Effect of tear film lipid parameters in contact lens wear comfort

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Publication Date:
2015

DOI:
https://doi.org/10.26190/unsworks/18142

EFFECT OF TEAR FILM LIPID PARAMETERS IN CONTACT LENS WEAR COMFORT

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Optometry and Vision Science

The University of New South Wales, Sydney, Australia

And

Brien Holden Vision Institute, Sydney, Australia

September 2014

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Abstract 350 words maximum: (PLEASE TYPE)

This thesis aimed to assess the effect of exogenous lipid supplements on ocular comfort during contact lens wear and the biology underpinning those effects. A reliable device to measure the tear evaporation rate was developed and validated. Tear collection methods were optimised to characterise the tear lipidome. A pilot study demonstrated that the tear film stability during short-term contact lens wear was associated with ocular comfort, the activity of phospholipase A_2 enzyme ($sPLA_2$) and the concentrations of a lipid aldehyde, malondialdehyde (MDA). After 6-8 hours of lens wear, as the tear film stability increased so did the mole% of wax esters in the total lipidome whereas the mole% of cholesterol esters decreased. Higher tear evaporation rates were associated with reduced levels of phospholipids. The study also provided preliminary evidence for the effectiveness of an exogenous lipid supplement in improving ocular comfort and tear film stability during short-term contact lens wear.

In the light of these preliminary findings, a double-masked, randomised crossover placebo controlled intervention study was conducted among habitual contact lens wearers to investigate various aspects of the lipid layer following the administration of two formulations of tear lipid supplements and their respective vehicle placebo. A transient improvement in tear film stability with the two lipid supplements was observed in symptomatic contact lens wearers. However, the improvement did not persist by the end of 2-week long lens wear. Between the two supplements, the anionic phospholipid emulsion drop showed a superior effect in ocular comfort, lipid layer appearance and tear evaporation rate in symptomatic wearers compared to the zwitterionic phospholipid spray. Improved contact lens wear comfort was related to increased tear film stability and lipid layer thickness, and a reduced tear evaporation rate, $sPLA_2$ concentration and activity in tears. Increased concentration of $sPLA_2$ was also associated with higher concentrations of degraded phospholipids and reduced levels of (O-acyl)-ω-hydroxy fatty acids in tears.

The study found significant associations between various aspects of the lipid layer and comfort of the eye. These associations suggests that alterations in lipid biochemistry might be modulating changes in clinical and functional aspects of lipid layer which triggers changes in lens wear comfort. The learnings may help to inform development of topical preparations to improve comfort. Thus, the thesis provided an evidence based assessment of the effect of topical lipid preparations on ocular comfort by a comprehensive exploration of the clinical, functional and biochemical aspects of tear lipid layer.

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ACKNOWLEDGEMENTS

My journey in pursuit of a PhD is ending. It has been a wonderful experience and I owe to the support and prayers of many people that made my journey comfortable. My gratitude is beyond these two pages, however, I will do my best to brief.

I thank my supervisors Prof Fiona Stapleton and Prof Mark D Willcox for their immense guidance throughout my candidature. Fiona, you have no idea how important your role was in moulding me into a better researcher from day one. Your expertise and in-depth knowledge in the field was a great advantage during my candidature. Your gentle approach and encouraging words was the energy drink at every difficulties of my journey. Mark, you are an element for inspiration. I have always looked up to you to build my confidence and discipline for the past few years. The weekly meetings with both of you were always an enlightenment. I find myself very lucky to have you both as the mentors in my journey. I thank Dr Klaus Ehrmann for providing me with all the technical assistance for my research. Klaus your simplicity have inspired me greatly. I thank you for all the unscheduled meetings and your brilliant solutions for all the problems; it was truly a delight to work with you. I would also like to thank Dr Simon Brown, Associate Prof Todd Mitchell and Amanda Scora at the University of Wollongong for their immense help with the mass spectrometer analysis.

I thank the University of New South Wales and the Brien Holden Vision Institute for the opportunity, financial support and resources for my candidature. I sincerely acknowledge the Cornea and Contact Lens Society of Australia and the Optometric Vision Research Foundation for providing the research scholarships during my PhD. Viv, you have supported me much more than your role as a postgraduate project manager and I thank you sincerely. Prof Eric Papas and Dr Judith Flanagan have been a

huge support during my candidature, especially during review periods. Eric, thank you for your critical reviewing of my documents and manuscripts. Judith, thank you so much for being the victim of my emotional breakout and always reserving a tissue box for me at your office. I thank Dr Thomas John and Dr Varghese Thomas for helping me with the statistical analysis. I thank the entire biological sciences faculty in level 3, especially Amali for introducing me to the world of biochemistry. I would like to express my gratitude to the staff at the clinical research and trials centre for the smooth running of my clinical trials. I thank the entire staff at I-media for their assistance in making beautiful posters and posting advertisements for participant recruitment.

I value friendship as one of the most beautiful relation in the world and I have been very fortunate to have many of them. I am grateful to my fellow postgraduates Andrea, Carolina, Simin, Waleed, Tina and Ling for a healthy and fun work environment. Maria, I owe you a lot. You not only have been an academic advisor throughout my candidature but also a good friend. Thank you Moneisha, Kalika, Swetha, Nisha, Lakshmi, Swathi, Preeji mam, Mou, Debarun, Ravi for all the get togethers we had, it was truly fun to entertain all of you. Jaya and Krupa chechi, thank you for those memorable moments in PG room. Arthi, I would like to thank you specially for taking care of me like a big sister. Vinod, thank you so much for helping me with the thesis formatting. Special mention to my dear Shailavi and Aarnav for bringing out the child in me. Thanks to Indrani and Eon for the beautiful lunches together and the friendship that grew within us, I will cherish those memories forever. Thank you Sneha, Sailesh, Ankita and Bawa for your love and thank you Rito for being there as my little sister. I would like to extend my gratitude to my favourites Tintu and Preethu for their love and care throughout.

Finally, I would like to thank, the most important segment, the reason of my existence, my family. Appa and amma, I truly admire you for the unconditional love and support and the freedom that you have given me my entire life. I thank you with the bottom of my heart for making me whatever I am now. Ouseppacha, thank you for the special bond that we share. Thank you Ammachi for your endless prayers for our wellbeing.

I have reserved this final section for the most important person, the love of my life, Rohit Pious. I have no idea how I would have survived without you. Mashumone, I am aware of the huge sacrifices you made, the patience you have shown, the many dinners you cooked and most importantly the moral support that you gave. My gratitude is beyond words, I thank you for being there as a strong, pillar of support. I owe you the most and yes, I can proudly say that we are made for each other.

To my little angel Nyra (who is just a week old), a big thank you for this amazing experience. Mamma and Dada feels so blessed with your arrival.

Above all, I bow down to my Guru…my Consciousness…The Almighty.

DEDICATION

I dedicate this thesis to,

Appa, Amma, Ouseppachan

&

Mashumon

ABSTRACT

This thesis aimed to assess the effect of exogenous lipid supplements on ocular comfort during contact lens wear and the biology underpinning those effects. A reliable device to measure the tear evaporation rate was developed and validated. Tear collection methods were optimised to characterise the tear lipidome. Electro-spray tandem mass spectrometry was used to analyse individual lipid components. A pilot study demonstrated that the tear film stability during short-term contact lens wear was associated with ocular comfort, the activity of phospholipase A_2 enzyme ($sPLA_2$) and the concentrations of a lipid aldehyde, malondialdehyde (MDA). After 6-8 hours of lens wear, as the tear film stability increased so did the mole% of wax esters in the total lipidome whereas the mole% of cholesterol esters decreased. Higher tear evaporation rates were associated with reduced levels of phospholipids. The study also provided preliminary evidence for the effectiveness of an exogenous lipid supplement in improving ocular comfort and tear film stability during short-term contact lens wear.

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increased tear film stability and lipid layer thickness, and a reduced tear evaporation rate, $sPLA_2$ concentration and activity in tears. Increased concentration of $sPLA_2$ was also associated with higher concentrations of degraded phospholipids and reduced levels of (O-acyl)-ω-hydroxy fatty acids in tears.

The study found significant associations between various aspects of the lipid layer and comfort of the eye. These associations suggests that alterations in lipid biochemistry might be modulating changes in clinical and functional aspects of lipid layer which triggers changes in lens wear comfort. The learnings may help to inform development of topical preparations to improve comfort. Thus, the thesis provided an evidence based assessment of the effect of topical lipid preparations on ocular comfort by a comprehensive exploration of the clinical, functional and biochemical aspects of tear lipid layer.

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CE	Cholesterol ester
$ChCl_3$	Chloroform
Chl	Free cholesterol
CLDEQ	Contact lens dry eye questionnaire
cm^2	Centimeter square
DE	Diesters
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immuno assay
ESI	Electrospray ionization
FFA	Free fatty acid
GC	Gas chromatography
g	Gravitational force
$g/cm^2/sec$	Gram per centimeter square per second
$g/m^2/h$	Gram per meter square per hour
HA	Hyaluronic acid
HEMA	Hydroxyethyl methacrylate
HNE	Hydroxy-nonenal
HPLC	High performance liquid chromatography
HREA	Human research ethics advisory
IBM	International business machines
LC	Liquid chromatography
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
MDA	Malondialdehyde
MeOH	Methanol
MS	Mass spectrometry
µg	Micrograms
µm	Micrometers

µl	Microliters
µM	Micromolar
$mg/cm^2/min$	Milligram per centimeter square per minute
ml	Milliliters
Mm	Millimolar
mOsms/l	Milliosmol per liter
nm	Nanometers
NISDT	Non-invasive surface drying time
NMR	Nuclear magnetic resonance
OAHFA	(O-acyl)-ω-hydroxy fatty acid
OCI	Ocular comfort index
pH	Measure of acidity or basicity
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PS	Phosphatidylserine
pg	Picograms
pmol/µl	Picomole per microliters
PMMA	Polymethyl methacrylate
r	Correlation coefficient
R^2	Coefficient of determination
ROS	Reactive oxygen species
SD	Standard deviation
SE	Standard error
$sPLA_2$	Secretory phospholipase enzyme
SM	Sphingomyelin
SPSS	Statistical package for social sciences
TAG	Triglyceride
TFOS	Tear film and ocular surface society
TLC	Thin layer chromatography
UNSW	The University of New South Wales
USA	The United States of America
Vol	Volume

WE	Wax ester
w/v	Weight per volume
~	Approximately
°C	Degree Celsius
>/<	Greater than/less than
×	Multiplication sign
%	Percentage
®	Registered sign
™	Trademark sign

CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

Since the era of thick glass shells (Efron *et al.* 1988) to the current flexible silicone hydrogels (Wichterle *et al.* 1960, Brennan *et al.* 2007), contact lenses have undergone immense advancements. Worldwide, approximately 140 million people use contact lenses for vision correction (Bennett 2011, Dumbleton *et al.* 2013) with 85% wearing hydrogel or silicone hydrogel soft lenses (Morgan *et al.* 2011). Despite the improved physiological characteristics of the new generation contact lenses, lens related discomfort and dryness remains unresolved leading to a significant proportion of wearers to discontinue lens wear (Pritchard *et al.* 1999, Begley *et al.* 2000, Ozkan *et al.* 2008, Maissa *et al.* 2012). Based on the report from the Tear Film and Ocular Surface society (TFOS) on contact lens discomfort (Dumbleton *et al.* 2013, Jones *et al.* 2013), factors that contribute to lens wear discomfort include lens characteristics such as wettability, ionicity, curvature and edge thickness, lens care regimen, individual characteristics such as tear film and lid interactions with lenses, and environmental factors such as humidity and air quality.

This thesis aims to elucidate the potential role of the tear lipid layer in contact lens wear discomfort. Chapter 1 is a literature review describing the significance of the tear lipid layer in ocular comfort during soft contact lens wears. This chapter also reviews the role of exogenous lipid supplements in modulating the clinical, functional and biochemical aspects of tear lipid layer in contact lens wearers.

1.2 TEAR FILM

The tear film is a thin transparent layer that lies over the anterior ocular surface. Several structural models of the tear film have been proposed (Ehlers 1965, Holly *et al.* 1977, Prydal *et al.* 1992, Dilly 1994, Creech *et al.* 1998). Although, Wolff (1946) described the tear film as a three-layered structure comprising an outer lipid layer, a middle aqueous component and an inner mucin layer, the current view of the tear film suggests an aqueous phase with a mixture of proteins and gel-forming mucins, and a thin lipid layer that outlines the aqueous phase (King-Smith *et al.* 2004). Evidence based on the reflection spectrum (King Smith *et al.* 2000), shows that the thickness of the tear film layer is approximately 3 μm. Apart from nourishing the avascular corneal surface by transferring atmospheric oxygen and other nutrients, the tear film protects the ocular surface against invasion by pathogens or other foreign substances (Lemp *et al.* 1992). The tear film also provides a quality optical surface and lubricates the ocular surface (Lemp *et al.* 1992).

1.2.1 THE MUCIN LAYER

The mucin layer, which is the deepest, is primarily secreted by goblet cells of the conjunctiva. The mucin layer is comprised with tightly bound proteins named glycoproteins as the inner layer and a looser mucin blanket as the top layer (Nichols *et al.* 1985). The distribution of unicellular goblet cells that produce secreted mucin is maximum around the nasal conjunctiva and minimum at the superior portion of the temporal bulbar conjunctiva (Kessing 1968). Mucins are spread by the mechanical action of the eyelids and play an important role in the viscosity of tears (Raj Kaura 1986). The major functions of the mucin layer include maintaining hydration of the tear film and minimising the friction between lid margins and conjunctiva by inherent

lubricating and anti-adhesive properties (Gipson 2004, Argueso 2013). Gel forming mucins also act as a barrier by preventing contact of the ocular surface by pathogens or foreign bodies (Gipson *et al.* 2003). The mucin layer traps foreign bodies and flushes them out through the lacrimal drainage system with the blinking mechanism (Gipson *et al.* 2003).

1.2.2 THE AQUEOUS LAYER

Approximately 90-95% of the aqueous is secreted by the acini and ducts of the main lacrimal gland, while the rest is produced by the accessory glands of Krause and Wolfring (Lemp *et al.* 1992). The aqueous layer is rich in electrolytes and hydrogen ions and these determine the tear pH and osmolality of tears. Antibacterial enzymes such as lysozyme and lactoferrin, immunoglobulins, and the lipid binding protein lipocalin (which has a significant role in providing tear film stability) are a few of the numerous protein molecules found in aqueous tears (Gachon *et al.* 1979). Metabolites such as glucose and urea which are transported from serum also contribute to aqueous layer composition (Schoenwald *et al.* 1998).

1.2.3 THE LIPID LAYER

The holocrine meibomian glands located in the tarsal plate of the upper and lower eyelid margins are the main source of the tear lipid layer (Lemp *et al.* 1992). A minor amount of lipid is secreted by the glands of Moll and Zeis (Lemp *et al.* 1992). The spreading of lipid components on to the ocular surface is aided by blinking and this forms the tear lipid layer (Tiffany 1997). However, the concentration of different components of the tear lipid layer might not be analogous to that of the lipid profile of the original meibum (Tiffany 1997) and this is further discussed in section 1.3.3.

Several models for the structure of the tear lipid layer have been proposed in the literature. A bilayer structure was initially described by Holly (1973) and further detailed by Shine and McCulley (1997) as having an inner thin polar phase and an outer thick non-polar phase (Figure 1-1). Wax and cholesterol esters form the bulk of the non-polar lipid layer along with triglycerides, free fatty acids and diesters. The non-polar phase depends highly on the structural consistency of polar phase comprising many subclasses of phospholipids, sphingolipids and a class of amphiphilic lipids called (O-acyl)-ω-hydroxy fatty acid (OAHFA) (McCulley *et al.* 1997, Lam *et al.* 2011).

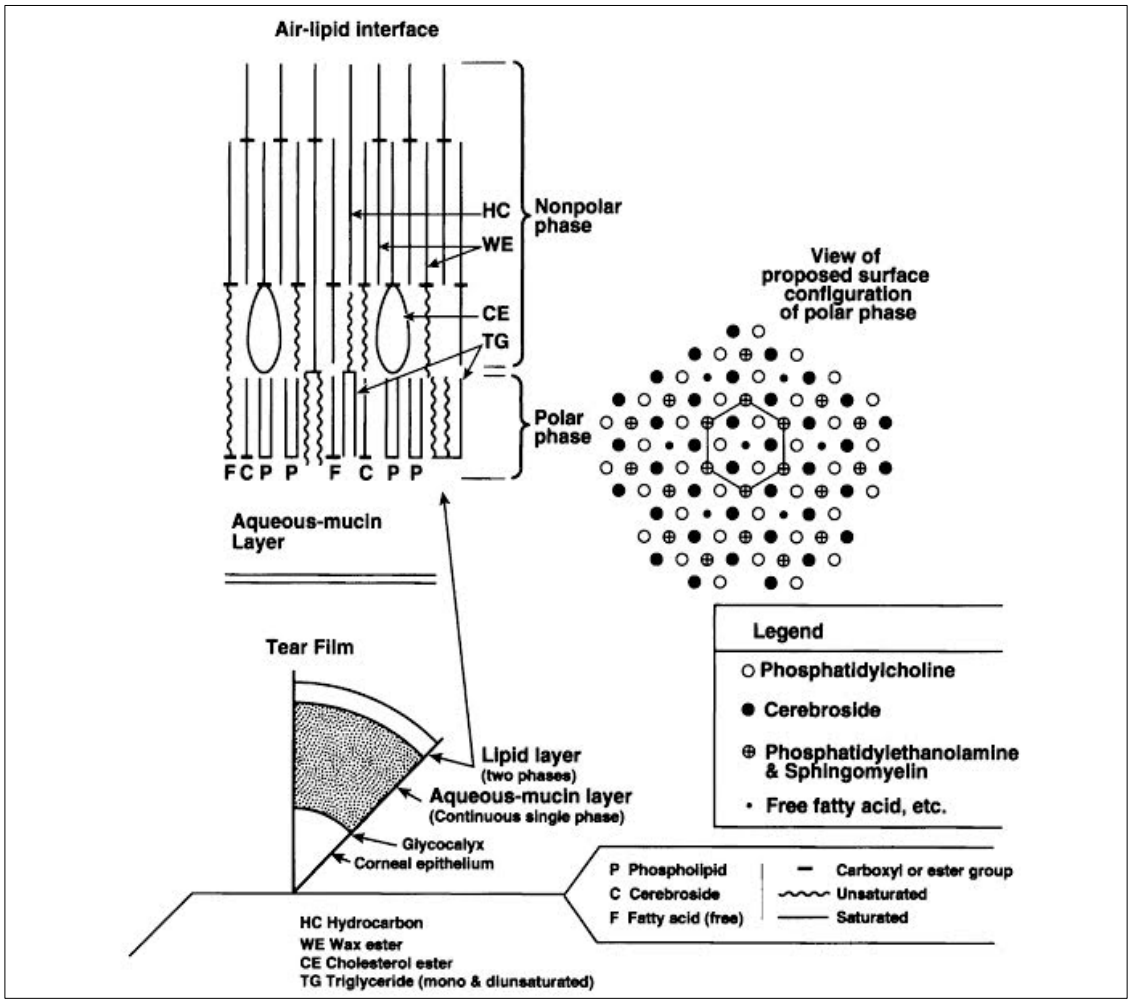


Figure 1-1 The bilayer tear lipid model proposed by McCulley and Shine (1997). Reproduced with permission

Evidence from models of the tear lipid layer using Langmuir troughs and pendant drops indicated that tear proteins such as tear lipocalin, lysozyme, lactoferrin and immunoglobulins are surface active and interact with the lipids in this layer (Miano *et al.* 2005, Tragoulias *et al.* 2005). Thus, a modified version of the two layered lipid layer with intercalated proteins in the polar lipid inner layer and a thicker outer lipid layer has been proposed (Butovich *et al.* 2008). Recently King-Smith *et al.* (2013) proposed a 'multilamellar sandwich model' of the tear lipid layer by comparing biological lipid layers involved in prevention of evaporation. In the sandwich model, there are dense arrays of long, saturated chains of non-polar components responsible for prevention of evaporation and a polar lipid interface between non-polar lipids and the aqueous layer (Figure 1-2).

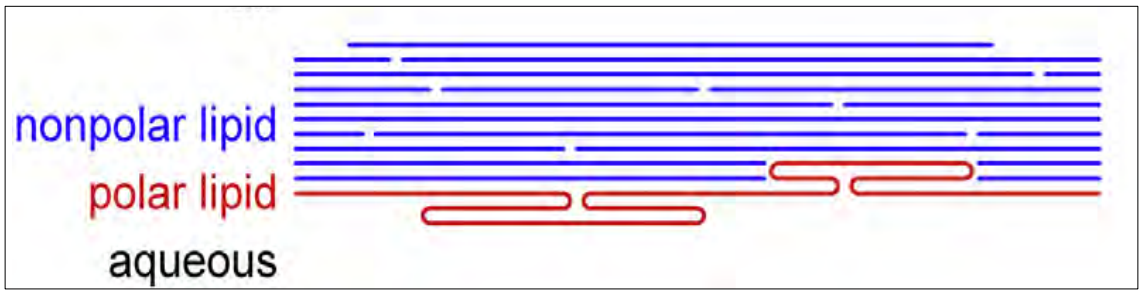


Figure 1-2 The multilamellar sandwich model proposed by King Smith *et al*. (2013). Reproduced with permission.

In contrast a further model has been proposed based on the rheological and structural properties of human and bovine meibomian lipids (Rosenfeld *et al.* 2013). Rosenfeld and colleagues stated that the tear lipid layer was composed of lipid components in the form of lamellar-crystallites immersed in a liquid phase in a random order. The proposed model includes tear proteins adsorbed at the aqueous-lipid interface that

shows liquid, air-lipid and aqueous-lipid interface properties (Figure 1-3). However, the authors recommend further validation for the proposed models.

