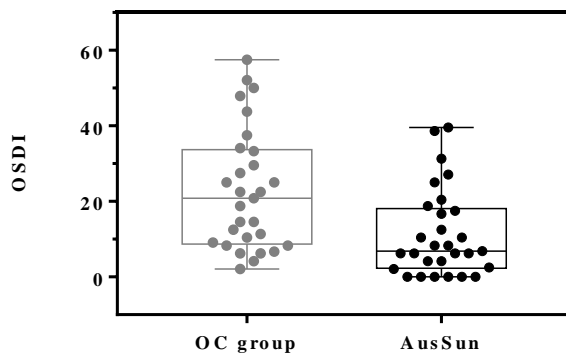
**Figure 4-1 Proportion of showing OSDI greater than 12 in participants with optimal serum vitamin D levels ( $> 75$ nmol/l).**

About 1/3 of the participants ( $n = 7$ ) with optimal vitamin D levels showed OSDI greater than 12.



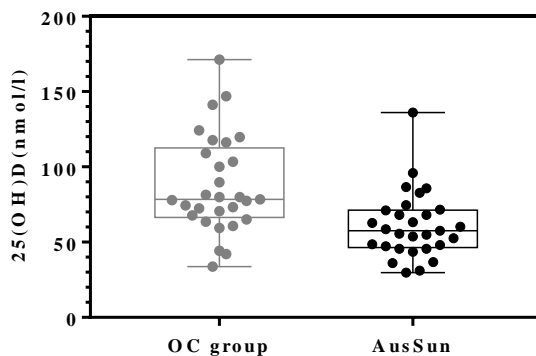
**Figure 4-2 Proportion of showing OSDI greater than 12 in participants with insufficient serum vitamin D levels (< 75 nmol/l).**

Half of the participants (n = 18) with deficient vitamin D levels showed OSDI greater than 12.



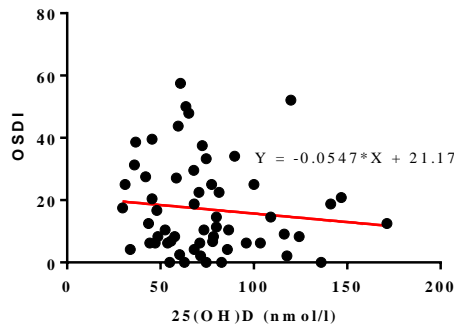
**Figure 4-3 The OSDI levels in the two groups**

The OSDI in OC group (Optometry Clinic group) (n = 29) was higher than AusSun group (n = 29) (p = 0.0028). Error bars are standard errors of the mean.

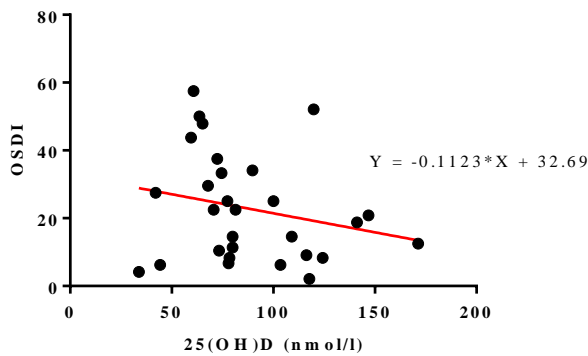


**Figure 4-4 The 25(OH) D levels in the two groups**

The serum vitamin D levels in OC group (Optometry Clinic group) (n = 29) were higher than AusSun group. Serum vitamin D levels in OC group and AusSun group (n = 29) were  $87.7 \pm 32.5$  nmol/ml and  $61.1 \pm 22.1$  nmol/ml, respectively (p < 0.001). Error bars are standard errors of the mean.

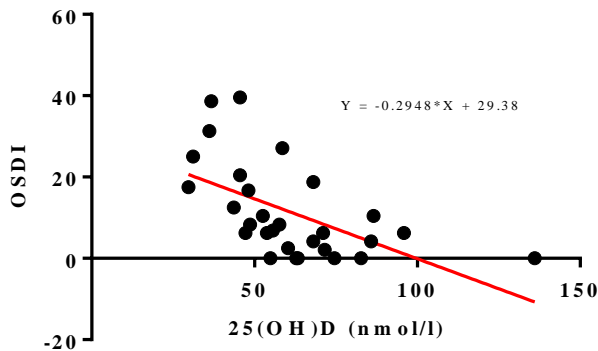


**Figure 4-5 The correlations between OSDI and 25(OH) D in all participants (n = 58)**  
 Serum vitamin D levels were not correlated with OSDI ( $r = -0.11$ ,  $p = 0.40$ ). Error bars are standard errors of the mean.



**Figure 4-6 The correlations between OSDI and 25(OH)D in the participants of Optometry Clinic group (n = 29)**

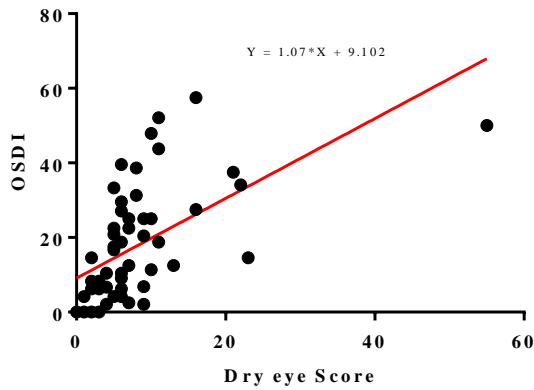
OSDI in the Optometry Clinic group did not show significant correlation with serum vitamin D levels ( $r = -0.23$ ,  $p = 0.23$ ).



**Figure 4-7 The correlations between OSDI and 25(OH) D in the participants of AusSun group (n = 29)**

OSDI in the AusSun group was significantly correlated with serum vitamin D levels ( $r = -0.56$ ,  $p = 0.0015$ ).

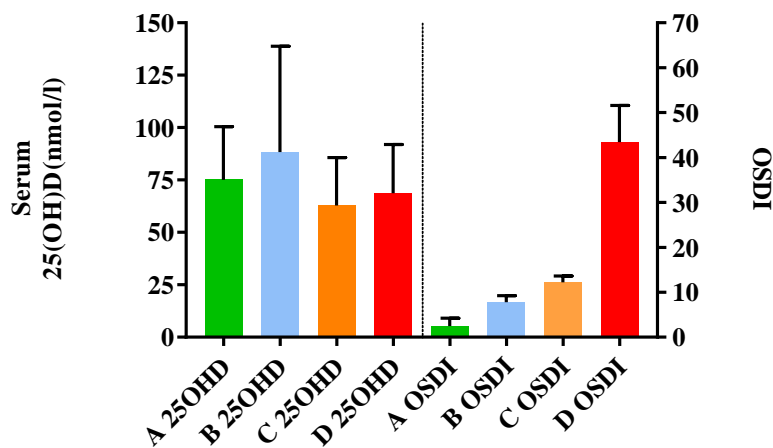




**Figure 4-8** The correlation between OSDI and dry eye score (n = 58)

A significant correlation was observed between dry eye score and OSDI ( $r = 0.58$ ,  $p < 0.001$ ).

**The association of OSDI and vitamin D levels with different severity of dry eye symptoms.** When further categorising the participants in accordance of the severity of dry eye as normal (OSDI  $\leq 12$ ), mild dry eye (OSDI 13-22), moderate dry eye (OSDI 23-32), and severe dry eye (OSDI  $\geq 33$ ), the means for OSDI in each group were  $5.35 \pm 3.67$ ,  $16.71 \pm 3.06$ ,  $26.16 \pm 2.99$ , and  $43.44 \pm 8.15$ ; the respective means of serum vitamin D levels were  $75.1 \pm 25.36$ ,  $88.32 \pm 50.44$ ,  $62.77 \pm 22.92$ , and  $68.75 \pm 23.22$  nmol/l ( $F = 33.3$ ,  $p < 0.001$ ) (see **Figure 4-9**). It is clear that with moderate and severe dry eye, the vitamin D levels were under optimal levels. However, no correlation was found in any of the severities with vitamin D levels.



**Figure 4-9** OSDI and 25(OH)D levels in different severity of dry eye.

The green bars show the means of OSDI and serum vitamin D levels in the individuals without dry eye, blue bars stand for the two values for mild dry eye, orange bars represent the means for moderate dry eye, and red bars show the values for severe dry eye ( $F= 33.3, p < 0.001$ ).

## Discussion

Dry eye is an irritating ocular condition affecting up to 1/3 of adult population, being associated with quality of life in later life, and it is commonly seen in older people. Of the 58 participants in this study, 50% had dry eye symptoms.

This study investigated the relationship between vitamin D levels and dry eye symptoms in people diagnosed with dry eye or with vitamin D insufficiency. We did not find any correlation between vitamin D levels and dry eye measurements. The participants of Optometry clinic group had higher OSDI indicating they had mild dry eye, while mean OSDI of participants of the AusSun group was within normal range. The Optometry clinic group with higher OSDI were also found to have higher serum vitamin D levels compared to the optimal status. For OSDI and serum vitamin D levels in AusSun group, a negative correlation was observed. This might be suggesting that OSDI could be predicted when there is a presence of vitamin D insufficiency. When observing participant in accordance with dry eye severity, it showed that people with moderate and severe dry eye were more likely to be vitamin D insufficient. A recent study reported that in their optimal vitamin D ( $81.8688 \pm 21.77$ ) participants, there was 15% exhibiting dry eye symptoms, while dry eye symptoms were observed in 100% in vitamin D deficient ( $28.70 \pm 4.49$  nmol/l) participants (Kurtul et al., 2015a). Of our studied population, 1/3 with optimal vitamin D levels showed at least mild dry eye symptoms ( $OSDI > 12$ ), while dry eye symptoms were observed in 50% of people with vitamin D deficiency.

The three important components of tear production, lacrimal gland, Meibomian glands, and goblet cells, directly influence the quality and quantity of tear. The association between changes in Meibomian glands and ageing has been suggested (Den et al., 2006). Changes in these components have been demonstrated in animals and human. In the rat model, it has been shown that there are changes such as acinar degeneration, nuclear abnormalities; increased collagen, and ductal dilation (El-Fadaly et al., 2014). Ageing Meibomian glands in both mice and human also showed reduced amount of cellular proliferation markers (Jester et al., 2011; Nien et al., 2009; Villani et al., 2013). Histological analysis in rats and mice and microscopic

examination in human all found that there is a decrease in the densities of goblet cells (Den et al., 2006; McClellan et al., 2014; Wei et al., 2011). Meibomian glands function as producing the lipid layer, while lacrimal gland produces the aqueous component, and the mucin layer, the component that is essential for adhering tear on the ocular surface, is produced by goblet cells. All the three components are important for appropriate tear quality and quantity. Changes in Meibomian glands, lacrimal glands, and goblet cells are known to be associated with the properties of tear film.

Decreased release of acetylcholine has been found in older animals using mice model, and both sympathetic and parasympathetic nerves showed reduction in the area surrounding the acinus (consists of lacrimal gland cells) (Ríos et al., 2005; Williams et al., 1994). These changes may contribute to lower volume of tear secretion as the level of stimulation has also been altered. A rat model also demonstrated that the stimulation of substance P, vasoactive intestinal peptide, histamine, and 5-hydroxytryptamine in ageing lacrimal glands resulted in lower responses - reduced peroxidase release and protein output from acinar cells, as compared to younger animal (Draper et al., 2003). The results showed that ageing is associated with altered ability of synthesising and secreting proteins by acinar cells.

Inflammation on the eye surface may also be associated with the ageing change, leading to the dryness of ocular surface. Almost 2/3 were categorised as vitamin D insufficiency (serum vitamin D levels < 75 nmol/l) in the studied population while only half had dry eye (OSDI > 12). The two groups in the study were of similar ages and therefore ageing lacrimal system should not be the principal contributing factor to the severer symptoms in the AusSun group. The mean vitamin D levels for all the participants and the Optometry Clinic group were  $74.37 \pm 30.62$  nmol/l and  $87.66 \pm 32.48$  nmol/l, respectively. A large cross-sectional study used a cut-off of 53 nmol/l for serum vitamin D levels and found no association between C-reactive protein (an inflammatory marker) and serum vitamin D in the group with higher serum vitamin D, however negative association was observed in people with low serum vitamin D concentration (Amer & Qayyum, 2012). Likewise, in our study, it appeared OSDI was not correlated with vitamin D levels when vitamin D levels were above or close to the optimal level (75 nmol/l). This might explain the reason that the AusSun had lower OSDI even with lower vitamin D levels, indicating

OSDI was more sensitive when the vitamin D levels were less than optimal. It has been reported that the prevalence of vitamin D insufficiency ( $< 50$  nmol/l) in adults ranged between 40% and 80% (depending on the age) (Prentice et al., 2008); in our study, vitamin D levels in 13 participants (22.41%) were under 50 nmol/l, and the proportion of those under 75 nmol/l accounted for 62.97% ( $n = 36$ ).

One of the limitations of the study was the vitamin D analysis methods. AusSun group was measured by the DiaSorin® Liaison semi-automated chemiluminescence assay (DiaSorin, Italy), while the serum vitamin D concentrations of the Optometry Clinic Group was measured using the 25 (OH) vitamin D ELISA (Sigma-Aldrich, St. Louis, MO, USA). With interest into what is the optimal vitamin D level for human health, a number of methods have been developed to measure vitamin D, for example, binding protein assays, immunoassays, radio-immunoassays, automated immunoassays, high performance liquid chromatography, and mass spectrometry. The principal challenge of using different assays for measuring 25(OH)D, is that it has been shown to have high degrees of variability and bias (Holmes et al., 2013).

In addition, since the recruiting period of the participants of Optometry Clinic group in the study was across from September 2014 to November 2015, one other thing worth noting is that the seasonal variation may potentially be a factor affecting the vitamin D levels in the participants. Kasahara and the colleagues (2013) analysed 3.44 million serum samples and have reported that serum vitamin D concentrations peaked in late summer and troughed in late winter. In Australia, the high ultra violet radiation months are from September to April. Under controlling variables such as gender, body mass index, and residing location etc., it has been shown that there could be a difference of 19.8 nmol/L in 25(OH)D between summer and winter (Kimlin et al., 2014), which is considered having a large impact in our study.

The study was not masked, and the absence of a control group might also affect the results of the study.

Kimlin et al. (2014) suggested that every percentage of clothing cover could reduce 25(OH)D by 0.5 nmol/L. While it is now appreciable the amount of sunlight directly links to vitamin D levels in the body, it was not feasible to survey the proportion of skin being exposed to sunlight - clothing style therefor was an important factor that could significantly affect the measured 25(OH)D.

Body fat is also one of the influencing factors to vitamin D levels in the human body. Snijder and the colleagues (2005) suggested that there is a 48 to 60 nmol/l decrease in vitamin D levels with a body fat increase of 20 - 35% in male, while a body fat increase of 32 - 51% can result in vitamin D reduction in the body by 40 to 55 nmol/l. Future studies should take consideration for this determinant given its significant effect on the vitamin D levels.

Further studies with more explicit design to ameliorate the estimate of both sunlight exposure time and intensity are warranted. A comprehensive assessment on the function of lacrimal units such as measuring the density of goblet cells, sensitivity of lacrimal glands to producing aqueous, and morphology of Meibomian glands, should assist researchers toward further understanding in the association between human vitamin D status and ocular dryness.

### **Conclusion**

Serum vitamin D levels were associated with subjective measurements - OSDI and dry eye score when the vitamin D levels were greatly below the optimal levels, indicating that people with insufficient vitamin D levels were more likely to have more and severer dry eye symptoms (higher OSDI). Also, OSDI might be better predicted in vitamin D insufficient individuals.

No direct association between 25(OH)D levels and the self-reported time spent outdoors was observed, it might be due to the small sample size and other potential influencing factors such as diet, clothing, and seasonal variability of sunlight intensity.

# Chapter 5: Experiment 2: Vitamin D Supplement and Dry Eye/Low Vitamin D

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## Abstract

The symptoms of dry eye negatively affect the quality of life in people suffering from dry eye. Economic burden of dry eye health care is vast. Studies have demonstrated the benefits of vitamin D to human bone health, however very little literature has investigated its function to eye health. Dry eye causes or can be caused by ocular surface inflammation. Vitamin D (a steroid like vitamin that can be synthesised by sun exposure) has been shown to have anti-inflammatory properties. In this study, we aimed to investigate whether a period of vitamin D supplement could improve the symptoms experienced by dry eye sufferers.

**Methods:** Thirty-two adults aged between 43 and 69 years were invited from a previous study. Twenty-five were recruited from the Optometry clinic of the Queensland University of Technology (QUT) with a previous diagnosis of dry eye. Seven adults with vitamin D insufficiency were selected from those who had participated in the AusSun study. Objective dry eye measurements in this study included tear meniscus height, non-invasive tear break up time, phenol red thread test, Schirmer test, and corneal staining grading. The Ocular Surface Disease Index and the Dry Eye Symptoms survey were used as subjective measures. Vitamin D levels of the participants in the AusSun study had been previously measured, whereas the blood samples were obtained for analysis from other participants.

**Results:** Vitamin D levels of participants rose by 29.08 nmol/l (from  $80.8 \pm 35.86$  at baseline nmol/l to  $109.9 \pm 49.95$  after the vitamin D treatment ( $n = 32$ ,  $P < 0.0001$ ). Oxford grading was significantly lower after the vitamin D treatment (After vs Before,  $0.31 \pm 0.54$  vs  $0.53 \pm 0.72$ ) ( $p = 0.03$ ). The OSDI reduced from  $21.01 \pm 14.14$  to  $10.45 \pm 10.44$  after the vitamin D treatment ( $p = 0.02$ ). No significant difference was found in the IL-6 levels ( $n = 25$ , baseline  $2.20 \pm 2.19$  pg/ml, after treatment  $4.86$

$\pm 10.09$  pg/ml) ( $p > 0.05$ ). Bulbar ( $r = 0.60$ ,  $p < 0.05$ ) and limbal ( $r = 0.53$ ,  $p < 0.05$ ) conjunctiva redness were correlated with age.

**Discussion:** Vitamin D levels were significantly raised after the vitamin D treatment. OSDI showed a reduction by 10.56. The results showed that a 60-day vitamin D supplement was able to lower dry eye symptoms but not the signs of dry eye.

## Introduction

Dry eye is an ocular condition that has prevalence for up to 33% of world population. Irritating symptoms from dry eye can negatively affect a dry eye sufferers' quality of work and life (Miljanović et al., 2007a). The reporting rates to eye care professionals in Western country such as France, the Germany, Italy, Spain, Sweden, and the UK have been estimated to be lower than 0.1% (Clegg et al., 2006). It has also been estimated that total annual cost of 1,000 dry eye patients managed by ophthalmologists ranged from 0.27 to 1.50 million in some Western countries (Clegg et al., 2006), the estimated total annual cost has also been estimated up to 55.4 billion (Yu et al., 2011). Therefore, the potential economic burden from managing dry eye cannot be overlooked.

T lymphocytes and elevated inflammatory cytokines present in the conjunctive and tears of dry eye patients (Massingale et al., 2009; Stern et al., 2002a), it therefore is believed that there is an inflammatory component in ocular dryness.

Currently, the available treatments for dry eye include pharmacological therapy such as tear replacement, topical cyclosporine, and complementary medicine, nonpharmacological treatments such as punctal occlusion or tarsorrhaphy (Reddy et al., 2004). However, adverse effects have been reported in nonpharmacological treatment, for example ocular inflammation can be promoted and tear production is lower following punctal occlusion (Yen et al., 2001). None of the current treatments for dry eye is using naturally synthesised nutrient such as vitamin D. Vitamin D, a lipophilic vitamin produced from skin after UV light exposure, and can be acquired naturally in some foods (Holick & Chen, 2008).

There is debate about the relationship between vitamin D and inflammation. While some studies have suggested elevated vitamin D levels reduced inflammation (Adorini & Penna, 2008; Marcotorchino et al., 2012; Patel et al., 2007), others hypothesize vitamin D reduction results from inflammation (Autier et al., 2014; Henriksen et al., 2014; Mangge et al., 2015). The metabolites of vitamin D and the analogues have been demonstrated to be effective in anti-inflammation in animals (Adzemovic et al., 2013; Erbaş et al., 2014; Ydmaz et al., 2013). A number of mechanisms of reducing inflammation have been proposed. One mechanism that is closely related to inflammation is that a switch from the more inflammatory T cells (T help 1) to the less inflammatory cells (T help 2), the switch effects lead to increase of anti-inflammatory cytokines and decrease of pro-inflammatory markers (Aranow, 2011; Guillot et al., 2010). To date, we have known serum calcium and phosphate levels are regulated by vitamin D, therefore vitamin D plays an important role in maintaining bone health (Cranney et al., 2008; Holick, 2004b). Few studies have investigated the other functions of vitamin D, for example, its involvement in epithelial cell health, modulation of immune system, and reduction of inflammation (Bikle, 2010). Studies have found that older people tend to have lower vitamin D levels and suggested higher supplementary dose to elderly (Wicherts et al., 2007). Although some evidence has indicated that vitamin D supplementation can improve neuromuscular function in older adults (Dhesi et al., 2004), and its association with peripheral vascular disease and Alzheimer disease (Cherniack et al., 2009; Holick, 2004a), the impact of vitamin D supplement to human's eye is not well understood. Few studies have evaluated the effect of vitamin D on dry eye. Sjogren's syndrome, usually accompanied with dry eye, has been found to be associated with reduced vitamin D levels (Bang, 1999), another recent study found that people with higher vitamin D levels had lower dry eye syndrome symptoms though there were no association between the severity of dry eye and vitamin D levels (Galor et al., 2014). In addition, it has also been demonstrated that the two commonly tests for dry eye - Schirmer score and tear break up time, were lower in vitamin D deficient people (Kurtul et al., 2015a). Yet, another recent study suggested there was no correlation between dry eye syndrome and serum vitamin D levels, though it was found that increased serum vitamin D levels had lower odds of dry eye syndromes (Jee et al., 2016). However, to our knowledge, no study has investigated whether vitamin D supplement for a period of time can relieve dry eye symptoms.



It has been suggested that a required vitamin D circulating level of 25-hydroxyvitamin D of  $> 75$  nmol/L, is able to maximise vitamin D's beneficial effects for health. A minimal dose of vitamin D (800 - 1000 IU) is suggested to achieve the benefit in both children and adults (Holick & Chen, 2008) in the absence of adequate sun exposure. A daily dosage of 1000 IU has also been suggested to maintain vitamin D serum level above 75 nmol/l (Dawson-Hughes et al., 2005; Holick et al., 2011).

Dry eye score assessed by the Dry Eye Questionnaire 5 could be reduced by 1.24 when there was an increase of 10 ng/mL in vitamin D in the human body (Galor et al., 2014).

We aimed to investigate whether an 8.6 week vitamin D (1000 IU/daily) supplement would improve the symptoms experienced by dry eye/insufficient vitamin D sufferers.

## Methods

This experiment involved recruiting a sub-set of participants from the previous study, 34 participants were invited for follow up visit if their vitamin D level were insufficient [lower than 75 nmol/L (the upper limit of vitamin D insufficiency)] (Dawson-Hughes et al., 2005; Holick et al., 2011) or they were classified as dry eye [(phenol red thread test  $< 6$ mm) (Bron, 2001), (tear meniscus height  $\leq 0.35$ mm) (Mainstone et al., 1996)], or OSDI  $\geq 13$ . A total of 32 participants (seven participants recruited from the AusSun study with low vitamin D serum levels in the Experiment 1, and 25 participants with dry eye symptoms were identified.), aged between 43 and 69 years ( $56.2 \pm 7.4$  years old), participated in this vitamin D supplement study. Each participant was asked to take one vitamin D supplement (1000IU, 60 capsules in a bottle, Swisse) orally daily. Measures and the serum level of vitamin D of the participants were performed and collected at baseline and the end of the treatment between the 50<sup>th</sup> and the 60<sup>th</sup> day since receiving the vitamin D supplement.

Dry eye assessment and vitamin D/Interleukin-6 measurements on the participants were identical as the first visit (see chapter 3 for details).