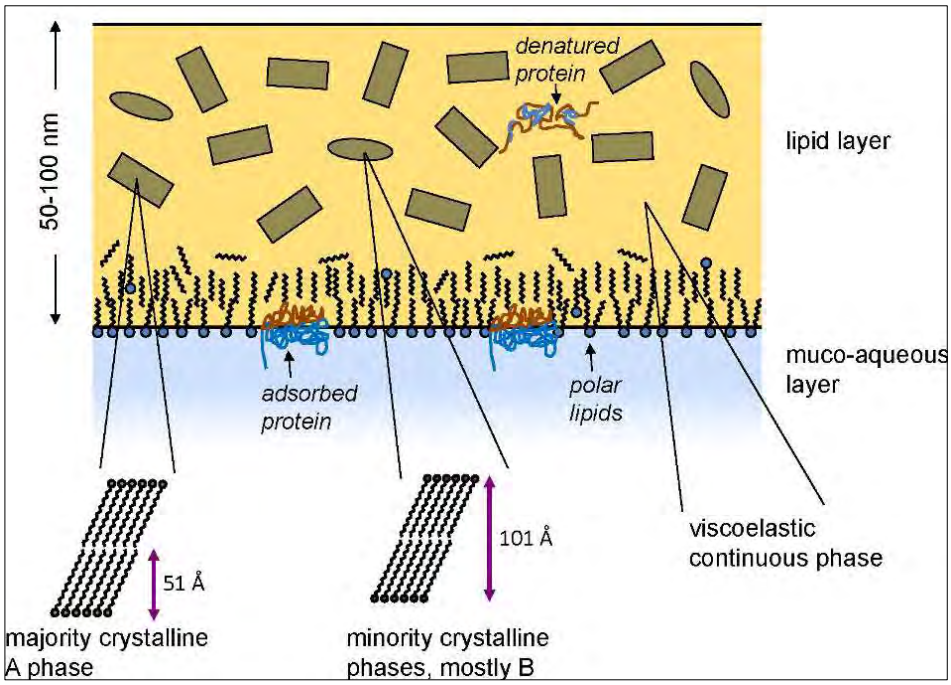


Figure 1-3 The viscoelastic duplex tear lipid model proposed by Rosenfeld *et al.* (2013). Reproduced with permission

The early work of Mishima *et al.* (1961) and Iwata *et al.* (1969) in rabbits suggested that the superficial lipid layer prevents or slows down tear evaporation. This has been studied subsequently *in-vivo* in humans by Craig *et al.* (1997) where they reported that an absent or a disrupted lipid layer increases tear evaporation rate by four fold. Other functions of the lipid layer include limiting the spillage of tears on to the eyelids (McDonald 1968) and protecting the tear film from the destabilizing effect of skin lipid contamination (Norn 1966).

1.3 CONTACT LENS WEAR AND THE LIPID LAYER

A contact lens on the eye compartmentalises the tear film into two layers; the pre-lens and post-lens tear films. While the pre-lens tear film is the distance between the air surface of tears and the contact lens surface, the post-lens tear film is defined as the distance from behind the posterior lens surface to the cornea (Holly 1981, King-Smith *et al.* 2004). An *in-vivo* wavelength - dependent interferometry technique has shown that the thickness of pre and post lens tear films to be 2.31 and 2.34 μm respectively (Nichols *et al.* 2003b). Maintaining the quality of pre and post lens tear film is critical for successful contact lens wear as the former provides contact lens hydration and a clear optical surface while the latter plays a key role in corneal oxygen transmission (Wagner *et al.* 1980, Guillon 1986, Tutt *et al.* 2000).

In the presence of contact lens wear, the aqueous and mucin layers undergo changes, as lens wear can alter mucin production (Versura *et al.* 1986), aqueous flow rate (Tomlinson 1992) and the concentration of certain tear immunoglobulins (Temel *et al.* 1991). Tear film alterations during lens wear might be associated with a variety of downstream changes such as lid wiper epitheliopathy (Korb *et al.* 2002b) and changes in tear osmolality (Stahl *et al.* 2009) and tear inflammatory mediators (Tan *et al.* 1997). This thesis however describes the changes to the tear film lipid layer only, when a contact lens is introduced to the ocular surface. The literature reviewed in the current chapter is based on a broad classification of lipid layer in terms of its clinical, functional and biochemical aspects. Clinical aspects of the tear lipid layer include the appearance of the lipid layer and its stability whereas preventing evaporation of aqueous tears refers to its function, and biochemical aspects refer to the concentrations of and change to the tear lipid components.

1.3.1 CLINICAL IMPLICATIONS

1.3.1.1 Lipid layer appearance

Hamano *et al.* (1980a) observed the interference patterns of a normal tear film lipid layer by using a bio-differential interference microscope. The three patterns seen were a network structured marble-like pattern, a wave-like structured flow pattern and amorphous pattern with no specific structure. They also observed an interference fringe pattern over the surface of a hard contact lens and a very short-lived interference pattern over a soft contact lens surface. With the development of the Tearscope by Guillon (1988) the lipid layer patterns were categorised into different grades such as open meshwork (grade 1), closed meshwork (grade 2), flow (grade 3), amorphous (grade 4) and colour fringe patterns (grade 5) in order of increasing visibility and thickness (Figure 1-4). Other patterns associated with ocular pathologies such as meibomian gland dysfunction and keratoconjunctivitis, or with eye make-up contaminations often appear as abnormal colour fringes which are generally referred as globular patterns (Guillon *et al.* 1994). A specular microscopic observation (Yokoi *et al.* 1996) of the central cornea of healthy and dry eye individuals provided tear lipid interference patterns which were classified into five grades based on the colour and uniformity. The five categories were, somewhat grey colour with uniform distribution (grade 1), somewhat grey colour with non-uniform distribution (grade 2), a few colours with non-uniform distribution (grade 3), many colours with non-uniform distribution (grade 4) and corneal surface partially exposed (grade 5) (Yokoi *et al.* 1996). The LipiView ocular surface interferometer is another instrument that utilises a specular reflection technique from which tear film interference patterns are captured and thickness estimated. In addition to viewing different interference images, blinking pattern can also be evaluated (Blackie *et al.* 2009, Korb *et al.* 2012b). It is challenging to confine lipid

layer patterns into a single grading system because of their heterogeneity. However, Remeseiro *et al.* (2012) examined the lipid layer grading system of Guillon and based on texture analysis and machine learning process, was able to assign lipid layer patterns that previously were not able to be classified into one of the six Guillon categories, thus validating the original classification system.

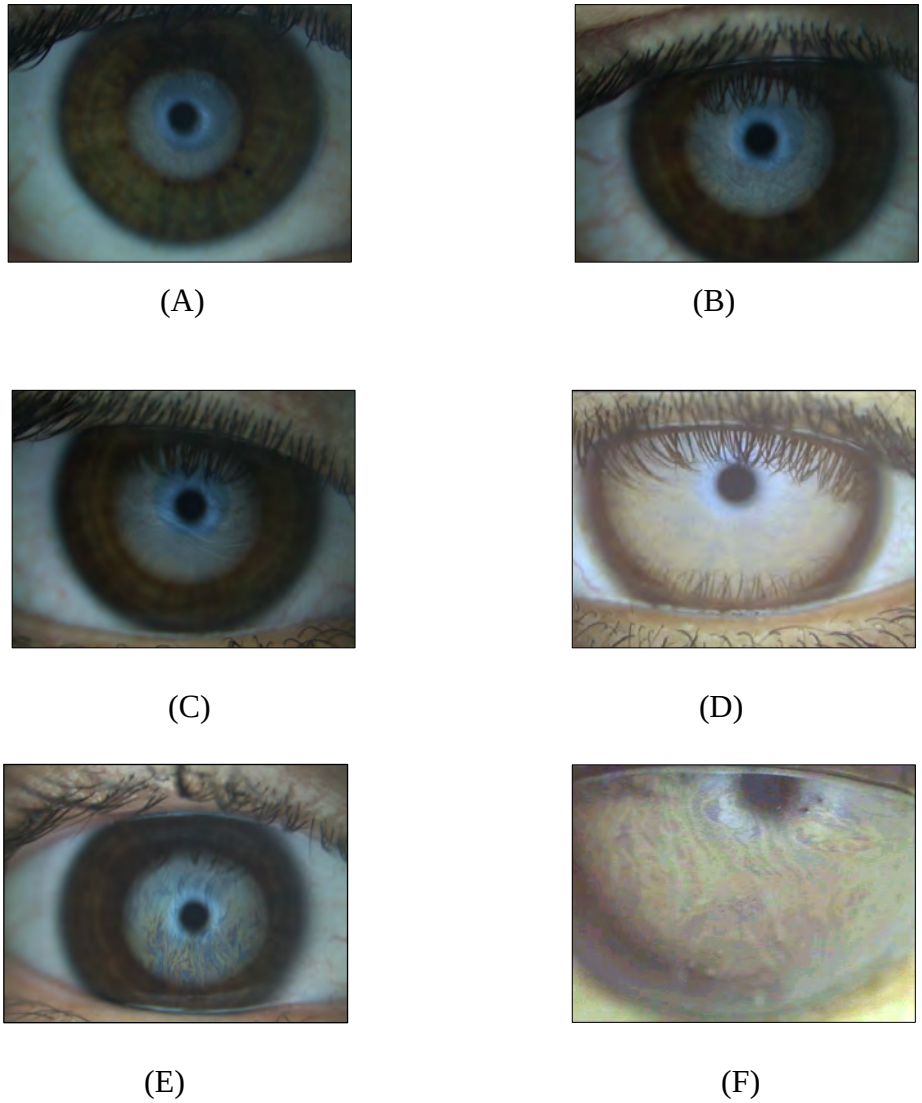


Figure 1-4 Tear lipid layer classification by Guillon. (A) Open Meshwork (~15 nm), (B) Close Meshwork (~30 nm), (C) Wave pattern (~30-80 nm), (D) Amorphous (~80 nm), (E) Colour fringes (~80-300 nm), (F) Abnormal colour fringes (~600 nm). Reproduced with permission from Guillon M and Remeseiro *et al*. (2012)

An association between the lipid layer pattern and tear film stability has been demonstrated in the absence of a contact lens, where the thin open meshwork pattern (grade 1) or no lipid layer (grade 0) was associated with poor tear film stability, and a thicker amorphous pattern (grade 4) was associated with enhanced tear film stability (Craig *et al.* 1997). A subjective improvement in ocular comfort with increased lipid layer thickness due to increased lipid flow secondary to an intervention with a latent heat device was reported in a non-contact lens wearing population (Mitra *et al.* 2005). The viscoelastic property of the tear lipid layer measured by observing the speed of tear lipid layer spreading has been proposed as a sensitive diagnostic test for aqueous deficient dry eye disease (Yokoi *et al.* 2008). The velocity of tear lipid layer spreading after a blink reduced proportionately with the decrease in tear volume (Yokoi *et al.* 2008).

Wearing a contact lens disturbs the lipid layer. The pre-lens lipid layer is altered over a soft hydrogel lens and is not visible over a rigid lens (Guillon 1986). Over a soft hydrogel lens, the lipid layer is commonly absent or shows an open meshwork appearance, which is the thinnest at approximately 15 nm (grade 1), whereas the pre-corneal lipid layer appearance is commonly a wave or amorphous pattern approximately 30-80 nm thick, (grade 3-4) (Guillon *et al.* 1994). A thickness dependent fringe imaging interferometer used to assess the lipid layer distribution of a large sample of contact lens wearers (n=360) showed thinner pre-lens lipid layer patterns among symptomatic lens wearers (based on the scores on the contact lens dry eye questionnaire) compared to asymptomatic wearers (Nichols *et al.* 2006). However, the non-linear clinical grading of the lipid layer pattern does not appear to be sufficiently sensitive to changes in contact lens comfort. Glasson *et al* (2003) found that there was no difference in the tear film lipid layer appearance/thickness between tolerant lens

wearers and intolerant lens wears in the absence of lens wear. Tolerance in this study was described as people who could not wear vifilcon A lenses for longer than 6 hours without reporting symptoms of discomfort. Similarly, the pre-lens (vifilcon A lenses worn for 6 hours) lipid layer appearance in these tolerant and intolerant contact lens wearers was similar, showing a closed meshwork appearance (approximately 30 nm) (Glasson *et al.* 2006). The pre-lens (various hydrogel lenses including some silicone hydrogel lenses) lipid layer grades did not change even when there was an improvement in contact lens comfort after oral intake of omega-6 capsules for 6 months (Kokke *et al.* 2008). The pre-corneal tear film lipid layer pattern before and after two weeks wear of a silicone hydrogel lens showed no significant difference in thickness using tear lipid interferometry (Dogru *et al.* 2011).

Conversely, in a study of the *in-vivo* wetting performance of a silicone hydrogel contact lens (galyfilcon A replaced 2 weekly) and a hydrogel contact lens (alphafilcon A replaced either 2 or 4 weekly), the pre-lens lipid layer, whilst still thin, was thicker and the lens wear comfort was better with the silicone hydrogel contact lens (Guillon *et al.* 2007). However, the authors of the former study state that the wetting performance of the silicone hydrogel lens in their study was solely the material property of that lens and is not generalisable for all silicone hydrogel lenses. By tracking the movement of reflective particles in the tear film, the speed and spread of the lipid layer over a silicone hydrogel lens surface (*in-vivo*) was assessed among habitual contact lens wearers (Varikooty *et al.* 2012). Lipid layer spread significantly reduced in speed following 8 hours of lens wear (Varikooty *et al.* 2012). The impaired spreading of lipid layer over a hydrophobic surface of a silicone hydrogel material could be attributed to the lack of tear volume during lens wear (Lorentz *et al.* 2007). A lower tear volume has been observed among a non-lens wearing dry eye population (Mainstone *et al.* 1996) and

symptomatic habitual contact lens wearers (Chen *et al.* 2011) and was associated with the discomfort and dryness symptoms of each group.

1.3.1.2 Tear film stability

Pre-corneal tear break up time is considered a clinical measure of tear film stability (Vanley *et al.* 1977, Craig *et al.* 2013). Tear break up time is defined as the interval between the formation of any tear film discontinuity in the form of a dry spot or streaks following a complete blink (Rengstorff 1974, Norn 1981). Although several theories and observations exist regarding the mechanism behind tear break up, the current consensus favours tear evaporation as the major factor contributing to tear film thinning and tear break up (Holly 1973, Korb *et al.* 1979, King-Smith *et al.* 2008, King Smith *et al.* 2009). A detailed review on tear evaporation rate and its implications during contact lens wear is discussed further (1.3.2). Though tear break up time is considered as a direct indicator of tear film stability, it seems variable within and between individuals depending upon the invasiveness of the technique used and the environment in which it is measured (Stodtmeister *et al.* 1983, Vitali *et al.* 1994, Cho *et al.* 1995, Maruyama *et al.* 2004, Abusharha *et al.* 2013). Tear break up time measured non-invasively and at a normal humidity (40%) was longer when compared to invasive techniques and at low humidity (5%) (Cho *et al.* 1995, Maruyama *et al.* 2004, Abusharha *et al.* 2013, Madden *et al.* 2013). The repeatability of three instruments (a videokeratoscope, a Tearscope and the traditional slit lamp method using topical sodium fluorescein) that measured tear break up time was compared at a temperature of 19-21℃ and humidity between 58-76%. The videokeratoscope was the least and the Tearscope was the most repeatable instrument (Elliott *et al.* 1998).

Tear film stability reduces with age and was observed to be lower in females compared to males (Patel *et al.* 1989, Cho *et al.* 1993b, Patel *et al.* 2000). However, Craig *et al.* (1998) did not observe any effect of age but observed that females had a lower tear break up time (23.8±22.1 seconds) than males (31.3±25.4 seconds) (p=0.02). Evidence suggests that tear film stability also depends on ethnicity (Cho *et al.* 1993a, Patel *et al.* 1995) with Chinese people exhibiting lower tear break up times (9.8±3.9 seconds) when compared to Africans (11.8±5.9 seconds), Indians (16.4±6.9 seconds) or Caucasians (19.9±8.3 seconds). In addition, tear break up time measured during the morning hours (9-10 am) was longer (6.6±2.6 seconds) than measured during the afternoon hours (5-6 pm) (5.4±2.3 seconds) among healthy individuals (Lira *et al.* 2011). Abnormalities of the ocular surface and eyelid pathologies also reduced tear film stability (Balogun *et al.* 2005, Bekibele *et al.* 2008, Tong *et al.* 2010).

Contact lens wear adversely affects tear film stability. Pre-lens tear break up time is referred to as the non-invasive surface drying time (NISDT) as it is the time recorded when an initial dry spot appears on the contact lens surface following a blink (Morris *et al.* 1998). The NISDT of a rigid gas permeable lens is 4-6 seconds whereas that of a soft contact lens surface is 7-9 seconds (Guillon *et al.* 1989b, Guillon *et al.* 1990). When the NISDT of five soft contact lens materials (polymacon, omafilcon A, phemfilcon A, balfilcon A and etafilcon A) were compared, it was significantly lower (p<0.05) compared to the pre-corneal tear break up time irrespective of the ionicity and water content of the hydrogel materials (Faber *et al.* 1991, Thai *et al.* 2004). On average, the pre-corneal tear break up time reduced from 21.0±19.7 seconds to an NISDT of 5.6±4.6 seconds equivalent to a 4 times reduction in stability (Thai *et al.* 2004). An association has been established between tear film lipid layer thickness and tear break

up time, where thicker lipid layers provide longer tear break up time than thinner lipid layer patterns in a non-contact lens wearing population (Craig *et al.* 1997). During contact lens wear, tear lipid layer thickness and tear film stability is significantly reduced (Guillon *et al.* 1997, Nichols *et al.* 2003a, Wang *et al.* 2003). The thinning of the lipid layer and impaired tear film stability might be due to lipid deposition on the contact lens surface leading to reduced wetting of the lens surface (Nichols *et al.* 2006).

Reduced tear film stability in contact lens wearers has been associated with decreased lens wear comfort in both hydrogel and silicone hydrogel lens wearers (Fonn *et al.* 1999, Glasson *et al.* 1999, Wolffsohn *et al.* 2010, Dogru *et al.* 2011, Sengor *et al.* 2011a). A few studies have reported improved tear film stability and reduced ocular dryness and discomfort symptoms with silicone hydrogel lens materials compared to hydrogel lenses (Santodomingo-Rubido *et al.* 2006, Kojima *et al.* 2011). Pre-corneal tear break up time and ocular symptomatology remained unchanged after 18 months of silicone hydrogel lens wear (lotrafilcon A and balafilcon A) among a group of neophyte silicone hydrogel contact lens wearers (Santodomingo-Rubido *et al.* 2006). Following exposure to an environment equivalent to a dry, windy and cool day, the pre-corneal tear break up time of hydrogel lens wearers (etafilcon A) significantly reduced (10.0±4.7 to 6.0±2.8 seconds) whereas that of silicone hydrogel lens wearers (narafilcon A) did not change significantly (7.9±3.9 to 7.2±3.2 seconds) (Kojima *et al.* 2011). However, Fonn *et al*. (2003) observed similar ocular dryness and discomfort symptoms of hydrogel and silicone hydrogel lens wearers. A NISDT of <3 seconds is considered to result in tear dysfunction and to cause contact lens-related dry eye symptoms (Hom *et al.* 2009). In a study where various clinical parameters for the predictive function in differentiating symptomatic and asymptomatic lens wearer were investigated, tear film stability and tear volume appeared to be the most important (Glasson *et al.* 1999).

Similarly, tear film stability was one of the major discriminants in contact lens-related dry eye among a group of new contact lens wearers (Pult *et al.* 2009).

1.3.2 FUNCTIONAL IMPLICATIONS

The principal function of the lipid layer is believed to be to retard the evaporation of tears from the aqueous phase. Along with the lipid layer, tear proteins and mucins might also play a role in preventing evaporation (Herok *et al.* 2009). Tear evaporation rate is considered as a functional indicator of the tear film lipid layer stability (Mathers 1993, Shimazaki *et al.* 1995, Mathers *et al.* 1998, Khanal *et al.* 2008). Higher tear evaporation rates are associated with increased tear thinning rate, resulting in ocular dryness and discomfort (Rolando *et al.* 1983b, Mathers *et al.* 1996a, Khanal *et al.* 2008).

Initial experiments to measure the rate of tear evaporation used rabbits subjected to invasive techniques such as pumping dry air close to the corneal surface under environmental conditions in which neither humidity nor temperature was controlled (Mishima *et al.* 1961, Iwata *et al.* 1969). Hamano *et al.* (1980b) measured the evaporation rate from humans using an instrument adapted from dermatology. However, this technique involved touching the corneal surface, which could possibly result in reflex tear secretion and overestimation of evaporation rates. Mathers (2004) and Herok *et al.* (2009) reviewed non-invasive and controlled measurement techniques of evaporation rates undertaken by several investigators including a pressure gradient technique and resistance hygrometry, using humidity and temperature sensors and infrared thermography (Hamano *et al.* 1981, Cedarstaff *et al.* 1983, Rolando *et al.* 1983a, Trees *et al.* 1990, Tsubota *et al.* 1992, Mathers *et al.* 1993, Goto *et al.* 2003, Arciniega *et al.* 2011b, Kojima *et al.* 2011, Petznick *et al.* 2013b). They concluded that in an atmosphere with a relative humidity of between 30-40%, the rate of tear film

evaporation in the absence of contact lens wear has a range from 4.1 to 15.6 $\times 10^{-7}$ g/cm^2/sec.

It is difficult to determine a “normal” tear evaporation rate since results vary with each technique and inter-subject variability is high. However, it has been argued that evaporimeters with ventilated chambers are more reliable for such measurements as they more closely mimic the natural air flow conditions in front of the ocular surface than do closed pre-ocular chambers (Kimball *et al.* 2010). Table 1-1 provides a summary of the evaporation rates measured in normal individuals, and techniques used to date (Hamano *et al.* 1981, Cedarstaff *et al.* 1983, Rolando *et al.* 1983a, Trees *et al.* 1990, Tsubota *et al.* 1992, Mathers *et al.* 1993, Craig *et al.* 1997, Goto *et al.* 2003, Thai *et al.* 2004, Guillon *et al.* 2008, Khanal *et al.* 2009, Arciniega *et al.* 2011b, Kojima *et al.* 2011, Petznick *et al.* 2013a).

Table 1-1 Review of techniques used for measuring tear evaporation rates

Author	Technique	ER±SD ($\times 10^{-7}$ g/cm^2/sec)	RH%
Hamano *et al.* (1981)	Open chamber with no temperature and humidity control	26.9±NR	NR
Tomlinson *et al.* (1983)	Closed ventilated chamber using resistance hygrometry	50±16.6	50%
Rolando *et al.* (1983)	Temperature and humidity sensors interconnected with a constant humidity-conditioning unit.	4.1±0.4	30%
Tsubota *et al.* (1992)	A temperature and humidity sensor placed inside the goggle chamber detecting the increasing rate of relative humidity inside the chamber.	15.6±3.8	40%
Mathers (1993)	Humidity sensor enclosed in an evaporation chamber. Humidity altered by alternatively pumping dry air into the chamber and then closing the chamber.	14.7±6.7	30%
Tomlinson *et al.* (1990)	Modified dermatologic evaporimeter (Servomed Evaporimeter EP1) with two sensors placed at a known distance from measurement surface.	12.5±1.8	NR
Craig *et al.* (1997)	Servomed Evaporimeter EP1	0.4±NR	50%
Goto *et al.* (2003)	Ventilated chamber with constant airflow of known water content.	4.1±1.4	NR
Thai *et al.* (2004)	Servomed Evaporimeter EP3	10.8±5.3	NR
Guillon *et al.* (2008)	Evaporimeter developed by Mathers.	15.1±7.3	30%
Khanal *et al.* (2009)	Servomed Evaporimeter EP1	5.8±2.8	NR
Kojima *et al.* (2011)	Quartz crystal humidity sensor	5.0±2.8	18%
Arciniega *et al.* (2011)	Evaporimeter similar to Mathers.	0.04±0.01*	30%
Petznick *et al.* (2013)	Infrared thermography in a controlled adverse environmental chamber	26±11.1	45%

*Value is expressed in µl/min/cm^2, ER: Evaporation Rate, SD: standard deviation, RH: relative humidity, NR: Not Reported.

Tear evaporation rate depends on many factors. Most studies have shown that in older age groups (>45 years) the evaporation rates were 1.5 times higher compared to younger adults and in women they were about 1.4 times higher than in men (Mathers *et al.* 1996b, Guillon *et al.* 2010, Tan *et al.* 2010). However, a study by Craig *et al*. (1998) showed that age and gender were not associated with evaporation rates. When the blink rate is higher than average and the area of the exposed ocular surface is large, there is an increased evaporation rate of tears from the ocular surface (Tsubota *et al.* 1995, Tan *et al.* 2010). Another factor that could increase evaporation rate is the increased cooling rate of the central cornea relative to limbus, often observed in dry eye populations (Morgan *et al.* 1995, Craig *et al.* 2000). An environment with low humidity is found to affect tear evaporation rate adversely by substantially increasing the rate (McCulley *et al.* 2006, Uchiyama *et al.* 2007, Abusharha *et al.* 2013). Tear evaporation rates of healthy individuals increased to 45.8% on average with a 10% reduction in relative humidity (McCulley *et al.* 2006). When a group of individuals with dry eye disease and meibomian gland dysfunction along with a control group with healthy eyes were exposed to a normal humidity range (40-45%) and a humidity range similar to that of an air cabin during flight (20-25%), the tear evaporation rate increased significantly up to 99.8% across all groups (Uchiyama *et al.* 2007). Increased tear evaporation rate followed by exposure to low humidity (5%) for 60 minutes was found to have significant association with reduced lipid layer thickness and tear film stability in a non-contact lens wearing population (Abusharha *et al.* 2013). Based on a meta analyses conducted on studies related to tear production, a 2-3 fold increase in tear evaporation rate is considered as the diagnostic cut-off for dry eye disease with a sensitivity of 45.5-61.2% and a specificity of 79.8-90.6% (Tomlinson *et al.* 2009).

Contact lens wear also has an influence on tear evaporation rate leading to ocular dryness and discomfort. According to the international dry eye workshop (Lemp *et al.* 2007), contact lens wear is considered to be one of the factors that can contribute to evaporative dry eye. Hamano *et al.* (1980b) who pioneered the measurement of tear evaporation rate in humans, observed similar tear evaporation rates with and without contact lens wear. This could be due to the differences in experimental set up used in measurement, which involved corneal contact, in which the evaporation rate from the cornea but not from the front surface of the eye was measured. In addition, there is no indication as to whether the temperature and humidity during measurement days were considered. However, other studies (Tomlinson *et al.* 1982, Cedarstaff *et al.* 1983, Thai *et al.* 2004, Guillon *et al.* 2008, Kojima *et al.* 2011) have shown that the evaporation rate is increased during lens wear (Table 1-2). Though the measured evaporation rates differ widely, perhaps due to the different techniques used, evaporation rate with contact lens wear is approximately 1.5 times higher with hydrogel lenses than the rate without contact lens wear.

Table 1-2 Review of tear evaporation rate (10^{-7}g/cm^2/sec) measured among soft contact lens wearers

Author	Non-contact lens wear±SD	Contact lens wear±SD	Change in evaporation rate	RH%	Lens type
Tomlinson *et al.* (1982)	50.0±16.6	233±68.3	4.7×	NR	Hydrogel
Tomlinson *et al.* (1983)	33.0±0.3*	71.0±0.3*	2.2×	NR	PMMA
Thai *et al.* (2004)	10.8±5.3	14.5±5.0	1.3×	NR	Hydrogel
Guillon *et al.* (2008)	15.1±7.3	23.5±6.8	1.6×	30%	Hydrogel
Guillon *et al.* (2008)	11.3±6.8	18.9±6.2	1.7×	40%	Hydrogel
Kojima *et al.* (2011)	5.0±2.8	9.1±3.1	1.8×	18%	Hydrogel
Kojima *et al.* (2011)	4.5±3.0	5.9 ±3.3	1.3×	18%	Silicone hydrogel

* Evaporation rate is measured in mg/cm^2/min, 4.7×: 4.7 times higher, NR: Not Reported SD: standard deviation, RH: relative humidity, PMMA: Polymethyl methacrylate.

Different contact lens materials such as hydrogel, silicone elastomer and PMMA (polymethyl methacrylate) can all disturb the lipid layer sufficiently to produce a significant increase in evaporation rate ($14.5\pm5\times10^{-7}$ g/cm^2/sec) which is about 1.3 times higher than the pre-corneal evaporation rate ($10.8\pm5.3\times10^{-7}$ g/cm^2/sec) (Thai *et al.* 2004). However, the increase in tear film evaporation is not affected by lens material type or water content of the lens material (Tomlinson *et al.* 1982, Cedarstaff *et al.* 1983, Thai *et al.* 2004). Tear evaporation rates in soft contact lens wearers including daily disposable lens wearers, frequent replacement lens wearers and conventional lens

wearers have been measured at two humidities (30% and 50%). The evaporation rate with any contact lens wear in a normal humid environment was equivalent to evaporation rate of a non-lens wearing eye in an atmosphere that was approximately 10% less humid (Guillon *et al.* 2008). A study performed by Kojima *et al.* (2011) reported an apparent association between marked ocular discomfort and higher tear evaporation rate among first time contact lens wearers. They observed that silicone hydrogel lens wearers (narafilcon A) had improved comfort and decreased evaporation rates compared to hydrogel lens wearers (etafilcon A), suggesting whilst silicone hydrogel lenses still increase evaporation and decrease comfort compared to no lens wear, they may offer some benefit compared to HEMA (hydroxyethyl methacrylate) based soft lenses.

1.3.3 BIOCHEMICAL IMPLICATIONS

The composition of meibum has been reported in animals (Baron *et al.* 1976, Tiffany 1979, Nicolaides *et al.* 1981) and humans (McCulley *et al.* 1997, Butovich 2009b, Chen *et al.* 2010) with slight variations based on the analytical techniques used. A recent review of analytical methods for meibum and tear lipids broadly divides the methods used into three techniques, chromatography, spectroscopy and techniques developed to analyse lipid deposits on contact lens surfaces (Pucker *et al.* 2012).

Chromatography is an analytical technique that separates components based on their distribution characteristics between a stationary and a mobile phase (Khandpur 2007). Gas chromatography (GC) and liquid chromatography (LC) are the two main types of chromatography. The former utilises a gas (nitrogen, helium or methane) and the latter uses a liquid (chloroform, acetonitrile, hexane, glacial acetic acid) as the mobile phase. Initial attempts to separate lipid components were made using paper chromatography

(Linton *et al.* 1961) wherein the concentrated meibum (sample) was applied on a filter paper and was allowed to move by dipping the filter paper in a mobile liquid phase. The components identified included neutral fats and limited phospholipids but no cholesterol or free fatty acids were detected. Thin layer chromatography (TLC) is more sensitive than paper chromatography and is carried out on a glass sheet, which is the stationary phase and with a solvent mixture acting as the mobile phase where a thin layer of sample is moved at varying speeds. TLC has been used to analyse meibum (Cory *et al.* 1973), tear lipids (Wollensak *et al.* 1990) and to separate lipid deposits recovered from the contact lens surface (Zhao *et al.* 2009, Babaei Omali *et al.* 2011). Even though the technique is simple and cost effective, large amounts of sample are required to perform the analysis and it is unable to separate the majority of individual lipid species. Tear lipid fatty acids have been analysed in a dry eye population using GC (Jarvinen *et al.* 2011) and lipid deposits on silicone and regular hydrogel lens materials have been analysed using high performance LC (Jones *et al.* 2003, Maziarz *et al.* 2006, Heynen *et al.* 2011).

Apart from TLC, GC and LC, few techniques have been developed solely for extraction and identification of lipid deposits on a contact lens surface. Total lipids were detected from a worn etafilcon A (44.1±8.2 μg/lens) and a polymacon (66.3±16.3 μg/lens) lens using a sulfo-phospho-vanillin reaction (Mochizuki *et al.* 2008). Similarly, an enzymatic digestion detected phospholipids from worn etafilcon A (1.8±0.4 μg/lens) and a polymacon lens (2.1±0.4 μg/lens) (Yamada *et al.* 2006). The levels of phosphatidylcholine (PC) and cholesterol ester (CE) have been detected from one hydrogel lens (etafilcon A) and eight silicone hydrogel lenses (balafilcon A, lotrafilcon A, lotrafilcon B, galyfilcon A, narafilcon A, senofilcon A, comfilcon A and enfilcon A) using a chloroform-methanol extraction (Pucker *et al.* 2010b, Pucker *et al.* 2010a). PC

was absorbed to the highest concentration and least by enfilcon A and narafilcon A respectively and CE was absorbed highest and least by narafilcon A and enfilcon A respectively. Adsorption of physiological concentrations of cholesterol and phosphatidylethanolamine (PE) on five silicone hydrogel lenses (balafilcon A, galyfilcon A, lotrafilcon A, lotrafilcon B and senofilcon A) were compared to a hydrogel lens (etafilcon A) *in-vitro* using a fluorescence quantification technique (Carney *et al.* 2008). The adsorption of lipids were least by the lotrafilcon lenses among silicone hydrogels and was in similar levels of adsorption by the hydrogel lens. Recently, mass spectrometry (MS) has been widely used to detect and quantify lipid extracts on contact lens surface and is explained further.

Spectroscopy is an analytical technique that uses the spectrum absorbed or transmitted through the sample (380-1015 nm) to detect and quantify lipid components (Pucker *et al.* 2012). Nuclear magnetic resonance (NMR) spectroscopy creates a magnetic field in which the nuclei of the specific molecule flips into a higher energy alignment and re-emits the radiation of that particular molecule's resonance frequency, enabling identification of individual molecules (Khandpur 2007). NMR spectroscopy has been used to detect phospholipids in rabbit meibum, to differentiate the meibum composition of steer and human, and to identify changes in human meibum related to age and meibomian gland pathology (Nicolaides *et al.* 1984, Greiner *et al.* 1996, Borchman *et al.* 2012a, Borchman *et al.* 2012b). Raman and infrared spectroscopy are two complimentary spectroscopic techniques. The former uses visible light (380-760 nm) and the latter infrared radiation (800-106 nm) to identify lipid components. Characterisation of human and mouse meibum (Oshima *et al.* 2009, Lin *et al.* 2011), quantification of human meibum and comparison of meibum and tear lipid composition

(Borchman *et al.* 2007, Borchman *et al.* 2010a, Borchman *et al.* 2010b) has been carried out using Raman spectroscopy and infrared spectroscopy respectively.

Mass spectrometry (MS) is one of the most sensitive analytical techniques currently used to detect and quantify a range of lipid classes with small (5 µl) amount of sample volume. In MS, the sample is bombarded with an electron beam to produce ionic fragments which then travel through electric and magnetic fields and components are separated based on their mass to charge ratio (Khandpur 2007). Structural elucidation and quantification of human meibum and tear lipid components have been analysed using MS alone (Ham *et al.* 2004, Nichols *et al.* 2007, Chen *et al.* 2010) or by coupling with TLC, GC and/or LC (Nicolaides *et al.* 1981, Stuchell *et al.* 1984, Dougherty *et al.* 1986a, Osgood *et al.* 1989, Shine *et al.* 1991, Mathers *et al.* 1998, Shine *et al.* 1998, Shine *et al.* 2003a, Butovich *et al.* 2007, Butovich 2008, Joffre *et al.* 2008, Butovich 2009a, Butovich *et al.* 2009, Saville *et al.* 2010, Lam *et al.* 2011, Brown *et al.* 2013). Recent advances in both the sensitivity and throughput of mass spectrometric lipid analysis have enabled the detection and quantification of over 17 lipid classes and about 600 molecular lipid species in tear film lipids (Brown *et al.* 2013, Lam *et al.* 2013) and also enabled quantification and detection of phospholipid and cholesterol extracts recovered from contact lens surface (Saville *et al.* 2010, Babaei Omali *et al.* 2011, Babaei Omali *et al.* 2012). Table 1-3 shows a list of lipid classes detected and quantified in human meibum and tear lipids among healthy individuals so far using the techniques mentioned above.

Table 1-3 Review of mean mole% of lipid classes detected and/or quantified in human meibum and tear lipidome (Table continued in the following page)

Author	Method	Sample	CE	WE	Chl	TAG	DE	FFA	PC	PE	PS	SM	LPC	LPE	Phl	OAHFA	PL
Andrews (1970)	TLC/GC	Tears	-	+	-	+	-	20.4	-	+	-	+	-	-	-	-	-
Cory *et al.* (1973)	TLC	Meibum	60.0		5.2	3.4	17.6	10.4	-	-	-	-	-	-	-	-	-
Tiffany (1978)	TLC/GC	Meibum	8-34	13-23	0-2	11-43	0-7	0-24	-	-	-	-	-	-	0-5	-	-
Nicolaides *et al.* (1981)	TLC/GC/MS	Meibum	27.3	32.3	1.6	3.7	7.7	2.0	-	-	-	-	-	-	-	-	14.8
Stuchell *et al.* (1984)	TLC	Tears	9.7	-	7.1	6.9	-	18.3	-	-	-	-	-	-	-	-	0.9
Wollensak *et al.* (1990)	TLC	Tears	45.0		15.0	-	-	-	-	-	-	-	-	-	15	-	-
Glasgow *et al.* (1995)	GC/MS	Tears	+	-	+	+	-	-	-	-	-	-	-	-	+	-	-
McCulley *et al.* (1997)	TLC/LC/GC/MS	Meibum	16.0	68.0	-	6.0	-	1.0	-	-	-	+	-	-	4.0	-	-
Mathers *et al.* (1998)	TLC/GC/MS	Meibum	39.4	45.2	1.2	3.1	2.3	2.8	-	-	-	-	-	-	-	-	-
Butovich *et al.* (2007 & 2009)	HPLC/MS	Meibum	31.0	+	-	+	-	-	-	-	-	-	-	-	-	+	-
Butovich (2008)	HPLC/MS	Tears	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Chen *et al.* (2010)	MS	Meibum	13.0	28.0	-	0.05	+	3.0	-	-	-	-	-	-	-	-	-
Rantamaki *et al.* (2011)	HPLC/MS	Tears	-	-	-	5.0	-	-	70.6	17.6	0.3	3.0	-	-	-	-	-
Rantamaki *et al.* (2011)	TLC	Tears	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Lam *et al.* (2011)	HPLC/MS	Meibum	66.8	25.2	-	4.0	-	-	0.19	0.15	-	0.03	+	+	0.3	3.5	-

Author	Method	Sample	CE	WE	Chl	TAG	DE	FFA	PC	PE	PS	SM	LPC	LPE	Phl	OAHFA	PL
Brown *et al.* (2013)	ESI/MS	Meibum	44.0	52.0	-	1.5	-	-	0.002	0.001	-	0.002	0.0004	-	0.006	3.1	-
Brown *et al.* (2013)	ESI/MS	Tears	39.0	43.0	-	2.1	-	-	2.28	1.2	1.02	1.8	5.76	-	12.0	4.4	-
Lam *et al.* (2013)	LC/MS	Tears	44.8	35.2	5.9	2.8	-	-	2.4	0.70	0.11	1.5	0.49	1.3	8.2	2.5	10.7
Lam *et al.* (2013)	LC/MS	Meibum	49.2	43.0	1.6	2.4	-	-	0.20	0.13	0.01	0.06	0.02	0.04	0.56	2.9	3.4

TLC: thin layer chromatography, HPLC: high performance liquid chromatography, GC: gas chromatography, ESI/MS: electron spray ionisation mass spectrometry, + lipid class detected, - no information, CE: cholesterol ester, WE: wax ester, Chl: free cholesterol, TAG: triglyceride, DE: diester, FFA: free fatty acid, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, SM: sphingomylein, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, Phl: total phospholipids, OAHFA: (O-acyl)-ω-hydroxy fatty acid, PL: total polar lipids.

Human meibum includes predominantly cholesterol and wax esters, followed by lesser amounts of triglycerides, diesters, free fatty acids and a small quantity of phospholipids and (O-acyl)-ω-hydroxy fatty acids. Studies have found substantial differences in composition between meibum and the tear lipidome. When the non-polar lipids of meibum and the tear lipidome were compared using HPLC/MS, components with higher polarities and lower molecular weights than in meibum was observed in the tear lipidome (Butovich 2008). Only trace amounts of phospholipids have been detected in meibum compared to the tear lipidome suggesting alternate sources for phospholipids such as the lacrimal gland, corneal or conjunctival epithelial cells (Butovich 2008). Several investigators confirmed this discrepancy (Wollensak *et al.* 1990, Farris 1994, Tiffany 1997, Borchman *et al.* 2007, Saville *et al.* 2010, Dean *et al.* 2012, Brown *et al.* 2013). It is speculated that the difference in meibum and tear lipidome composition might be due to the ability of lid bacteria to produce lipases that can modify tear film phospholipids. Hence, when meibum spreads over the tear film to form the lipid layer, the resultant phospholipids might differ in composition to those fresh from the acini of the glands (Tiffany 1997). Phosphatidylcholine (PC) and sphingomylein (SM) are the two major polar lipid species present in meibum (Shine *et al.* 2003a). The concentration of PC to SM in meibum is 5:1 whereas in tears it is 1:1, which further supports the disparity in phospholipid concentrations between meibum and tear lipidome (Saville *et al.* 2010). Recently a new class of fatty acids called (O-acyl)-ω-hydroxy fatty acid (OAHFA) have been identified and quantified in meibum (Butovich *et al.* 2009) and in tear lipidome (Brown *et al.* 2013) and was found to be 61% higher in the tear lipidome. Table 1-4 provides a comparison of meibum and tear lipid composition based on the current literature (Andrews 1970, Tiffany 1978, Nicolaides *et al.* 1981, Wollensak *et al.*

1990, McCulley *et al.* 1997, Lam *et al.* 2011, Rantamaki *et al.* 2011, Brown *et al.* 2013, Lam *et al.* 2013).

Table 1-4 Comparison of lipid classes (mole% in total lipidome) in human meibum and tears

Lipid Classes	Meibum	Tears
Wax ester (WE)	13-68%	21-43%
Cholesterol ester (CE)	8-66%	0-55%
Free cholesterol (Chl)	0-15%	0-8%
Triglyceride (TAG)	3-43%	0-5%
Diester (DE)	0-18%	Not detected
Free fatty acid (FFA)	0-24%	0-18%
Phosphatidylcholine (PC)	0-0.2%	0-70%
Phosphatidylethanolamine (PE)	0-0.15%	0-18%
Sphingomyelin (SM)	0-0.6%	0-3%
(O-acyl)-ω-hydroxy fatty acid (OAHFA)	2.9-3.5%	1.8-4.4%
Lysophosphatidylcholine (LPC)	0.0004-0.02%	0.49-5.7%
Lysophosphatidylethanolamine (LPE)	0-0.04%	0.88-1.3%

To explore the possible association between contact lens wear and lipid layer composition, the structure and biochemical implications of each lipid class with contact lens wear is reviewed below.

1.3.3.1 Cholesterol ester (CE)

CEs are non-polar in nature and constitute approximately 0-66 mole% of total human tear lipidome or meibum. The structure of the CE (Figure 1-5) includes long saturated or unsaturated fatty acid chains (Butovich 2009a) ranging between C_{18} to C_{34} and

approximately 56 individual CE species have been detected and quantified in meibum and tears (Brown *et al.* 2013).