## Results

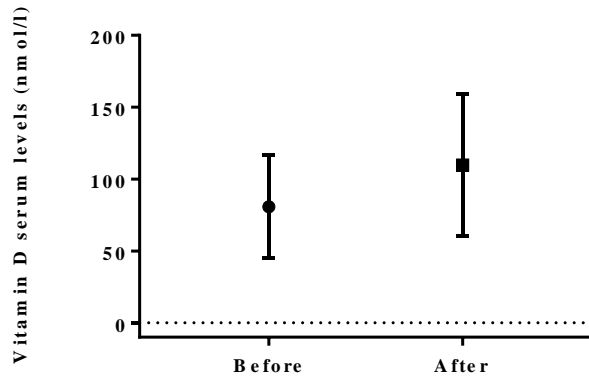
A summary table for dry eye measurements before and after the vitamin D treatment is included (see **Table 5-1**). More females (62.50%) than males participated in the experiment. Their serum vitamin D levels rose by 36% from  $80.8 \pm 35.9$  nmol/L at baseline to  $109.9 \pm 50.0$  after the vitamin D treatment ( $n = 32$ ,  $P < 0.0001$ ) (see **Figure 5-1**). Tear meniscus height slightly increased by 0.03 mm (After vs Before,  $0.31 \pm 0.11$  vs  $0.28 \pm 0.09$ ) ( $p > 0.05$ ), while NITBUT increased by 0.77 seconds (After vs Before,  $10.26 \pm 5.4$  vs  $12.2 \pm 6.7$ ) ( $p > 0.05$ ). Both of the redness scores for bulbar and limbal conjunctiva were higher by 0.06 (After vs Before,  $1.31 \pm 0.45$  vs  $1.25 \pm 0.39$ ) ( $p > 0.05$ ), and 0.023 (After vs Before,  $0.87 \pm 0.38$  vs  $0.85 \pm 0.36$ ) ( $p > 0.05$ ), respectively. Schirmer's test in the 7 participants of AusSun group showed a decrease of 1.14 mm (After vs Before,  $21 \pm 12.49$  vs  $22 \pm 10.79$ ) ( $p > 0.05$ ). Slight increase was observed in phenol red thread test in the participants of Optometry Clinic group (After vs Before,  $7 \pm 5.28$  vs  $6.82 \pm 5.51$ ) ( $p > 0.05$ ). Oxford grading was significantly lower after the vitamin D treatment (After vs Before,  $0.31 \pm 0.54$  vs  $0.53 \pm 0.72$ ) ( $p = 0.03$ ) (see **Figure 5-2**). The OSDI was significantly lower, at baseline it was  $21.01 \pm 14.14$  and after the vitamin D treatment it dropped to  $10.45 \pm 10.44$  ( $p = 0.02$ ) (see **Figure 5-3**). There was no significant difference found in the IL-6 levels ( $n = 25$ , baseline  $2.20 \pm 2.19$  pg/ml, after treatment  $4.86 \pm 10.09$  pg/ml) ( $p > 0.05$ ). Bulbar ( $r = 0.60$ ,  $p < 0.05$ ) and limbal ( $r = 0.53$ ,  $p < 0.05$ ) conjunctiva redness were correlated with age (see **Figure 5-4**).

**Table 5-1 Dry eye measurements before and after the vitamin D treatment**

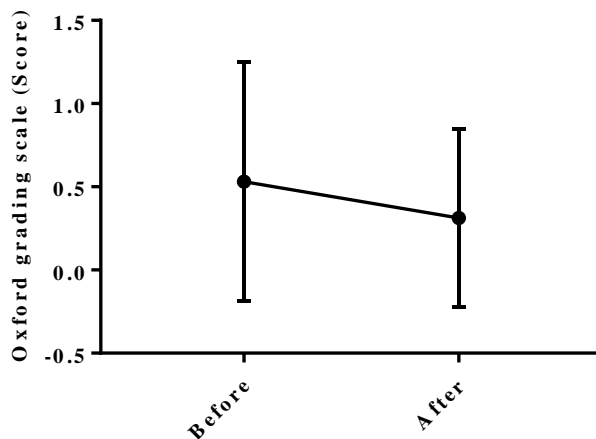
	Before		After		Mean difference (After - Before)	Adjusted P
	Mean $\pm$ SD	Range (Min - Max)	Mean $\pm$ SD	Range (Min - Max)		
Age (year)	$56.0 \pm 7.3$	43 - 69	$56.2 \pm 7.4$	43 - 69	0.16	> 0.99
Tear meniscus	$0.28 \pm 0.09$	0.15 - 0.57	$0.31 \pm 0.11$	0.07 - 0.57	0.03	> 0.99

height (mm)						
OSDI	21.01 ± 14.14	2.08 - 57.5	10.45 ± 10.44	0 - 40.63	-10.56	<b>0.02</b>
NITBUT (s)	12.2 ± 6.7	2.13 - 24.46	10.26 ± 5.4	2.87 - 23.52	0.77	> 0.99
Bulbar Conjunctiva Redness	1.25 ± 0.39	0.5 - 2.05	1.31 ± 0.45	0.35 - 2.05	0.06	> 0.99
Limbal Conjunctiva Redness	0.85 ± 0.36	0.35 – 1.4	0.87 ± 0.38	0.15 – 1.8	0.023	> 0.99
AusSun Schirmer Test (mm)	22 ± 10.79	10 - 35	21 ± 12.49	5 -34	-1.14	> 0.99
Optometry Clinic Phenol Red Thread Test (mm)	6.82 ± 5.51	-1 -18	7 ± 5.28	1 - 23	0.18	<b>0.03</b>
Oxford Grading	0.53 ± 0.72	0 - 2	0.31 ± 0.54	0 - 2	-0.22	> 0.99
Serum 25 (OH) D (nmol/l)	80.8 ± 35.86	29.7 – 171.2	109.9 ± 49.55	28.36 - 265	29.08	<b>&lt; 0.0001</b>
Optometry Clinic IL-6 (pg/ml)	2.2 ± 2.19	0.13 - 10.41	4.03 ± 9.02	0.34 - 52.28	2.65	> 0.99

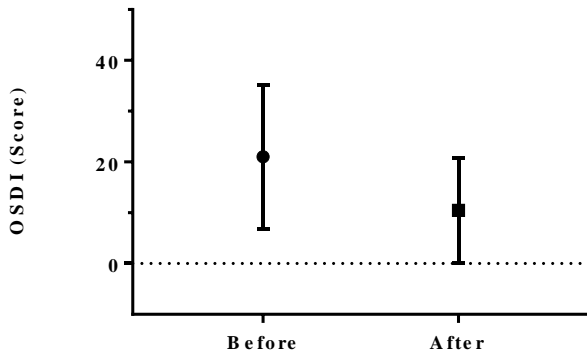
## 5.1 DRY EYE MEASUREMENTS BEFORE AND AFTER THE VITAMIN D TREATMENT



**Figure 5-1 Vitamin D levels in serum before and after the vitamin D supplement.** Vitamin D levels increased by 29.08 nmol/l after the vitamin D supplement period (from  $80.8 \pm 35.86$  at baseline nmol/l to  $109.9 \pm 49.95$ ) ( $p < 0.0001$ ). Error bars are standard errors of the mean.

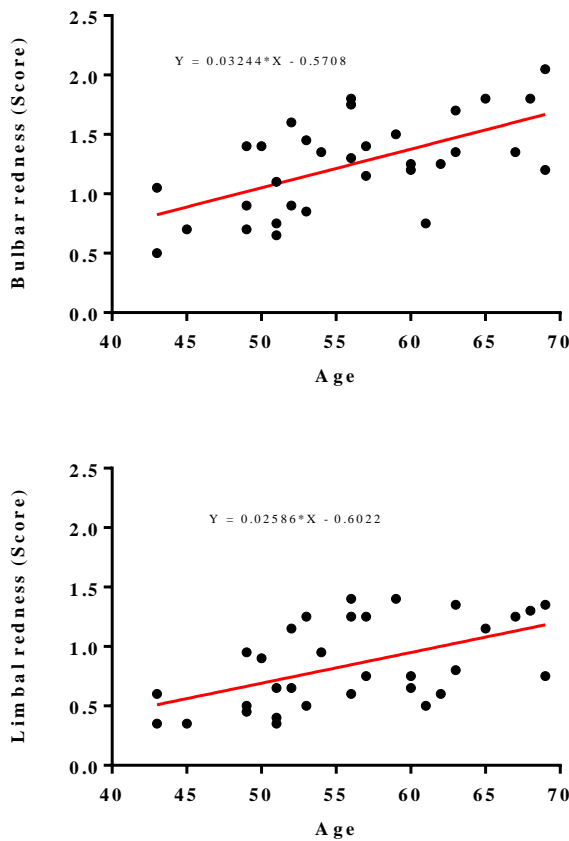


**Figure 5-2 Oxford grading scale before and after vitamin D treatment** The rankings of Oxford grading scale were  $0.53 \pm 0.72$  and  $0.31 \pm 0.54$  for before and after vitamin D treatment, respectively ( $p = 0.03$ ). Error bars are standard errors of the mean. Error bars are standard errors of the mean.



**Figure 5-3 OSDI before and after the vitamin D treatment**

Before the vitamin D treatment, OSDI was  $21.01 \pm 14.14$  and reduced to  $10.45 \pm 10.44$  after ( $p = 0.02$ ). Error bars are standard errors of the mean.



**Figure 5-4 The correlation between the index of bulbar conjunctiva and limbal conjunctiva redness**

The top graph shows the correlation between bulbar conjunctival redness and age (redness index =  $0.03244 * \text{age} - 0.5708$ ), while the bottom reveals a slightly lower correlation between limbal conjunctival redness and age (redness index =  $0.02586 * \text{age} - 0.6022$ ). Error bars are standard errors of the mean.

## Discussion

An early study has shown that an 11-week 1000IU/daily vitamin D supplement in the end of winter rose the serum vitamin D levels by 48% in adults aged between 18 and 84 years (Holick et al., 2008). The results of this present study suggested that an 8-week 1000IU/daily vitamin D supplement D increased serum vitamin D level by 36% in serum concentration. The lower increase in serum vitamin D levels might be the baseline mean value (80.8 nmol/l) was higher as compared to the mean baseline value (equal to 48.92 nmol/l) in the study conducted by Holic and the colleagues. Another influencing factor might be the widespread age range of the participants in their study, while there were only people aged between 43 and 69 included in our study. It has been suggested older people have relatively lower vitamin D levels in the body (Lund & Sørensen, 1979), in addition, older people require higher dose of vitamin D to achieve optimal vitamin D status (Holick, 2007).

Studies have been disputing the relationship between vitamin D and inflammation. Whether lower vitamin D levels is the consequence of inflammation, or inflammation results in lower vitamin D have been discussed greatly in recent years. Some hypothesised that inflammation inducing oxidative biological environment decreases vitamin D levels via disturbing synthesis of vitamin D in the liver (Autier et al., 2014; Henriksen et al., 2014; Mangge et al., 2015), while it has been hypothesised that increasing vitamin D leads to inflammation reduction (Adorini & Penna, 2008; Guillot et al., 2010; Marcotorchino et al., 2012).

In animals, it has been demonstrated the vitamin D metabolite [1,25(OH)<sub>2</sub>D<sub>3</sub>] reduces inflammation (Erbaş et al., 2014; Ydmaz et al., 2013) Also, anti-inflammatory properties of 1,25(OH)<sub>2</sub>D<sub>3</sub> has been observed in vitro studies (Feng et al., 2013; Guo et al., 2013a; Korf et al., 2012). One of the anti-inflammatory mechanisms of 1,25(OH)<sub>2</sub>D<sub>3</sub> is that the effect of anti-inflammation is a switch between T helpers (i.e. 1,25(OH)<sub>2</sub>D<sub>3</sub> switches the response to the less inflammatory T helper 2 from the greater inflammatory T helper 1) (Guillot et al., 2010). The switching mechanism leads to increase of anti-inflammatory cytokine and reduction of pro-inflammatory markers (Aranow, 2011).

Interleukin-6, a cytokine not only involves in inflammation responses but also regulates metabolic, regenerative, and neural process (Scheller et al., 2011). In most inflammatory states, increases of cytokines such as IL-6, IL-1, and TNF $\alpha$  are

observed (Muylle et al., 1993). IL-6, commonly acts as a pro-inflammatory, however it also involves many regenerative and anti-inflammatory activities (Scheller et al., 2011; Tilg et al., 1994). During IL-6 mediated responses, the two key signalling pathways have been identified, the classic- and trans-signalling (Scheller et al., 2011). Increased levels of IL-6 following partial hepatectomy has indicated the important role of IL-6 in the regenerative activities (Trautwein et al., 1996). In animal model using mice, it has also been shown that IL-6 deficient animals were impaired in liver regeneration (Cressman et al., 1996). Liver damage induced by concanavalin A (a carbohydrate-binding protein) was reduced via blocking classic IL-6 signalling (Malchow et al., 2011). In addition, IL-6 deficient animals (mouse models) were protected in experimental chronic disease such as arthritis (Nowell et al., 2003; Nowell et al., 2009). IL-6 trans-signalling also demonstrated less ascites formation in mice (Lo et al., 2011). Higher inflammatory score was observed in IL-6 deficient mice treated with dextran sodium sulfate than wild type, suggesting the positive effects of IL-6 in anti-inflammation and regeneration of intestinal epithelial cells (Grivennikov et al., 2009). Tears of human following photorefractive keratectomy also contained higher IL-6 levels and it has been suggested IL-6 can reduce the production of metalloproteinase-2 (Malecaze et al., 1997). IL-6 was also found to be higher in people with dysfunctional tear syndrome and it was also suggested IL-6 is an important mediator in existing inflammation (Lam et al., 2009). The results of these studies might suggest IL-6 has potential of anti-inflammatory effects through trans-signalling, having potential to regenerate epithelium in ocular surface. The increased vitamin D levels in our study might increase the levels of IL-6, resulting in anti-inflammatory effects via trans-signalling pathway in the long term.

Another possible consequence of higher IL-6 has been suggested, the pro-inflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ) secreted by lacrimal glandular cells, can disrupt the release of acetylcholine from cholinergic nerves (Stern et al., 2004). If the turnover of dopamine can be manipulated by higher dose of vitamin D (Kesby et al., 2009), acetylcholine might be released in higher amount, normal response/reflex to stimulation on the lacrimal system should prevent the consequence of ageing. In our study, the dose used was only for maintaining optimal skeleton health and might not have reached a level to assist large release of acetylcholine.

However, our results showed that the increased level of IL-6 was not significant in the serum samples, this might be due to IL-6 has the highest expression at the site of inflammation (Gabay, 2006). Perhaps higher dose and longer treating period of vitamin D supplement may observe significance; further investigation concerning the role of IL-6 on ocular surface is warranted.

One of the limitations for this study was that participants did not take the vitamin D supplement during the same period, which the measured vitamin D levels might be affected by varied sunlight exposure during the treating period. Also, compliance could not be well controlled though a reminder text/email was given to participants each week, this might have influenced the results of vitamin D levels measured.

### **Conclusion**

A 60 day 1000 IU vitamin D supplement increased the vitamin D levels in human body and was able to improve dry eye symptoms assessed by OSDI. Also, the vitamin D supplement might have effects on improving the quality of tear on ocular surface but not tear quantity.

Subjective dry eye assessment as OSDI might be more sensitive than observing signs of dry eyes when there was only minor improvement on dry eye symptoms.



# Chapter 6: Experiment 3: The Association between Sunlight Exposure/Vitamin D and Myopia/Accommodation in Young Adults

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## Abstract

Myopia is a common refractive error that has a prevalence of approximately 20% in the world, and population suffering from this condition is still increasing. Recent studies have indicated that vitamin D and dopamine levels in myopes are lower than in non-myopes, and myopes tend to spend less time outdoors. Although the exact pathogenesis of myopia remains unclear, it has been suggested dopamine can affect the development and onset of myopia. Vitamin D, as a steroid like hormone in human body, has been shown to be able to alter the behaviour of dopamine in animals. Thus, we proposed the higher cutaneous vitamin D synthesis resulting from more sunlight exposure may be able to affect myopia development. We aimed to investigate whether there was an association between vitamin D levels, dopamine and myopia in young adults.

**Methods:** Fifty-five adults aged between 18 and 25 years were recruited from Queensland University of Technology and the University of Queensland. All participants completed a sunlight exposure questionnaire and had saliva samples taken for measurement of vitamin D and dopamine; the levels of vitamin D binding protein were measured in 40 participants. Ocular biometry including axial length, anterior chamber depth, lens thickness, aqueous depth, central corneal thickness, corneal refraction, pupil diameter was assessed by Lenstar. Choroidal thickness was measured by Optical Coherence Tomography. Binocular vision, accommodative functions including tonic accommodation, phoria, near induced temporary myopia, negative relative accommodation, positive relative accommodation, and accommodation accuracy were assessed.

**Results:** Choroidal thickness was correlated with spherical equivalent refraction ( $r = 0.62$ ,  $p < 0.0001$ ) and negatively correlated with axial length ( $r = -0.77$ ,  $p < 0.0001$ ). Dopamine levels were correlated with negative relative accommodation ( $r = 0.52$ ,  $p < 0.0001$ ). People with poor accommodation had lower dopamine levels ( $p < 0.05$ ). High myopes had higher pre-task TA than emmetropes ( $p = 0.03$ ), while no difference was found in post-task difference ( $p > 0.05$ ). Myopes had myopic TA shifts while emmetropes had hyperopic TA shifts ( $p < 0.05$ ). Emmetropes were more likely to have poor accommodation (of participants with poor accommodation 43.59% were emmetropes, while in participants with normal accommodation, 18.75% were emmetropes). Emmetropes had higher levels of vitamin D binding protein ( $p < 0.05$ ), with no significant difference comparing to low myopes ( $p > 0.05$ ) and higher than high myopes ( $p = 0.002$ ). Difference was also observed between emmetropes, progressing myopes, and non-progressing myopes ( $p = 0.01$ ). People with light colour iris also has higher vitamin D binding protein than those with dark iris ( $p = 0.02$ ), however no significant difference in the levels vitamin D binding protein when comparing people with similar refractive errors ( $p > 0.05$ ). People with poor accommodation had higher levels of vitamin D binding protein ( $p < 0.001$ ). Low myopes reported least time spent outdoors, difference was found between low myopes and high myopes ( $p = 0.0003$ ).

**Discussion:** In the study, the number of myopes was more than two fold of emmetropes. Although both the averages of self-reported time spent outdoors and the actiwatch measurements in the young emmetropes were higher than myopes, the difference might be more significant when the sample size was more even between the groups. As the finding of earlier studies, refraction was correlated with axial length and choroidal thickness, myopic eyes had longer axial length and thinner choroidal thickness (Chen et al., 2009; Li et al., 2011; Mutti et al., 2007b).

Study using human ocular tissue has shown that UV-B exposure to the eye could increase the level of vitamin D metabolites in corneal epithelial cells (Lin et al., 2012), implying the benefits of vitamin D<sub>3</sub> to the human eye could be acquired from the UV-B exposure of sunlight. The synthesis and transport of vitamin D, might be via routes other than blood circulation with UV-B exposure to the eye.

The results showed emmetropes were more likely to have difficulty accommodating. Dopamine levels were correlated with NRA but PRA; also, when categorising participants as normal and poor accommodation, people with normal accommodative function had higher dopamine levels. This may be suggesting at performing near task, the higher dopamine in the human body assists accommodation system more easily toward hyperopic shift via beta activity.

The results showed that pre-near task TA in high myope was higher than emmetropes. However, TA in post-near task did not show significant difference between emmetropes, low myopes, and high myopes. This might be due to over-accommodation of high myopes in the first TA measurement, and myopic eyes might need adaptive task such as near work to regain the equilibrium between sympathetic and parasympathetic inputs. There was a myopic shift at post-near task TA measurement in high myopes, though it was not significant, the shift was toward myopic in high myopes in our study, with a higher pre-task tonic accommodation as compared to both emmetropes and low myopes. The results showed that people with lighter iris colour had higher vitamin D binding protein, implying that the levels of vitamin D binding protein and vitamin D might be associated with genetic variation. Furthermore, lighter iris coloured people tend to have lighter skin pigmentation, and may therefore be more sensitive to UV exposures.

The higher concentrations of DPB in the emmetrope group might be indicating the circulating vitamin D metabolites - 25(OH)D could be better maintained and circulated in the human body Whether the large variation between serum and saliva was due to the lipophilic property of this plasma proteins and if vitamin D metabolite is well soluble in aqueous medium, requires further investigation. Also, whether the transport of vitamin D metabolites can cross the barrier between human blood and aqueous humour requires further investigation.

## **Introduction**

Myopia, is one of the leading causes of blindness in developing countries (Congdon et al., 2003), and the most common cause of visual impairment in global adult and children population (Foster & Jiang, 2014; Kleinstein et al., 2003; Lam et al., 2004). The current prevalence of myopia is estimated to be ~ 20% and the figure

has been projected to rise to 50% by the year 2050 (Foster & Jiang, 2014). Although myopia is correctable by optical appliances such as glasses and/or contact lens, long term implications including economic burden in health care systems and pathological changes in the eye cannot be neglected. To date, the pathogeny of myopia remains unclear. Two principal theories, genetic factors and environmental effects, have been believed to be associated extensively investigated (Feldkämper & Schaeffel, 2004; Hammond et al., 2001; Morgan & Rose, 2005).

Increasing incidence of school myopia has particularly caught the concerns of health/eye research as it is believed to be highly likely developing high myopia in the subsequent lifetime. During the last decades, researchers have been attempting to investigate the relationship between myopia onset/development and outdoor activity (French et al., 2013a; Li et al., 2015a; Sherwin et al., 2012c). While childhood onset myopia may be well explained by inheritance (Wojciechowski, 2011; Young et al., 2007), environmental effects being a major influencing factor in increasing myopia prevalence should not be excluded.

Increasing evidence indicates that myopia development can be inhibited by sunlight exposure. The major hypothesis is that higher intensity of light drives greater dopamine release, which inhibits myopia onset/development (Rose et al., 2008b). Animal models showed high intensity of light only inhibited FDM, while no effect in LIM was observed (Ashby et al., 2009b; Rose et al., 2008b; Siegart Jr et al., 2012; Smith et al., 2013; Smith et al., 2012b), the difference might be suggesting there are different pathogenesis behind these two types of myopia. The mechanism underlying LIM might be of higher relevance with human myopia while it is now known axial elongation compensates hyperopic defocus induced by negative lenses (Smith et al., 2009).

Although many studies have reported myopes spent less time outdoors (Dirani et al., 2009; Guggenheim et al., 2012c; Rose et al., 2008c), Mutti and Marks (2011) suggested serum vitamin D levels were independent factors in myopia development. If vitamin D levels are independent of other potential factors toward myopia development, the question remains would be - what the mechanism of higher vitamin D levels is to prevent myopia development. Mutti (2013) raised a hypothesis that lower crystalline lens power and thickness would help well match axial elongation in emmetropes. The underlying mechanism is that the stretching of ciliary muscle, lens,

and lens zonules makes lower dioptric power, maintaining optical system emmetropic. It has been suggested that vitamin D assisted bladder function via producing hypertrophy and impaired contraction in rats and humans (Dallosso et al., 2004; Schröder et al., 2006). Raised serum vitamin D levels might assist in stretching crystalline lens by a more flexible ciliary muscle, thereby postponing the onset of myopia. Under such mechanical stretch, vitamin D should not have effects on axial length elongation and this might explain why slower axial elongation was not observed in existing myopes with greater time spending outdoors while it was associated with slower growth rate of axial length in non-myopes (Li et al., 2015a).

To date, there have been different opinions concerning the protective effect of spending more time outdoors against myopia development. For example, physical activity was also found to be associated with myopia progression (Jacobsen et al., 2008). Also, the effect of greater time spending outdoors results from longer and stronger sunlight exposure is higher vitamin D synthesis from cutaneous production. Therefore, whether the protective effect is from physical activity, higher intensity of light, more vitamin D production, or all the factors can affect myopia development, requires more detailed consideration and investigation. However, there has been no literature to date discussing the correlation between retinal and salivary dopamine levels; measured salivary dopamine levels might not reflect local dopamine circulation in the retina.