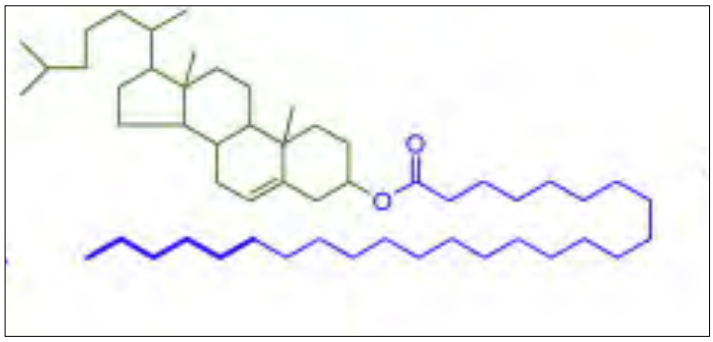


Figure 1-5 A typical structure of cholesterol ester. Reproduced with permission Butovich (2013)

Compositional differences in CEs in human meibum of patients with dry eye disease, chronic blepharitis, meibomian gland dysfunction and meibomian gland related keratoconjunctivitis have been observed compared to healthy individuals (Shine *et al.* 1991, Mathers *et al.* 1998, Borchman *et al.* 2010a, Lam *et al.* 2011, Shrestha *et al.* 2011). Higher levels of CEs detected using HPLC/MS was observed in the meibum of patients diagnosed with moderate dry eye disease compared to patients with mild dry eyes (Lam *et al.* 2011). Similarly, in patients with seborrheic meibomian gland dysfunction, the mole% of CE detected using TLC and GC/MS was higher compared to meibum collected from healthy individuals (42.7%vs. 39.1 %) (Mathers *et al.* 1998). Lower levels of unsaturated and branched fatty acids detected using TLC and LC/GC/MS have been observed in the CE profile of patients with meibomian keratoconjunctivitis (Shine *et al.* 1991). However, similar concentrations of CEs were observed in meibum collected from infants and patients with meibomian gland dysfunction indicating that CE alone might not be a biochemical marker for disease diagnosis (Shrestha *et al.* 2011).

The level of cholesterol (CE + free cholesterol) in tears decreased immediately with lens wear (1.91±1.9 mg/ml) compared to no lens wear (1.95±1.5 mg/ml), but recovered to habitual levels within two weeks of ceasing lens wear (Young *et al.* 1973). A higher level of cholesterol esters detected using HPLC was significantly associated with thin lipid layer patterns and increased dryness symptoms in symptomatic contact lens wearers (Guillon *et al.* 2002).

1.3.3.2 Wax ester (WE)

Similar to CEs, wax esters are non-polar in nature, form the bulk of meibum and tear lipids (13-68%), and consist of a double bond between a fatty acid and a fatty alcohol. A detailed structural (Figure 1-6) elucidation of WE in human meibum using GC/LC/MS revealed about 100 individual saturated and unsaturated WE species with 82% unsaturated fatty acids in the total WE pool (Butovich *et al.* 2012).

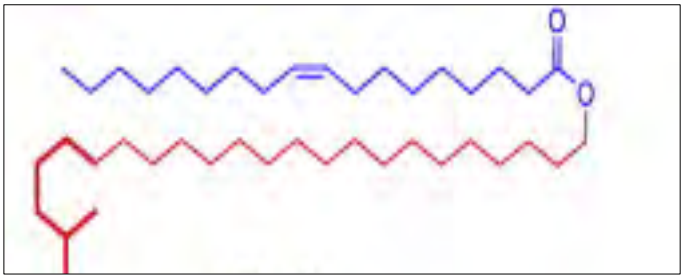


Figure 1-6 A typical wax ester structure. Reproduced with permission Butovich (2013)

Compositional differences in wax esters among patients with chronic blepharitis and meibomian gland dysfunction have been observed (Dougherty *et al.* 1991, Mathers *et al.* 1998, Joffre *et al.* 2008). Branched fatty acids were higher and saturated fatty acids were lower in meibum of patients with meibomian gland dysfunction using GC/MS (Joffre *et al.* 2008) whereas higher levels of monounsaturated wax fatty acids were

observed in meibum of patients with chronic blepharitis compared to healthy individuals using GC/LC (Dougherty *et al.* 1991). Similarly, higher levels of WE using TLC and GC/MS, was observed in meibum of patients with obstructive meibomian gland dysfunction (48.8%) compared to healthy individuals (45.2%) (Mathers *et al.* 1998). The impact of contact lens wear on wax ester composition is yet to be established.

1.3.3.3 Triglyceride (TAG)

TAG is a non-polar lipid component formed by the esterification of glycerol with three fatty acids (Apps *et al.* 1992), and constitutes a relatively small percentage of the total meibum or tear lipidome 0-5.0%, except in one study by Tiffany, (1978) where the mole% of TAG in total meibum composition were reported to range from 11-48%. Approximately 16 individual molecular species of TAG have been identified and quantified in the tear lipidome (Brown *et al.* 2013) (Figure 1-7).

Figure 1-7 A typical triglyceride structure. Reproduced with permission Butovich (2013)

Compositional differences of TAG in meibum of patients with dry eye disease, meibomian gland dysfunction and blepharitis have been observed (Shine *et al.* 1996, Mathers *et al.* 1998, Lam *et al.* 2011). An HPLC/MS quantification of triglycerides in

meibum samples of dry eye patients showed higher levels of TAG with disease severity (Lam *et al.* 2011) whereas lower levels of TAG (using TLC and GC/MS) was found in patients with obstructive meibomian gland dysfunction (2.2%) compared to healthy individuals (3.1%) (Mathers *et al.* 1998). A detailed GC/MS analysis of TAGs in meibum of different types of chronic blepharitis patients revealed differences in iso and anteiso fatty acid groups compared to healthy meibum (Shine *et al.* 1996). In the same study, lower levels of unsaturated and branched fatty acids of the TAGs were observed in patients with meibomian keratoconjunctivitis. The impact of contact lens wear on TAG composition is yet to be established however, in one study (Guillon *et al.* 2002) lower levels of TAG in the tear lipidome (using HPLC) in symptomatic contact lens wearers were associated with reduced tear film stability.

1.3.3.4 Free cholesterol (Chl) and Free fatty acid (FFA)

Only minor amounts of Chl (0-8%) and FFAs (0-3%) are found (using MS analysis) in human meibum and tear lipidome. Higher levels of Chl and FFA have been observed in patients with acne rosacea with meibomian gland dysfunction and chronic blepharitis and levels were significantly reduced following a 6 month treatment with oral minocycline (Shine *et al.* 2003b). A role for bacterial lipases that may hydrolyse CE has been postulated in patients with chronic blepharitis (Dougherty *et al.* 1986b). In inflammatory diseases such as meibomian gland dysfunction and chronic blepharitis, these lipases could catalyse the hydrolysis of cholesterol esters resulting in higher levels of Chl in meibum and tears. Higher levels of Chl and FFA have the potential to destabilise the tear film layer (Arciniega *et al.* 2011a, Arciniega *et al.* 2013). *In-vitro* observations using Langmuir troughs have shown disintegration of meibomian lipid films with the addition of >2.5 μM FFA (Arciniega *et al.* 2011a) and rigid and

destabilised lipid films with the addition of Chl at different concentrations (Arciniega *et al.* 2013). The impact of contact lens wear on Chl and FFA composition is yet to be established.

1.3.3.5 Phospholipids

Phospholipids are polar in nature and include a range of lipid classes such as phosphatidylcholine (PC) (Figure 1-8), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), sphingomylein (SM) and more. The presence of phospholipids in human meibum is still questioned as some reports have not detected them (Cory *et al.* 1973, Chen *et al.* 2010) or detected only trace amounts (0-5%) (Tiffany 1978, McCulley *et al.* 1997, Shine *et al.* 2003a, Butovich *et al.* 2007, Lam *et al.* 2011, Brown *et al.* 2013). However, tear phospholipids have been reported (5-15%) and about 50 individual molecular species have been identified and quantified so far (Wollensak *et al.* 1990, Saville *et al.* 2010, Rantamaki *et al.* 2011, Brown *et al.* 2013, Lam *et al.* 2013).

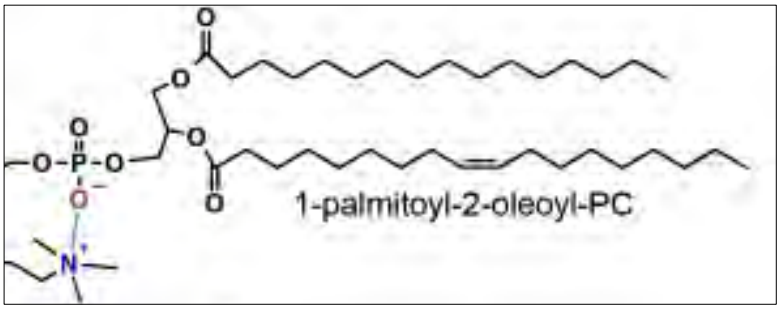


Figure 1-8 A typical phospholipid structure. Reproduced with permission Butovich (2013)

McCulley and Shine have performed detailed investigations on patients with chronic blepharitis and its associations with phospholipid composition in meibum (Shine *et al.*

1998, Shine *et al.* 2003a, Shine *et al.* 2004). Significantly lower levels of PE ($p<0.05$) and SM ($p<0.05$) were observed in meibum of chronic blepharitis patients with dry eye (4.6% and 7.2%) compared to chronic blepharitis alone (11.2% and 12.6%) (Shine *et al.* 1998). They also suggested that the polar lipid composition was most affected in meibomian keratoconjunctivitis rather than the non-polar lipids (Shine *et al.* 2004).

Wear of daily disposable lenses (etafilcon A) reduces tear phospholipid concentrations (162±33 µg/ml) in tears compared to tears of the same subjects when not wearing lenses (220±35 µg/ml) (Yamada *et al.* 2006). Also, reduced tear phospholipids have been associated with shorter tear breakup times among soft contact lens wearers (Guillon *et al.* 2002). Phospholipase enzymes cleave the ester bond of glycerophospholipids at their sn-2 position yielding free fatty acids. An increased activity of the enzyme secretory phospholipase A_2 ($sPLA_2$) could be a reason for the reduction in phospholipid concentration in tears during lens wear (Yamada *et al.* 2006).

Phospholipase enzymes can be broadly classified into two groups 'secretory' and 'cytosolic' and are further subcategorised into groups I, II, III and beyond based on their amino acid sequence (Dennis 1994). Nevalainen *et al.* (1994) were the first to detect $sPLA_2$ in human tears and lacrimal glands and reported its possible anti-inflammatory functions. In tears, the normal $sPLA_2$ concentration is 54.5±33.9 µg/ml, but this is higher (79.6±29.6 µg/ml) among the young (20-29 years), and reduced (32.4±27.8 µg/ml) in an older population (>70 years). During contact lens wear (experienced soft contact lens wearers for a minimum of 2 years), the concentration of $sPLA_2$ in tears was decreased (56.3±30.0 µg/ml) compared to its concentration in people who did not wear contact lenses (95.2±48.2 µg/ml) (Aho *et al.* 2003).

The activity of $sPLA_2$ produces free fatty acids such as arachidonic acid. Arachidonic acid catabolism leads to the formation of phospholipid aldehydes, which are the by-products of lipid peroxidation (Adibhatla *et al.* 2008). Excess amounts of degraded lipids can alter cellular function in many human diseases (Adibhatla *et al.* 2003). Adverse effects of degraded lipids have been implicated in systemic diseases (Bowton *et al.* 1997, Cai *et al.* 1999, Bidgood *et al.* 2000, Hurt-Camejo *et al.* 2001), inflammation of the ocular surface (Wei *et al.* 2011) and in contact lens intolerance (Glasson *et al.* 2002). Higher levels of the lipid aldehyde, malondialdehyde (MDA) (0.05±0.03 mM/μl), have been found in tears of elderly (65-85 years) compared to younger (18-30 years) populations (0.03±0.02 mM/μl) (Benlloch-Navarro *et al.* 2013). Glasson *et al.* (2002) found higher levels of MDA as well as another lipid aldehyde, 4-hydroxy-2(E)-nonenal (4-HNE), in the tears of symptomatic contact lens wearers (0.84±1.0 μM) compared to asymptomatic wearers (0.15±0.15 μM). The difference in the magnitude of MDA levels in both studies could be due to the analytical techniques used, where the former used HPLC (Benlloch-Navarro *et al.* 2013) and the latter used a colorimetric assay (Glasson *et al.* 2002) to detect MDA and 4-HNE in tear lipidome.

1.3.3.6 (O-acyl)-ω-hydroxy fatty acid (OAHFA)

The presence of OAHFA was first reported by Nicolaides *et al.*(1984) and its structural elucidation in human meibum (Figure 1-9) was first reported by Butovich *et al.* (2009). OAHFAs are polar in nature and constitute 3.5% of meibum and 4.4% of the tear lipidome. More than 80 individual OAHFA species have been detected and quantified in tears (Brown *et al.* 2013). Because of their amphiphilic and surfactant properties, OAHFAs are likely to play an important role in maintaining the structural stability of the tear lipid layer along with phospholipids (Butovich *et al.* 2009, Lam *et al.* 2011).

Lam *et al.* (2011) reported reduced concentrations of OAHFAs in meibum with increased disease severity in a dry eye population. The effect of contact lenses on OAHFAs in tears has not been reported.

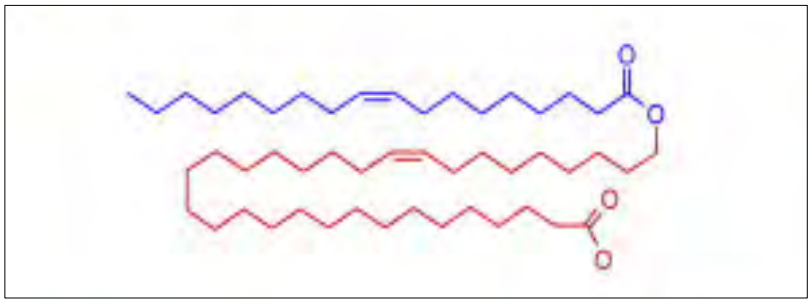


Figure 1-9 A typical (O-acyl)-ω-hydroxy fatty acid structure. Reproduced with permission (Butovich 2013)

Table 1-5 provides a comparison of lipid class concentrations in normal tears and during contact lens wear (Young *et al.* 1973, Aho *et al.* 2003, Yamada *et al.* 2006).

Table 1-5 Concentration of lipid classes in tears with and without contact lens wear

Lipid classes	Without contact lens	With contact lens	*p* value	Lens type
Total Cholesterol (mg %)* Young *et al.* (1973)	195±1.5	192±1.9	<0.05	NR
Total Phospholipids (µg/ml) Yamada *et al.* (2006)	220±35	162±33	0.002	Hydrogel
$sPLA_2$ Activity (mmol/min/mL) Yamada *et al.* (2006)	0.47±0.2	0.52±0.2	NS	Hydrogel
$sPLA_2$ Concentration (µg/ml) Aho *et al.* (2003)	92.3±48.2	54.1±24.3	0.002	Hydrogel

*mg%: total cholesterol in tears per 100 ml, NR: not reported, NS: not significant

1.3.3.7 Lipid deposits

Lipid deposits on the surface of contact lenses are also a possible cause for lens related ocular discomfort (Tripathi *et al.* 1991, Zhao *et al.* 2010). The rate of deposition mainly

depends on the lens material used, duration of lens wear and wearing schedule (Maissa *et al.* 1998, Panaser *et al.* 2012). Silicone hydrogel lens materials are prone to higher lipid deposition than hydrogel lenses, which is further worsened by wearing the lenses for longer hours (Panaser *et al.* 2012). However, higher lipid deposition cannot be attributed to all silicone hydrogel materials as some deposit less or equivalent lipid levels when compared to hydrogel lens materials (Carney *et al.* 2008, Nichols 2013). Contact lens wear tends to be associated with increasing ocular discomfort over the course of the day, and this end of the day discomfort might be associated with increased lipid deposition (Fonn *et al.* 2003). However, an analysis of the amount of cholesterol extracted from silicone hydrogel lenses worn on a daily wear schedule found only weak associations in univariate analysis for comfort on insertion of lenses ($r=0.17$, $p<0.01$) and overall comfort during lens wear ($r=0.14$, $p=0.03$) (Zhao *et al.* 2010) (higher amounts of cholesterol associated with lower comfort scores). Cholesterol, cholesteryl and wax esters are the principal lipid classes found in lipid deposits from soft contact lenses (Hart *et al.* 1986, Zhao *et al.* 2009, Babaei Omali *et al.* 2011) although lenses can also deposit phospholipids (Saville *et al.* 2010, Babaei Omali *et al.* 2012). Hume *et al.* (2004) recovered $sPLA_2$ from both ionic (etafilcon A) and non-ionic (polymacon) hydrogel contact lens materials worn on daily wear or extended wear schedules. There was higher enzyme deposition and higher enzyme activity on etafilcon A lenses worn on both daily and extended wear schedules. As described in section 1.3.3.5, higher enzyme activity is associated with increased lipid degradation and has been linked to increased ocular discomfort (Panaser *et al.* 2012).

1.4 LIPID LAYER SUPPLEMENTS

From a recent review (Alves *et al.* 2013), the most common intervention used by dry eye sufferers are exogenous tear supplements. Traditionally, tear supplements are classified based on chemical composition, physical properties and type of additives (Murube *et al.* 1998a, Murube *et al.* 1998b). In the current chapter, artificial tears are classified as either aqueous supplements or lipid supplements, based on the dry eye subtype for which they are usually prescribed. As the name indicates, aqueous supplements address aqueous deficient dry eye and lipid supplements focus on the deficiency in tear lipids or the evaporative dry eye subtype.

Preservative free tear formulations with a bicarbonate buffering agent have shown improved ocular surface health (Gilbard *et al.* 1989) and are widely used as aqueous supplements by dry eye populations (Pflugfelder *et al.* 2000). The corneal epithelial permeability of aqueous deficient dry eyes was significantly reduced by the use of an artificial tear formulation based on 1.4% polyvinyl alcohol with 0.5% chlorobutanol as the preservative agent but not with 0.005% benzalkonium chloride as the preservative (Gobbels *et al.* 1991). The toxic effects of benzalkonium chloride have been shown in animal models and in human eyes (Burstein 1984). Tear formulations based on 0.4% sodium hyaluronate (HA) compared to 0.3% hydroxypropyl methylcellulose showed clinically significant improvement in symptoms and clinical signs among a mild to moderate dry eye population (Iester *et al.* 2000), whereas the HA solution showed an equally protective effect on ocular surface signs and symptoms when compared to 0.5-1% carboxymethylcellulose (Brignole *et al.* 2005, Lee *et al.* 2011) and 0.9% sodium chloride (saline) (Aragona *et al.* 2002). Synthetic polymers can be used as gel formulations, the most commonly used being Carbomer 940 P (Leibowitz *et al.* 1984,

Marner *et al.* 1996, Murube *et al.* 1998b) and guar based polysaccharides such as hydroxypropyl guar in conjunction with preservatives and essential minerals. These have shown improved ocular surface health in patients with severe keratoconjunctivitis sicca (Cheng *et al.* 2002, Christensen *et al.* 2004). Though aqueous supplements are widely used, their short retention time on eyes remains as a major disadvantage. Moreover, aqueous supplements have failed to show improvement of signs and symptoms over a saline solution during contact lens wear (Efron *et al.* 1991).

Recently, the focus has been shifted to address the deficiency in tear film lipids using lipid based emulsion drops or sprays. Although the possible effects of lipid based supplements have been discussed for two decades (Rieger 1990), a quantitative and qualitative effect was apparent when castor oil was used as an emulsion to help distribute cyclosporine A in an aqueous solution. These oil in water emulsions showed significant therapeutic benefits among a moderate to severe dry eye population (Sall *et al.* 2000) along with the anti-inflammatory effects of cyclosporine A (Laibovitz *et al.* 1993). Subsequently, several studies used castor oil with water emulsion supplements among cohorts of healthy (Pearce *et al.* 2002), mild to moderate dry eye (Di Pascuale *et al.* 2004, Khanal *et al.* 2007, Maissa *et al.* 2010) and meibomian gland dysfunction patients (Goto *et al.* 2002). When compared to an aqueous supplement (hypermellose 0.3%), the castor oil and water emulsion significantly reduced tear evaporation rates in healthy eyes (Pearce *et al.* 2002). Di Pascuale *et al*. (2004) observed improved lipid layer thickness among healthy and aqueous deficient dry eyes soon after the use of an emulsion eye drop and a symptomatic improvement among the severe disease group but there were no changes with the use of a non-preserved saline drop. The efficacy of oil emulsion drops were further supported by Maissa *et al*. (2010) where they showed the retention of castor oil emulsion in tears for up to 4 hours after the initial instillation

using high performance liquid chromatography. A double-masked crossover trial comparing the oil emulsion drop and a placebo eye drop showed significant improvement in symptoms and clinical signs of meibomian gland dysfunction (Goto *et al.* 2002).

Phospholipid supplements delivered in the form of liposomes have been a recent breakthrough for the treatment of ocular surface disorders. A phospholipid eye spray containing phosphatidylcholine (PC) (Tears again, BioRevive®) when compared to a saline spray significantly improved tear break up time and decreased lid associated inflammation in a dry eye population (Lee *et al.* 2004). Similar improvements have been shown among an evaporative dry eye population when the phospholipid spray was compared to an eye gel containing triglycerides (Dausch *et al.* 2006). The effectiveness of the eyelid spray was further confirmed in a group of contact lens wearers including soft and rigid lens wearers, where a significant improvement in tear break up time, Schirmer's test, decreased lid parallel conjunctival folds and eyelid inflammation was observed when compared to a hyaluronate based tear supplement (Kunzel 2008). In all these studies, the phospholipid spray showed significant symptomatic improvement and subjective preference over other supplements. Craig *et al*. (2010) observed improved tear break up time and subjective comfort from baseline when a phospholipid spray was compared to a contralateral saline spray in healthy eyes. Preliminary evidence for the beneficial effects of the same spray was also observed in silicone hydrogel contact lens wearers (Craig 2010).

The effectiveness of anionic based phospholipid emulsion drops have been studied by Korb *et al.* (2002a). They compared an anionic (negatively charged, phosphatidylglycerol (PG)) and a zwitterionic (neutral charge, phosphatidylcholine

(PC)) phospholipid class. The PG drop significantly improved lipid layer thickness and in conjunction with a metastable oil emulsion, the drop retained the thickness up to 80% for 60 minutes whereas PC drop did not change the thickness in a healthy population. A non-phospholipid based emulsion drop (Soothe, Bausch & Lomb, NY, USA) when compared to an anionic based (PG) emulsion drop (Systane, Balance Alcon, Texas, USA) showed a significant improvement in lipid layer thickness from baseline in subjects who had dry eye symptoms (Scaffidi *et al.* 2007). However, when the duration of effect was compared, the PG drop significantly improved the lipid layer thickness (60 to 90 nm) for two hours whereas the non-phospholipid drop did not (60 to 75 nm) (Christensen *et al.* 2010).

A systematic review of lipid supplements concluded that lipid based emulsion drops and sprays showed beneficial effects on the tear lipid layer by increasing the layer thickness and slowing down the tear evaporation rate among a dry eye population (Lee *et al.* 2012). Similar preliminary evidence with contact lens wearers were observed when exogenous lipid supplements were compared to aqueous based tear supplements (Lee *et al.* 2012).

1.5 RATIONALE

There is evidence to suggest that tear film instability due to contact lens wear is a contributing factor to lens wear discomfort. Numerous commercially available tear lipid supplements have shown symptomatic improvements among dry eye populations and a few among contact lens wearers. However, an evidence based assessment of whether exogenous lipid supplements can improve ocular comfort during contact lens wear and the underlying biological reason for the improvement is yet to be performed.

1.6 THESIS OBJECTIVES

The thesis aims to,

1. Determine and develop appropriate methods to measure the clinical, functional and biochemical aspects of the tear lipid layer.
2. Evaluate the effect of short-term contact lens wear on the tear lipid layer and to determine associations, if any, between the clinical, functional and biochemical aspects of the tear lipid layer or with contact lens discomfort.
3. Assess the underlying effect of exogenous lipid supplements on tear lipid layer in habitual contact lens wearers and determine associations between the clinical, functional and biochemical aspects of tear lipid layer or with contact lens discomfort.

1.7 THESIS HYPOTHESES

The thesis tests the hypotheses that improved contact lens comfort is associated with a thicker tear lipid layer and decreased lipid degradation resulting in reduced tear evaporation. Use of an exogenous tear lipid supplement will increase lipid thickness, reduce lipid degradation, increase tear stability, reduce tear evaporation and consequently improve contact lens comfort.

1.8 THESIS OVERVIEW

The thesis is structured as described below. In brief, the initial three chapters describe method developments, which are followed by a main study chapter and the thesis ends with a summary chapter.

CHAPTER 2 describes the validation of a modified dermatologic instrument to measure the evaporation rate of tears. Reducing the tear evaporation rate is a major function of the tear lipid layer. This chapter addresses the first objective in developing and validating a device to measure this functional aspect of the tear lipid layer.

CHAPTER 3 addresses the first and second objectives of the thesis. The chapter includes a prospective study that compares the clinical and biochemical aspects of the tear lipid layer among symptomatic and asymptomatic contact lens wearers. This preliminary study also includes a randomised crossover trial that explores the effect of an exogenous lipid supplement on the tear lipid layer of symptomatic contact lens wearers.

CHAPTER 4 validates an appropriate tear collection method to perform tear lipid analysis. Collection of basal tears using microcapillary tubes often returns low tear volumes and is a time consuming process. Since a minimum of 20 μl of tears are required to conduct all biochemical analyses, the chapter evaluates alternative options of tear collection to obtain increased tear volume at a faster rate.

CHAPTER 5 describes a double-masked, randomised crossover placebo controlled intervention study among habitual contact lens wearers. In the study, a comprehensive analysis of the clinical, functional and biochemical aspects of the tear film lipid layer in contact lens wearers is performed using two formulations of exogenous lipid supplements and their respective vehicle placebo. The study also explores associations between various aspects of the tear lipid layer and the lens wearer’s discomfort.

CHAPTER 6 summarises the key findings of the thesis, along with the limitations, concludes the outcomes relative to the objectives of the thesis and provides direction for future studies.

CHAPTER 2. VALIDATION OF THE VAPOMETER

2.1 INTRODUCTION

The initial objective of the thesis was to determine and develop appropriate methods to measure the clinical, functional and biochemical parameters of the tear lipid layer. The current chapter addresses the development of an instrument to measure the functional impact of the tear lipid layer, which is to reduce the rate of evaporation of tears.

Increased tear evaporation rate is associated with thinning of the tear film, (Kimball *et al.* 2010) reduced lipid layer integrity (Craig *et al.* 1997) and development of dry eye disease (Rolando *et al.* 1983b, Khanal *et al.* 2008). Contact lens wear, irrespective of the material or water content, significantly increases tear evaporation rates (Tomlinson *et al.* 1982, Cedarstaff *et al.* 1983, Thai *et al.* 2004, Guillon *et al.* 2008). Kojima *et al.* (2011) reported an apparent association between marked ocular discomfort and higher tear evaporation rates among first time contact lens wearers and observed that silicone hydrogel lens wearers (narafilcon A) had improved comfort and decreased evaporation rates compared to hydrogel lens wearers (etafilcon A). Hence, measurement of tear evaporation rate acts as a physiological indicator of tear film stability, which might assist in characterising dry eye subtypes, estimating treatment efficacy and in understanding contact lens-related dryness and discomfort (Rolando *et al.* 1983a, Tsubota *et al.* 1992, Shimazaki *et al.* 1998, Guillon *et al.* 2005).

Previous attempts (Tomlinson *et al.* 1982, Rolando *et al.* 1983a, Trees *et al.* 1990, Tsubota *et al.* 1992, Mathers *et al.* 1993, Kojima *et al.* 2011, Petznick *et al.* 2013b) to measure tear evaporation rates have produced a wide range of values, presumably due to the differences in measurement techniques used. The rate of tear film evaporation

ranged from 4.1 to 50.6×10^{-7} g/cm^2/sec with a relative humidity of between 30-50%. Some of the earlier techniques used dermatologic instruments, often modified with a goggle cup with the results corrected for individual variations in ocular surface dimensions (Trees *et al.* 1990, Mathers *et al.* 1993). Other techniques have included resistance hygrometry, thermal analysers and other types of humidity sensors, which were also affected by the area of surface exposure, ambient airflow, and blinking due to the long duration of measurement (Rolando *et al.* 1983a, Trees *et al.* 1990, Yamada *et al.* 1990, Tsubota *et al.* 1992, Mathers 1993, Mathers *et al.* 1993, Shimazaki *et al.* 1995, Mathers *et al.* 1996a, Mathers *et al.* 1998, Craig *et al.* 2000, Goto *et al.* 2003, Tan *et al.* 2010, Petznick *et al.* 2013b). The currently available evaporimeters are research prototypes and there is no commercially available device for measuring tear evaporation rate.

This chapter describes the calibration and validation procedures performed using a commercially available dermatologic instrument for use in tear evaporation measurement. The instrument selected in this study, the VapoMeter (Delfin Technologies Inc., Kuopio, Finland), is commercially available and is validated for skin measurement (De Paepe *et al.* 2005). With minimal modifications, it was adapted for measuring absolute tear evaporation rates. The chapter describes the development of a reliable method to measure the rate of tear evaporation and the establishment of repeatability and diurnal variability of the technique with and without contact lens wear.

2.2 METHODS

2.2.1 STUDY PARTICIPANTS

Fifteen participants (7 men, 8 women) with a mean age of 25.9±4.4 years, with healthy eyes and no exposure to previous contact lens wear were recruited. The sample size of

fifteen participants was calculated to determine a significant difference of 36 g/m^2h between repeat measurements at the 5% level of significance corrected for multiple comparisons and with 80% power. The within subject standard deviation of 34 g/m^2h in tear evaporation used in this calculation was based on a pilot study (tear evaporation rate of five participants were measured at three time points on a day with and without lens wear). The expected difference of 36 g/m^2h was based on the change in evaporation rate with contact lens wear observed in the pilot study. The Human Research Ethics Advisory Committee of the University of New South Wales, Sydney approved the study and it was conducted in accordance with the Declaration of Helsinki (HREA approval no: AD11061). All participants signed written informed consent before commencing the study.

2.2.2 THE INSTRUMENT

The instrument has a temperature and a humidity sensor that first detects the ambient environmental conditions. After the instrument is applied, the change in temperature and humidity within a small volume sealed on one side by the skin is detected. Based on the change in relative humidity and temperature within the measurement interval, the evaporation rate (g/m^2h) is automatically calculated and displayed on the instrument's screen (Nuutinen *et al.* 2003). Since the measurement area is enclosed during the measurement period, evaporation rate is unaffected by ambient airflow. A swim goggle was attached to the probe of the instrument such that the goggle cup adhered well to the surrounding eye socket margins of the participant. This approach isolated the eye from the external environment and maintained the instrument as a closed chamber device. (Figure 2-1)

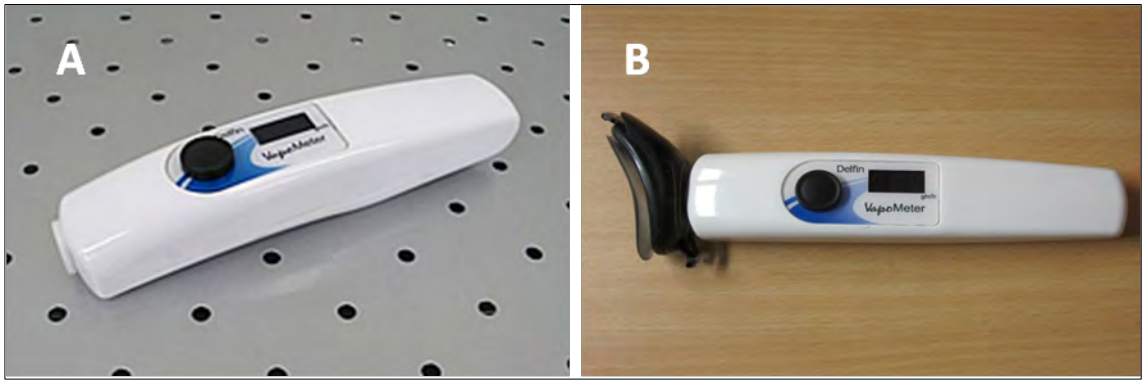


Figure 2-1 Instrument (A) and its modified version (B) with a goggle cup

2.2.2.1 Instrument calibration

The calibration procedure entailed two steps. Firstly, an evaporation source was built and validated that provided a range of evaporation rates in units of gram water per hour. In the second step, the modified VapoMeter was placed over the evaporation source and instrument readings were taken for a range of different volumes under the cup. From this, the instrument readings could be compared with the known evaporation rates. By dividing the converted evaporation rate by the exposed ocular surface area, absolute tear evaporation rates in grams per hour per meter square were obtained.

For the evaporation sources, a stainless steel model eye was constructed (Figure 2-2) with a range of inbuilt cylindrical holes with known volume and area. The shape of the model eye corresponded to the curvature of the goggle cup. The cylindrical holes could be filled with water to simulate different evaporation areas and thereby provide known evaporation rates in g/m^2h. All calibration experiments were performed at a constant ambient humidity (40±10%) and temperature (20°C±1°C).

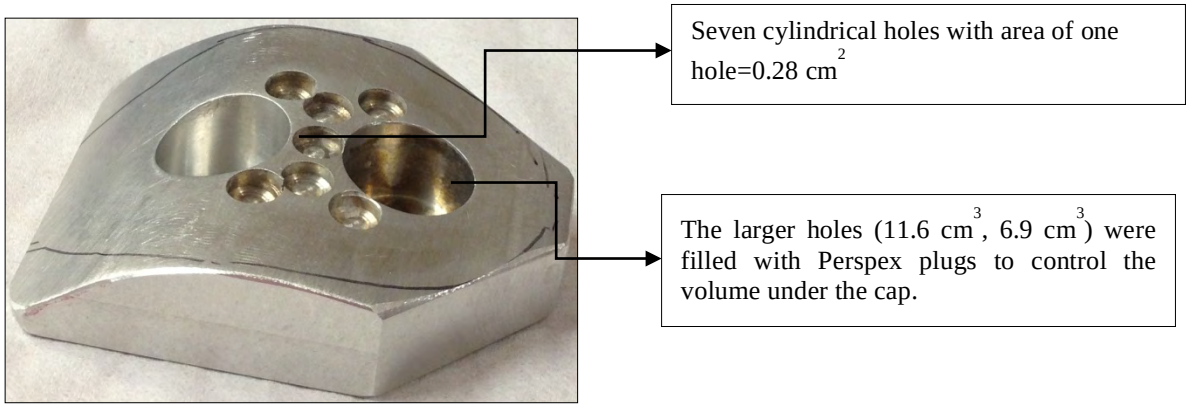


Figure 2-2 Model eye used for the calibration of the VapoMeter

2.2.2.1.1 Establishing absolute evaporation rates

One (0.28 cm^2 exposed surface area), four (1.12 cm^2) or seven (1.96 cm^2) of the cylindrical holes of the model eye were filled with water to simulate three different surface areas. The model eye was warmed to a constant temperature of 34°C on a temperature-regulated hot plate. The loss of water in grams per unit was recorded at 0, 0.5 and at 1 hour by weighing the model eye including the remaining water on an analytical balance (Scientech Inc., USA).

2.2.2.1.2 Effect of chamber volume

The next experiment was to compare the absolute evaporation rates to the readings from the modified instrument for different volumes under the cup. The model eye was warmed to 34°C and the required number of cylindrical holes was filled with water and the volume under the cup selected by inserting various Perspex plugs in the unfilled holes for each of the measurement series. The modified instrument with the cup was placed on the model eye and 5 repeat readings taken. Five volumes were tested (5.0, 8.0,

9.8, 10.8 and 13.0 cm^3) which matched the volume range of study participants (5.0-11.0 cm^3).

2.2.3 STUDY PROTOCOL

Participants were seated upright on a chair and were provided with a distance fixation target. To minimise the effect of skin evaporation, petroleum jelly (Vaseline®, Unilever, Australia) was applied over the upper eyelid and the surrounding areas. The VapoMeter was then placed over the participant's randomly selected eye and a non-invasive measurement of tear evaporation was taken within 10 seconds. Participants were instructed not to blink during open eye measurement and to maintain a normal straight gaze at the fixation target. Evaporation rates with the eyes closed were also taken, in order to account for the residual skin evaporation from eyelids and surrounding skin tissue. The study was conducted on two separate days in a random order, one during bilateral contact lens wear (-0.50 D Focus Dailies, Ciba Vision, Australia) and the second with no lens wear. The contact lenses were inserted at least 60 minutes before the first measurement. Both right and left eye measurements were taken three times each after 2.5±0.5 hours of waking, in the morning (8:30-10:30 am), at noon (12:30-2:30 pm) and in the evening (4:30-6:30 pm) on two separate days.

2.2.4 MEASUREMENT OF AREA AND VOLUME

Open eye images of each participant were taken using a camera attached to a slit lamp bio-microscope (Righton RS-1000 zoom photo slit lamp). Using a graphic editing program (Adobe Photoshop CS6, Adobe Systems Inc., San Jose, CA), the area of the exposed ocular surface was calculated. The variation in external ocular and orbit anatomy controlled the total volume enclosed by the goggles of each individual. The total volume was determined by asking the participants to lean back and tilt their heads

up with their eyes closed. The goggle cup was then placed over their eye sockets. A syringe with its scale marked in millilitres was used to fill the cup with saline *in-situ*. The fluid volume within the cup was recorded. Both area and volume measurements were recorded in a randomised order, either from right or left eye after recording the tear evaporation measurement.

2.2.5 STATISTICAL ANALYSIS

Associations between absolute evaporation rates, and the areas and volumes of the model eye were assessed using linear regression analysis. The impact of humidity, temperature, contact lens wear, time of day, and day and their interactions on the adjusted evaporation measurements were analysed using linear mixed model accounting for within subject repeated observations. Bland-Altman plots were used to assess the within subject variance in evaporation between lens wear and non-lens wearing conditions during morning, noon and evening. The coefficient of repeatability (1.96×within subject standard deviation) was estimated for each of the above conditions. The association between evaporation with and without lens wear was assessed using the Pearson correlation test. Data analysis was performed using Statistical Package for Social Sciences (IBM SPSS Statistics 19, New York). A p value of <0.05 was considered statistically significant.

2.3 RESULTS

2.3.1 INSTRUMENT CALIBRATION

The measured evaporation rates were directly proportional to the exposed water area of the model eye ($r=0.99, p<0.01$). The experiment to determine the absolute evaporation rates resulted in a linear relationship between the mass of evaporated water and time

(Figure 2-3). The linear equations obtained for the areas of 0.28 cm^2, 1.12 cm^2 and 1.96 cm^2 were a_1=-0.06b_1+77.3 (r=0.99, p<0.01), a_2=-0.17b_2+77.5 (r=0.99, p<0.01) and a_3=-0.29 b_3+77.7 (r=0.99, p<0.01) respectively where 'b' is the lapsed time in hours and 'a' is the mass of water in grams. The absolute evaporation rates (g/h) were calculated using the slope of the linear equations.

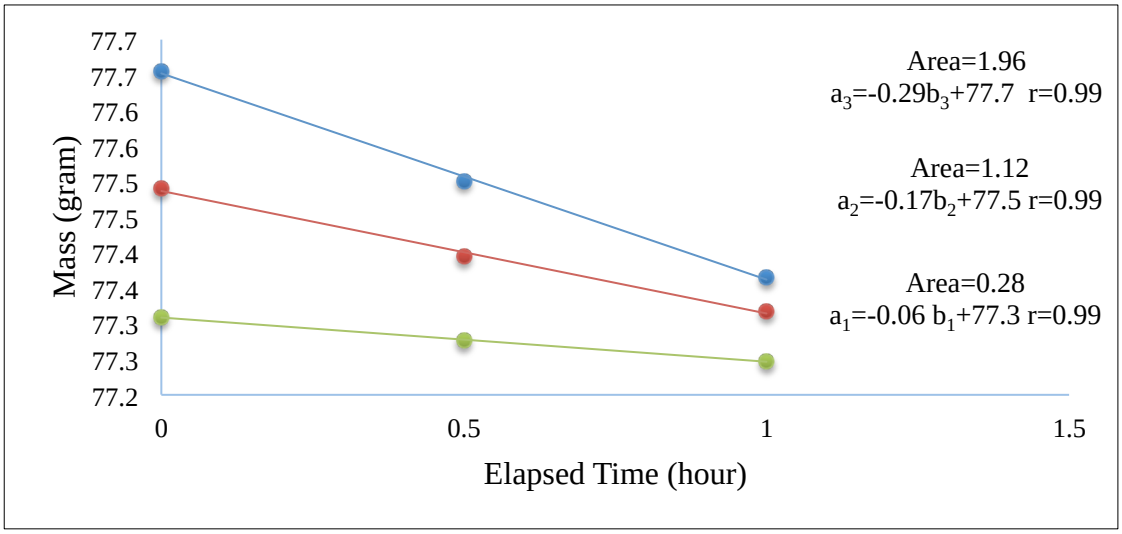


Figure 2-3 Mass of water over time for the measurement areas 0.28, 1.12 and 1.96 cm^2

The second experiment resulted in five straight lines for five different volumes showing a significant inverse association between the absolute evaporation rates and the chamber volumes (Figure 2-4). The linear equations obtained were c_1=1255.7d_1 (r=0.99, p<0.01), c_2=999.7d_2 (r=0.99, p<0.01), c_3=840.7d_3 (r=0.99, p<0.01), c_4=740d_4 (r=0.98, p<0.01), c_5=566.3d_5 (r=0.97, p<0.01) for the volumes 5, 8, 9.75, 10.97 and 13 cm^3 respectively. Table 2-1 shows the averaged instrument readings of five repeats for each of the measurement conditions. The absolute evaporation rates are the slopes of the fitted linear lines of Figure 2-3. However, the absolute evaporation rate given by the area 1.96 cm^2 was much higher than the expected tear evaporation rate and hence that area

was not included in the second experiment. A third data point with the area of 0.56 cm^2 was therefore obtained by the interpolation of the two adjacent areas 0.28 cm^2 and 1.12 cm^2. The data are plotted in Figure 2-4, including the linear equations for the absolute evaporation rate for the five different volumes. The calibration steps showed that the larger the exposed water area and the smaller the chamber volume, the higher was the rate of evaporation from the model eye.

Table 2-1 Instrument readings (averaged for five repeats with standard deviations) for five different volumes using the model eye

Area (cm^2)	ER (g/h)	Volume (cm^3)				
		13.0	10.97	9.75	8.0	5.0
		ER±SD				
1.12	0.172	88.7±6.2	116.8±8.8	147.3±6.8	168.8±5.6	220.1±4.2
0.56*	0.117	73.4	101.1	96.4	125.0	150.6
0.28	0.062	45.9±3.6	47.8±0.7	48.6±0.7	55.7±1.4	59.5±1.2
0	0	0.0	0.0	0.0	0.0	0.0

ER: Evaporation rate of the water, Area: area of the water reservoirs on the model eye surface, Volume: enclosed volume within the goggle cap and the model eye surface, * the area of 0.56 cm^2 was obtained by interpolation of the two neighbouring rows.

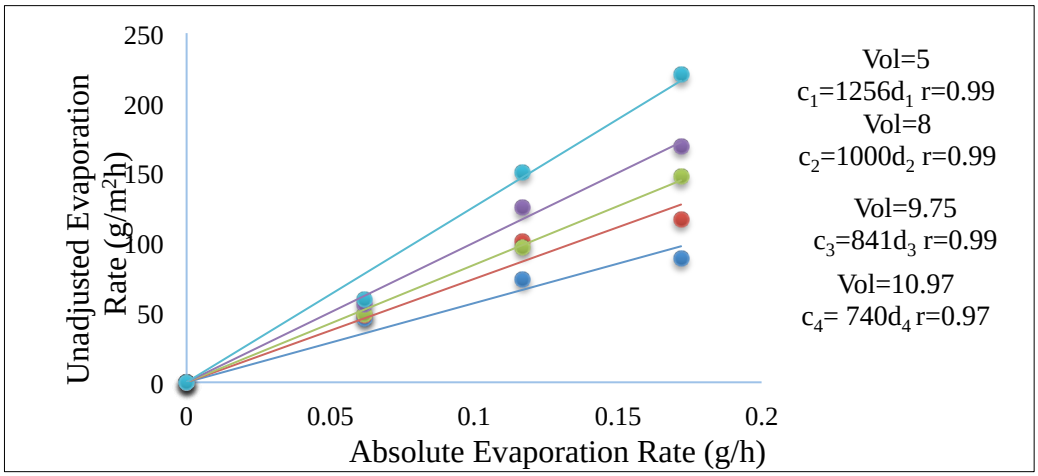


Figure 2-4 Absolute evaporation rates (g/h) for five different volumes plotted against the unadjusted evaporation rates

The correction factors obtained from Figure 2-4 for specific volumes were then plotted against the volumes tested (Figure 2-5) for linear interpolation. Thus for a given participant volume, a correction factor was derived using the linear equation e=-86.5f+1689 obtained from Figure 2-5; where 'f' is the volume under the cup and 'e' is the correction factor for that volume.