We aimed to investigate whether there is association between vitamin D levels and accommodative function and refraction in young adults aged between 18 and 25 years.

## Methods

Fifty-five participants aged between 18 and 25 years were recruited from Queensland University of Technology and the University of Queensland. Inclusion criteria were young people without any eye conditions except for refractive errors and/or accommodative problems. The results of observing accommodation function in childhood (Anderson et al., 2008) have suggested that minus-lens-induced accommodative amplitude is relatively stable at a mean magnitude of approximately 7 D have no significant decline until the third decade of life. Fiftieth percentile for

accommodative amplitude also peaked between the age of 10 and 14 (8.50 D), and showed significant drop beyond the age of 25 (6.00 D) (León et al., 2016). The target age(s) was set between the maturity and notable decrease of accommodation function for more objective observation. All participants were asked to complete an online questionnaire that consists of sunlight exposure behaviour, ethnicity, and dietary vitamin D intake, as well as family history and progression of myopia. They also had saliva samples taken for measurement of vitamin D and dopamine.

A number of measurements were conducted in the subsequent sequence. Optical coherence tomography (RS-3000 Advance, NIDEK) was performed and the subfoveal choroidal thickness was measured using the digital ruler right under the fovea for each participant. Each had 120 images taken for accurate averaging of the thickness measurement. All the participants then underwent binocular auto-refraction measured by the Natural Vision Auto Refkeratometer (Nvision-K 5001 Shin-Nippon) to minimise instrument myopia, using NVRI 3 meter LogMAR chart (Bailey-Lovie) (equivalent to 6 m Snellen values) as the visual target. In the third procedure, intraocular pressures of each eye were measured by hand-held tonometer (Icare® TA01) without anaesthetic. Participants were asked to relax and look straight ahead on distant visual target. The tip of the probe from the cornea was approximately 4-8 mm. The tip then hit central cornea after pressing the measurement button lightly. The average intraocular pressure was shown on the screen after six consecutive readings in the tonometer. As the first optical biometer on the market that is capable of measuring the thickness of the crystalline lens, Lenstar (LS-900, HAGG-STREIT, USA) was utilised for all optic measurements, including the axial length, anterior chamber depth, aqueous depth, central corneal thickness, corneal astigmatism, and pupil diameter. Refractive errors were also corrected by contact lens (ACUVUE QASYS, Base curve 8.4, Diameter 14.0) in accordance with participants' spherical equivalence. Subsequently, a 6 prism diopter lens was placed in front of the participant's right eye and Howell Dywer phoria cards were placed at near of 33 cm and at 3 m for distance to measure the distant and near phoria of the participants. The relative accommodation (NRA/PRA) was measured through phoropter at 40 cm. The participants were asked if the letters on the near target are clear at the beginning of test. Negative relative accommodation (NRA) was performed first by adding lenses binocularly, +0.25 D for each step until the participant reported the first sustained

blur. The adding plus power was recorded as NRA. Following NRA, PRA was performed after returning the power of phoropter to zero. Minus lenses were added until the participant reported the first sustained blur. The total adding minus power was recorded as PRA. To assess the accommodation accuracy, a metered rod was fixed on the top of the Auto Refkeratometer to avoid varied size of retinal image, and the size of letters on the near targets was adjusted for three different distances of 25, 33, and 40 cm respectively. Near induced transient myopia (NITM) was immediately measured distance refractive state after the near task, which required the participants to stare at the computer screen with a 40 cm eye-screen distance for a constant five minutes. Lastly, tonic accommodation was measured in a completed dark room with a fixation of red light dot shined 6 meters away on the wall at the same level of the participants' eyes. Ten consecutive readings were recorded as soon as the room lighting was switched off and the average of the readings was used as the measurement.

**Actiwatch measurements:** ten randomly selected participants (five each from myope and emmetrope groups) were instructed to wear an Actiwatch on his/her non-dominant hand continuously for 7 days. The Actiwatch was portable, waterproof, and had an inbuilt light sensor that recorded the intensity and duration of light exposure to illuminance in lux every 30 seconds from 6am on the first day to 5:59am on the last day. The participants were told to wear the Actiwatch all the time with the light sensor facing upward and not to cover it. The measuring period was from 08<sup>th</sup>/Oct/2015 to 10<sup>th</sup>/Nov/2015, and the sunrise/sunset times of each day were 05:20am/5:51pm on 08<sup>th</sup>/Oct/2015 with a day length of 12:31 hours, and 04:51am/6:13pm on 10<sup>th</sup>/Nov/2015 with a day length of 13:21 hours (acquired from <http://www.timeanddate.com>). The average day length over the 34 days was 12.945 hours.

Statistical data analysis was conducted using one-way ANOVA and Spearman's rank correlation analyses by GraphPad Prism (GraphPad Software, Inc., CA, USA). A p-value < 0.05 is indicative of significance.

## Result

There were 55 participants (age  $22.2 \pm 2.2$  years) included in this study, female (65.45%) was more than male. Thirty-five participants were classified as myopes, whereas 20 were emmetropic. The measurements of ocular biometry, binocular vision and accommodative functions, as well as dopamine and vitamin D related levels are included in the following summary tables (**Table 6-1**, **Table 6-2**, and **Table 6-3**).

**Table 6-1 Summary of measurements of ocular biometry**

	Mean $\pm$ SD	Range (Min - Max)
Refraction (SE)	$-2.21 \pm 2.16$	-7 - 0.75
IOP (right eye)	$13.24 \pm 3.27$	6 - 21
Axial length (mm)	$24.64 \pm 1.22$	22.52 - 27.52
Anterior chamber depth (mm)	$3.67 \pm 0.30$	2.71 - 4.21
Lens thickness (mm)	$3.56 \pm 0.20$	3.2 - 4.17
Aqueous depth (mm)	$3.12 \pm 0.28$	2.59 - 3.69
CCT ( $\mu\text{m}$ )	$550.6 \pm 36.48$	471 - 264
Corneal astigmatism	$0.89 \pm 0.46$	0 -1.77
Pupil diameter (mm)	$6.02 \pm 0.93$	4.23 - 8.11
Choroidal thickness ( $\mu\text{m}$ )	$302.6 \pm 73.75$	171 - 468



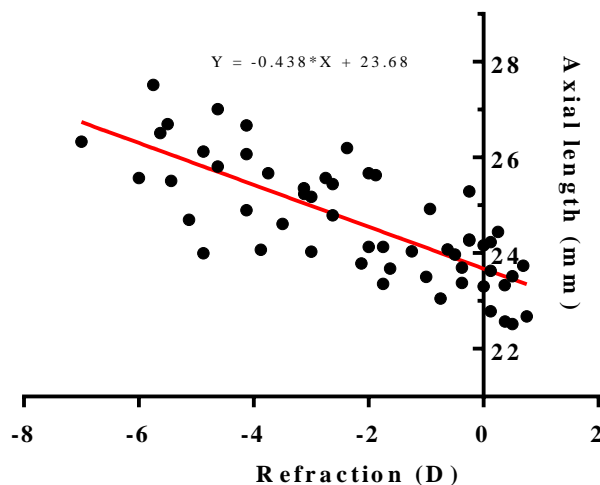
**Table 6-2 Summary of measurements in binocular vision and accommodative functions**

	Mean $\pm$ SD	Range (Min - Max)
Pre-near task TA	0.29 $\pm$ 0.60	-0.75 - 2.19
Post-near task TA	0.27 $\pm$ 0.59	-0.73 - 2.48
Distance phoria	-0.83 $\pm$ 2.11	-8 - 3
Near phoria	-2.94 $\pm$ 4.31	-16 - 5
NITM	-0.33 $\pm$ 0.90	-4.27 - 0.9
NRA	2.06 $\pm$ 0.61	1 - 4.5
PRA	-1.72 $\pm$ 0.89	-5 - 0.5
AE@40cm	0.97 $\pm$ 0.37	-0.06 - 1.94
AE@33cm	1.10 $\pm$ 0.40	0.14 - 2.12
AE@25cm	1.42 $\pm$ 0.54	0.46 - 3.55
Post-near task AE@25cm	1.21 $\pm$ 0.58	-0.29 $\pm$ 3.19

**Table 6-3 Measurements of salivary DBP, dopamine, and vitamin D levels**

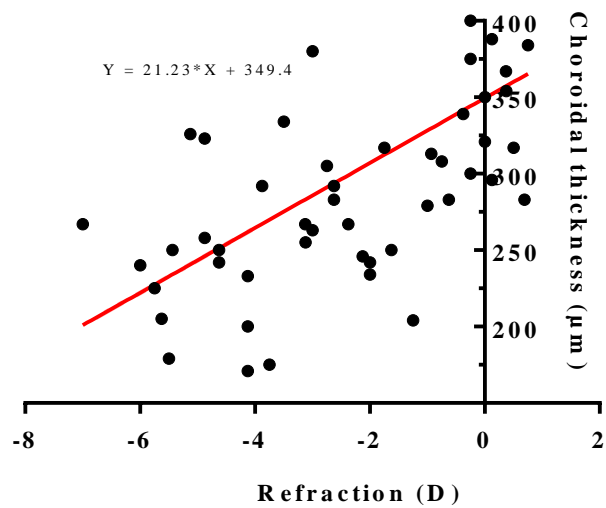
	Mean $\pm$ SD	Range (Min - Max)
Dopamine (nmol/l)	0.94 $\pm$ 1.67	0 - 850
Vitamin D binding protein (ng/ml)	87.99 $\pm$ 77.93	7.84 - 348.3
Vitamin D (nmol/l)	Not available	Not available

**The association between different accommodative functions in myopes and emmetropes.** Among the 10 participants involving in the Actiwatch measurements for the time spent outdoor (higher than 10,000 lux), there was no significant difference between two groups (myope  $51.0 \pm 42.48$  minutes vs emmetrope  $63.6 \pm 68.88$  minutes) ( $p = 0.7367$ ). Spherical equivalent refraction was correlated with axial length ( $r = -0.77$ ,  $p < 0.0001$ ) (see **Figure 6-1**) and choroidal thickness ( $r = 0.62$ ,  $p < 0.0001$ ) (see **Figure 6-2**). There was a correlation between axial length and choroidal thickness ( $r^2 = 0.33$ ,  $p < 0.0001$ ) (see **Figure 6-3**), and between axial length and anterior chamber depth ( $r = 0.48$ ,  $p = 0.0002$ ) (see **Figure 6-4**). Aqueous depth was correlated with lens thickness ( $r = -0.50$ ,  $p = 0.0001$ ) (see **Figure 6-5**). NRA was correlated with dopamine levels ( $r = 0.52$ ,  $p < 0.0001$ ) (see **Figure 6-6**). The dopamine levels was  $1.1 \pm 1.8$  nmol/l for the myope group, while in the emmetrope group it was  $0.57 \pm 0.95$  nmol/l ( $p = 0.33$ ), when comparing people with normal and poor accommodation, the former ones had higher dopamine levels  $1.81 \pm 2.45$  nmol/l vs  $0.52 \pm 0.88$  nmol/l ( $p < 0.05$ ) (see **Figure 6-7**).



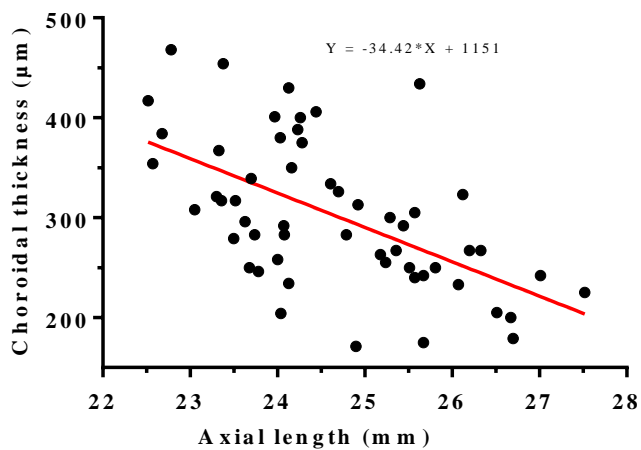
**Figure 6-1 The correlation between spherical equivalent refraction and axial length.**

Spherical equivalent refraction was correlated with axial length ( $r = -0.77$ ,  $p < 0.0001$ ).



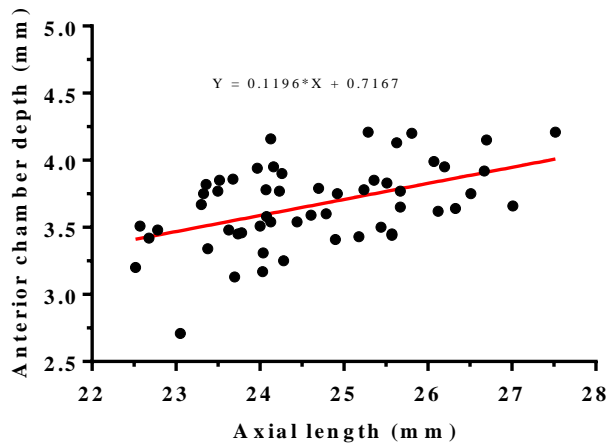
**Figure 6-2** The correlation between spherical equivalent refraction and choroidal thickness.

Spherical equivalent refraction was correlated and choroidal thickness ( $r = 0.62$ ,  $p < 0.0001$ ).

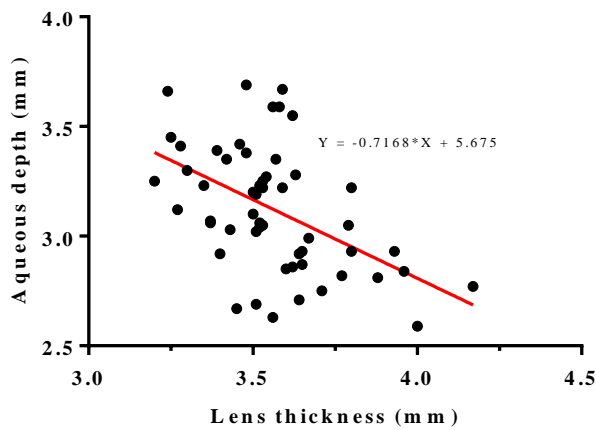


**Figure 6-3** The correlation between axial length and choroidal thickness.

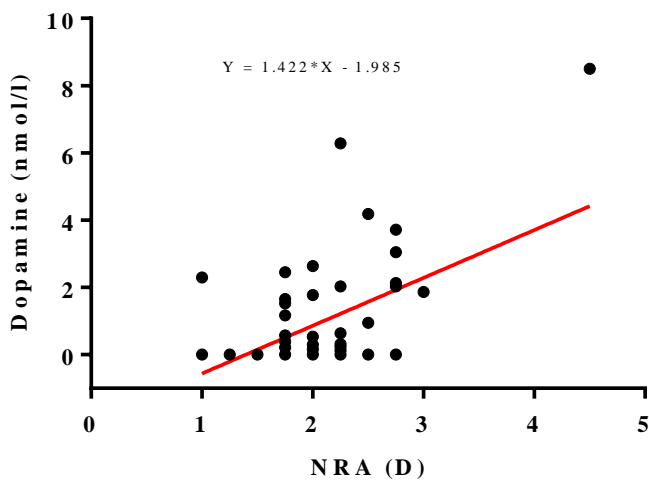
Axial length was correlated with choroidal thickness ( $r = -0.57$ ,  $p < 0.0001$ ).



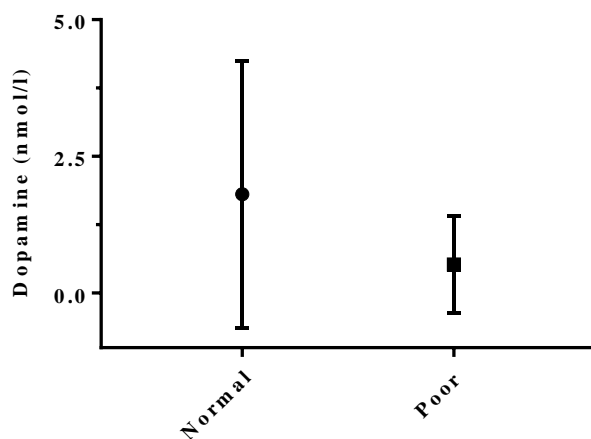
**Figure 6-4 The correlation between axial length and anterior chamber depth**  
Axial length and anterior chamber depth ( $r = 0.48$ ,  $p = 0.0002$ ).



**Figure 6-5 The correlation between aqueous depth and lens thickness**  
Aqueous depth was correlated with lens thickness ( $r = -0.50$ ,  $p = 0.0001$ ).



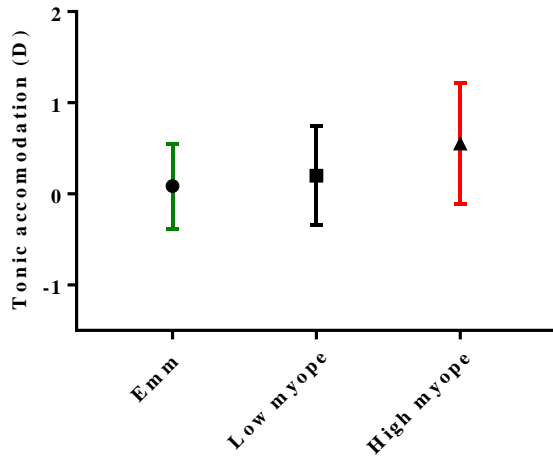
**Figure 6-6 The correlation between negative relative accommodation and dopamine.**  
NRA was correlated with dopamine levels ( $r = 0.52$ ,  $p < 0.0001$ ).



**Figure 6-7 Dopamine levels in young people with normal and poor accommodation**

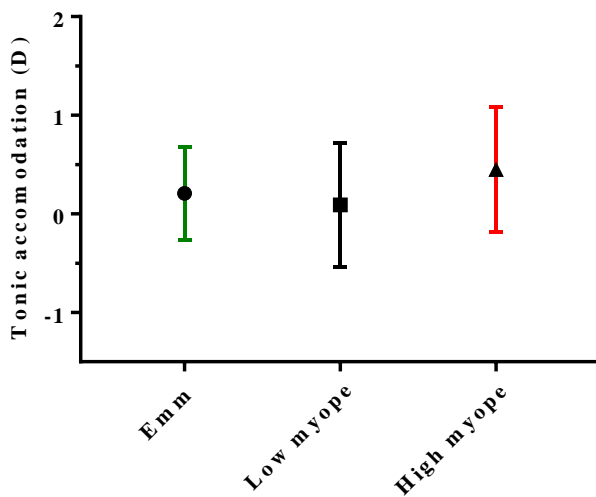
The group with poor accommodation had dopamine levels of  $0.52 \pm 0.88$  nmol/l, while it was  $1.81 \pm 2.45$  nmol/l for people with normal accommodation ( $p < 0.05$ ).

**The association between emmetropes, low myopes and high myopes in pre- and post-near task TA measurements.** When further categorised the participants as emmetropes (spherical equivalent refraction between + 0.50 and -0.749 D), low myopes (spherical equivalent refraction less than - 0.75 D and greater than -3.00 D), and high myopes (spherical equivalent refraction  $\leq -3.00$  D), there were 20, 14, and 21 in the three categorised groups, respectively. The first TA measurement (before all other measurements) showed that high myopes had higher values than emmetropes ( $p = 0.03$ ) (see **Figure 6-8**) while the second TA did not show significant difference between the groups ( $p > 0.05$ ) (see **Figure 6-9**). No significant difference was found when comparing the first and second TA measurements in any of the groups ( $p > 0.05$ ), with the emmetropes showing hyperopic shift, while TA in both low myopes and high myopes had myopic shift (see **Figure 6-10**). TA shift showed significant difference between emmetropes, low myopes, and high myopes ( $p < 0.05$ ) (see **Figure 6-11**).



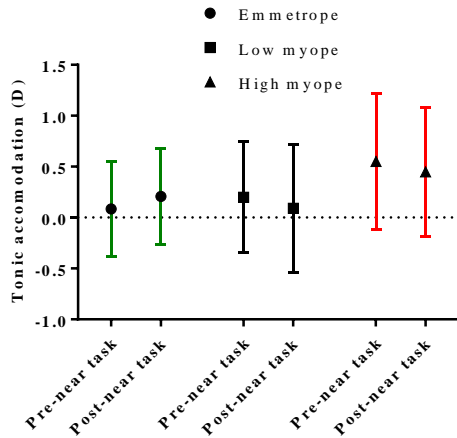
**Figure 6-8 First tonic accommodation (pre-near task) in emmetropes, low myopes, and high myopes.**

First TA for emmetropes, low myopes, and high myopes were  $0.08 \pm 0.47$  D,  $0.20 \pm 0.55$  D, and  $0.55 \pm 0.67$  D, respectively. First TA in high myopes was significantly higher than that in emmetropes ( $p = 0.03$ ).



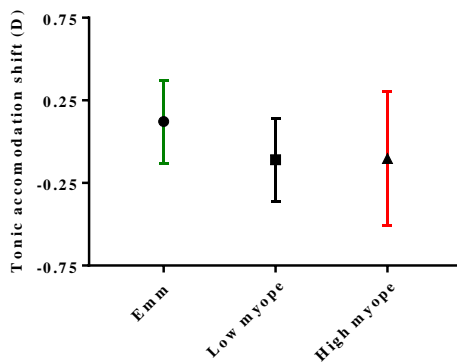
**Figure 6-9 Second tonic accommodation (post-near task) in emmetropes, low myopes, and high myopes**

Second TA in emmetropes, low myopes, and high myopes did not show difference ( $p > 0.05$ ).



**Figure 6-10 Pre- and post-near task TA measurements in emmetropes, low myope, and high myopes.**

No significant difference was observed between the first and second TA measurements in emmetropes, low myopes, and high myopes ( $p > 0.05$ ).

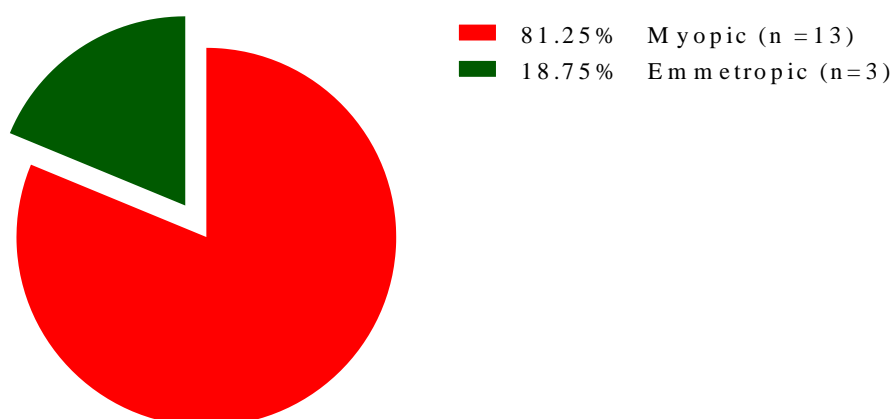


**Figure 6-11 Tonic accommodation shift in emmetrope, low myope, and high myope groups.**

There was significant difference in TA shift between the groups. For emmetrope it was  $0.12 \pm 0.25$  D, and  $-0.11 \pm 0.25$  D and  $-0.10 \pm 0.41$  D for low myope and high myope, respectively ( $p < 0.05$ ).