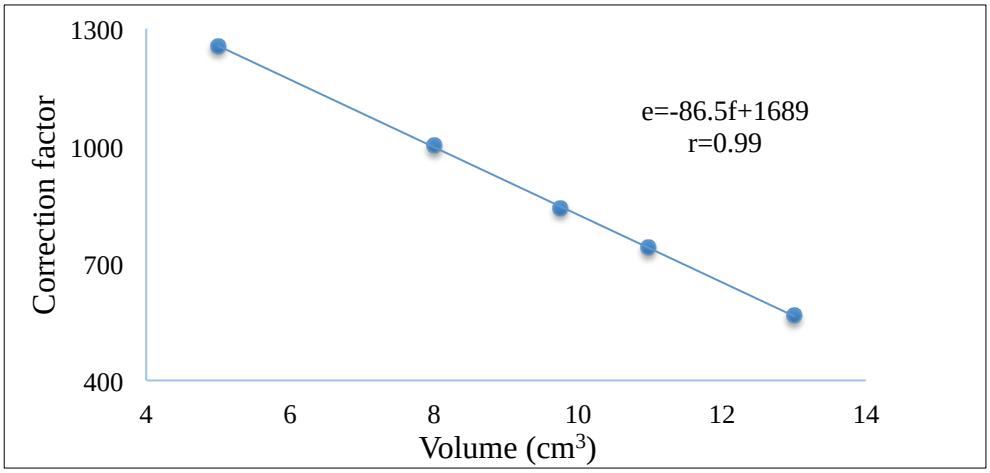


Figure 2-5 Linear relationship between the correction factors and the volumes tested in the model eye

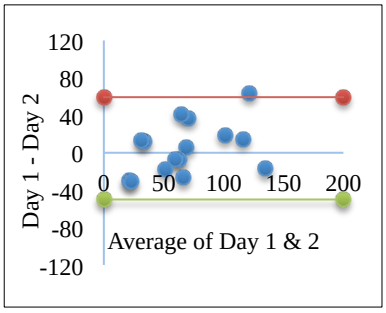
In a last step, the volume corrected evaporation rates were divided by the area of the exposed ocular surface for each participant to obtain the absolute evaporation rate for that particular measurement.

2.3.2 INSTRUMENT REPEATABILITY

Prior to assessing the repeatability of the instrument, the variation in humidity and temperature were assessed between two days and three time points. A general linear model that determined the consistency of ambient humidity and temperature showed that humidity (p=0.01) and temperature (p<0.001) were significantly affected by diurnal variation but did not vary day to day (p=0.63, p=0.65). These analyses indicated that repeatability of the instrument would be best analysed separately at morning, noon and evening conditions and this would ensure that conditions remained stable while

measuring repeatability. Additionally, repeatability was assessed separately for lens-wear and no lens wear conditions as lens wear influences tear evaporation rate (Tomlinson *et al.* 1982, Thai *et al.* 2004, Guillon *et al.* 2008, Kojima *et al.* 2011).

Figure 2-6 shows the limits of agreement obtained from the Bland-Altman plots for no lens wear and lens wear conditions at morning, noon and at evening. Almost 95% of the differences between days when not wearing lenses fell between +59.4 g/m^2h and -50.1 g/m^2h in the morning, +53.8 g/m^2h and -58.3 g/m^2h at noon, and +23.1 g/m^2h and -45.9 g/m^2h in the evening. Similarly, when wearing lenses the difference lay between +97.4 g/m^2h and -96.6 g/m^2h, +87.6 g/m^2h and -79.6 g/m^2h and +118.9 g/m^2h and -87.3 g/m^2h during morning, noon and evening respectively. The coefficients of repeatability for measuring evaporation rate were 55.9, 57.2 and 35.2 g/m^2h in the absence of contact lens wear and 99.0, 85.3 and 95.0 g/m^2h with contact lens wear during morning, noon and evening respectively. The intraclass correlation values for tear evaporation rate measured at day 1 and day 2 were 0.85, 0.84 and 0.93 in the absence of contact lens wear and 0.73, 0.83 and 0.72 with contact lens wear for morning, noon and evening measurements respectively.

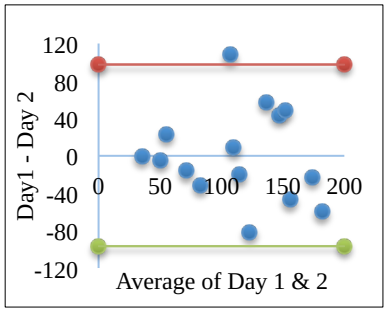


Upper Limit =+97.4

113.0±52.1

Lower Limit = -96.6

A) Morning with lens wear

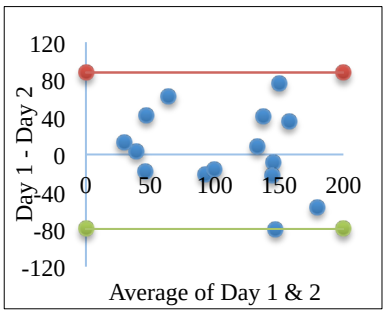


Upper Limit=+59.4

68.4±37.9

Lower Limit = -50.1

B) Morning with no lens wear

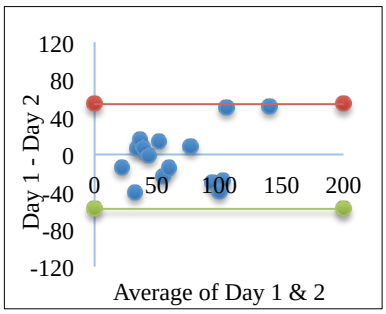


Upper Limit =+87.6

107.7±54.6

Lower Limit = -79.6

C) Noon with lens wear

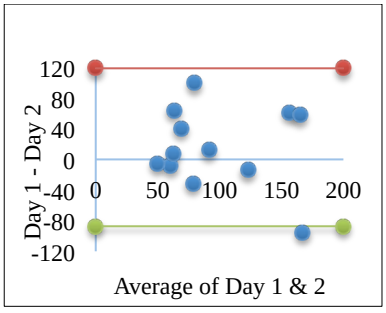


Upper Limit =+53.8

66.3±37.6

Lower Limit = -58.3

D) Noon with no lens wear

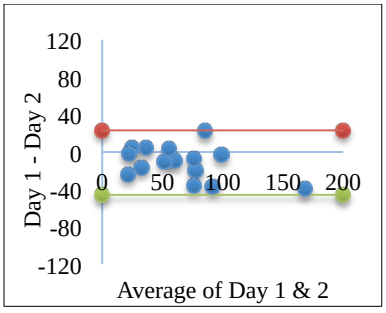


Upper Limit =+118.9

101.5±50.3

Lower Limit = -87.3

E) Evening with lens wear



Upper Limit =+23.1

65.3±39.4

Lower Limit = -45.9

F) Evening with no lens wear

Figure 2-6 Bland-Altman plots of tear evaporation rates at morning, noon, and evening with lens wear and no lens wear conditions for measurements on day 1 and 2

2.3.3 INSTRUMENT VALIDITY

To assess the validity of the measurements, tear evaporation was measured on a third day and at a further three time points without control of temperature and humidity. A linear mixed model was obtained to assess whether evaporation rate was a function of temperature, humidity, diurnal and day variation and contact lens wear (Table 2-2). Evaporation rate was log transformed prior to analysis to ensure an equality of variance between lens wear and no lens wear groups (p=0.70). Lower humidity (p<0.001) and contact lens wear (p<0.01) significantly increased the tear evaporation rate whereas day (p=0.85) and diurnal variation (p=0.65) and temperature (p=0.54) did not. Contact lens wear did not interact with humidity, temperature and time (p>0.05).

Based on this model, the estimated mean evaporation rate at an average humidity of 56% and temperature of 24°C was 55.6 g/m^{2}h (95% CI: 40.8-75.8) with no lens wear and 90.6 g/m^{2}h (95% CI: 66.5-123.6) with lens wear.

Table 2-2 Linear mixed model to assess the influence of environment and contact lens wear on tear evaporation rate

Parameter	Overall significance	Slope coefficient	Standard. error	Significance
Intercept		3.9	0.56	**<0.001**
Day 1	0.853	0.02	0.05	0.75
Day 2		0.03	0.05	0.57
Day 3		0(ref)	0(ref)	
Morning	0.654	0.04	0.06	0.501
Noon		-0.01	0.05	0.866
Evening		0(ref)	0(ref)	
Humidity	0.007	0.01	0.002	**0.007**
Temperature	0.542	-0.01	0.02	0.542
Contact lens	<0.001	0.49	0.04	**<0.001**
No contact lens		0(ref)	0(ref)	

Dependent Variable: Evaporation Rate (Log transformed)

There was a strong association (r=0.90, p<0.05) between evaporation in the lens wearing and non-lens wearing eye. Figure 2-7 shows a linear association for lens wearing and non-lens wearing eye obtained from the Bland-Altman plot indicating that higher evaporation rates resulted in greater difference in tear evaporation rates between the lens wear and no lens-wearing eye.

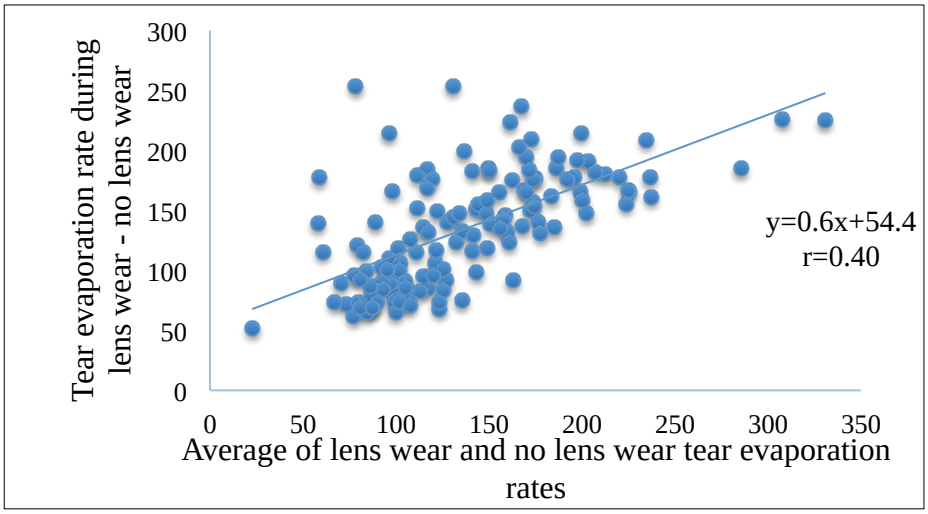


Figure 2-7 Bland-Altman plot for the tear evaporation rates in lens wearing and non-lens wearing eye

2.4 DISCUSSION

This study reports the repeatability and validation of a modified dermatologic instrument for measuring tear evaporation rates. The short measurement time (<10 seconds) of the instrument overcomes several limitations experienced by previous modified dermatologic evaporimeters used for the measurement of tear evaporation (Hamano *et al.* 1980b, Trees *et al.* 1990). The short measurement time limited the impact of blinking on lipid layer stability and its influence on normal tear evaporation rate (Benedetto *et al.* 1984, Bron *et al.* 2004, Tan *et al.* 2010) as the measurement time was close to the normal blink interval (Carney *et al.* 1982). Although the use of an

unventilated system isolated the ocular surface from the effect of ambient airflows (Cedarstaff *et al.* 1983, Goto *et al.* 2003), Kimball *et al*. (2010) observed that the tear thinning rates for the central cornea were in close agreement to the tear evaporation rate measured using ventilated chambers. Kimball and colleagues concluded that a mild airflow over the ocular surface closely resembles the natural environment experienced by the eye. However, for a short period the effect of closed unventilated system is unlikely to disrupt habitual evaporation rates profoundly.

Evaporation rate is dependent on humidity (Tsubota *et al.* 1992, Abusharha *et al.* 2013) and hence it was important to monitor humidity during measurements. Early evaporimeters required either an additional environmental control or incorporation of humidity sensors to their evaporimeter set up (Cedarstaff *et al.* 1983, Trees *et al.* 1990, Mathers *et al.* 1993). The VapoMeter automatically records ambient humidity and temperature, and compensates for environmental variations. However, due to the instrument modifications, the manufacturer's calibration was no longer reliable. For the purposes of these experiments, a range of 20°C±1°C and 40±10% relative humidity was used. The data shows that it is important to report the evaporation rates relative to the particular humidity and temperature.

The area of the exposed ocular surface and the volume under the cup influenced the rate of evaporation (Doyle 1953, Tsubota *et al.* 1995). Most previous evaporimeters have considered the area of the exposed ocular surface while reporting their tear evaporation rates, but neglected the potential influence of the variations in chamber volume induced by the differences in individual external orbital anatomy. The current study has demonstrated the impact of both volume and surface area by reducing the variance of

the data significantly. For each participant the area and volume of the exposed ocular surface were recorded and these data were used to adjust the tear evaporation rates.

Previous studies have accounted for the peri-orbital skin evaporation rate confounding tear evaporation rate measurements by either recording the closed eye measurements (Cedarstaff *et al.* 1983, Trees *et al.* 1990, Mathers *et al.* 1993, Wojtowicz *et al.* 2009, Arciniega *et al.* 2011b) or by using petroleum jelly (Rolando *et al.* 1983a). The current study combined both procedures to minimise the measurement error due to skin evaporation. The decision to combine both procedures was based upon a pilot study conducted on five subjects. When both methods were used, the evaporation rate from the peri-orbital skin was reduced by 64.1±7%, whereas the naked skin measurements significantly underestimated tear evaporation rates. By including both procedures, a more accurate representation of tear film evaporation is likely to be obtained.

Short-term lens wear significantly increased tear evaporation rates in all the participants, which is in agreement with most of the previous findings (Tomlinson *et al.* 1982, Thai *et al.* 2004, Guillon *et al.* 2008, Kojima *et al.* 2011). In contrast Hamano *et al.* (1981) found similar evaporation rates with and without contact lens wear. However, the pressure gradient technique he used was partially invasive and could have influenced the results. In addition, tear evaporation rate only from the corneal surface was measured but not from the entire exposed ocular surface, possibly excluding the evaporation rate from the lens edge. Tear evaporation rates in the present study are comparable to the previous reports and the relative change in evaporation rate with contact lens wear in our study is about 1.6 times, which falls within the range (1.3-4.7 times) of previously reported values (Iskeleli *et al.* 2002).

Although the data obtained from Bland-Altman plots question the reliability of the instrument, the intraclass correlation showed good-excellent agreement for tear evaporation rate measured on the same person on two different days. It should be noted that the tear film is a dynamic system and therefore the instrument is likely to pick up true biological variability.

In the current study, tear evaporation rates with and without lens wear showed a significant direct association. This finding indicates that an individual's tear film characteristics rather than a lens might be an overriding influence on tear film evaporation. In a similar manner, Glasson *et al.* (2003) found decreased tear volume and tear stability among symptomatic compared to asymptomatic lens wearers in the absence of lens wear suggesting that the person's tear film stability predisposes them to symptoms during lens wear.

Tear evaporation rates in the present study showed no association with day or diurnal variations when humidity and temperature were controlled. Hence, it is important to record the ambient humidity and temperature at the time of the measurement. Tomlinson *et al.* (1992) observed reduced tear evaporation rate on waking and an increased rate after 2 hours which remained constant for the remainder of the day. The lower morning evaporation rate was attributed to an intact lipid layer soon after waking (Guillon *et al.* 1989a, Tomlinson *et al.* 1992). Similarly McCulley *et al.* (2009) examined the effect of time and day on tear evaporation rate and found that day had no influence but evaporation rates were higher and less variable during afternoon hours than in the morning.

2.5 CONCLUSION

The calibration procedures using the model eye resulted in obtaining absolute evaporation rates (g/m^2h). Though the accuracy of the instrument is somewhat affected by ambient humidity, the instrument minimises the effects of blinking, ambient airflow and instrument handling. However, the current study successfully established the repeatability and validity of the modified instrument thereby addressing the initial objective of the thesis. A reliable device to measure the functional aspect of the tear lipid layer was validated and could be confidently used in the main study of this thesis. Further studies with the use of VapoMeter could test the association between evaporation rate and different factors including comfort in which case, it is recommended to allow the subject to blink normally order to represent a normal behaviour.

CHAPTER 3. TEAR FILM LIPIDS DURING SHORT-TERM CONTACT LENS WEAR

3.1 INTRODUCTION

The previous chapter validated a modified dermatologic instrument, which could be used to measure the functional aspect of the tear lipid layer. Appropriate methods to measure the clinical and biochemical aspects of the tear lipid layer are yet to be determined in this thesis. Chapter 3 therefore addresses the method development for measuring the clinical and biochemical aspects of the tear lipid layer, which includes a preliminary clinical study. In addition, the chapter addresses the second objective of the thesis, which was to determine the effects of an exogenous lipid supplement on clinical and biochemical aspects of tear lipid layer in symptomatic contact lens wearers during short-term contact lens wear.

This chapter describes a two-stage study where stage 1 was a prospective study and stage 2 was a single masked randomised crossover study. The prospective study compared the clinical and biochemical aspects of the tear lipid layer in symptomatic and asymptomatic contact lens wearers. A thicker lipid layer is associated with increased stability of the tear film and increased inter-blink period, and a decreased tear evaporation rate (Craig *et al.* 1997). Reduced concentrations of phospholipids and cholesterol have been observed in tears of soft contact lens wearers (Young *et al.* 1973, Yamada *et al.* 2006). Yamada *et al.* (2006) proposed that deposition of enzymes on contact lenses that are able to degrade lipids catalyses the release of free fatty acids including arachidonic acid. Increased levels of $sPLA_2$ activity have been reported in many inflammatory diseases (Bowton *et al.* 1997, Cai *et al.* 1999, Bidgood *et al.* 2000, Hurt-Camejo *et al.* 2001) including ocular surface inflammation in dry eye (Wei *et al.*

2011), and in contact lens intolerance (Glasson *et al.* 2002). Fatty acid catabolism can lead to the formation of phospholipid derived aldehydes, which are the by-products of lipid peroxidation (Adibhatla *et al.* 2008). Excess amounts of lipid peroxides can alter the cellular function in many human diseases (Adibhatla *et al.* 2003). Glasson et al. (2002) found higher levels of lipid derived aldehydes in the tears of symptomatic contact lens wearers compared to asymptomatic wearers.

This chapter also describes a randomised crossover trial with an exogenous lipid supplement and a saline control among symptomatic lens wearers. The most common intervention used by dry eye sufferers in attempts to alleviate their symptoms is exogenous tear supplements (Alves *et al.* 2013). Supplements containing a water emulsion of castor oil in healthy (Pearce *et al.* 2002), mild to moderate dry eye (Di Pascuale *et al.* 2004, Khanal *et al.* 2007, Maissa *et al.* 2010) and meibomian gland dysfunction patients (Goto *et al.* 2002) have improved clinical symptoms and signs such as increased lipid layer thickness and reduced tear evaporation rate. A phospholipid eye spray (Tears Again, BioRevive®) when compared to a saline-based placebo spray significantly improved tear break up time and decreased inflammation of eyelid margins in a dry eye population (Lee *et al.* 2004) and in contact lens wearers when compared to a hyaluronate based tear supplement (Kunzel 2008). Craig *et al.* (2010) observed an improved tear break up time in healthy eyes and improved subjective comfort from baseline after use of the phospholipid spray compared to a saline-based placebo spray. Preliminary evidence on the beneficial effects of the spray in silicone hydrogel contact lens wearers has also reported (Craig 2010).

The purpose of this study was to evaluate the effect of short-term contact lens wear on the clinical and biochemical aspects of the tear lipid layer and thereby determine

appropriate methods to measure these parameters. The effect of an exogenous lipid supplement on the tear lipid layer of the symptomatic contact lens wearers was also explored.

3.2 METHODS

3.2.1 STUDY PARTICIPANTS

Participants with a history of soft contact lens wear were recruited through email and poster advertisements. The study protocol was approved by the Human Research Ethics Advisory panel at the University of New South Wales and was conducted in accordance with the Declaration of Helsinki (HREA approval no: 11008). All study participants prior to study commencement signed consent forms. Twenty participants (18 females and 2 males) with a mean age of 25±4 years were enrolled. The sample size was based on detecting a change in tear break up time (TBUT) (Craig *et al.* 2010). In the current study, the non-invasive surface drying time (NISDT) is defined as equivalent to the stability of tear film measured over the lens surface. Since a contact lens significantly reduces tear film stability, it is assumed that the standard deviation (SD) of NISDT is numerically lower than TBUT (Faber *et al.* 1991). Therefore SD for the current study was assumed to be 6 seconds. To determine a significant change in NISDT of 6 ± 6 seconds over 2 hours with 80% power and 5% level of significance, the sample size was estimated to be 10 participants in each group.

3.2.2 STUDY PROTOCOL

The study had two stages. In stage 1, participants were asked to wear soft hydrogel lenses (Focus Dailies, Ciba Vision; Atlanta.) bilaterally for 6-8 hours. At the end of the lens wear, with lenses *in-situ*, clinical parameters such as lipid layer thickness and

stability were assessed and basal tears were collected using micro capillary tubes (see below). Tear samples were assayed for the concentration and activity of secretory phospholipase enzyme ($sPLA_2$) and the concentration of malondialdehyde (MDA, a lipid peroxidation product). Mass spectrometric analysis was conducted to characterise the tear lipidome. In stage 2, symptomatic lens wearers (n=10) were subjected to a single masked, randomised crossover trial where a liposomal spray (Tears Again, BioRevive®) or a placebo saline spray was sprayed over the upper eyelids of each subject during their down gaze and during lens wear. The washout period was 48 hours between experiments. Lipid layer appearance and stability along with ocular comfort scores using a numeric rating scale were obtained soon after the instillation (0^{th} hour) and 2 and 6 hours after the initial instillation. Each visit occurred at the same time of the day for each stage for each participant. Clinical and biochemical procedures performed in each stage are explained below.