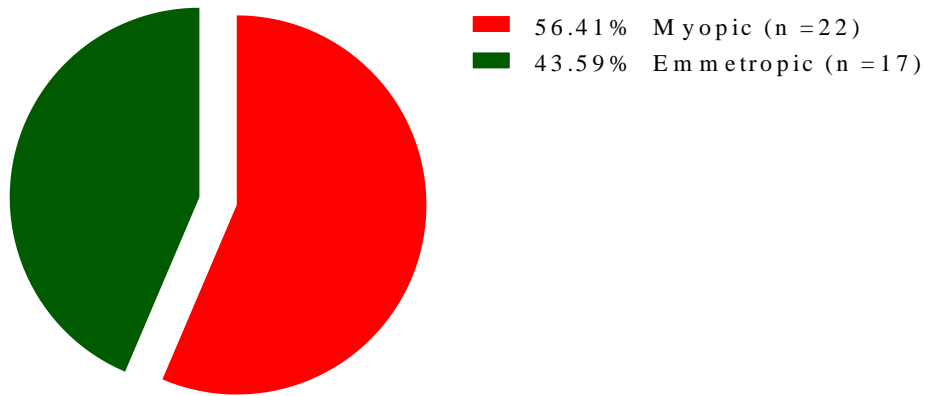
**The association between different accommodative functions and vitamin D binding protein in emmetropes, non-progressing myopes and progressing myopes.** Of the individuals with poor accommodative function, emmetropes comprised 43.59%, while there was only 18.75% in the group of normal accommodation (see **Figure 6-12** and **Figure 6-13**). The choroidal thickness in each group was significantly different to each other, the thickness for emmetropes, low myopes, and high myopes were  $360.6 \pm 54.76$   $\mu\text{m}$ ,  $292.6 \pm 67.25$   $\mu\text{m}$ , and  $254 \pm 54.69$   $\mu\text{m}$ , respectively ( $p < 0.001$ ) (see **Figure 6-14**). Choroidal thickness showed

significant difference between each group (emmetropes vs non-progressing myopes vs progressing myopes). Choroidal thickness in emmetropes, non-progressing myopes, and progressing myopes were  $360.6 \pm 76 \mu\text{m}$ ,  $258.8 \pm 53.5 \mu\text{m}$ , and  $260.5 \pm 43.39 \mu\text{m}$ , indicating a significant difference between emmetropes and progressing myopes ( $p < 0.05$ ), also between emmetropes and stable myopes ( $p < 0.05$ ), while no difference between progressing and non-progressing myopes was observed ( $p > 0.05$ ) (see **Figure 6-15**). The concentrations of vitamin D binding protein (DBP), were  $70.7 \pm 69.5 \text{ ng/ml}$  for the myope group and  $123.9 \pm 84.9 \text{ ng/ml}$  for the emmetrope group, respectively ( $p < 0.05$ ) (see **Figure 6-16**). No significant difference was found when comparing DBP between emmetropes and low myopes ( $p = 0.78$ ), while it was lower in high myopes by comparison to emmetropes ( $p = 0.0001$ ) and to low myopes ( $p = 0.0017$ ) (see **Figure 6-17**). DBP concentrations were  $98.05 \pm 58.85 \text{ ng/ml}$ ,  $35.68 \pm 15.43 \text{ ng/ml}$ , and  $75.11 \pm 48.37 \text{ ng/ml}$  for emmetropes, non-progressing myopes, and progressing myopes, respectively, while a significant difference was observed between emmetropes and both myope groups ( $p = 0.01$ ) (see **Figure 6-18**). In additional, the individuals with darker colour (brown) iris had lower DBP than those with lighter iris colour ( $75.93 \pm 64.88 \text{ ng/ml}$  vs  $156.4 \pm 114 \text{ ng/ml}$ ) ( $p = 0.0177$ ) (see **Figure 6-19**). However, it shows no significant finding when equal sample size was made by picking from those dark coloured iris participants who had similar refractive errors ( $71.48 \pm 31.65 \text{ ng/ml}$  vs  $156.4 \pm 114 \text{ ng/ml}$ ) ( $p > 0.05$ ) (see **Figure 6-20**).

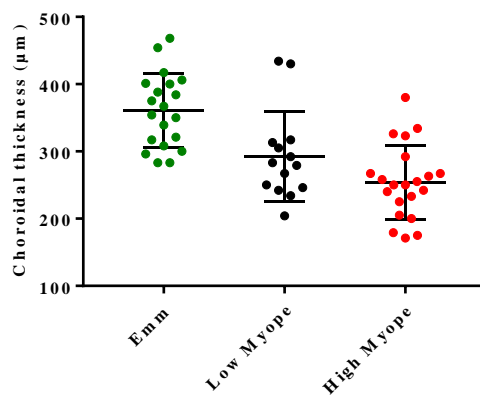


**Figure 6-12** The proportions of myopes and emmetropes in normal accommodation group



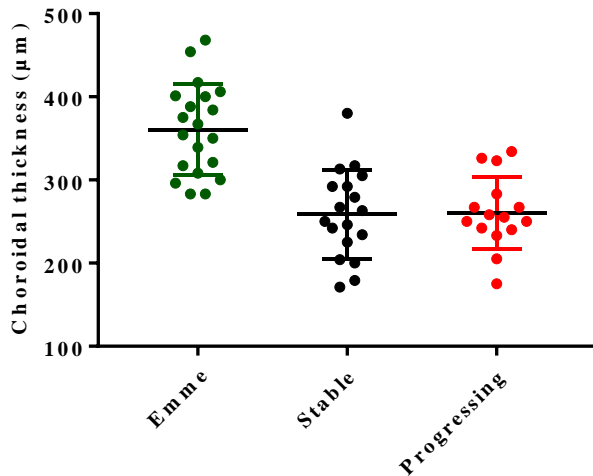


**Figure 6-13** The proportions of myopes and emmetropes in poor accommodation group



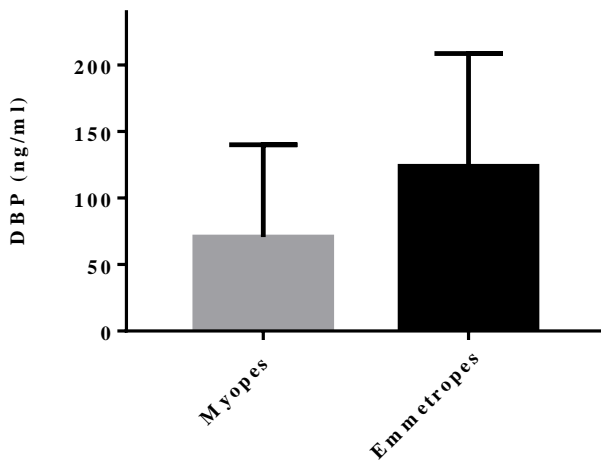
**Figure 6-14** Choroidal thickness in emmetrope, low myope, and high myope groups

The choroidal thickness in emmetropes was significantly higher than both low myopes ( $p < 0.0001$ ) and high myopes ( $p < 0.0001$ ), it was also thicker in low myopes as compared to high myopes ( $p = 0.032$ ). Choroidal thickness for emmetropes was  $360.6 \pm 54.76 \mu\text{m}$ , and  $292.6 \pm 67.25 \mu\text{m}$  in low myopes,  $254 \pm 54.69 \mu\text{m}$  in high myopes.



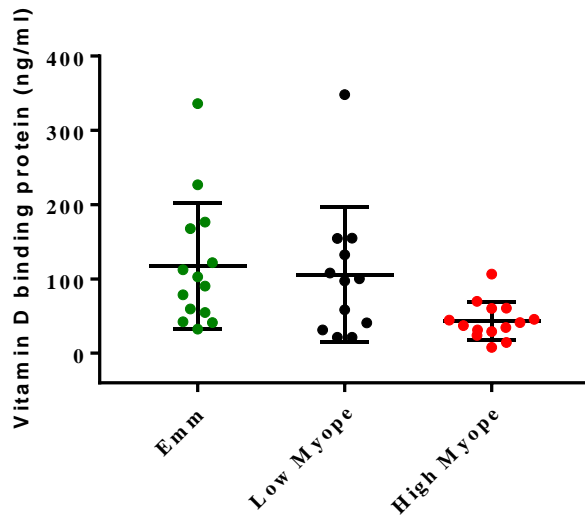
**Figure 6-15 Choroidal thickness in emmetropes, stable myopes, and progressing myopes.**

Choroidal thickness in emmetropes, stable myopes, and progressing myopes were  $360.6 \pm 76$   $\mu\text{m}$ ,  $258.8 \pm 53.5$   $\mu\text{m}$ , and  $260.5 \pm 43.39$   $\mu\text{m}$ , significant different difference was found between emmetropes and progressing myopes ( $p < 0.05$ ), also between emmetropes and non-progressing myopes ( $p < 0.05$ ), while no difference between progressing and non-progressing myopes was found ( $p > 0.05$ ).



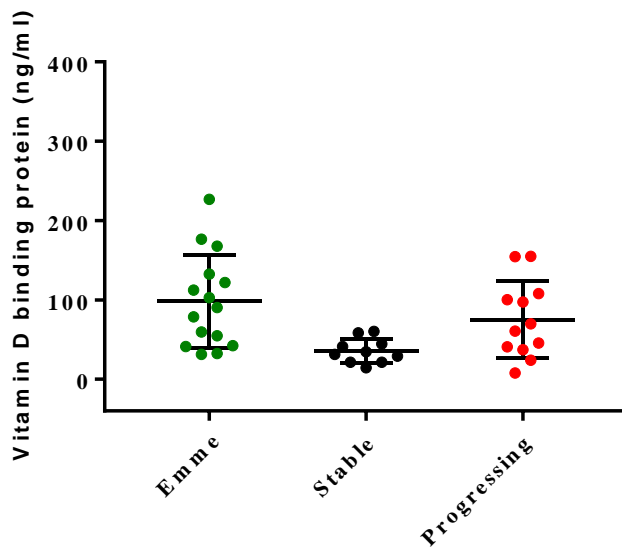
**Figure 6-16 Vitamin D binding protein in myopes and emmetropes**

Vitamin D binding protein was higher in emmetropes ( $p < 0.05$ )



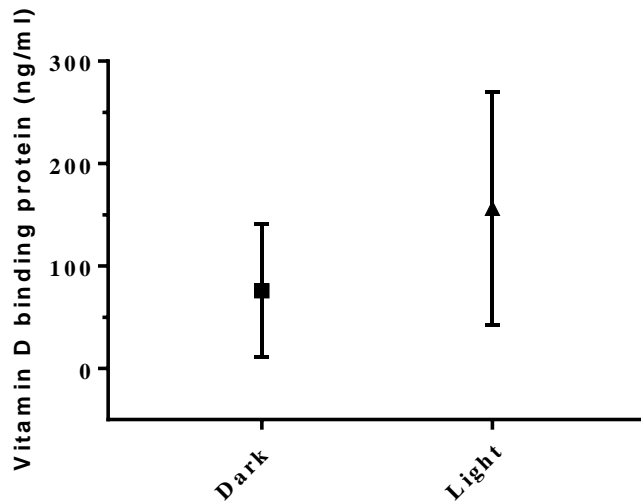
**Figure 6-17 The levels of vitamin D binding protein in emmetropes, low myopes, and high myopes.**

DBP levels were  $117.4 \pm 85.14$  ng/ml,  $105.8 \pm 90.8$  ng/ml, and  $43.39 \pm 25.13$  ng/ml for emmetropes, low myopes, and high myopes. No significant difference was found between emmetropes and low myopes ( $p > 0.05$ ), while the levels were both higher in emmetropes (mean difference 65.76,  $p = 0.0017$ ) and low myopes (mean difference 64.55,  $p = 0.0001$ ) as compared to high myopes.



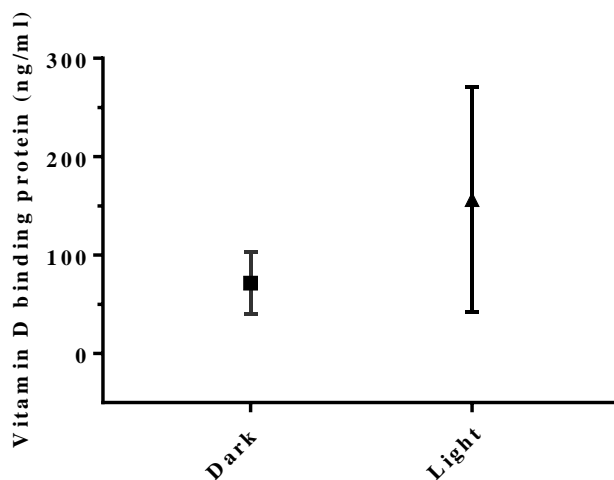
**Figure 6-18 The levels of vitamin D binding protein in emmetropes, stable myopes, and progressing myopes.**

Difference of the levels of vitamin D binding protein was observed between each group ( $p = 0.01$ ).



**Figure 6-19 The levels of vitamin D binding protein in individuals with dark and light coloured iris.**

Participants with light colour (hazel, green, blue) as compared to dark (brown) showed higher levels of DBP (n 34 vs 6) (dark 75.93 ± 64.88 ng/ml vs light 156.4 ± 114 ng/ml) ( $p = 0.0177$ ).

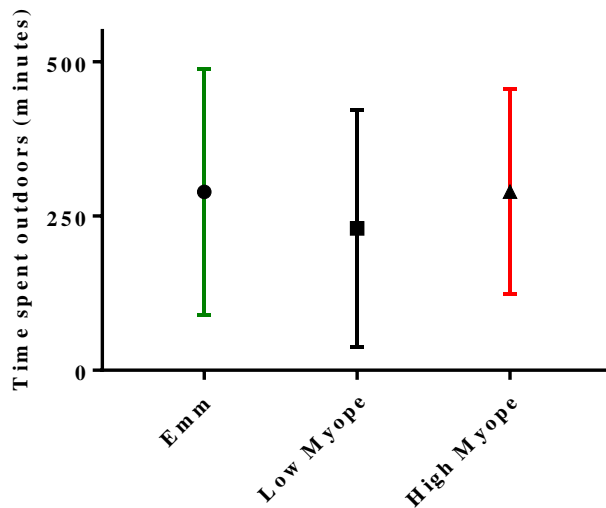


**Figure 6-20 The levels of vitamin D binding protein in dark and light coloured individuals with similar refractive error.**

When the refractive errors were similar between young people with dark and light coloured iris, the difference of DBP was insignificant (71.48 ± 31.65 ng/ml vs 156.4 ± 114 ng/ml) ( $p > 0.05$ ).

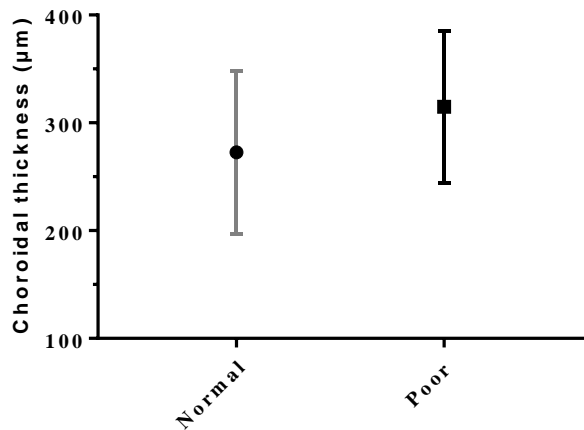
**The comparison in time spent outdoors between emmetropes and myopes.**

There was no significant difference between emmetrope (n = 17) group and myope group (n = 38) in the self-reported weekly time spent outdoors ( $257 \pm 173.9$  minutes vs  $311.9 \pm 205$  minutes) ( $p = 0.31$ ), while differences were found between emmetropes and low myopes ( $p = 0.0004$ ), and between low myopes and high myopes ( $p = 0.0003$ ) (see **Figure 6-21**). When further categorising participants with better accommodation and poor accommodation (defined as a lag at 25 cm after near task higher than 1.00 D), both choroidal thickness and the vitamin D binding protein levels in eyes showed difference between the two groups ( $p < 0.001$ ) (see **Figure 6-22** and **Figure 6-23**).



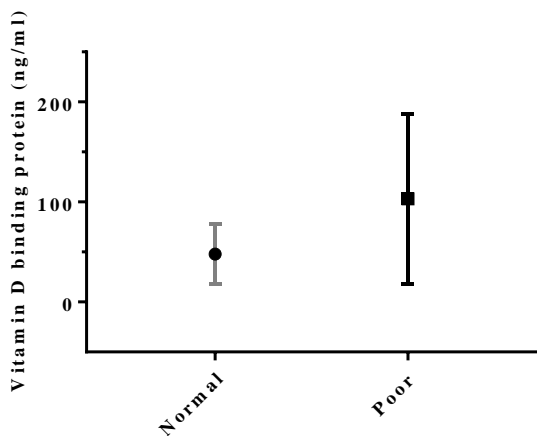
**Figure 6-21 Time spent outdoors during a typical week**

Emmetropes reported the highest amount of time spent outdoors ( $302.5 \pm 202.8$  minutes), whereas low myopes spent least time outdoor ( $229.9 \pm 181.9$  minutes), for high myopes an amount of  $285.8 \pm 169.7$  minutes was reported. Significant difference was found between low myopes and high myopes ( $p = 0.0003$ ).



**Figure 6-22 Choroidal thickness between eyes with better and poor accommodation accuracy at (25 cm) after near task.**

A lag higher than 1.00 D (poor) showed thicker choroidal thickness ( $314.9 \pm 70.35 \mu\text{m}$ ) as compared to that in eyes with normal accommodation accuracy ( $272.5 \pm 75.37 \mu\text{m}$ ) ( $p < 0.001$ ).



**Figure 6-23 The levels of vitamin D binding protein in eyes with normal and poor accommodation accuracy (at 25cm) after near task.**

The levels of DBP were higher in eyes with poor accommodation  $103.2 \pm 85.24 \text{ ng/ml}$ , while it was  $47.93 \pm 30.08 \text{ ng/ml}$  in the eyes with normal accommodation accuracy ( $p < 0.001$ ).

## Discussion

In the study, the number of myopes ( $n = 40$ ) was more than two fold of emmetropes ( $n = 15$ ).

Although the averages of self-reported time spent outdoor and the actiwatch measurement in the young emmetropes were higher than myopes by 55 minutes and 12.6 minutes respectively, the difference might be more significant when the sample size was more even between the groups. As the finding of earlier studies, refraction was correlated with axial length and choroidal thickness, myopic eyes had longer axial length and thinner choroidal thickness (Chen et al., 2009; Li et al., 2011; Mutti et al., 2007b).

The supply of aqueous humour is secreted from ciliary epithelium via circulating blood. One of the important functions of the aqueous humour circulation to the anterior section of the eye is that it supplies oxygen and nutrients to the intraocular tissues (Civan, 1997). The two body fluids - the blood and the aqueous humour have different chemical composition though the aqueous humour is derived from the blood. The most manifest difference between the blood plasma and aqueous humour is the concentration of the protein, which is vastly higher by 400 to 1400 fold in the plasma (Davson, 1990), meaning virtual absence of protein in aqueous humour. Given the functions of DPB are binding and transporting vitamin D metabolites, this might explain the reason an 8-week vitamin D<sub>3</sub> supplement in a previous study has shown non-detectable vitamin D<sub>3</sub> level in all the tissues of the rabbit eyes (Lin et al., 2012). In their study, they also found that both doses of 10 mJ/cm<sup>2</sup> and 20 mJ/cm<sup>2</sup> UV-B exposure to the increased the levels 25(OH)D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> in the human corneal epithelial cells, and there was a dose dependant relationship, the higher amount of UV-B exposure to the animals, the higher the vitamin D metabolites. To ocular tissues, the addition of being incapable to circulate the carrier - DPB to aqueous humour form the blood plasma, and of the ability to synthesise vitamin D<sub>3</sub>, might be implying the benefits of vitamin D<sub>3</sub> to the human eye could only be acquired from the UV-B exposure of sunlight. The synthesis and transport of vitamin D, might be via routes other than blood circulation with UV-B exposure to the eye.

Choroidal thickness, when looking the onset of myopia, was thinnest in early onset myopes, while the thickest was emmetropic eyes. No difference in choroidal

thickness was found between progressing and non-progressing myopes. Interestingly, choroidal thickness was higher in people with poorer accommodative function, while it is widely accepted that emmetropes have thicker choroidal thickness. This might be indicating emmetropes are more likely to have difficulty accommodating, and choroidal thickness is not associated with accommodation in young adults, who are relatively stable in refractive development.

Dopamine levels were correlated with NRA but PRA; also, when categorising participants as normal and poor accommodation, people with normal accommodative function had higher dopamine levels. This may be suggesting at performing near task, the higher dopamine in the human body assists accommodation system more easily toward hyperopic shift via beta activity. As it is now known that membranes of human iris-ciliary body are predominantly of the beta-2 adrenoceptors (Wax & Molinoff, 1987), the underlying mechanism resulting in higher NRA might be the relaxing status was better achieved by higher dopamine levels binding on beta-2 adrenoceptors. Previous study has shown that non-selective beta-agonists, such as isoprenaline, can induce hyperopic shifts in accommodation (Gilmartin, 1986).

Although it has been suggested that TA is stable due to its resting position in accommodative system (Johnson et al., 1984), it has also been demonstrated that visual stimulus prior to the measurement can affect TA (Tan & O'Leary, 1986). Loman and the colleagues (2002) suggested both longer hours of near work and darkness increased the incidence of myopia progression. The results showed that pre-near task TA in high myope was higher than emmetropes. However, TA in post-near task did not show significant difference between emmetropes, low myopes, and high myopes. This might be due to over-accommodation of high myopes in the first TA measurement, and myopic eyes might need adaptive task such as near work to regain the equilibrium between sympathetic and parasympathetic inputs. There was a myopic shift at second TA measurement in high myopes, though it was not significant. Ebenholtz and the colleagues (1985) suggested that the magnitude of the shifts in tonic accommodation in emmetropes had a negative correlation with the pre-task tonic accommodation; Thus, a relatively higher pre-task tonic accommodation should lead to a smaller myopic shift of the tonic position. However, the shift was toward myopic in high myopes in our study, with a higher pre-task tonic accommodation as compared to both emmetropes and low myopes.



The results showed that people with lighter iris colour had higher vitamin D binding protein. Implying that the levels of vitamin D binding protein and vitamin D is are associated with genetic variation.

The higher concentrations of DPB in the emmetrope group might be indicating the circulating vitamin D metabolites - 25(OH)D could be better maintained and circulated in the human body. The term, vitamin D binding protein, was originally given because of its property - only specifically binds to vitamin D and its metabolite. It has been suggested that the concentration of vitamin D binding protein in human serum is 2403.85 ng/ml (Bouillon et al., 1977), our findings suggested salivary concentration of vitamin D binding protein was only between 1/7 and 1/307 of that in serum. Whether the large variation between serum and saliva was due to the lipophilic property of this plasma proteins and if vitamin D metabolite is well soluble in aqueous medium, requires further investigation. With the higher DPB levels in the saliva of emmetropes, whether the transport of vitamin D metabolites can cross the barrier between human blood and aqueous humour requires further investigation. Future studies might consider conducting a visual based test that estimates dopamine levels in the retina, as there is no current way of getting a retinal sample for dopamine analysis in normal young adult human eyes.

### **Conclusion**

The amount of sunlight exposure time was not different between emmetropes and myopes. Dopamine levels were not different between emmetropes and myopes. People with poor accommodation had higher dopamine levels, suggesting dopamine can affect accommodative function. High myopes had higher tonic accommodation and greater TA shifts. The levels of vitamin D binding protein may affect myopia development via accommodative function.

# Chapter 7: Experiment 4: Vitamin D Supplement and Refraction/Accommodation in Young Adults

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## Abstract

Although myopia is an optically correctable refractive error, the consequences of myopia in the long term cannot be neglected. Previous studies have demonstrated use of dopamine agonists can inhibit myopia development, while dopamine antagonists have contrary effects. Study using animals also showed that vitamin D deficiency during developing period can alter dopamine levels. In addition, exposure to high light levels has been demonstrated to have inhibitory effects on myopia development. Although it has been found in past studies that myopes have lower vitamin D levels in the body, to date it remains unclear whether the protective effects are from the bright light or the cutaneous synthesised vitamin D from exposure to sunlight. Poor accommodative function is believed to be associated with myopia progression/development. In this study, we aimed to investigate whether a period of vitamin D supplement could improve the function of accommodation/refraction in young adults.

**Methods:** Thirty-three adults aged between 18 and 25 years were invited from a previous study. All participants were given vitamin D supplement for 60 days and asked to return for the identical accommodative function assessments. Saliva samples were taken from each participant for measuring the levels of vitamin D and dopamine.

**Result:** Of the 33 returned for this study, tonic accommodation shift was significantly lower and accommodation at 25 cm was significantly more accurate. A 3.8 % (10.27 20.4  $\mu\text{m}$ ) increase in choroidal thickness was observed. The level of

salivary dopamine showed no significant difference after the vitamin D treatment. In further observing myopes and emmetropes.

**Discussion:** Varied degree of accommodative hysteresis has been suggested (Ebenholtz, 1985). Some studies have reported that the onset of myopia is one of the influencing factors for the degree of accommodative hysteresis, however a consistent and definite conclusion to date has not yet been confirmed. Although pre-and post-task TA measurements at both visits did not show significant difference, less TA shifts were observed after the vitamin D. In addition, the accommodation shifts at 25 cm remained relatively stable after the vitamin D treatment. An 8.6 week 1000 IU vitamin D treatment might have an assisting effect in maintaining accommodation function such as TA and accommodation accuracy.

The results of the present study showed that choroidal thickness increased by 3.8% after the vitamin D treatment while the refractive errors remained the same as baseline measurement. Furthermore, our results showed that early onset myopes had thicker choroidal thickness than late onset myopes. Further investigation would be required to investigate whether the thicker choroidal thickness in human was the result of developed myopia or the cause toward myopia.

Central corneal thickness (CCT) showed no difference between each group and the two visits. A negligible increase in dopamine levels was observed after the vitamin D treatment.

## **Introduction**

Myopia is a type of the refractive errors when light focuses in front of the retina, leading to blurred distant vision. From a long term perspective, myopia can result in vision threatening diseases such as glaucoma (Shim et al., 2016), retinal degeneration (Shim et al., 2016), and cataract (McCarty et al., 1999). It has been recently estimated that the current global prevalence of myopia is 22.9% and it will rise to 49.8% by the year 2050 (Holden et al., 2016).

Although it is believed that myopia is a complicated and multifactorial ocular condition, the required effort for near work is believed to be associated with myopia

development, as the association has been suggested in a number of studies (Hepsen et al., 2001; Ip et al., 2008b; Saw et al., 2002; Tan et al., 2000). Difference in accommodative functions/response would be expected if prolonged near work leading to spasm of accommodation is the trigger toward myopia development/progression.

Studies investigating pharmacological and anatomical aspects have demonstrated that sympathetic and parasympathetic receptors are present in the ciliary muscle of both monkey and human (Hurwitz et al., 1972; Törnqvist, 1966), and beta receptors in sympathetic innervation are dominant in mediating and inhibiting the action of ciliary muscles (Hurwitz et al., 1972; Törnqvist, 1966; Wax & Molinoff, 1987; Yörnqvist, 1967). Pharmacological investigation has discovered that the majority (90%) of beta adrenoceptors in ciliary body and iris are beta-2 subtype, while beta-1 receptors account for 10% of total beta-adrenoceptors (Zetterström & Hahnenberger, 1988). When beta-adrenoceptors in ciliary body are activated, relaxation of ciliary muscle occurs, resulting in decrease in eye's accommodation (Tachado et al., 1989)

The role of dopamine as an adrenergic neurotransmitter in mammal has been described (Hornykiewicz, 1966; Miller et al., 1974). Early work showed that lower levels of retinal dopamine concentration were found in vision deprived chicks (Stone et al., 1989a). Also, Iuvone and the colleagues (1989) reported accompanying tyrosine hydroxylase (rate limiting enzyme in synthesising dopamine) reduction in vision deprived monkey. Investigations in other species such as tree shrew and guinea also demonstrated reduced dopamine levels in form deprived animals' retina (Dong et al., 2011a; McBrien et al., 2001). Because of large variability of dopamine levels between individuals, one could not firmly conclude there was a correlation between dopamine levels and myopia development until an inverted correlation between initial dopamine levels and the degree of deprivation myopia was shown (Ohngemach et al., 1997; Schaeffel et al., 1995). Recent studies have found that there is association between myopia development and time spending outdoors, for example, Jin and the colleague (2015) applied two 20-minute recess programs outside classroom during the day on school children between the age of 6 and 14 found that increased outdoor time helped prevent the onset and development of myopia. Similar intervention of additional time spending outdoors in young children

also showed there is reduced incidence rate of myopia in the following 3 years (He et al., 2015b). Quantifying objective biomarkers of sunlight exposure in the eye was also found to be negatively correlated with the degree of myopia in young adults (McKnight et al., 2012; Sherwin et al., 2012b). Intensive near work did not seem to compromise the protective effect of sunlight in young children (Dirani et al., 2009; Wu et al., 2010). Both the intensity and duration of sunlight are different between summer and winter; however, it is difficult to distinguish whether the protective effect of outdoor activity is due to higher sunlight exposure or less near work committed. In recent studies, myopes have been reported to have lower vitamin D levels (Kwon et al., 2016), and the effect of vitamin D was believed to be independent of outdoor exposure (Tideman et al., 2016a). The results were in line with the hypothesis that different duration of time outdoors might result in different circulating vitamin D levels between non-myopes and myopes (Mutti & Marks, 2011). High intensity of ambient light, including sunlight and artificial light, reduced FDM and LIM in chicks and tree shrews (Ashby et al., 2009b; Siegwart Jr et al., 2012), while the protective effect of high illuminance light was only demonstrated in FDM of monkeys (Smith et al., 2012b). In addition, circadian disruptions using indoor light intensity (700 lux) at nights has been shown to increase both axial length and choroidal thickness in chicks (Nickla & Totonelly, 2016). Thus, the results of this studies are consistent with the hypothesis that the inhibitory myopia effects of outdoor activity are due to eye exposing to high intensity of light. Also, it might be implying that light exposure has to correspond with ocular growth rhythms. While some may argue the protective effects were due to UVA/UVB (the portions of sunlight that contain shorter wavelength), however high intensity laboratory light without UV wavelength below 400 nm has also demonstrated an inhibitory effects toward myopia development (Ashby et al., 2009a). Moreover, spiperone (a dopamine D2 antagonist) vitreous injection was found to nullify the inhibitory effects of myopia development resulted from strong light exposure (Ashby & Schaeffel, 2010b), enhancing the hypothesis that dopamine release plays a role in myopia development/inhibition. Therefore, the inhibitory effects toward myopia development might be associated with the increased dopamine levels following strong light exposure (Rose et al., 2008c).

The importance of the rate limiting enzyme - tyrosine hydroxylase to dopamine synthesis is well recognised, which Puchacz et al. (1996) demonstrated vitamin D metabolites can increase the expressions of tyrosine hydroxylase gene in mice. In addition, dopamine turnover in rat can be altered when there is presence of vitamin D deficiency during development (Kesby et al., 2009). If dopamine behaviour (i.e. increased dopamine release) could be partially altered by vitamin D supplement, the effects of myopia inhibition would be observed.

We aimed to investigate whether an 8.6 week 1000 IU vitamin D supplement could affect accommodation/ refraction in young adults aged between 18 and 25.

### **Methods**

Participants were invited for follow up refraction/accommodation assessments from the stage 1. Participants (n=33, aged between 18 and 25 years ( $22.79.1 \pm 2.27$  years) with myopia/poor accommodation (spherical equivalent refraction  $\leq -0.75$  D, NRA  $\leq +1.50$  D or PRA  $\geq -1.50$  D, or a lag of accommodation  $\geq 1.00$ D at near of 25, 33, or 40 cm) were identified and given 1000 IU/daily vitamin D supplement, the treatment period lasted for 60 days, during the treatment the participants were asked to take one 1000 IU of vitamin D<sub>3</sub> (Swisse, 60 capsules in total) orally each day; following that, the participants returned for the same assessment as stage 1 (details see Chapter 3). The recruitment and data collecting period was from October 2015 to November 2016, all the participants had their saliva samples collected for vitamin D and dopamine levels measurements for both visits between October 2015 and November 2016.

Two-way ANOVA and paired t test were used to analyse the data collected from the two visits.

All experiments were conducted with ethics approval (approval number 1500000591) in accordance with the Declaration of Helsinki and the requirement of the Queensland University of Technology Human Research Ethics Committee. Written informed consent was obtained from all the participants.

## Results

There were 33 participants (male 30.3%, female 69.7%) returned and completed the assessments. Summaries of measurements for the two visits, comparing the effects of vitamin D treatment in ocular biometry, as well as binocular vision and accommodative functions are shown in **Table 7-1** and **Table 7-2**.

**Table 7-1 Summary of measurements of ocular biometry before and after vitamin D treatment**

	Before (Mean ± SD)	Range (Min - Max)	After (Mean ± SD)	Range (Min - Max)	Mean difference (After - Before)	Adjusted P
SE refraction	-3.07± 1.8	0.75 - (-6)	-3.16 ± 1.84	0.88 - (-6)	-0.09	> 0.99
IOP (RE)	13.42 ± 3.23	6 - 19	13.15 ± 3.54	7 - 19	-0.27	> 0.99
Axial length (µm)	25.08 ± 1.18	22.68 - 27.52	25.02 ± 1.10	22.68 - 26.99	-0.06	> 0.99
ACD	3.65 ± 0.31	2.71 - 4.21	3.65 ± 0.26	3.17 - 4.25	-0.0015	> 0.99
LT	3.59 ± 0.22	3.2 - 4.2	3.58 - 0.24	3.19 - 4.24	-0.0039	> 0.99
AD	3.10 ± 0.27	2.59 - 3.66	3.11 ± 0.27	2.58 - 3.72	0.0064	> 0.99
CCT (µm)	561.3 ± 32.84	482 - 624	561 ± 32.09	482 - 617	-0.33	> 0.99
Corneal refraction	0.94 ± 0.47	0.19 - 1.77	0.95 ± 0.39	0.38 - 1.76	-0.0012	> 0.99
Pupil diameter (mm)	5.99 ± 1.01	4.23 - 7.89	5.98 ± 1.05	4.22 - 8.52	-0.0061	> 0.99
CT (µm)	269.8 ±	171 - 434	280.1 ± 56.85	182 - 429	10.27	= 0.049

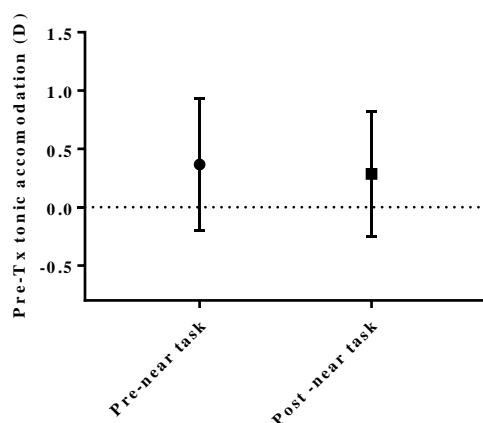
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**Table 7-2 Summary of measurements of binocular vision and accommodative functions before and after vitamin D treatment**

	<b>Before (Mean ± SD)</b>	<b>Range (Min - Max)</b>	<b>After (Mean ± SD)</b>	<b>Range (Min - Max)</b>	<b>Mean difference (After - Before)</b>	<b>Adjusted P</b>
Pre-near task TA	0.37 ± 0.57	-0.43 - 1.73	0.54 ± 0.52	-0.33 - 2.19	0.17	> 0.99
Post-near task TA	0.29 ± 0.53	-0.4 - 1.67	0.53 ± 0.44	-0.26 - 1.63	0.25	> 0.99
D phoria	-1.22 ± 2.46	-8 - 3	-1.14 ± 2.77	-8 - 6	0.0781	> 0.99
N phoria	-3.15 ± 4.83	-16 - 5	-3.36 ± 4.94	-14 - 7	-0.2109	> 0.99
AE@40cm	0.96 ± 0.41	-0.06 - 1.94	1.05 ± 0.52	0.18 - 2.91	0.0912	> 0.99
AE@33cm	1.09 ± 0.46	0.14 - 2.12	1.11 ± 0.41	0.25 - 1.92	0.0165	> 0.99
Pre-near task AE@25cm	1.42 ± 0.56	0.46 - 3.55	1.49 ± 0.52	0.42 - 2.84	0.0630	> 0.99
Post-near task@ 25cm	1.10 ± 0.56	-0.29 - 2.03	1.47 ± 0.68	0.21 - 3.96	0.3632	> 0.99
NITM	-0.39 ± 0.72	-2.68 - 0.63	-0.34 ± 0.85	-3.84 - 0.7	0.05	> 0.99
NRA	2.09 ± 0.43	1.25 - 2.75	2.11 ± 0.61	0.75 - 3	0.0152	> 0.99
PRA	-1.63 ± 0.68	-3.5 - (-0.5)	-1.76 ± 0.75	-3.25 - (-0.5)	-0.1288	> 0.99

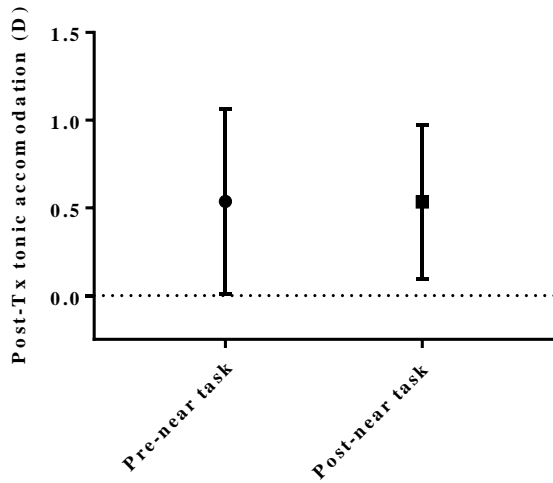


**Effect of vitamin D supplementation on different accommodative functions assessed by tonic accommodation.** The measurements of TA at pre-treatment visit was  $0.37 \pm 0.57$  D for the pre-near task measurement, while for the post-treatment it was  $0.29 \pm 0.53$  D ( $p > 0.05$ ) (see **Figure 7-1**). After the vitamin D treatment, the pre-near task measurement was  $0.54 \pm 0.52$  D, and the post-near task was  $0.53 \pm 0.44$  D ( $p > 0.05$ ) (see **Figure 7-2**). The post-near task TA measurements before and after the vitamin D treatment were  $0.29 \pm 0.53$  D and  $0.53 \pm 0.44$  D, respectively ( $p = 0.0063$ ) (see **Figure 7-3**). Tonic accommodation shift before the vitamin D treatment was  $-0.5373 \pm 0.5248$  D, and after the vitamin D treatment it became  $-0.0036 \pm 0.4113$  D (mean difference  $0.53 \pm 0.44$  D) ( $p < 0.05$ ) (see **Figure 7-4**). Accommodation accuracy at 25 cm at the first visit showed a significant difference, with  $1.42 \pm 0.56$  D before the near task and decreased to  $1.10 \pm 0.56$  ( $p = 0.0054$ ) after the task (see **Figure 7-5**). While a significant difference was not observed between the pre-near task ( $1.49 \pm 0.52$  D) and post-near task measurement at the second visit ( $1.47 \pm 0.68$  D) ( $p > 0.05$ ) (see **Figure 7-6**). Choroidal thickness increased after the vitamin D treatment by  $10.27 \pm 20.4$   $\mu\text{m}$  ( $269.8 \pm 60.2$   $\mu\text{m}$  vs  $280.1 \pm 56.85$   $\mu\text{m}$ ) ( $p < 0.05$ ) (see **Figure 7-7**). In both visits, CCT was thicker in EOM than LOM ( $p = 0.01$  for the first visit,  $p = 0.0064$  for the second visit) (see **Figure 7-8**).



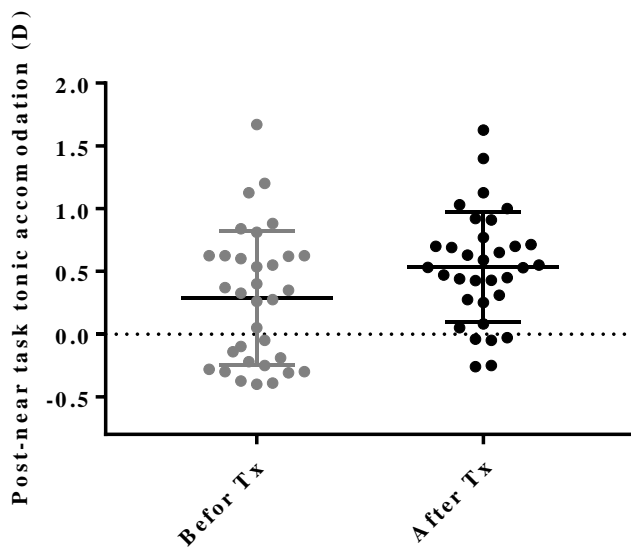
**Figure 7-1 Tonic accommodation before and after near task at the first visit.**

The first measurement was  $0.37 \pm 0.57$  D, while the second was  $0.29 \pm 0.53$  D ( $p > 0.05$ ).



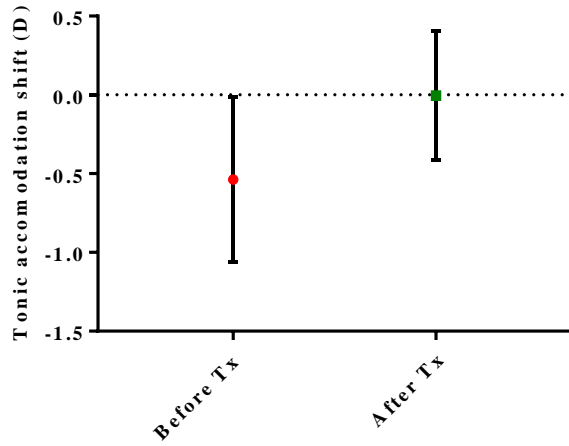
**Figure 7-2 Tonic accommodation before and after near task at the second visit.**

After the vitamin D treatment, the first TA was  $0.54 \pm 0.52$  D, and the second was  $0.53 \pm 0.44$  D.



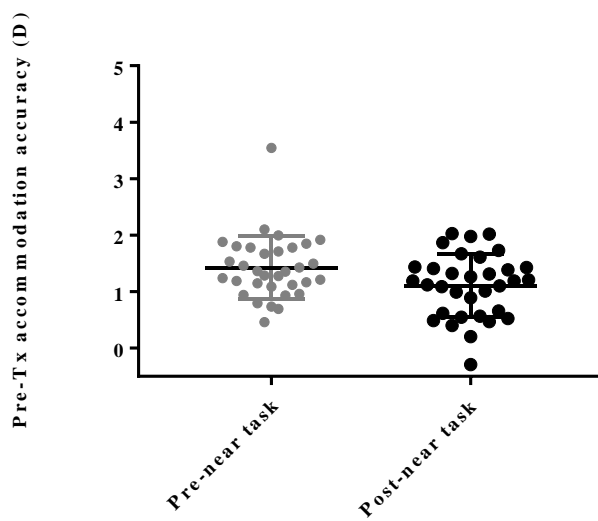
**Figure 7-3 The measurements for the second tonic accommodation in the two visits**

The measurement of the second tonic accommodation in the follow up visit was higher (mean difference  $0.25 \pm 0.49$ ), though it was statistically insignificant ( $p > 0.05$ ).



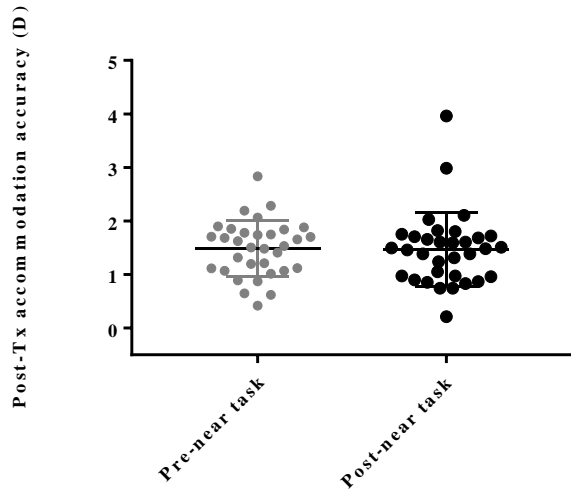
**Figure 7-4 Tonic accommodation shift before and after the vitamin D treatment**

For the pre-treatment, the TA shift was  $-0.5373 \pm 0.5248$  D, and after the vitamin D treatment it was  $-0.0036 \pm 0.4113$  D (mean difference  $0.53 \pm 0.44$  D) ( $p < 0.05$ )



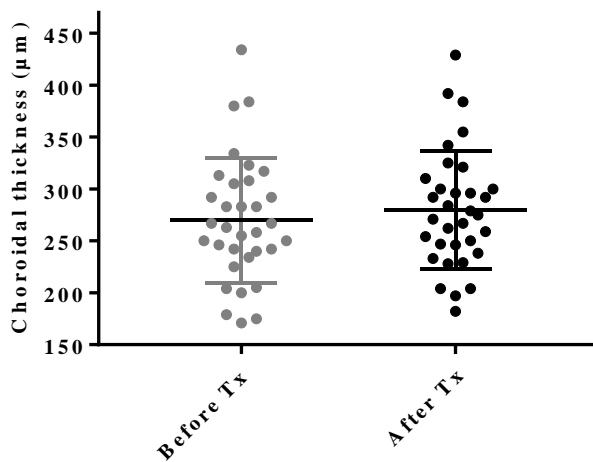
**Figure 7-5 Accommodation accuracy at 25cm before and after near task at the first visit**

After the near task, accommodation accuracy showed a myopic shift by  $-0.32 \pm 0.46$  D ( $p = 0.0054$ ) as compared to the first measurement.



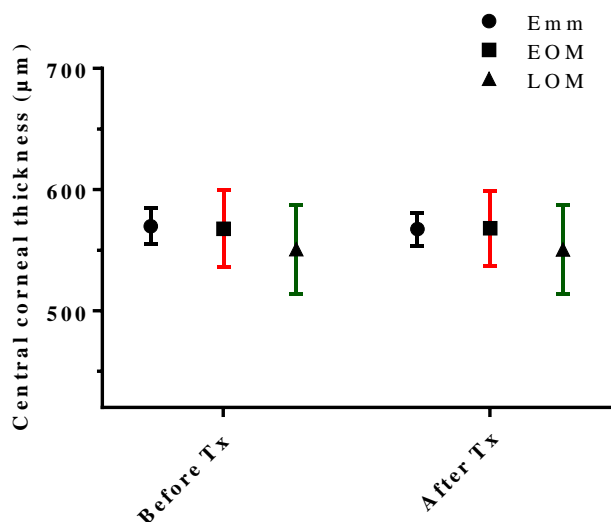
**Figure 7-6 Accommodation accuracy of the 1<sup>st</sup> and 2<sup>nd</sup> measurements at the visit after the vitamin D treatment.**

Before the near task, accommodation accuracy (1<sup>st</sup>) was  $1.49 \pm 0.52$  D, while it was  $1.47 \pm 0.68$  D after the near task (2<sup>nd</sup>) ( $p = 0.80$ ).



**Figure 7-7 Choroidal thickness before and after vitamin D treatment**

After the vitamin D treatment, choroidal thickness increased by  $10.27 \pm 20.4$   $\mu\text{m}$  ( $p = 0.0491$ ).



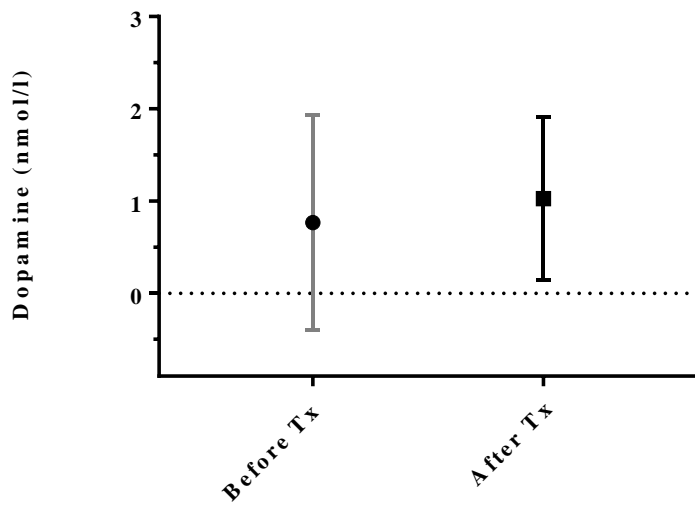
**Figure 7-8 Central corneal thickness in emmetropes, early onset myopes, and late onset myopes.**

For each group the measurements (before vs after) were  $569.8 \pm 15.11 \mu\text{m}$  vs  $567.3 \pm 13.6 \mu\text{m}$ ,  $567.7 \pm 31.94$  vs  $567.9 \pm 30.65 \mu\text{m}$ ,  $550.8 \pm 36.71$  vs  $550.5 \pm 36.44$  for emmetropes, early onset myopes, and late onset myopes, respectively. EOM had higher CCT than LOM at both the first visit ( $p = 0.01$ ) and the second visit ( $p = 0.0064$ ).