3.2.2.1 Questionnaires

The questionnaire used in this study was the 12-item Contact Lens Dry Eye Questionnaire (CLDEQ). A numerical rating scale and a visual analogue scale were also used to assess ocular comfort.

3.2.2.1.1 The Contact Lens Dry Eye Questionnaire

The CLDEQ-12, developed and validated by Begley *et al*. (2000) is used as a screening questionnaire among contact lens wearers to diagnose contact lens-related dry eye. The questionnaire consists of a self-diagnosis question and nine habitual symptom subscales with frequency and intensity at morning, noon and at the end of the day. The analysis of CLDEQ involves an algorithm that produces a dichotomous outcome for the diagnosis of contact lens related dry eye (Nichols *et al.* 2002). If a participant ticked ‘yes’ to the

self-diagnosis question for dry eye and scored >0.13 on the CLDEQ, he or she was categorised as a symptomatic contact lens wearer. On the other hand, if a participant ticked 'no' or 'unsure' to the self-diagnosis question for dry eye, they were categorised to the symptomatic group only if their scores were >1.27 and >1.44 respectively.

3.2.2.1.2 Rating scales

Rating scales were used in stage 2 to record the ocular comfort scores immediately and 2 and 6 hours after the initial instillation of the liposomal or the saline spray. The numerical rating scale ranged from 0 to 10 points in 0.50 steps with 0 being 'poor' and 10 being 'excellent' in terms of ocular comfort. Participants were asked to volunteer a number between 0 and 10 for each eye separately. The visual analogue scale was a vertically oriented 20 centimetres straight line with two anchors on both ends with 1 being 'poor' and 20 being 'excellent' in terms of ocular comfort. Subjects were asked to volunteer a mark anywhere on the line respective to their general comfort in both eyes together.

3.2.2.2 Clinical Tests

3.2.2.2.1 Lipid layer grade

Tear lipid patterns were assessed non-invasively using the Tearscope Plus^{TM} (Keeler Ltd., Berkshire, UK) in conjunction with a slit lamp bio-microscope and then graded into one of five categories based on the order of their increasing lipid layer thickness (Guillon *et al.* 1988). The absence of a lipid layer was graded as 0, open or tight meshwork interference patterns were graded as 1 or 2, a wavy appearance as 3, and amorphous or colour fringes as 4 or 5 respectively.

3.2.2.2.2 Tear film stability

Tear break up time defined as 'the interval between the formation of any tear film discontinuity as in the form of a dry spot or streaks followed by a complete blink' (Norn 1969, Rengstorff 1974) is often recorded as a measurement of tear film stability (Vanley *et al.* 1977, Cho *et al.* 1992). The time recorded when an initial dry spot appears on the contact lens surface following a blink is referred as non-invasive surface drying time (NISDT) (Morris *et al.* 1998). In the current study, NISDT was assessed non-invasively using the Keeler Tearscope as a measure of tear film stability over the lens surface. Participants were asked to blink normally three times and then to refrain from blinking until the examiner noticed the development of an initial dry spot on the contact lens surface. A minimum of three readings was recorded for each eye and they were averaged at the conclusion of the experiment. Lipid layer grading and NISDT were taken for both right and left eyes in a random order.

3.2.2.3 Biochemical Tests

Basal (non-stimulated) tear samples were collected using disposable micro capillary tubes (Blaubrand® intraMARK, Wertheim, Germany). Up to 5 µl of tears were collected from each eye, by asking the participants to tilt their heads to one side and resting the capillary tube gently on the lower lid in the temporal region of a randomly selected eye. Care was taken in collecting only basal tears and not reflex tears. Precautions included providing regular breaks in between tear collection and avoiding contact of microcapillary tube with the conjunctiva. If reflex tearing occurred, tear collection was stopped immediately and then continued with a fresh capillary tube after a few minutes break. The time allotted for tear collection was 30 minutes for all participants. On average, 8±2 µl of basal tears were collected from each participant.

However, tear collection rate was slower in symptomatic contact lens wearers (0.26 µl/min) than asymptomatic wearers (0.5 µl/min). Following collection, tears from both eyes were pooled, centrifuged at 5000 g, for 10 min to remove any cellular debris, and placed into smaller aliquots and stored at -80°C until analysis.

3.2.2.3.1 Secretory phospholipase A_2 ($sPLA_2$)

The concentration of phospholipase A_2 in tear samples was quantified using a double antibody sandwich ELISA. Samples and standards were prepared according to the manufacturer's instructions (Cayman Chemical Company, MI USA). The concentration of $sPLA_2$ standards ranged from 0-1000 pg/ml and the minimum detectable level was 15.6 pg/ml. Tear samples were diluted in EIA buffer (1:100000). After incubation, Ellman's reagent was added to each well including samples and standards resulting in a yellow colour reaction. Absorbance was read at 405 nm after 2 hours. The intensity of this colour, determined spectrophotometrically, was proportional to the concentration of $sPLA_2$. Plotting absorbance versus concentration of standards generated a standard curve and the equation was used to calculate the concentration of each tear sample.

The catalytic activity of $sPLA_2$ was measured using the 1, 2-dithio analogue of diheptanoyl phosphatidylcholine, which serves as a substrate for most PLA_2 enzymes/isoforms (Hendrickson *et al.* 1983). Upon hydrolysis of this substrate by the enzyme, the assay used 5, 5'-dithio-*bis*-(2-nitrobenzoic acid) to detect free thiols. Sample and standard preparations were prepared according to the manufacturer's instructions (Cayman Chemical Company, MI USA). Tear samples were diluted using the assay buffer (25 mM Tris-HCl, pH 7.5; 1:10). The detection range of this assay was 0.02 to 0.2 µmol/min/ml of $sPLA_2$ activity. The substrate was added to each well and carefully shaken and the absorbance was read every minute at 414 nm for at least

five minutes. By plotting the absorbance values as a function of time, the catalytic activity of $sPLA_2$ was calculated.

3.2.2.3.2 Lipid peroxides

Derived from polyunsaturated fatty acids, lipid peroxides decompose to form compounds such as malondialdehyde (MDA). Measurement of these reactive carbonyl compounds is widely used as an indicator of lipid peroxidation (Esterbauer *et al.* 1991). The assay is based on the reaction of a chromogenic reagent N-methyl-2-phenylindole with MDA at 45°C, yielding a stable carbocyanine dye. The standards and reagents were prepared as per manufacturer's instructions (Biokits.com Bioxytech® MDA-586™, Dublin, USA). Samples were diluted in the ratio 1:10 using 20 mM Tris-HCl buffer and with the recommended reagents were incubated at 45°C for an hour and then centrifuged at 10,000 g for 10 minutes to obtain a clear supernatant. Absorbance was read at 586 nm, plotting absorbance versus concentration of standards drew a standard curve, and the equation was used to calculate the concentration of MDA in each tear sample.

3.2.2.3.3 Lipid classes

Tear lipid extraction was performed as described previously using a methyl-tert-butyl-ether/methanol/ammonium acetate two-phase extraction (Brown *et al.* 2013). An internal standard mix (5 µl) along with methanol (MeOH, 60 µl) and methyl-*tert* butyl ether (MTBE 200 µl) with 0.01% BHT was added to each tear sample (5 µl). The internal standard mixture contained a mixture of phospholipid standards (Avanti Polar Lipids, Alabama, USA), cholesterol and wax ester standards (Nu-Chek Prep, MN, USA), a triglyceride standard (CDN isotopes, Quebec, Canada) and an (O-acyl)-ω-hydroxy fatty acid standard (Brown *et al.* 2013). In each sample the internal

standard mixture included 30 pmol (O-acyl)-ω-hydroxy fatty acid 18:1/16:0, 2.4 pmol phosphatidylcholine 19:0/19:0, 2.4 pmol dihydrosphingomyelin 12:0, 10 pmol of D_5 triglyceride 16:0/16:0/16:0, 20 pmol wax ester 16:0/16:0, 200 pmol of 18:1/18:0, and 80 pmol of cholesterol ester 14:0 (Brown *et al.* 2013). The samples were then mixed thoroughly using an orbital shaker at low speed for 30 minutes. Aqueous ammonium acetate (0.15 M) was added to each sample, which was vortexed and centrifuged for 5 minutes at 2000 g. The upper non-polar phase was then carefully extracted without disturbing the lower polar phase. The extracted lipid was dried under nitrogen at 37°C and reconstituted with 100 µl of 2:1 MeOH/$CHCl_3$ containing 5 mM ammonium acetate. The samples were stored at -80°C until mass spectrometric analysis. Tear extracts were characterised by targeted chip-based nanoelectrospray tandem mass spectrometry (ESI/MS) (Brown *et al.* 2013). 199 individual lipid species were quantified by comparison to the class specific internal standards. Lipid species were quantified in the classes cholesterol ester (CE), wax ester (WE), triglyceride (TAG), phosphatidylcholine (PC), sphingomyelin (SM) and (O-acyl)-ω-hydroxy fatty acid (OAHFA). In order to normalise for individual variation in lipid concentration, mole% of each lipid species in total lipidome was calculated.

3.2.3 STATISTICAL ANALYSIS

A paired *t* test and Wilcoxon sign rank test was conducted between the right and left eyes for NISDT and lipid layer grades respectively. There was no significant difference between the eyes and hence right eye results are presented. In stage 1, comparisons between symptomatic and asymptomatic groups were performed using the Student *t* test or Mann-Whitney U test. Continuous variables such as NISDT and mole% of each lipid class was compared using the Student *t* test whereas a categorical variable such as lipid

layer grade was compared using Mann-Whitney U test. Associations between variables were examined using either Pearson or Spearman correlation coefficient test where appropriate. In stage 2, a repeated measures ANOVA was conducted to compare the variables at three time points. A Bonferroni correction was applied to control the overall Type 1 error rate. Statistical analyses were performed using SPSS (IBM SPSS Statistics 19, New York) and a *p* value of <0.05 was considered statistically significant.

3.3 RESULTS

3.3.1 STAGE 1

The CLDEQ-12 identified 10 symptomatic and 10 asymptomatic contact lens wearers based on their scoring as described in section 3.2.2.1.1. Table 3-1 shows the mean CLDEQ scores obtained for symptomatic and asymptomatic contact lens wearers.

Table 3-1 The Contact Lens Dry Eye Questionnaire (CLDEQ) scores of symptomatic and asymptomatic contact lens wearers

Group	Age (years)	CLDEQ Score
	Mean±standard deviation	
Asymptomatic	25.2±4.5	1.14±0.5
Symptomatic	24.8±3.4	0.45±0.4

All measurements and observations were recorded after 6-8 hours of lens wear with contact lenses on the eye. The appearance of the lipid layer was not significantly different (p>0.05) between symptomatic and asymptomatic contact lens wearers with a closed meshwork pattern (grade 2) being the most common lipid layer pattern in both groups. NISDT was significantly (p=0.01) lower in symptomatic contact lens wearers (4.5±0.6 seconds) compared to asymptomatic contact lens wearers (9.9±3.1 seconds). Mass spectrometric analysis quantified 199 molecular species in six lipid classes. Table

3-2 shows the mean proportion of mole% of the six lipid classes quantified. A detailed table with the mole% of 199 individual molecular species detected in stage 1 is described in Appendix 1.

Table 3-2 Proportion of lipid classes (mean±SD) in total tear lipidome of contact lens wearers at the end of 6-8 hours of lens wear with no intervention.

Lipid Classes	Symptomatic	Asymptomatic	*p* value
Cholesterol esters	53.4 ± 5.3 %	49.4 ± 1.3 %	0.28
Wax esters	38.9 ± 3.9 %	43.1 ± 0.8 %	0.07
Triacylglycerols	1.4 ± 0.3 %	0.9 ± 0.1 %	0.07
(O-acyl)-ώ-hydroxy fatty acids	4.2 ± 0.4 %	3.9 ± 0.9 %	0.90
Phosphatidylcholine	0.78 ± 0.3 %	0.97 ± 0.3 %	0.48
Sphingomyelin	1.3 ± 0.9 %	1.5 ± 0.8 %	0.72

SD: Standard deviation

Cholesterol esters predominated whereas phospholipid classes such as phosphatidylcholine and sphingomyelin were at low concentrations in the tears of both symptomatic and asymptomatic groups. The proportion of wax esters in the total lipidome was slightly but not statistically significantly (p=0.07) lower in symptomatic wearers whereas the proportion of triglycerides was slightly but not significantly higher (p=0.07) than in the tears of asymptomatic wearers. Lipid ratios were compared between symptomatic and asymptomatic wearers (Table 3-3). As the ratio analysis included repeated measures, a Bonferroni correction was applied to each comparison. The ratio of wax esters to triglycerides in symptomatic contact lens wearers (30.4:1) was significantly lower (p=0.03) than in asymptomatic contact lens wearers (49.3:1).

Table 3-3 Lipid ratios in total tear lipidome (mean±SD) of symptomatic and asymptomatic lens wearers

Lipid Classes	Symptomatic: Asymptomatic	*p* value
WE: CE	0.72: 0.87	0.15
WE:TAG	30.4: 49.3	**0.03**
WE:OAHFA	9.5: 12.4	0.28
WE:PL	17.2: 17.1	0.98
CE:TAG	43.2:56.6	0.15
CE:OAHFA	13.6:14.2	0.48
CE:PL	25.0:19.8	0.85
TAG:OAHFA	0.32:0.26	0.28
TAG:PL	0.63:0.34	0.62
OAHFA:PL	1.5: 79	0.85

*SD: standard deviation, WE: wax ester, CE: cholesterol ester, TAG: triglycerides, OAHFA: (O-acyl)-ω-hydroxy fatty acid, PL: total phospholipids

Table 3-4 shows the concentration and activity of $sPLA_2$ and the concentration of the lipid peroxide MDA in the tears of symptomatic and asymptomatic lens wearers. $sPLA_2$ and MDA concentration were not different. While $sPLA_2$ activity appeared to increase in the tears of symptomatic lens wearers, the differences did not reach statistical significance. An association between each lipid class and NISDT was observed and the significant associations are reported. As the NISDT increased so did the percentage of wax esters in the total lipidome (r=0.83, p=0.01), whereas the percentage of cholesterol esters decreased (r=-0.77, p=0.04). Phospholipase enzyme activity was associated with increased MDA (r=0.80, p=0.01) and shorter NISDT (r=-0.91, p=0.001). There were no associations with any other lipid classes.

Table 3-4 The concentration and activity of phospholipase enzyme ($sPLA_2$), and the concentration of malondialdehyde (MDA) in tears of contact lens wearers

Biochemical variables	Symptomatic	Asymptomatic	*p* value
	Mean±standard deviation*		
$sPLA_2$ Concentration (µg/ml)	26.1±14	25.7±7.5	0.93
$sPLA_2$ Activity (mmol/min/ml)	0.08±0.02	0.05±0.005	0.12
MDA Concentration (mM)	0.003±0.002	0.002±0.001	0.75

3.3.2 STAGE 2

The mean NISDT of symptomatic lens wearers treated with the liposomal spray immediately after and after 2 and 6 hours of instillation were 9.5±2.2, 7.9±2.5 and 5.8±2.3 seconds and with the placebo spray were 8.8±1.8, 6.5±3.1 and 5.9±2.1 seconds respectively (Figure 1). The NISDT of those treated with the liposomal spray was significantly different (p=0.03) between 0 and 6 hours but not between 0 and 2 hours (p=0.34) whereas the NISDT of the lens wearers treated with the placebo spray was significantly different between 0 and 2 hours (p=0.05), and 0 and 6 hours (p<0.01).

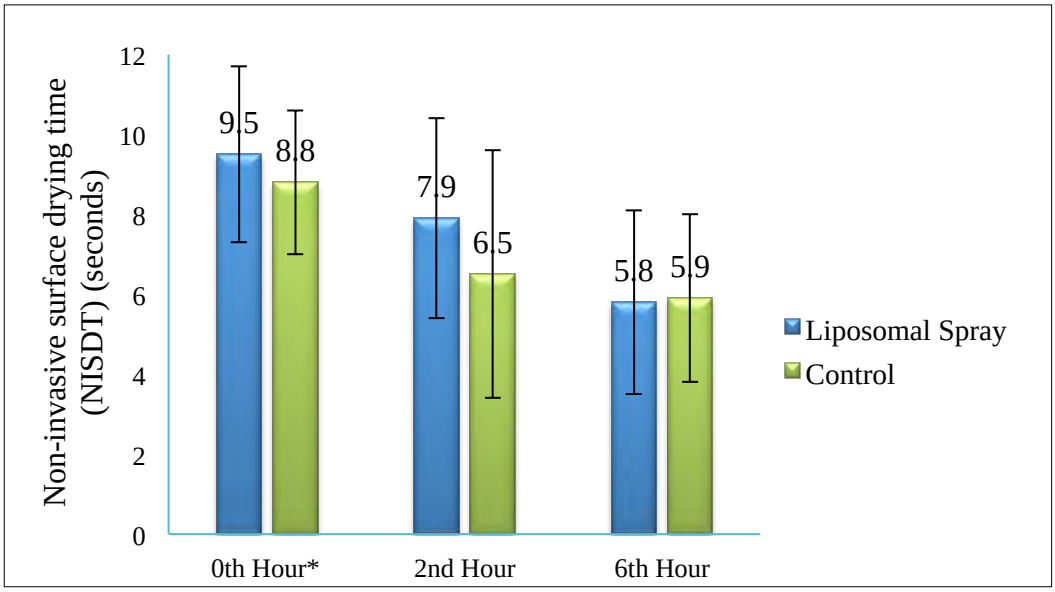


Figure 3-1 Comparison of non-invasive surface drying time immediately after instillation*, 2 and 6 hours followed by a single dose application of the liposomal spray and the placebo spray

Table 3-5 shows the mean±SD ocular comfort scores, concentration and activity of $sPLA_2$ and the concentration of MDA in symptomatic wearers. The ocular comfort scores significantly reduced with time for the placebo spray (p=0.02) but did not reduce with the liposomal spray (p=0.12). There was a strong positive association between the two types of rating scales used (numerical rating scale and visual analogue scale) (r=0.77, p<0.001). A survey regarding the preference of using both scales showed a preference for the numerical rating scale by 70% of study participants. Hence, the numerical rating scale will be used in the future studies to record ocular comfort. As the NISDT increased, there were improved ocular comfort scores for those subjects treated with the liposomal spray (r=0.50, p=0.005) but not with the placebo spray. Use of the liposomal spray did not change the activity of $sPLA_2$ or the concentration of MDA compared to the saline spray although the concentration of $sPLA_2$ slightly but not significantly reduced (p=0.09) at 6 hours following the initial dose of the liposomal spray.

Table 3-5 The ocular comfort scores, concentration and activity of phospholipase enzyme ($sPLA_2$) and the concentration of malondialdehyde (MDA) in symptomatic contact lens wearers following a single application of the liposomal spray and the placebo spray

Variables	0th hour		2nd hour		6th hour		*p* value	
	Mean±standard deviation							
	Liposomal spray	Placebo spray	Liposomal spray	Placebo spray	Liposomal spray	Placebo spray	Liposomal spray	Placebo spray
Numerical rating scale scores	7.8±0.9	8.8±0.7	8.1±1.1	8.2±0.6	7.1±1.7	7.7±1.0	0.12	**0.02**
Visual analogue scale scores	72.9±23.4	88.3±9.7	83.2±14.0	81.5±5.8	67.7±21.9	76.8±9.2	0.13	**0.005**
$sPLA_2$ Concentration (µg/ml)	30.8±16	29.7±21	38.3±21	34.4±15	20.5 ±9	34.9±12	0.09	0.73
$sPLA_2$ Act (µmol/min/ml)	0.08±0.07	0.03±0.01	0.07±0.05	0.08±0.02	0.04±0.03	0.07±0.05	0.69	0.20
#MDA (mM)	0.03±0.02	0.03±0.02	0.03±0.01	0.04±0.03	0.01±0.001	0.02±0.01	0.15	0.11

Samples were pooled to detect MDA.

3.4 DISCUSSION

This chapter illustrated methods for measuring clinical and biochemical aspects of the tear film. The study explored a potential role of the tear film in maintaining the ocular comfort in a small group of symptomatic and asymptomatic lens wearers during short-term contact lens wear. The clinical and biochemical effectiveness of a liposomal spray in contact lens wear was also observed in the second stage of the study.

Contact lens wearers with lower tear volume and reduced tear break up time are more susceptible to contact lens intolerance (Fanti *et al.* 1980, Glasson *et al.* 2006). NISDT measured in the current study refers to the stability of tear film over the lens surface and was significantly lower among symptomatic contact lens wearers. Interestingly, when symptomatic wearers were treated with a liposomal spray, their NISDT did not change until 2 hours after the initial instillation when compared to a placebo saline spray. The absence of significant differences could be due to the small sample size (n=10) and limited power. It can also be speculated that the spray does not affect the lipid layer of tears immediately; probably the spray is not sufficiently migrating into the tear film from the eyelid and hence needs some time to interact significantly with this layer. Improved clinical symptoms and ocular comfort when using the same liposomal spray have been shown previously among healthy and dry eye population and among contact lens wearers (Lee *et al.* 2004, Kunzel 2008, Craig 2010).

Bowman *et al*. (1987) found increased levels of cholesterol esters and decreased levels of wax esters in meibum of patients with meibomian gland dysfunction. They suggested that the differences observed in ester composition could increase the melting point of meibomian gland secretions that might lead to blocked glands and a destabilized tear film. Similarly, McCulley *et al*. (1991) observed alterations in wax and cholesterol ester

fractions of patients with chronic blepharitis where they proposed that the differences observed in ester fractions were either regulated by the meibomian gland or due to the activity of bacterial lipases. Approximately 50% of patients with chronic blepharitis and 33% of patients with meibomian gland dysfunction have an associated dry eye disease (Bowman *et al.* 1987, Osgood *et al.* 1989, Dougherty *et al.* 1991, Shine *et al.* 1991). The current study found that a higher proportion of wax esters and lower proportion of cholesterol esters in the tear film resulted in improved tear film stability, and further supports the previous observations.