**Effect of vitamin D supplementation on dopamine levels.** The dopamine levels were  $0.76 \pm 1.2 \text{ nmol/l}$  at baseline and  $1.03 \pm 0.84 \text{ nmol/l}$  after the vitamin D treatment ( $p = 0.30$ ) (see **Table 7-3** and **Figure 7-9**). There was no significant difference in dopamine levels between before and after the vitamin D supplement treatment in the myope group (mean difference  $0.13 \pm 1.45 \text{ nmol/l}$ ,  $p = 0.62$ ) (see **Figure 7-10**), neither in the emmetrope group (mean difference  $1.17 \pm 0.81 \text{ nmol/l}$ ,  $p = 0.063$ ) (see **Figure 7-11**).

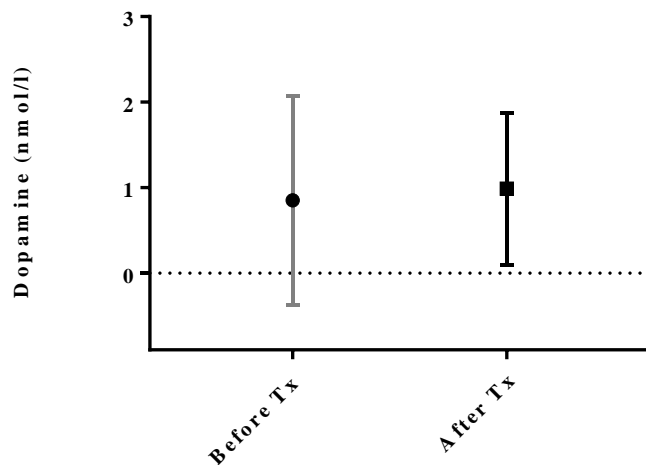
**Table 7-3 Measurements of salivary dopamine and vitamin D levels**

	Before (Mean ± SD)	Range (Min - Max)	After (Mean ± SD)	Range (Min - Max)	Mean difference (After - Before)	Adjusted P
Dopamine (nmol/l)	$0.76 \pm 1.17$	0 - 4.18	$1.03 \pm 0.88$	0 - 3.21	0.26	> 0.99
Vitamin D (nmol/l)	Not available		Not available			



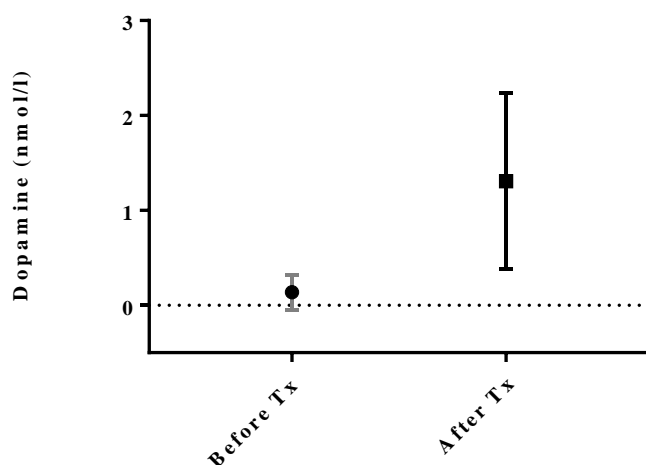
**Figure 7-9 Salivary dopamine levels before and after the vitamin D treatment.**

The dopamine levels before and after the vitamin D treatment were  $0.76 \pm 1.17$  nmol/l and  $1.03 \pm 0.88$  nmol/l, the mean difference was  $0.26 \pm 1.42$  nmol/l ( $p = 0.30$ ).



**Figure 7-10 Dopamine levels before and after the vitamin D treatment in myopes.**

No significant difference in dopamine levels between before and after the vitamin D supplement treatment in the myope group (mean difference  $0.13 \pm 1.45$  nmol/l,  $p = 0.62$ )



**Figure 7-11 Dopamine levels before and after the vitamin D treatment in emmetropes**

No significant difference in dopamine levels between before and after the vitamin D supplement treatment the emmetrope group (mean difference  $1.17 \pm 0.81$  nmol/l,  $p = 0.063$ )

### Discussion

Accommodation shifts could go toward the required direction (myopic) after a period of sustained near work (Ebenholtz, 1983). The temporary near work induced myopic shift in TA and accommodation is known as accommodative hysteresis. Varied degree of accommodative hysteresis has been suggested (Ebenholtz, 1985). Some studies have reported that onset of myopia is one of the influencing factors for the degree of accommodative hysteresis. However, no consistency and a definite conclusion to date have been confirmed from the results of previous studies. For example, studies using young adults aged between 18 and 38 found that late onset myopes had higher accommodative hysteresis (McBrien & Millodot, 1988; Woung et al., 1993), however Morse and Smith (1993) did not find difference in accommodative hysteresis between different onset myopes. The findings in children and teenagers were different than that in adults, early onset myopes showed higher accommodative hysteresis (Gwiazda et al., 1995; Woung et al., 1998). Although pre- and post-task TA measurements at both visits did not show significant difference, the results showed that TA after the vitamin D treatment had less shifts as compared to TA shift in the first visit. Also, the accommodation accuracy at 25 cm at the first visit showed significant myopic shifts, while it remained relatively stable after the vitamin D treatment. This might be implying that an 8.6 week 1000 IU vitamin D treatment

had an assistant effect in maintaining accommodation function such as TA and accommodation accuracy.

It is well known that choroidal thickness in myopes is thinner, studies using primate have demonstrated that choroidal thickness changes with refractive state (Brown et al., 2009; Hung et al., 2000b). In human, it has also been found that subfoveal choroidal thickness was lower by 15  $\mu\text{m}$  for every a dioptre increase of myopic refractive error, or lower by 32  $\mu\text{m}$  for every 1 mm increase in axial length (Wei et al., 2013). The results of the present study showed that choroidal thickness increased by 3.8% after the vitamin D treatment while the refractive errors remained the same as baseline measurement. It was not feasible to control all the influencing factors such as participants' awareness to the potential importance of sunlight exposure, whether the increase of choroidal thickness was affected by vitamin D supplement or greater sunlight exposure remains unknown. Also earlier studies have demonstrated that choroidal thickness fluctuates during the day between 25.9–103  $\mu\text{m}$  (Brown et al., 2009; Tan et al., 2012), though some also suggested there is no significant fluctuation in choroidal thickness during the day (Pollithy et al., 2015). Controlling participants' timing for visit during the study would not be attainable. In addition, our results showed that early onset myopes had thicker choroidal thickness. Whether the thicker choroidal thickness in human was the result of developed myopia or the cause toward myopia, requires further investigation. Another recent study investigating young female found that the amplitude of change in choroidal thickness during a day was greater in the dominant eyes (Zhao et al., 2016), in future studies dominant eye should be noted considering varied magnitude in diurnal change.

Central corneal thickness (CCT) showed no difference between each group and the two visits. Chen and the colleagues (2009) found no association between CCT and refractive error and other ocular parameters. Thus, CCT should be considered as an independent ocular biometry that is unrelated to other ocular measures.

A negligible increase in dopamine levels was observed after the vitamin D treatment. There was no significant difference between the two visits when observing myopes and emmetropes, though the dopamine levels increased in both groups. Since



the increases were minor, a higher dosage of vitamin D might determine whether oral vitamin D supplement could promote the release of dopamine.

### **Conclusion**

A 60 day 1000 IU vitamin D supplement can help accommodative function remain relatively stable, particularly when the stimulus is greater. The vitamin D supplement also thickens the choroidal. Whether this improved function has dopaminergic involvement requires further investigation.

## Chapter 8: Discussion and Conclusions

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The main outcomes of this project showed that there was only correlation between dry score assessed by OSDI and serum vitamin D levels when the vitamin D levels were below optimal ( $< 75$  nmol/l). An 8.6 week vitamin D oral supplement in old adults increased serum vitamin D levels by 36%. Both the OSDI and the severity of eye dryness measured by ocular surface staining reduced significantly after the vitamin D treatment. The vitamin D did not alter the levels of interleukin-6.

In the refraction/ accommodation assessments in the young adults, there was a positive correlation between dopamine levels and NRA. People with lower dopamine levels showed relatively poorer accommodative function. High myopic individuals had higher pre-near task TA. The direction of TA depended on the refractive error, myopes had myopic TA shift while hyperopes had hyperopic TA shift. DPB levels showed no significant difference between low myopes and high myopes, while it was higher in emmetropes by comparison to high myopes. Higher levels of DBP were observed in people with light coloured iris and poorer accommodation. TA shift significantly decreased after the 8.6 week vitamin D supplement. Accommodation accuracy at 25 cm between pre-and post-near task showed relative more stability after the vitamin D treatment. Choroidal thickness increased by nearly 4% after the vitamin D treatment. There was a negligible increase in dopamine levels after the vitamin D treatment, though not significant, emmetropes showed higher dopamine increase., however this might be due to its smaller number as compared to myopes.

### Vitamin D levels and Dry eye

Dry eye affects about 1/3 of the world's adult population and it has negative impacts on the quality of life (see **Chapter 2:**). Although the dry eye symptoms are highly prevalent among older people, only a minority of the randomly recruited elderly participants in the AusSun study had dry eye (tested by the OSDI), whereas only patients with dry eye from the Optometry clinic were recruited. Prentice and colleagues (2008) suggested that the prevalence of vitamin D insufficiency were between 40% and 80%; our results showed a 22.4% ( $n = 13$ ) ( $< 50$  nmol/l) to 63%

(n = 36) (< 75 nmol/l) prevalence of vitamin D insufficiency. Lower prevalence might be due to the small sample size. Conflicting with the hypothesis that vitamin D levels would be lower in individuals with dry eye, the Optometry clinic group had the higher average serum vitamin D levels. The most likely explanation for this is the different techniques used to measure vitamin D levels [i.e. the Liaison semiautomated chemiluminescence assay (DiaSorin, S.p.A., Saluggia, Italy) in the AusSun study vs ELISA (Sigma-Aldrich, St. Louis, MO, USA) used in the Optometry Group]. Currently available vitamin D immunoassays can also be inaccurate (Heijboer et al., 2012). Thus, we were not able to collapse the two groups' data into one and they had to be separately analysed. There was a significant negative correlation between OSDI and vitamin D levels in the AusSun group, suggesting that dry eye symptoms were associated with lower vitamin D levels (serum vitamin D levels < 75nmol/l). This association was not found in the optometry group where all participants had dry eye and the severity based on OSDI score did not vary much (OSDI 2.08 to 57.5 vs OSDI 0 to 39.58 in AusSun group). In addition, the data of the Optometry group clearly shows that individuals can have dry eye even in the presence of normal vitamin D levels; whether their dry eye conditions would have been worse if their levels were abnormal was not addressed in this study. This finding is in agreement with the results of a recent study showing all vitamin D deficient participants had dry eye symptoms while the symptoms were only observed in 15% of the participants with optimal vitamin D levels (Kurtul et al., 2015a).

The limitation of the studies is that the vitamin D analysis methods used in Experiment 1 and Experiment 2 for AusSun group was the DiaSorin® Liaison semi-automated chemiluminescence assay (DiaSorin, Italy), while the serum vitamin D levels for the Optometry Clinic Group were analysed with the 25 (OH) vitamin D ELISA (Sigma-Aldrich, St. Louis, MO, USA). Variability on testing vitamin D level measurement using different assays has been suggested (Holmes et al., 2013).

It is also interesting to note that the inflammatory biomarker - C-reactive protein was associated with vitamin D levels. A negative correlation was observed in people with lower vitamin D levels (< 21 ng/ml) but not in those with higher vitamin D levels (> 21 ng/ml) (Amer & Qayyum, 2012), this again implies significant findings only present in individuals with lower vitamin D levels. Yildirim and colleagues (2016) have demonstrated positive correlation between vitamin D levels and Schirmer's test/ TBUT (the lower the vitamin D the lower tear production and its

stability) and negative correlation between vitamin D levels and OSDI score.

In addition to the indication of lower vitamin D levels in dry eye patients, studies have also suggested the increased expression of inflammatory cytokines including IL-1, IL-6, IL-8, and TNF- $\alpha$  (Jones et al., 1994; Pflugfelder et al., 1999; Raphael et al., 1988). When compared to healthy eyes, the levels of IL-6 showed most significant increase in dry eyes (Pflugfelder et al., 1999), therefore having a crucial evaluating power in the assessment of dry eye. The levels of IL-6 were associated with some dry eye symptoms such as burn, discomfort soreness, irritation, grittiness, feeling of something in the eye, pain, and crustiness.

### **Impact of vitamin D supplements on dry eye**

The literatures provided mixed outcomes of the effect of vitamin D on the tears and dry eye. For example, Jee et al. (2016) reported no effect, whereas Yildirim et al. (2016) and Kurtul (2015b) suggested good improvements. Jin et al (2016) observed that TBUT was shorter in people with vitamin D deficiency, but did not measure a significant impact on OSDI scores or fluorescein staining (Jin et al., 2016).

A vitamin D supplementation study was conducted recently in Korea (Bae et al., 2016), 105 participants were given a dosage of 200,000 IU vitamin D intramuscular injection and dry eye assessment was measured with fluorescein staining score (FSS), OSDI, visual analogue pain score (VAS-pain), Schirmer's test, eyelid margin hyperemia, and TBUT before and after the supplement. Dry eye symptoms were reassessed at 2 weeks and 6 weeks of treatment with the results showing that OSDI and VAS were improved. TBUT increased in both males and females, while improvements in FSS and Schirmer's test were only observed in females. When categorising the participants into older and younger groups, improvement in FSS and Schirmer's test were only observed in the younger group. For the older group, prolonged reduction in eyelid margin hyperemia was observed as compared to the younger group. They concluded that vitamin D supplementation improves the symptoms of dry eye syndrome and is effective in treating people with dry eye.

In this study, a 60 day (8.6 weeks) 1000 IU/daily vitamin D supplement increased the vitamin D levels by 36%, whereas Holick et al (2008) reported 11-weeks of 1000 IU/daily could increase the serum vitamin D levels by nearly 50% in adults (aged between 18 to 84 years) (Holick et al., 2008). The lower increase here

may be due to the shorter treating period or the fact that the mean baseline vitamin D levels were higher (lower values have more potential to increase). It has also been shown that older people require higher doses of vitamin D to achieve optimal vitamin D levels (Holick, 2007), we did not include participants aged less than 40 years which might have better vitamin D absorption.

Oxford grading scale in the present study decreased from 0.53 to 0.31, indicating the vitamin D treatment might have improved the intact of epithelial surface (Bron et al., 2003).

IL-6 is a cytokine not only involves in human inflammatory responses but also plays a role in regulating metabolic, regenerative, and neural process (Scheller et al., 2011). In the serum samples collected in both visits, IL-6 levels were not significant and this might be that IL-6 only shows highest expression at the site of inflammation (Gabay, 2006). Increase of IL-6 has been observed in most inflammatory states (Muylle et al., 1993), however IL-6 not only promotes inflammation but also involves in some anti-inflammatory activities (Tilg et al., 1994). For example, it has been demonstrated in mouse model that IL-6 deficient animals had higher severity of inflammation (Grivennikov et al., 2009). In human it has also been demonstrated that IL-6 can reduce the inflammatory marker - metalloprotein-2 (Malecaze et al., 1997). In existing inflammation, IL-6 has also been found as one of the important mediators (Lam et al., 2009). Although there was no significant increase in IL-6 after the vitamin D treatment, which might be resulted from the limited samples in the Optometry group as the baseline IL-6 measurements for AusSun group were not available, the approximate two fold increase of IL-6 might have contributed to less severe dry eye symptoms (lower OSDI) and higher inflammatory sign such as ocular redness.

### **Vitamin D and Myopia**

In recent years, the association between vitamin D levels and myopia development has been addressed by a number of studies. Most of them were cross-sectional. Mutti and Marks (2011) were the first to examine the association between myopia and vitamin D levels in human, they measured vitamin D levels in 22 young people (13 to 25 years old) and found myopes had lower vitamin D levels in serum. However, the small sample size renders definitive conclusions difficult. The results of population based studies conducted in Korea (Choi et al., 2014) and Australia

(Yazar et al., 2014) in adolescents (13 to 18 years old) and young adults (20 years old) showed increased risk of myopia with reduced vitamin D levels. Recently in young children (6 years), it was reported that higher vitamin D levels were associated with reduced myopia risk (Tideman et al., 2016a). Although another study found no independent association between vitamin D levels and the incidence of myopia (Guggenheim et al., 2014). The conclusion in Choi et al.'s study has to be carefully made considering its limitations. For example, unlike most other studies of vitamin D, the amount of sunlight exposure and time spent outdoors were not included in the analysis. Also, the study participants were not cyclopleged, which might generate errors in classifying refractive errors. By comparison, cycloplegic refraction was performed in Yazar et al.'s study and the results suggested that children with vitamin D levels lower than 50 nmol/l had two fold increase in the odds ratio of developing myopia compared to those who had vitamin D levels higher than 50 nmol/l. Sunlight exposure was taken into account and quantified in this study using UV autofluorescence (UVAF). Not only did the results show lower likelihood with increased vitamin D levels but also higher serum vitamin D levels with raised UVAF. This is crucial when assessing whether vitamin D is an independent influencing factor in myopia development. Use of cycloplegic would affect a number of measurements; in our study, the participants were not cyclopleged as their accommodative functions were to be assessed, also Kara and colleagues (2014) have demonstrated reduced choroidal thickness after cycloplegic instillation (1% tropicamide).

A recent study examined 3168 individuals aged above 65 and suggested that there was an association between increased UVB exposure and lower myopia, but none were found between vitamin D levels and myopia (Williams et al., 2017). However, Choi and colleagues (2014) found that vitamin D, as a biomarker and byproduct of UVB, was associated with myopia. Furthermore, a recent study suggested an independent role of vitamin D in ocular growth in young children (Tideman et al., 2016b). Therefore, it is believed that elderly people have lower vitamin D levels compared to younger people. Limitations of the study include the recruitment of solely older participants, and since the proportion of myopes was only ~ 1/8 of the samples, recruiting younger and more even size of participants with and without myopes may be more justified to conclude the association between vitamin D levels and refractive errors.

As previous myopia studies have found, choroidal thickness changes with refractive error, myopes have longer axial length and thinner choroidal thickness (Chen et al., 2009; Li et al., 2011; Mutti et al., 2007b). It has been suggested that every diopter of myopic increase and every 1 mm axial length increase see reductions of 15  $\mu\text{m}$  and 32  $\mu\text{m}$  in choroidal thickness, respectively (Wei et al., 2013). It is also noteworthy that old people with vitamin D insufficiency ( $< 50\text{nmol/l}$ ) had lower macular thickness (Graffe et al., 2014).

The results of the present study showed that choroidal thickness was positively correlated with refraction and negatively correlated with axial length. Choroidal thickness increased by nearly 4% after the vitamin D treatment with no change in refraction and axial length.

Neither self-reported time spent outdoor nor the Actiwatch measurement found significant difference between emmetropes and myopes. This might be due to the number of emmetropes is smaller than that of myopes.

### **Vitamin D/dopamine and accommodation/myopia**

Variability in retinal dopamine levels between individuals can be more than 200%, while there is large variation in deprivation myopia among individual [see review (Feldkaemper & Schaeffel, 2013)]. The degree and susceptibility of deprivation myopia in animals therefore might be determined by dopamine levels (Li et al., 1992). Ohngemach and colleagues (1997) demonstrated in chick models that animals with lower dopamine levels developed higher deprivation myopia. Dopamine may be involved in ocular growth was also demonstrated in human: multifocal electroretinogram responses showed delayed times in myopes as compared to emmetropes, suggesting different retinal function between myopes and emmetropes (Chen et al., 2006). One of the functions of retinal dopamine is to reorganise the size of receptive field occurring with changes in retinal illuminance (Feldkaemper & Schaeffel, 2013). Also, Witkovsky (2004) suggested spatial and dynamic properties of retinal ganglion cell response can be modified by dopamine, resulting in contrast sensitivity changes in retina.

The dopamine levels were correlated with NRA and participants with normal accommodation had higher dopamine levels, implying accommodation system might be assisted with higher dopamine, possibly via beta activity considering human iris-ciliary body is predominantly of the beta-2 adrenoceptors (Wax & Molinoff, 1987).

In Experiment 3, pre-near task TA was higher in myopes as compared to emmetropes, while no significant difference in post-near task measurement between emmetropes, low myope, and high myopes. Myopes had myopic shift in the post-near task TA. In emmetropes, it has been suggested the magnitude of TA shifts had negative correlation with pre-task TA (Ebenholtz, 1985), which was observed in the emmetropes of the present study. However, a relatively large myopic TA shift was observed in the high myopes, this might be suggesting the equilibrium between sympathetic and parasympathetic input differs between emmetropes and myopes.

For TA measurements, as 10 consecutive reading had to be noted from the screen of Auto Refkeratometer, the light projected from the screen might have given the participants a clue for the distance from the eye to the fixating red light, this might have impacted the results of measurements.

### **Factors that affect vitamin D levels**

As described in Chapter 2, there are both external and internal factors which can affect the level of vitamin D in the human body either through its production, absorption, or metabolism. Variability in the outcomes of the study due to these factors was limited by the participants all residing in Brisbane and only participants with good general health being involved. The age of participants did vary with the conditions under investigation, those in the dry eye study had wider spread and were older (40 to 70 years) and those in the accommodation/myopia student were younger (18 to 25 years).

### **General Limitations**

#### **Sample size:**

The most significant factor limiting these experiments may be the sample size; the first stages of the two main studies only included 58 and 55, respectively, the relatively small sample size might have influenced the results, showing non-significance between a number of variables. Likewise, it might be one of the factors influencing the Actiwatch measurements in Experiment 3 (5 emmetropes vs 5 myopes), resulting in a non-significant difference between myopes and emmetropes. Furthermore, the recruiting period for dry eye study was across 2012 and 2014 (during November 2012 and July 2014), between 2015 and 2016 (during October 2015 and November 2016) for myopia study. The time for blood samples collection



lasted across seasons, which might have affected both baseline and post-vitamin D treatment measurements, considering the peak levels during a year in late summer and lowest in late winter (Kasahara et al., 2013). A difference of nearly 20 nmol/l in serum vitamin D levels between summer and winter has also been reported (Kimlin et al., 2014). The measurements of ocular biometry and possibly accommodation in Experiment 3 and 4 for myopia study might have also been impacted due to the extended recruiting period. For example, axial elongation (Fujiwara et al., 2012) and vitreous chamber depth (Fulk et al., 2002) have been reported to be slower in summer months. Another factor that might also affect the measured vitamin D levels was the amount of skin being exposed to sunlight, which was directly influenced by clothing style (Kimlin et al., 2014). Reduction of vitamin D levels is also inversely associated with body fat in both male and female, with higher impact in male (Snijder et al., 2005).

#### **Impact of diurnal variations:**

Right eyes of all participants were used to assess choroidal thickness. Choroidal thickness not only varies during the day (Brown et al., 2009), the amplitude of choroidal thickness has also been found to be greater in the dominant eyes during the day (Zhao et al., 2016). Change in systolic blood pressure has also been found to be associated with choroidal thickness, this might have to be taken into consideration in the future study (Tan et al., 2012).

Sunlight exposure in the present also relied on self-reported time spent outdoors, though actiwatch was worn in 5 emmetropes and 5 myopes in Experiment 3. The size might be too small to observe significant difference in between the groups.

#### **Future studies**

##### **Vitamin D and neurotransmitters:**

Tyrosine hydroxylase (the enzyme that is required to synthesise dopamine precursor) can be activated by active vitamin D metabolite (calcitriol) (Puchacz et al., 1996), the activation of tyrosine hydroxylase increases the availability of dopamine, adrenaline, and noradrenaline. Cholinergic function can also be enhanced by increasing the required enzyme for acetylcholine synthesis (acetylcholine

transferase), and decreasing the enzyme that limits acetylcholine synapse transmission (acetylcholine esterase) (Sonnenberg et al., 1986). The involvements of dopamine, noradrenaline, and acetylcholine are well recognised in systemic diseases such as Alzheimer's disease (Seeman et al., 1987), attention deficient/hyperactivity (Krause et al., 2000; LaHoste et al., 1996), mood disorder (Drevets & Furey, 2010). Recently, a study found that children with high myopes had worse sleep quality (including sleep duration, bed time, sleep efficacy) (Ayaki et al., 2016), indicating there may be an involvement between nocturnal rhythm in ocular growth and emotion. Tonic accommodation might alter when an individual's mood is high (Miller, 1978a), this might be suggesting that there is link between temporary changes of these neurotransmitters and accommodation status, potentially being capable of changing refractive status/development. Further studies are suggested to investigate the potential role of these neurotransmitters influenced by vitamin D levels and supplements on ocular growth/ refractive development are warrant.

### **Dry eye and ageing:**

The three important components of tear production, lacrimal gland, Meibomian glands, and goblet cells, directly influence the quality and quantity of tear. The association between changes in Meibomian glands and ageing has been suggested (Den et al., 2006). Changes in these components have been demonstrated in animals and human. In the rat model it has been shown that there are changes such as acinar degeneration, nuclear abnormalities; increased collagen, and ductal dilation (El-Fadaly et al., 2014). Ageing Meibomian glands in both mice and human also showed reduced amount of cellular proliferation markers (Jester et al., 2011; Nien et al., 2009; Villani et al., 2013). Histological analysis in rats and mice and microscopic examination in human all found that there is decrease in the densities of goblet cells (Den et al., 2006; McClellan et al., 2014; Wei et al., 2011). Meibomian glands function as producing the lipid layer; lacrimal gland produces the aqueous component, while the mucin layer, the component that is essential for adhering tear on the ocular surface, is produced by goblet cells. All the three components are important for appropriate tear quality and quantity. Changes in Meibomian glands, lacrimal glands, and goblet cells are known to be associated with the properties of tear film.

Decreased release of acetylcholine has been found in older animals using mice model, and both sympathetic and parasympathetic nerves showed reduction in the area surrounding the acinus (consists of lacrimal gland cells) (Ríos et al., 2005; Williams et al., 1994). These changes may contribute to lower volume of tear secretion due to level of stimulation is also altered. A rat model also demonstrated that the stimulation of substance P, vasoactive intestinal peptide, histamine, and 5-hydroxytryptamine in ageing lacrimal glands resulted in lower responses - reduced peroxidase release and protein output from acinar cells, as compared to younger animal (Draper et al., 2003). The results showed that ageing is associated with altered ability of synthesising and secreting proteins by acinar cells.

Inflammation on the eye surface may also be associated with the ageing change, leading to the dryness of ocular surface. Further studies focusing on the association between vitamin D levels/treatment and acetylcholine are required to further investigate the role of vitamin D in ocular surface.

### **Dopamine in the eye:**

It has been suggested that the rise of dopamine appears most significant in the first hour of light exposure (Megaw et al., 2001). Although there was no significant difference in dopamine levels between emmetropes and myopes, we found dopamine levels in people with normal accommodation were more than 3 fold higher as compared to those with poor accommodation. This might be indicating that accommodative functions are associated with not only the total levels of dopamine but also the frequency of dopamine release (i.e. the duration of higher dopamine levels that can be maintained in performing normal accommodation). Further studies need to be conducted to confirm whether higher vitamin D levels/vitamin D supplements can lead to higher dopamine levels, maintaining in higher frequency of dopamine release in people with normal accommodation.

### **Vitamin D binding protein:**

The results of Experiment 3 showed that in the myopes had lower vitamin D binding protein as compared to the emmetropes in the young people. The principal biological function of DPB is to bind, transport, and solubilise the two vitamin D

metabolites – 25(OH)D and 1,25(OH)D. The circulating concentrations of DPB in the plasma are higher than the total vitamin D metabolites by 20 fold (White & Cooke, 2000). The vast difference between DPB and vitamin D metabolites, therefore renders most 25(OH)D and 1,25(OH)D are bound to DBP. A study using a DBP- deficient mouse model found although the appearance and size of the animals were did not differ to the control, both lower serum 25(OH)D and 1,25(OH)D levels were observed (Safadi et al., 1999). In the mouse model, it is also suggested the DBP is helped in prolonging the half-life of the circulating vitamin D metabolites.

The greater prolongation of the vitamin D metabolites in the emmetropes might help stimulate dopamine release in the human body via a higher frequency, partially contributed to myopia inhibition. Due to lack of sensitivity in our salivary vitamin D analysis, whether there was an association between the levels of vitamin D and dopamine/ DBP could not be concluded. The post-treatment dopamine levels only showed significant rise in emmetropes, indicating dopamine levels might be more prone to rise by vitamin D supplement in emmetropes.

### **Conclusion**

Vitamin D levels were associated with the Ocular Surface Disease Index and dry eye score in older individuals with vitamin D insufficiency. Self-reported time spent outdoors was not associated with vitamin D levels. A 60 day 1000 IU vitamin D supplement increased vitamin D levels by 36% and improved dry eye symptoms. The 60 day vitamin D supplement helped improve the quality of tear but had no effect on tear quantity.

Neither self-reported time spent outdoor or actiwatch measurements showed difference between emmetropes and myopes. No significant difference in dopamine levels between emmetropes and myopes, however dopamine levels were higher in people with poor accommodation, implying accommodative function might be affected by dopamine. The higher TA and TA shifts in high myopes suggested relatively unstable equilibrium between sympathetic and parasympathetic systems in high myopes. Higher DBP in people with poor accommodation might suggest DBP can affect myopia development via accommodative function. A 60 day vitamin D supplement assisted accommodative function in remaining relatively stable and

thicken the choroid. Further investigation needs to be conducted to confirm whether the effects on accommodation and choroid result from altered dopaminergic activity caused by more sunlight exposure or increased vitamin D levels by supplement.

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# Appendices

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## Appendix A Ethics Approval Forms

	<b>PARTICIPANT INFORMATION FOR QUT RESEARCH PROJECT</b>
<b>Investigation of the association between vitamin D levels and dry eye</b>	
<b>QUT Ethics Approval Number 140000555</b>	

### RESEARCH TEAM

Principal Researcher: Chih-Huang Yang, PhD Student  
Associate Researchers: Associate Prof Katrina Schmid, Prof Michael Kimlin, Dr Julie Albietz  
**School of Optometry and Vision Science, Faculty of Health  
Queensland University of Technology (QUT)**

### DESCRIPTION

This project is being undertaken as part of the PhD for Chih-Huang Yang.

The purpose of this project is to investigate the association between Vitamin D levels and the health of the surface of the eye. We predict that vitamin D levels may be low in people with dry eye.

You are invited to participate in this project because you are within the target age range (40 to 70 years) and have been diagnosed as having dry eye.

### PARTICIPATION

The study involves one visit of approximately 1 hour duration to assess the health of the surface of your eyes.

The eye tests include:

1. Questionnaires about the symptoms of "dry eye", eye irritation, eye history, allergies and medical history.
2. The tears, the eyelids, blinking patterns, and the surface tissues of the eye will be examined under magnification.
3. Your ability to make tears will be measured by placing a piece of thread under the outer aspect of the lower lid.
4. A surface dye (fluorescein) will be instilled into the eye to assess for dry eye related eye surface damage.
5. Your blood will be collected for analysing the levels of vitamin D and interleukin-6.

6. Photographs of your eye/s under magnification may be taken. It will not be possible to identify you from these photographs of your eye/s. These photographs may be used in research publications and in presentations at scientific meetings.

All of these assessments are routinely used to evaluate and monitor eye surface health during eye examinations. It is possible you will have previously had all or most of these tests performed on your eyes at one time or another. There is minimal to no discomfort during and after these tests, aside from the test to measure tear production that will involve mild temporary irritation of the front surface of the eyes. No adverse effects have been documented regarding any of these tests. There will be no drops used to dilate your pupils and blur the vision so you may drive afterwards if you wish. The eye drops used in the assessments for this study may have been instilled in your eyes during previous eye health examinations, if you suspect any previous allergy problems with eye staining drops or any of the eye tests please inform the investigators.

Your participation in this project is entirely voluntary. If you agree to participate you do not have to complete any question(s) you are uncomfortable answering. Your decision to participate or not participate will in no way impact upon your current or future relationship with QUT or with associated external organisation. If you do agree to participate you can withdraw from the project without comment or penalty. Any identifiable information already obtained from you will be destroyed.

#### **EXPECTED BENEFITS**

This study is for the purpose of research rather than treatment and may be of no direct benefit to you. However, it may increase the understanding for the impact of vitamin D levels on the health of the ocular surface and dry eye.

To reimburse some of your travel costs we will provide you with a \$10 gift card.

#### **RISKS**

There are minimal risks associated with your participation in this project. These include stinging feeling when inserting the thread, shining bright lights towards the eyes or minor discomfort when collecting your blood.

## PRIVACY AND CONFIDENTIALITY

All comments and responses will be treated confidentially unless required by law. The names of individuals, all comments and responses will be treated confidentially. Any data collected will be stored securely as per QUT's Management of research data policy.

**Confidentiality of all information, including personal details provided for the purpose of the study will be safeguarded, but can be released to medical or other eye care practitioners on your written consent.**

Only the student researchers and supervisors will have access to the data. Only average data will be used in any published reports and this will not personally identify you. Please note that non-identifiable data collected in this project may be used as comparative data in future projects.

## CONSENT TO PARTICIPATE

The signed consent form is an indication of your consent to participate in this project.

## QUESTIONS / FURTHER INFORMATION ABOUT THE PROJECT

If have any questions or require further information please contact one of the research team members below.

Katrina L. Schmid	07 3138 6150	<a href="mailto:k.schmid@qut.edu.au">k.schmid@qut.edu.au</a>
Chih Huang Yang	07 3138 6221	<a href="mailto:chih.yang@student.qut.edu.au">chih.yang@student.qut.edu.au</a>

## CONCERNS / COMPLAINTS REGARDING THE CONDUCT OF THE PROJECT

QUT is committed to research integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Unit on 07 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au). The QUT Research Ethics Unit is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

***Thank you for helping with this research project. Please keep this sheet for your information.***

**Investigation of the association between vitamin D levels and dry eye**

**QUT Ethics Approval Number 1400000555**

**RESEARCHER**

Principal Researcher: Chih-Huang Yang, PhD Student

Associate Researchers: Associate Prof Katrina Schmid, Prof Michael Kimlin, Dr Julie Albiez

**School of Optometry and Vision Science**

**Faculty of Health, Queensland University of Technology (QUT)**

**STATEMENT OF CONSENT**

**By signing below, you are indicating that you:**


- Have read and understood the information document regarding this project.
- Have had any questions answered to your satisfaction.
- Understand that if you have any additional questions you can contact the research team.
- Understand that the project is for the purpose of research and there may be no clinical benefit to you.
- Understand that your vitamin D levels and sunlight exposure data collected in the Seasonal D study will be accessed in this project.
- Have been informed of the possible side effects of the tests.
- That the confidentiality of the information you will provide will be safeguarded. Only average data will be used in any published reports and this will not personally identify you.
- That non-identifiable data collected in this project may be used as comparative data in future projects.
- **Understand participation is entirely voluntary and you are free to withdraw at any time without comment or penalty.**
- Understand that you can contact the Research Ethics Unit on 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au) if you have concerns about the ethical conduct of the project.
- Have been assured that data collected from the Seasonal D study will not contain any personal information other than details about your vitamin D status relevant to the research project. The data obtained from the Seasonal D study will be coded in the results database to protect your identity.
- Agree to participate in the project and give permission for the research investigators to obtain data relevant to the research project from the Seasonal D study.

Name \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_

*Please return this sheet to the investigator.*

 Queensland University of Technology Brisbane Australia	<b>WITHDRAWAL OF CONSENT FOR QUT RESEARCH PROJECT</b>
<b>Investigation of the association between vitamin D levels and dry eye</b>	
QUT Ethics Approval Number <b>140000555</b>	

**RESEARCH TEAM CONTACTS**

Chih Huang Yang – PhD student	Katrina Schmid – Associate Professor
Optometry and Vision Science / Portfolio / Domain	Optometry and Vision Science/ Portfolio / Domain
Health Faculty	Health Faculty
Phone	Phone
Email chih.yang@student.qut.edu.au	Email k.schmid@qut.edu.au

I hereby wish to WITHDRAW my consent to participate in the research project named above.


Name \_\_\_\_\_

Signature \_\_\_\_\_

---

**Date** \_\_\_\_\_

I understand that this withdrawal WILL NOT jeopardise my relationship with Queensland University of Technology.

	<b>PARTICIPANT INFORMATION FOR QUT RESEARCH PROJECT – Phase 2 –</b>
<b>Investigation of the association between vitamin D levels and dry eye</b>	
<b>QUT Ethics Approval Number 140000555</b>	

**RESEARCH TEAM**

Principal           Chih-Huang Yang, PhD Student  
Researcher:  
Associate           Associate Professor Katrina Schmid, Professor Michael Kimlin, Dr  
Researchers:       Julie Albietz  
**School of Optometry and Vision Science, Faculty of Health  
Queensland University of Technology (QUT)**

**DESCRIPTION**

This project is being undertaken as part of the PhD study of Chih-Huang Yang.

You are invited to participate in this project because in the earlier phase of the study it was revealed you have low vitamin D and/or dry eye. We invite you to participate in the next phase which involves taking vitamin D supplement provided by us for 60 days.

The purpose of this phase is to investigate the effect of vitamin D supplements on the ocular dryness. We propose that raised vitamin D levels may improve eye dryness.

**PARTICIPATION**

Before the following eye tests you are required to take vitamin D supplement daily (1,000 international unit) for 60 days.

The study involves 1 visit and takes approximately 30 minutes duration to assess the health of the surface of your eyes and includes:

1. Questionnaires about the symptoms of "dry eye", eye irritation, eye history, allergies and medical history will be completed.
2. The tears, the eyelids, blinking patterns, and the surface tissues of the eye will be examined under magnification.

3. Your ability to make tears will be measured by placing a thin strip of filter paper under the outer aspect of the lower lid.
4. A surface dye (fluorescein) will be instilled into the eye to assess for dry eye related eye surface damage.
5. Photographs of your eye/s under magnification may be taken. It will not be possible to identify you from these photographs of your eye/s. These photographs may be used in research publications and in presentations at scientific meetings.

All of these assessments are routinely used to evaluate and monitor eye surface health during eye examinations. You will have previously had all or most of these tests performed on your eyes at one time or another. There is minimal to no discomfort during and after these tests, aside from the test to measure tear production that will involve mild temporary irritation of the front surface of the eyes. No adverse effects have been documented regarding any of these tests. There will be no drops used to dilate your pupils and blur the vision so you may drive afterwards if you wish. The eye drops used in the assessments for this study will have been instilled in your eyes during previous eye health examinations, however if you have had any previous allergy problems with eye staining drops or any of the eye tests please inform the investigators.

We will contact you by email or text every two weeks to remind you to take your Vitamin D table and to check if you are having any issues. Please advise on the consent form whether you prefer to receive a text or email, and provide your contact details.

Your participation in this project is entirely voluntary. If you agree to participate you do not have to complete any question(s) you are uncomfortable answering. Your decision to participate or not participate will in no way impact upon your current or future relationship with QUT or with associated external organisation. If you do agree to participate you can withdraw from the project without comment or penalty. Any identifiable information already obtained from you will be destroyed.

#### **EXPECTED BENEFITS**

This study is for the purpose of research rather than treatment and may be of no direct benefit to you. However, it may increase the understanding for the impact of vitamin D levels on the health of the ocular surface and dry eye.

To reimburse some of your travel costs we will provide you with a \$10 gift card.

## RISKS

There are minimal risks associated with your participation in this project. These include a stinging feeling when inserting paper strips or shining bright lights towards the eyes.

The main consequence of taking too much vitamin D is a build-up of calcium in your blood (hypercalcemia), which can cause poor appetite, nausea, vomiting, weakness, frequent urination and kidney problems. However these only occur when the amount taken is 50,000 international units (IU) daily D for several months.

## PRIVACY AND CONFIDENTIALITY

All comments and responses are anonymous and will be treated confidentially unless required by law. Any data collected will be stored securely as per QUT's Management of research data policy.

**Confidentiality of all information, including personal details provided for the purpose of this study will be safeguarded, but can be released to medical or other eye care practitioners on your written consent.**

Only the student researchers and supervisors will have access to the data. Only average data will be used in any published reports and this will not personally identify you. Please note that non-identifiable data collected in this project may be used as comparative data in future projects.

## CONSENT TO PARTICIPATE

We would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

## QUESTIONS / FURTHER INFORMATION ABOUT THE PROJECT

If have any questions or require further information please contact one of the research team members below.

Katrina L. Schmid	3138 6150	<a href="mailto:k.schmid@qut.edu.au">k.schmid@qut.edu.au</a>
Chih Huang Yang	3138 6221	<a href="mailto:chih.yang@student.qut.edu.au">chih.yang@student.qut.edu.au</a>

## CONCERNS / COMPLAINTS REGARDING THE CONDUCT OF THE PROJECT

QUT is committed to research integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Unit on 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au). The QUT Research Ethics Unit is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

***Thank you for helping with this research project. Please keep this sheet for your information.***





**Investigation of the association between vitamin D levels and dry eye**

**QUT Ethics Approval Number 1400000555**

**RESEARCHER**

Principal

Researcher: Chih-Huang Yang, PhD Student

Associate Associate Professor Katrina Schmid, Professor Michael Kimlin,

Researchers: Dr Julie Albietz

**School of Optometry and Vision Science, Faculty of Health  
Queensland University of Technology (QUT)**

**STATEMENT OF CONSENT**

**By signing below, you are indicating that you:**

- Have read and understood the information document regarding this project.
- Have had any questions answered to your satisfaction.
- Understand that if you have any additional questions you can contact the research team.
- Understand that the project is for the purpose of research and there may be no clinical benefit to you.
- Have been informed of the possible side effects of the tests.
- That non-identifiable data collected in this project may be used as comparative data in future projects.
- Understand that you can contact the Research Ethics Unit on 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au) if you have concerns about the ethical conduct of the project.
- That the confidentiality of the information you will provide will be safeguarded. Only average data will be used in any published reports and this will not personally identify you.

**Name**

\_\_\_\_\_ **Please indicate and include your preferred contact details below.**

**Email**

\_\_\_\_\_  
\_\_\_\_\_

Text \_\_\_\_\_

Signature \_\_\_\_\_

Date \_\_\_\_\_

- Understand that non-identifiable data collected in this project may be used as comparative data in future projects.
- Understand participation is entirely voluntary. You are free to withdraw at any time without comment or penalty.
- **Agree to take vitamin D supplement daily (1,000 international unit) for 60 days which has been provided.**
- Agree to participate in this phase of the project.

*Please return this sheet to the investigator.*



**Investigation of the association between vitamin D levels and  
accommodation accuracy / refraction**

**QUT Ethics Approval Number 1500000591**

**RESEARCH TEAM**

Principal	Chih-Huang Yang	PhD Student
Researcher:		
Associate	Associate Professor Katrina Schmid	Principal Supervisor
Researchers:		
	Prof Michael Kimlin	Associate Supervisor
	<b>Faculty of Health, Queensland University of Technology (QUT)</b>	

**DESCRIPTION**

This project is being undertaken as part of PhD for Chih-Huang Yang.

The purpose of this project is to investigate the association between Vitamin D levels and accommodation (the eye’s focusing ability) /refraction (the status and amount of eye’s short-sightedness / long-sightedness) of the eye.

You are invited to participate in this project because you are within the target age range (18 to 25 years).

**PARTICIPATION**

The study involves at least one visit of approximately 50 minute duration to assess the accommodation/refraction of your eyes (with contact lens correcting your vision if required) and collect a spit (saliva) sample for vitamin D, dopamine, and insulin growth factor-1 analysis. Then, if your accommodation/refraction/vitamin D levels are suitable you will be asked to come back for the vitamin D supplement study. In the supplement study, you will be required to take 1000 IU vitamin D tablet/daily for 60 days) and have another assessment visit of 50 minutes duration. The eye tests include:

1. Biometric ocular measures using a special instrument named the Lenstar (this is an optical instrument that does not touch the eye), the measures include the eye’s axial length, central corneal (of the eye’s front surface) thickness, corneal refractive power, retinal (light sensing layer) thickness, and pupil size.
2. The latent horizontal misalignment in the position of the two eyes (usually not observable) using specially designed charts named Howell phoria cards.
3. How accurate your eyes can focus at distances of 40, 33, and 25cm.
4. The resting positions of your eyes’ accommodation system.
5. The limit your eyes can increase and decrease the focus ability.

Most of these assessments are routinely used to evaluate the accommodative functions of the eye during eye examinations. It is possible you will have previously had some of these tests performed on your eyes at one time or another. There is minimal to no discomfort during and after these tests, aside from the transient blur caused by testing your eyes' focus limit. No adverse effects have been documented regarding any of these tests.

Your participation in this project is entirely voluntary. If you agree to participate you do not have to complete any question(s) you are uncomfortable answering. Your decision to participate or not participate will in no way impact upon your current or future relationship with QUT (for example your grades) or with associated external organisation. If you do agree to participate you can withdraw from the project without comment or penalty. Any identifiable information already obtained from you will be destroyed.

### **EXPECTED BENEFITS**

It is expected that this project will not directly benefit you. However, it may increase the understanding for the impact of vitamin D levels on the accommodative function/refraction of the eyes.

To compensate you for your contribution should you choose to participate the research team will provide you with a \$10 gift card.

### **RISKS**

There are minimal risks associated with your participation in this project. These include the transient blur caused by testing eyes' focus ability limit. The examination will take approximately 50 minutes but please allow 1 hour of your time for the appointment.

The dose 1000IU/daily vitamin D is believed to maintain an optimal status (above 50mol/L) in the human body and should not cause any adverse effect. The main consequence of taking too much vitamin D is a build-up of calcium in your blood (hypercalcemia), which can cause poor appetite, nausea, vomiting, weakness, frequent urination and kidney problems. However these only occur when the amount taken is 50,000 international units (IU) daily D for several months.

### **PRIVACY AND CONFIDENTIALITY**

All comments and responses will be treated confidentially unless required by law. The names of individuals, all comments and responses will be treated confidentially.

Any data collected will be stored securely as per QUT's Management of research data policy. The saliva samples collected from you will be disposed after they are analysed.

**Confidentiality of all information, including personal details provided for the purpose of the study will be safeguarded, but can be released to medical or other eye care practitioners with your written consent.**

Only the student researchers and supervisors will have access to the data. Only average data will

be used in any published reports and this will not personally identify you. Please note that non-identifiable data collected in this project may be used as comparative data in future projects.

### **CONSENT TO PARTICIPATE**

We would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

### **QUESTIONS / FURTHER INFORMATION ABOUT THE PROJECT**

If have any questions or require further information please contact one of the researchers listed below.

Katrina Schmid	3138 6150	<a href="mailto:k.schmid@qut.edu.au">k.schmid@qut.edu.au</a>
Chih Huang Yang	3138 6221	<a href="mailto:chih.yang@student.qut.edu.au">chih.yang@student.qut.edu.au</a>

### **CONCERNS / COMPLAINTS REGARDING THE CONDUCT OF THE PROJECT**

QUT is committed to research integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Unit on 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au). The QUT Research Ethics Unit is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

***Thank you for helping with this research project. Please keep this sheet for your information.***

**Investigation of the association between vitamin D levels and  
accommodation accuracy / refraction**

**QUT Ethics Approval Number 1500000591**

**RESEARCHER**

Katrina	3138	
Schmid	6150	<a href="mailto:k.schmid@qut.edu.au">k.schmid@qut.edu.au</a>
Chih Huang	3138	
Yang	6221	<a href="mailto:chih.yang@student.qut.edu.au">chih.yang@student.qut.edu.au</a>

**Faculty of Health, Queensland University of  
Technology (QUT)**

**STATEMENT OF CONSENT**

**By signing below, you are indicating that you:**

- Have read and understood the information document regarding this project.
- Have had any questions answered to your satisfaction.
- Understand that if you have any additional questions you can contact the research team.
- Understand that you can contact the Research Ethics Unit on 3138 5123 or email [ethicscontact@qut.edu.au](mailto:ethicscontact@qut.edu.au) if you have concerns about the ethical conduct of the project.
- Understand that the project is for the purpose of research and there may be no clinical benefit to you.
- Understand that your sunlight exposure data will be collected.
- Understand that a saliva sample will be collected from you.
- Have been informed of the possible side effects of the tests.
- That the confidentiality of the information you will provide will be safeguarded. Only average data will be used in any published reports and this will not personally identify you.
- Understand that non-identifiable data collected in this project may be used as comparative data in future projects.
- **Understand participation is entirely voluntary. You are free to withdraw at any time without comment or penalty.**
- Agree to participate in the project.

**Name** \_\_\_\_\_

**Signature** \_\_\_\_\_

**Date** \_\_\_\_\_

*Please return this sheet to the investigator.*

# Ocular Surface Disease Index<sup>®</sup> (OSDI<sup>®</sup>)<sup>2</sup>

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

Have you experienced any of the following <i>during the last week</i> ?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? . . .	4	3	2	1	0
2. Eyes that feel gritty? . . . . .	4	3	2	1	0
3. Painful or sore eyes? . . . . .	4	3	2	1	0
4. Blurred vision? . . . . .	4	3	2	1	0
5. Poor vision? . . . . .	4	3	2	1	0

Subtotal score for answers 1 to 5

Have problems with your eyes limited you in performing any of the following <i>during the last week</i> ?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading? . . . . .	4	3	2	1	0	N/A
7. Driving at night? . . . . .	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV? . . . . .	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9

Have your eyes felt uncomfortable in any of the following situations <i>during the last week</i> ?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions? . . . . .	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned? . . .	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12

Add subtotals A, B, and C to obtain D   
(D = sum of scores for all questions answered)

Total number of questions answered   
(do not include questions answered N/A)

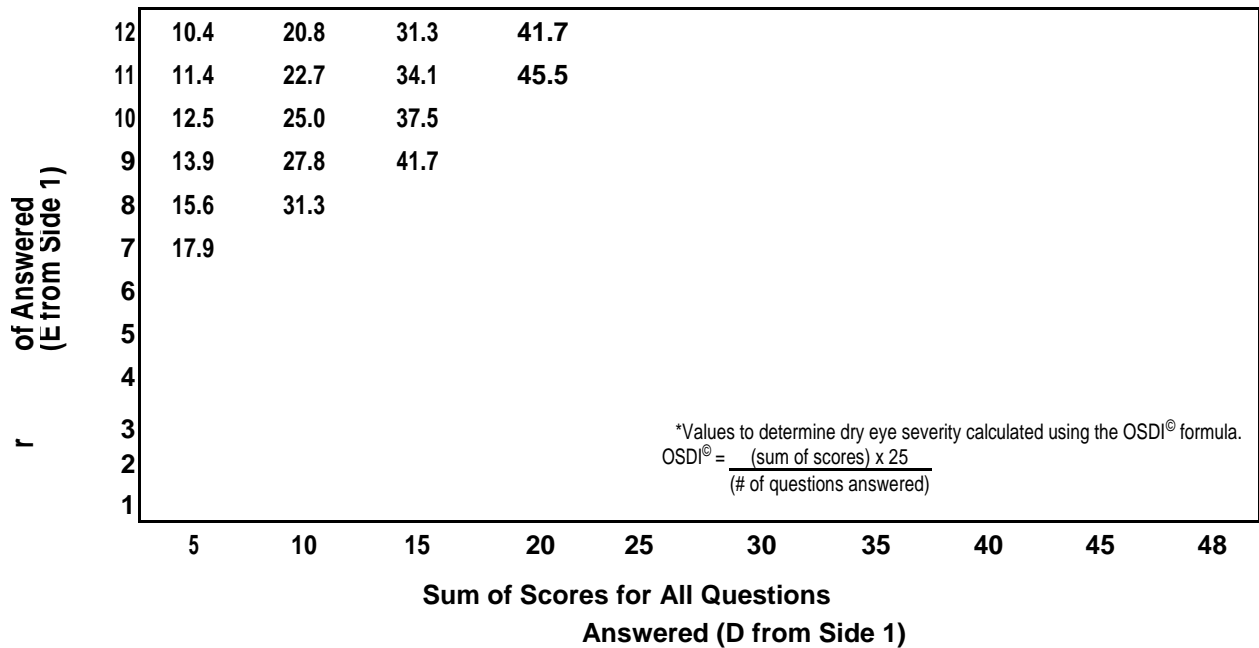


## Evaluating the OSDI<sup>®</sup> Score<sup>1</sup>

The OSDI<sup>®</sup> is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI<sup>®</sup> is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

## Assessing Your Patient's Dry Eye Disease<sup>1, 2</sup>

Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.\* Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.



Normal                      Mild                      Moderate                      Severe

Patient's Name: \_\_\_\_\_ Date: \_\_\_\_\_

How long has the patient experienced dry eye disease? \_\_\_\_\_

Eye Care Professional's Comments: \_\_\_\_\_

1. Data on file, Allergan, Inc.
2. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615-621

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## Appendix B Sunlight Exposure Questionnaire

### Sunlight Exposure Questionnaire

QUT Ethics Approval Number 1400000555  
Investigation of the association between vitamin D levels and dry eye

\*Required

1. **Participant ID \***

Please type in your participant ID

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2. **Date of Birth \***

(mm/dd/yyyy)

*Example: 15 December 2012*

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3. **City/ Town of Birth \***

Please type in the place you were born

4. **Country of Birth \***

Please type in the country you were born

---

5. **If you were not born Australia, in what year did you come to Australia?**

Please type in the year you came to Australia

6. **How would you describe your ancestry? \***

Please select one of the answers below  
*Mark only one oval.*

- Australian Aboriginal
- North-West European (including British, Irish, Western European, Northern European)
- Southern European (including Spanish, Italian, Portuguese)
- Torres Strait Islander
- New Zealander/Maori
- Melanesian and Papuan
- Micronesian
- Polynesian
- South-East Asian (including Burmese, Thai, Vietnamese, Indonesian, Malay etc)
- South Eastern European (including Albanian, Croatian, Greek, Cypriot etc)
- North East Asian (including Chinese, Japanese, Korean, Mongolian etc)
- Central Asian (including Afghan, Georgian, Kazakh, Turkmen etc)
- North American Aboriginal
- Central or South American
- Caribbean Islander
- Central, West, South and East African
- Mixed Race
- Other

7. **Please select your gender \***

Please select one of the answers below  
*Mark only one oval.*

- Male
- Female

8. **Which of the following best describes the occupation you had for the longest period? \***

Please select one of the answers below  
*Mark only one oval.*

- Manager
- Professional
- Technicians and Trades Workers
- Community and Personal Service Workers
- Clerical and Administrative Workers
- Sales Workers
- Machinery Operators and Drivers
- Labourer or related worker
- Other

9. **Which of the following best describes your current employment status? \***

Please select one of the answers below  
Mark only one oval.

- Unemployed
- Home duties
- Part time work - employed/self employed
- Full time work - employed/self employed
- Student
- Sole parent pension
- Disability pension
- Retired
- Other

10. **Which of the following best describes your current, main occupation? \***

Please select one of the answers below  
Mark only one oval.

- Mainly indoors (e.g. office worker)
- Half indoors and half outdoors (e.g. physical education teacher)
- Mainly outdoors (e.g. gardener)

11. **What is your natural eye colour? \***

Please select one of the answers below  
Mark only one oval.

- Blue, light blue, grey blue, blue-green
- Green
- Hazel, green hazel
- Brown, dark brown

12. **What is your natural hair colour (when you were 18 years old)? \***

Please select one of the answers below  
Mark only one oval.

- Red
- Blond
- Chestnut or dark blond
- Brown
- Black

13. **What is your natural (untanned) skin colour (on non-sun exposed skin)? \***

Please select one of the answers below  
Mark only one oval.

- Fair
- Medium
- Olive
- Dark/Black

14. **How does your skin react if you were to sit in the sun in your current area of residence, in the middle of the day, for the first time in summer, without sunscreen? \***

Please select one of the answers below  
*Mark only one oval.*

- Never burn  
 Burn after more than 2 hour sun exposure  
 Burn after 1 - 2 hours  
 Burn after 1/2 - 1 hour  
 Burn within half an hour

15. **How does your skin react if you were to sit in the sun in your current area of residence, for one hour in the middle of the day, for the first time in summer, without sunscreen? \***

Please select one of the answers below  
*Mark only one oval.*

- Burn then peel  
 Burn then tan  
 Tan only

16. **At the end of the summer or after a two week holiday in the sun, what kind of tan would you have? \***

Please select one of the answers below  
*Mark only one oval.*

- A dark tan  
 A medium tan  
 A light tan  
 Practically no tan

17. **About how many night shifts have you worked in the past month? \***

Please select one of the answers below  
*Mark only one oval.*

- 0  
 1 night shift  
 2 night shifts  
 3 night shifts  
 4 night shifts  
 5-7 night shifts  
 8-10 night shifts  
 11-13 night shifts  
 14-16 night shifts  
 17-19 night shifts  
 20+ night shifts

**We are interested in the CLOTHING AND SUN PROTECTION that you have worn in the PAST MONTH when you were outside. Did you wear or use...**

---

18. \*

Please tick the corresponding option - only one per line  
 Mark only one oval per row.

	Never	Rarely	Sometimes	Often	Almost always	Always
Hat, cap or other head covering?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Long sleeves?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Clothing that covers most of your legs?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sunglasses?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Umbrella in the sun?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

19. **Have you used sunscreen in the PAST MONTH, including moisturiser and make-up with sun protection factor (SPF)? \***

Please select one of the answers below  
 Mark only one oval.

- Yes  
 No

20. **What is the sun-protection factor (SPF) of the sunscreen that you have used most often in the PAST MONTH? Consider any product you may use that have a SPF.**

Please mark the corresponding circle – only one per line; if never used please leave blank  
 Mark only one oval per row.

	SPF15	SPF30	SPF50
Sunscreen	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moisturiser	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Make-up	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Please check the number of serves that you have consumed for each food in the PAST MONTH**

---

21. **Fresh salmon (1 serve = 120g cooked, or about palm size) \***

type in the number of serves you had in the past month

.....

22. **Salmon, tinned (1 serve=100g)**

type in the number of serves you had in the past month

.....

23. **Fresh tuna (1 serve = 120g cooked, or about palm size)**

type in the number of serves you had in the past month

.....

24. **Tuna, tinned (1 serve=100g)**  
type in the number of serves you had in the past month

25. **Sardines (1 serve = 60g about ½ tin)**  
type in the number of serves you had in the past month

---

26. **Mackerel or Herring (1 serve = 100g)**  
type in the number of serves you had in the past month

---

27. **Milk or yoghurt fortified with vitamin D (1 serve = 250mL, 1 cup milk or yoghurt)**  
type in the number of serves you had in the past month

---

28. **Milk (1 serve = 250mL or 1 cup)**  
type in the number of serves you had in the past month

29. **Yoghurt (1 serve = 125g-160g)**  
type in the number of serves you had in the past month

---

30. **Cheese (1 serve = 40g, 2 slices)**  
type in the number of serves you had in the past month

---

*Skip to question 31.*

### **Time Outdoors In The Sun & Clothing Habits**

Select the box which best represents the amount of time that you spent in the sun during each one hour interval shown below



**31. Typical work/school day spent outdoor**

Still thinking in the PAST MONTH, please select the box which best represents the amount of time that you spent in the sun during each one hour interval in a typical week day  
*Mark only one oval per row.*

	0 mins	< 15 mins	15 - 29 mins	30 - 44 mins	45 - 60 mins
5 - 6 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7 - 8 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8 - 9 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9 -10 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10 - 11 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11 -12 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12 - 1 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
1 - 2 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2 - 3 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3 - 4 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4 - 5 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5 - 6 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**32. Typical NON-work/school day spent outdoor**

Still thinking in the PAST MONTH, please select the box which best represents the amount of time that you spent in the sun during each one hour interval in a typical weekend day  
*Mark only one oval per row.*

	0 mins	< 15 mins	15 - 29 mins	30 - 44 mins	45 - 60 mins
5 - 6 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7 - 8 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8 - 9 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9 -10 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10 - 11 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11 -12 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12 - 1 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
1 - 2 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2 - 3 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3 - 4 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4 - 5 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5 - 6 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Type of Clothing Worn**

---

Please select a number in each line

**Clothing Guide**

SUN DIARY CLOTHING GUIDE							
UPPER BODY	NO CLOTHING ON UPPER BODY						
	0 No upper clothing	1 Bikini	2 Swimsuit	3 Crop top	4 Singlet top	5 Short-sleeved top	6 Long-sleeved top
LOWER BODY	NO CLOTHING ON LOWER BODY						
	0 No lower clothing	1 Speedos/briefs	2 Shorts or short skirt	3 Medium shorts or 3/4 pants	4 Long trousers/jeans	5 Medium skirt	6 Long skirt
HEADWEAR	NO HEADWEAR						
	0 No headwear	1 Beanie	2 Cap	3 Legionnaire's cap	4 Bucket hat	5 Wide-brimmed hat	6 Veil/burkha
FOOTWEAR	NO FOOTWEAR/HANDWEAR						
	0 No footwear	1 Thong/open sandals	2 Semi-enclosed shoes	3 Enclosed shoes	4 Workboots	5 Long socks	HANDWEAR Gloves

33. **Thinking in the PAST MONTH, Please give a number for the type of clothing worn in a typical WEEK day**

Please refer to the above clothing guide  
Mark only one oval per row.

	0	1	2	3	4	5	6
Upper Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lower Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Headwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Footwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

34. **Thinking in the PAST MONTH, please give a number for the type of clothing worn in a typical WEEKEND day**

Please refer to the above clothing guide  
Mark only one oval per row.

	0	1	2	3	4	5	6
Upper Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lower Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Headwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Footwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## Sunlight Exposure Questionnaire A/R study

QUT Ethics Approval Number 1500000591

Investigation of the association between vitamin D levels and accommodation accuracy/refraction

\*Required

**1. Participant ID \***

Please type in your participant ID

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**2. Date of Birth \***

(mm/dd/yyyy)

---

*Example: 15 December 2012*

**3. City/ Town of Birth \***

Please type in the place you were born

**4. Country of Birth \***

Please type in the country you were born

---

**5. If you were not born Australia, in what year did you come to Australia?**

Please type in the year you came to Australia

6. **How would you describe your ancestry? \***

Please select one of the answers below  
*Mark only one oval.*

- Australian Aboriginal
- North-West European (including British, Irish, Western European, Northern European)
- Southern European (including Spanish, Italian, Portuguese)
- Torres Strait Islander
- New Zealander/Maori
- Melanesian and Papuan
- Micronesian
- Polynesian
- South-East Asian (including Burmese, Thai, Vietnamese, Indonesian, Malay etc)
- South Eastern European (including Albanian, Croatian, Greek, Cypriot etc)
- North East Asian (including Chinese, Japanese, Korean, Mongolian etc)
- Central Asian (including Afghan, Georgian, Kazakh, Turkmen etc)
- North American Aboriginal
- Central or South American
- Caribbean Islander
- Central, West, South and East African
- Mixed Race
- Other

7. **Please select your gender \***

Please select one of the answers below  
*Mark only one oval.*

- Male
- Female

8. **Which of the following best describes the occupation you had for the longest period? \***

Please select one of the answers below  
*Mark only one oval.*

- Manager
- Professional
- Technicians and Trades Workers
- Community and Personal Service Workers
- Clerical and Administrative Workers
- Sales Workers
- Machinery Operators and Drivers
- Labourer or related worker
- Other

9. **Which of the following best describes your current employment status? \***

Please select one of the answers below  
Mark only one oval.

- Unemployed
- Home duties
- Part time work - employed/self employed
- Full time work - employed/self employed
- Student
- Sole parent pension
- Disability pension
- Retired
- Other

10. **Which of the following best describes your current, main occupation? \***

Please select one of the answers below  
Mark only one oval.

- Mainly indoors (e.g. office worker)
- Half indoors and half outdoors (e.g. physical education teacher)
- Mainly outdoors (e.g. gardener)

11. **What is your natural eye colour? \***

Please select one of the answers below  
Mark only one oval.

- Blue, light blue, grey blue, blue-green
- Green
- Hazel, green hazel
- Brown, dark brown

12. **What is your natural hair colour (when you were 18 years old)? \***

Please select one of the answers below  
Mark only one oval.

- Red
- Blond
- Chestnut or dark blond
- Brown
- Black

13. **What is your natural (untanned) skin colour (on non-sun exposed skin)? \***

Please select one of the answers below  
Mark only one oval.

- Fair
- Medium
- Olive
- Dark/Black

14. **How does your skin react if you were to sit in the sun in your current area of residence, in the middle of the day, for the first time in summer, without sunscreen? \***

Please select one of the answers below  
Mark only one oval.

- Never burn  
 Burn after more than 2 hour sun exposure  
 Burn after 1 - 2 hours  
 Burn after 1/2 - 1 hour  
 Burn within half an hour

15. **How does your skin react if you were to sit in the sun in your current area of residence, for one hour in the middle of the day, for the first time in summer, without sunscreen? \***

Please select one of the answers below  
Mark only one oval.

- Burn then peel  
 Burn then tan  
 Tan only

16. **At the end of the summer or after a two week holiday in the sun, what kind of tan would you have? \***

Please select one of the answers below  
Mark only one oval.

- A dark tan  
 A medium tan  
 A light tan  
 Practically no tan

17. **About how many night shifts have you worked in the past month? \***

Please select one of the answers below  
Mark only one oval.

- 0  
 1 night shift  
 2 night shifts  
 3 night shifts  
 4 night shifts  
 5-7 night shifts  
 8-10 night shifts  
 11-13 night shifts  
 14-16 night shifts  
 17-19 night shifts  
 20+ night shifts

**We are interested in the CLOTHING AND SUN PROTECTION that you have worn in the PAST MONTH when you were outside. Did you wear or use...**

---

18. \*

Please tick the corresponding option - only one per line  
 Mark only one oval per row.

	Never	Rarely	Sometimes	Often	Almost always	Always
Hat, cap or other head covering?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Long sleeves?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Clothing that covers most of your legs?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sunglasses?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Umbrella in the sun?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

19. **Have you used sunscreen in the PAST MONTH, including moisturiser and make-up with sun protection factor (SPF)? \***

Please select one of the answers below  
 Mark only one oval.

- Yes  
 No

20. **What is the sun-protection factor (SPF) of the sunscreen that you have used most often in the PAST MONTH? Consider any product you may use that have a SPF.**

Please mark the corresponding circle – only one per line; if never used please leave blank  
 Mark only one oval per row.

	SPF15	SPF30	SPF50
Sunscreen	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Moisturiser	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Make-up	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Please check the number of serves that you have consumed for each food in the PAST MONTH**

---

21. **Fresh salmon (1 serve = 120g cooked, or about palm size) \***

type in the number of serves you had in the past month

.....

22. **Salmon, tinned (1 serve=100g)**

type in the number of serves you had in the past month

.....

23. **Fresh tuna (1 serve = 120g cooked, or about palm size)**

type in the number of serves you had in the past month

.....

24. **Tuna, tinned (1 serve=100g)**  
type in the number of serves you had in the past month

25. **Sardines (1 serve = 60g about ½ tin)**  
type in the number of serves you had in the past month

26. **Mackerel or Herring (1 serve = 100g)**  
type in the number of serves you had in the past month

27. **Milk or yoghurt fortified with vitamin D (1 serve = 250mL, 1 cup milk or yoghurt)**  
type in the number of serves you had in the past month

28. **Milk (1 serve = 250mL or 1 cup)**  
type in the number of serves you had in the past month

29. **Yoghurt (1 serve = 125g-160g)**  
type in the number of serves you had in the past month

30. **Cheese (1 serve = 40g, 2 slices)**  
type in the number of serves you had in the past month

*Skip to question 31.*

### **Time Outdoors In The Sun & Clothing Habits**

Select the box which best represents the amount of time that you spent in the sun during each one hour interval shown below



**31. Typical work/school day spent outdoor**

Still thinking in the PAST MONTH, please select the box which best represents the amount of time that you spent in the sun during each one hour interval in a typical week day  
*Mark only one oval per row.*

	0 mins	< 15 mins	15 - 29 mins	30 - 44 mins	45 - 60 mins
5 - 6 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7 - 8 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8 - 9 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9 -10 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10 - 11 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11 -12 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12 - 1 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
1 - 2 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2 - 3 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3 - 4 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4 - 5 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5 - 6 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**32. Typical NON-work/school day spent outdoor**

Still thinking in the PAST MONTH, please select the box which best represents the amount of time that you spent in the sun during each one hour interval in a typical weekend day  
*Mark only one oval per row.*

	0 mins	< 15 mins	15 - 29 mins	30 - 44 mins	45 - 60 mins
5 - 6 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7 - 8 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8 - 9 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9 -10 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10 - 11 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11 -12 am	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12 - 1 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
1 - 2 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2 - 3 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3 - 4 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4 - 5 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5 - 6 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6 - 7 pm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Type of Clothing Worn**

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Please select a number in each line

**Clothing Guide**

SUN DIARY CLOTHING GUIDE							
UPPER BODY	NO CLOTHING ON UPPER BODY						
	0 No upper clothing	1 Bikini	2 Swimsuit	3 Crop top	4 Singlet top	5 Short-sleeved top	6 Long-sleeved top
LOWER BODY	NO CLOTHING ON LOWER BODY						
	0 No lower clothing	1 Speedos/briefs	2 Shorts or short skirt	3 Medium shorts or 3/4 pants	4 Long trousers/jeans	5 Medium skirt	6 Long skirt
HEADWEAR	NO HEADWEAR						
	0 No headwear	1 Beanie	2 Cap	3 Legionnaire's cap	4 Bucket hat	5 Wide-brimmed hat	6 Veil/burkha
FOOTWEAR	NO FOOTWEAR/HANDWEAR						
	0 No footwear	1 Thong/open sandals	2 Semi-enclosed shoes	3 Enclosed shoes	4 Workboots	5 Long socks	HANDWEAR Gloves

**33. Thinking in the PAST MONTH, Please give a number for the type of clothing worn in a typical WEEK day**

Please refer to the above clothing guide  
Mark only one oval per row.

	0	1	2	3	4	5	6
Upper Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lower Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Headwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Footwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**34. Thinking in the PAST MONTH, please give a number for the type of clothing worn in a typical WEEKEND day**

Please refer to the above clothing guide  
Mark only one oval per row.

	0	1	2	3	4	5	6
Upper Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lower Body	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Headwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Footwear	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**35. At what age you noticed or were diagnosed with short-sightedness? \***

Please leave it as "0" if you are not short-sighted

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**36. Please state how many people in your immediate family have short-sightedness.**

If 1 of the parents have, please type in "1", if both have, enter "2"; if any siblings please also note.

.....

.....

.....

37. **What is the progression of your short-sightedness like each year? \***

Please tick "0" if you are not short-sighted or you are stable in progression  
*Tick all that apply.*

- 0
  - 0.25D to -0.75D
  - 1.00D to -1.50D
  - more than -1.50D
-