The effect of lipid ratios in regulating the stability and distribution of lipid films have been observed *in-vitro* using Langmuir films, X-ray diffraction and coarse-grained simulations (Smaby *et al.* 1987, Kulovesi *et al.* 2012, Telenius *et al.* 2012). Neutral lipid classes (cholesterol esters, triglycerides and fatty acids) increase tear film stability (Telenius *et al.* 2012), however a decreased phospholipid to neutral lipid ratio can reduce the stability of the lipid film *in-vitro* (Kulovesi *et al.* 2012). In higher (5.0-7.5 mole%) concentrations of triglycerides, the addition of cholesteryl oleate reduced lipase activity whereas in lower (1.0-2.5 mole%) concentrations of triglycerides, cholesterol oleate enhanced the enzyme activity on a monolayer made of egg phosphatidylcholine (Demel *et al.* 1985, Jackson *et al.* 1985). In the current study, lipid ratios were compared between symptomatic and asymptomatic wearers *in-vivo*, and the asymptomatic group had a higher wax ester to triglyceride ratio of (49.3:1) in their tear lipidome compared to symptomatic wearers (30.4:1). This further emphasizes the significance of lipid ratios in the stability of the tear lipid layer and their association with ocular comfort.

Increased $sPLA_2$ activity results in free fatty acid formation. Arachidonic acid is one of the free fatty acids that can be further metabolised and in the process lead to the formation

of reactive oxygen species (ROS). ROS react with other lipids generating lipid aldehydes (Kudo *et al.* 2002, Adibhatla *et al.* 2008). One of the major by-products of lipid peroxidation is MDA, which in the current study was found to have an association with increased $sPLA_2$ activity and reduced NISDT. Increased enzyme activity and degraded lipids has been observed among symptomatic lens wearers (Glasson *et al.* 2002) and also a reduced tear film stability has been associated with reduced levels of phospholipids in tears (Guillon *et al.* 2002).

In the current study, there was no statistically significant difference in enzyme activity and lipid degradation between symptomatic and asymptomatic wearers. Measuring the concentration of MDA was challenging due to the low levels of MDA in tears and the requirement of large sample volume for detection. In stage 2, due to insufficient tear volume from symptomatic wearers, pooled tear samples were used to detect MDA. The current study results of MDA concentration ranged from 0.003-0.04 mM and was much higher than reported concentration for MDA along with another lipid aldehyde, 4-hydroxy-2(E)-nonenal in symptomatic contact lens wearers (0.84±1.0 μM) (Glasson *et al.* 2002). However, the current results were similar to a recent report on the levels of MDA in an elderly population (0.05±0.03 mM/μl) using a high performance liquid chromatography technique (Benlloch-Navarro *et al.* 2013). These differences could arise due to the different analytical kits used to detect MDA. The method described in section 3.2.2.3.2 will be used to detect MDA in this thesis as this was found to be sufficiently sensitive to detect the aldehyde at a tear dilution of 1:10.

3.5 CONCLUSION

This pilot study confirmed that contact lens wear causes clinical and biochemical changes to the tear lipid layer. Though the study provided preliminary evidence for a clinical and

biochemical basis to the effectiveness of an exogenous lipid supplement used during contact lens wears, there was no association between the improved ocular comfort and phospholipase activity or lipid degradation. This could be due to the small sample size (n=10). To obtain a significant difference between the treatment and control, a sample size of 24 is required based on the current data of $sPLA_2$ activity at 6 hours after the treatment with a clinically significant difference of 0.03±0.05 μmol/min/ml at 0.05% significance level with 80% power. Insufficient tear volume obtained from symptomatic lens wearers precluded mass spectrometer analysis of tear lipidome in stage 2. In addition, to detect MDA, pooled tear samples were used rather than individual samples. A minimum of 20 μl of basal tears is required to conduct the ELISAs and lipidome analysis. Basal tear collection using microcapillary tubes often generates lower volume of tear samples and is tedious and time consuming. The subsequent chapter will evaluate alternative methods of tear collection for tear lipid analysis.

CHAPTER 4. BASAL, REFLEX AND FLUSH TEARS

4.1 INTRODUCTION

A major limitation in the preliminary study described in CHAPTER 3 was the limited volume of tear samples, which precluded comprehensive analysis of the tear lipidome. CHAPTER 4 therefore considers alternative methods of tear collection. Basal (non-stimulated) tears collected using microcapillary tubes is generally time consuming and tedious for both the examiner and patient. An alternative option is to stimulate reflex tearing to provide increased volume of tears at a faster rate (Van Haeringen 1981, Fullard *et al.* 1991). Bjerrum *et al.* (1994) introduced a technique named 'flush tears' where tear samples are collected by flushing the eye with saline solution. This has made the collection process quicker.

In basal and reflex tears, the major lacrimal gland proteins such as lysozyme and lactoferrin are present at similar concentrations, and so are independent of the tear flow rate (Berta 1986). However, the concentration of serum proteins is reduced during reflex tearing, as is the concentration of secretory Immunoglobulin A (Berta 1986, Fullard *et al.* 1990). A range of tear proteins can be detected in basal but not in reflex tears (Berta 1986, Fullard *et al.* 1990). A significantly lower concentration of secretory phospholipase A_2 ($sPLA_2$) has been observed in reflex tears compared to basal tears (Aho *et al.* 2002). Conversely, if the tear film is supplemented by the addition of an aliquot of buffer to facilitate collection, the concentration of the majority of proteins does not change compared to the concentration of proteins obtained from undiluted tear samples using gel electrophoresis after taking into account the dilution effect of the flush buffer (Bjerrum *et al.* 1994, Markoulli *et al.* 2011). Flush tears have also been

used to quantify mucins in tears from patients with Sjögren syndrome (Garcher *et al.* 1998, Argueso *et al.* 2002, Spurr-Michaud *et al.* 2007).

The current study looked at optimising tear collection using microcapillary method for tear lipid analysis. In order to determine whether flush or reflex tear samples can be used as an alternative for basal tears, a randomised cross-sectional study was conducted to characterise the tear lipidome in basal, reflex and flush tears using mass spectrometry. This study aimed to compare the concentrations and mole% of various lipid classes in basal, reflex and flush tear samples collected using glass microcapillary tubes.

4.2 METHODS

4.2.1 STUDY PARTICIPANTS

Ten participants with no history of ocular surface disease or contact lens wear were recruited. The sample size was based on one of the outcome variables, cholesterol esters (mole%). The expected standard deviation was 18.9% to detect a difference of 21.8% at a 0.05 significance level with 80% power. The calculation was based on a pilot study, where basal, reflex and flush tear samples were collected from a group of 4 healthy individuals between 12-2 pm. Ethics approval was obtained from Human Research Ethics Advisory committee at the University of New South Wales, Sydney, Australia (HREA approval no: 13190). The study was conducted in accordance with the tenets of the Declaration of Helsinki. Signed informed consent was obtained from each participant.

4.2.2 STUDY PROTOCOL

The tears of the ten participants were sampled using either basal, reflex or flush tear collection techniques. Tear collection was carried out on three occasions for each participant with the order of collection randomised and allowing at least 24 hours between each collection method. Participants were instructed to tilt their head to one side and a glass micro capillary tube was gently rested on the lateral canthus of the eye and up to 10 µl of tear samples collected from each eye at the same time of the day. Basal tears were collected with caution to prevent reflex lacrimation. Reflex tears were stimulated with a sneeze reflex by gently inserting a sterile cotton bud in to the nasal passage or by self-induced yawning. Flush tears were collected following instillation of a 40 µl drop of unit dose saline (unpreserved sodium chloride for injection BP 0.9% (w/v), AstraZeneca, North Ryde, Australia) into the inferior palpebral fornix using a plastic eppendorf pipette (Markoulli *et al.* 2011). Subjects were instructed to gently close and rotate their eyes and the residual saline was immediately collected using glass capillary tubes.

4.2.2.1 Lipid extraction

The lipid extraction procedure was described in Chapter 3, section 3.2.2.3.3. In brief, an internal standard mix (5 µl) along with methanol (MeOH, 60 µl) and methyl-*tert* butyl ether (MTBE, 200 µl) with 0.01% butylated hydroxytoluene was added to each tear sample (20 µl of basal, reflex and flush samples). The samples were then mixed thoroughly using an orbital shaker at low speed for 30 minutes. Aqueous ammonium acetate (0.15 M) was added to each sample, which was vortexed and centrifuged for 5 minutes at 2000 g. The upper non-polar phase was then carefully extracted without disturbing the lower polar phase. The extracted lipid was dried under nitrogen at 37°C

and reconstituted with 100 µl of 2:1 MeOH/$CHCl_3$ containing 5 mM ammonium acetate. The samples were stored at -80°C until mass spectrometric analysis. The period for storage and sampling were same across each sample.

4.2.2.2 Mass spectrometry

The non-polar lipid classes cholesterol ester (CE), free cholesterol (Chl), wax ester (WE) and triglyceride (TAG) and polar lipids phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) and (O-acyl)-ω-hydroxy fatty acid (OAHFA) were analysed using electrospray ionization tandem mass spectrometry (ESI/MS) as described in section 3.2.2.3.3 (Brown *et al.* 2013).

4.2.3 STATISTICAL ANALYSIS

A Friedman's ANOVA was used to assess whether the concentration of each lipid component and the mole% of each lipid class in the total lipidome varied with tear collection method. Statistical Package for Social Sciences (IBM SPSS Statistics 19, New York) was used for data analysis. A p value of <0.05 was considered statistically significant.

4.3 RESULTS

Ten lipid classes consisting of 123 molecular species were detected in tear samples. All lipid classes except PE were detected from every participant. PE was only detected in basal tears of 4 participants. In addition, those lipid classes of lower abundance (TAG, OAHFA, PE and LPE) were not detected in more than 50% of reflex or flush tear samples. Total lipid concentration differed between each collection technique. Table 4-1 shows the mean concentration of each lipid class in basal, reflex and flush tears. The

concentration (mean±SD pmol/µl) of all non-polar lipid classes (CE, Chl, WE & TAG) and most polar lipid classes (PC, PE, SM and OAHFA) were higher in basal tears, with CE (220.6±202 pmol/µl) being the most abundant and PE (0.1±0.09 pmol/µl) being the least abundant lipid class. The day to day variations in tear lipid profile have been previously indicated to be low (Brown *et al.* 2012). The standard deviation for each lipid class across each collection technique clearly indicates the inter-subject variability (Table 4-1). Hence, the concentration of each lipid class was normalised to mole% for better interpretation.

Table 4-1 Mean concentration of lipid classes in basal, reflex and flush tear samples

Lipid Classes	Basal			Reflex			Flush			*p* value
	Mean±SD	Median	IQR	Mean±SD	Median	IQR	Mean±SD	Median	IQR	
CE	220.6±202	178.1	158.4	45.1±55.1	27.9	26.7	15.7±16.7	13.5	9.6	**0.001**
WE	118.9±111	81.8	56.4	25.1±31.7	15.5	11.3	12.1±10.9	8.3	4.6	**0.004**
Chl	22.5±12.6	16.6	5.2	11.8±5.4	9.8	7.9	6.6±3.6	5.9	2.5	**0.002**
TAG	2.7±2.1	1.6	2.7	0.5±0.7	0.12	0.72	0.3±0.3*	0.22*	0.46*	**0.002**
OAHFA	7.9±9.2	4.3	3.3	0.9±1.2	0.35	0.72	0.3±0.4*	0.14*	0.34*	**0.001**
PC	1.5±0.7	1.6	1.2	1.2±0.5	1.1	0.64	0.6±0.3	0.5	0.27	**0.002**
PE	0.1±0.1*	0.1*	0.2*	0.1±0.1*	0.1*	0.1*	0.04±0.06*	0.00*	0.06*	0.12
SM	1.9±0.5	1.9	0.8	1.5±0.7	1.6	1.0	0.7±0.5	0.61	0.68	**0.03**
LPC	5.7±4.3	3.5	6.7	10.6±6.1	9.5	8.9	3.7±2.6	3.2	3.5	**0.003**
LPE	1.5±1.6	1.0	1.6	1.9±2.1*	1.2*	3.5*	0.3±0.3*	0.00*	0.65*	**0.007**

*Indicates that the lipid class was detected in less than 50% of the total samples under respective collection method. SD: standard deviation, IQR: interquartile range, CE: cholesterol ester, Chl: free cholesterol, WE: wax ester, TAG: triglyceride, PC: phosphatidylcholine, PE: phosphatidylethanolamine, SM; sphingomyelin, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OAHFA: (O-acyl)-ω-hydroxy fatty acid

The mole% of each lipid class substantially varied with collection technique. Non-polar lipids (mean±SD%) formed the bulk of total lipidome in basal (92.8±5.8%), reflex (71.8±23.8%) and flush (83.6±11.3%) tear samples. Figure 4-1 and Figure 4-2 shows the mean proportion of non-polar and polar lipid classes of the total lipidome in tear samples collected with the different techniques. The proportion of CE in the total lipidome was lower in reflex 35.7±19.2%, (p=0.02) and flush 33.0±18.9%, (p=0.02) tears than basal tear samples 54.8±9.3%. The mole% of Chl in the total lipidome was higher (p=0.01) in reflex tears 17.2±10.6% compared to basal tears 8.2±5.2% whereas the mole% of WE was lower (18.6±11.4% vs. 29.1±4.5%, p=0.03). The mole% of total diacyl phospholipids (PC+PE+SM) in the total lipidome were higher in reflex (5.1±4.5%, p=0.03) and was slightly but not significantly higher in flush tears (4.7±4.7%, p=0.06) compared to basal tears (1.5±1.2%). Similarly, the mole% of total lysophospholipids (LPC+LPE) in the lipidome accounted for 22.5±21% and 11.2±7.8% in reflex and flush tear samples but only 3.9±4.0% in basal tear samples. These differences were statistically significant between basal and reflex (p=0.02) but not between reflex and flush samples (p>0.05). The concentration (pmol/μl) and mole% of the 123 individual molecular species detected and quantified in basal, reflex and flush tears is shown in Appendix 2.

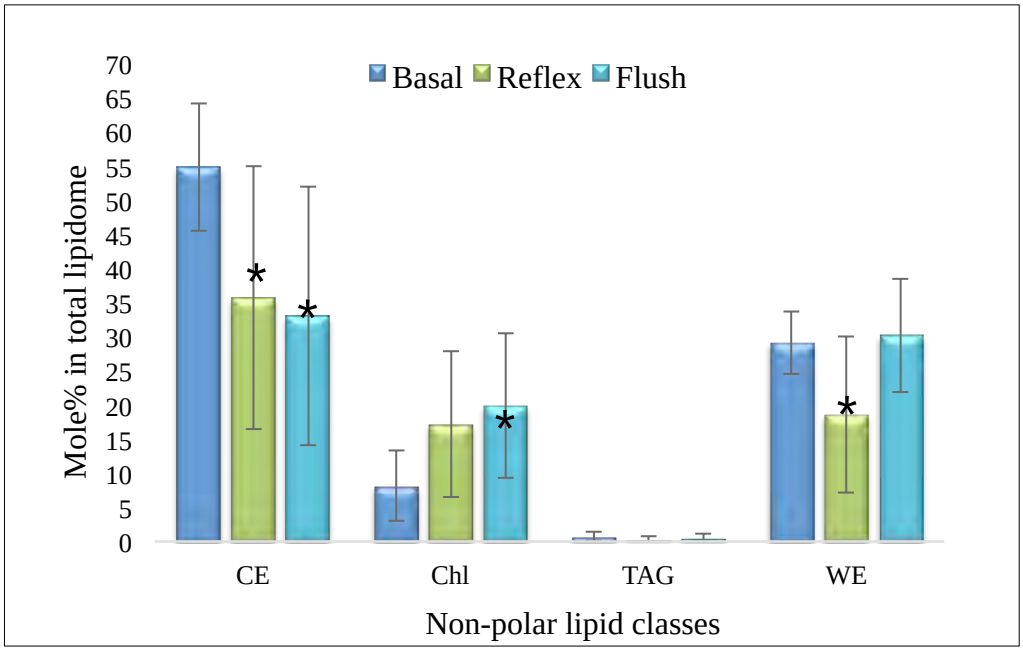


Figure 4-1 Relative proportion of non-polar lipids (CE: Cholesterol ester, Chl: Free cholesterol, TAG: Triglycerides and WE: Wax ester) in total lipidome of basal, reflex and flush tear samples. Error bars represents standard deviation. * indicates a *p* value of <0.05, basal tears vs reflex or flush tears.

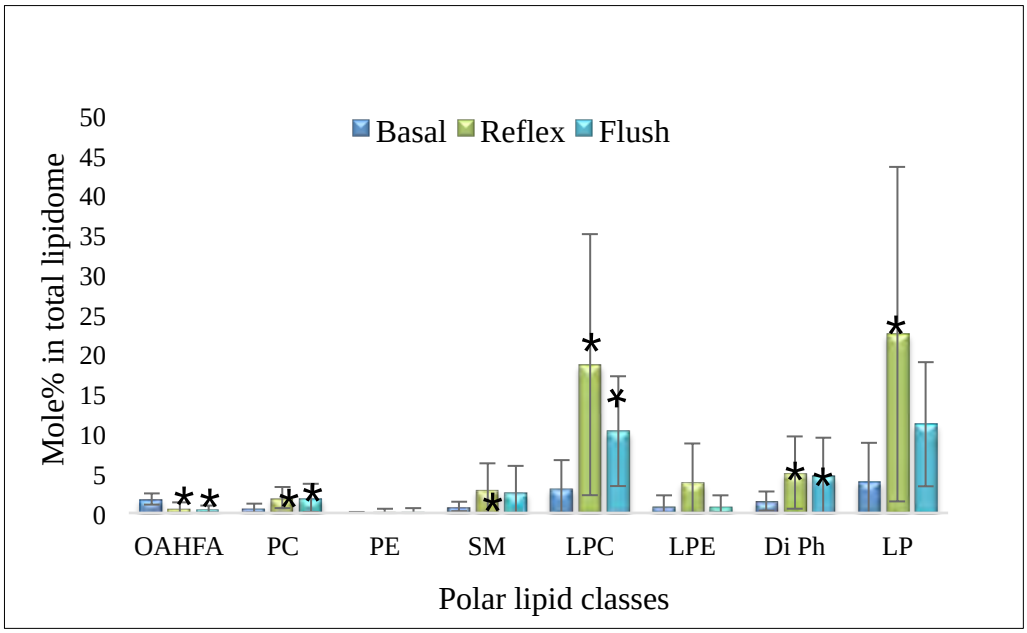


Figure 4-2 Relative proportion of polar lipids (OAHFA: (O-acyl)-ω-hydroxy fatty acid, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, SM: Sphingomyelin, LPC: Lysophosphatidylcholine and LPE: Lysophosphatidylethanolamine, Di Ph: PC+PE+SM, LP: LPC+LPE) in total lipidome of basal, reflex and flush tear samples. Error bars represents standard deviation. * indicates a *p* value of <0.05, basal tears vs reflex or flush tears.

4.4 DISCUSSION

This chapter attempted to consider reflex or flush tear samples using microcapillary tubes as an alternative for basal tears for the characterisation of the tear lipidome. The profile of the tear film lipid in basal, reflex and flush tear samples was compared. Total lipid concentration, concentrations of each lipid class and the mole% of each lipid class in the total lipidome changed with tear collection method. In the current study, an equal volume (20 µl) of basal, reflex and flush tears were used for tear lipidome analysis. No correction was performed for the additional volume of saline in flush tears as the excess fluid dilutes the lipid components as soon as tears are stimulated or flushed.

The reduced concentration of both non-polar and polar lipids in reflex and flush tears was probably due to the dilution of lipid components caused by the increased rate of tear flow while reflexing (18.1±1.9 µl/min) and flushing tears (26.9±1.4 µl/min) than basal (4.5±0.6 µl/min). Aho *et al*. (2002) observed a similar dilution effect of reflex tearing (stimulated by making the participants breathe onion vapour and illuminating their eyes with bright light) in the concentration of phospholipase enzyme, where the concentration was significantly lower ($p<0.05$) in reflex tears 53.4±31.4 mg/ml than in basal tears 75.2±31.3 mg/ml of healthy individuals.

CE and WE were the major components of tear lipidome in basal, reflex and flush tears of healthy individuals, as previously observed by a number of investigators analysing basal tears (Andrews 1970, Nicolaides *et al.* 1981, Wollensak *et al.* 1990, Ham *et al.* 2004, Butovich *et al.* 2007, Chen *et al.* 2010). Interestingly, phospholipid classes such as PC and LPC were higher in proportion in reflex and flush tears than in basal tear samples in the current study. It is argued that only trace amounts of PC (<0.015%) are present in meibum whereas a range of phospholipid classes can be detected and

quantified in the tear lipidome (Butovich *et al.* 2007). Several investigators (Wollensak *et al.* 1990, Farris 1994, Tiffany 1997, Nagyova *et al.* 1999, Borchman *et al.* 2007, Saville *et al.* 2010, Brown *et al.* 2013) confirmed this discrepancy and proposed the possibility of alternative sources for tear phospholipids such as lacrimal gland or conjunctival or corneal epithelial cells.

The proportion of LPC in the current study is concordant with previous studies (Rantamaki *et al.* 2011, Dean *et al.* 2012, Brown *et al.* 2013) where LPC was the most abundant phospholipid class found in tear samples. The presence of LPC is most likely due to the degradation of PC in tears (Rantamaki *et al.* 2011, Brown *et al.* 2013). Evidence suggests that the hydrolysis of *sn*-2 acyl chain of PC is due to the activity of $sPLA_2$ in tears (Saari *et al.* 2001). Tear lipocalin, the principal lipid binding protein in tears (Glasgow *et al.* 1995) was found to have lower affinity towards LPC binding compared to PC (Rantamaki *et al.* 2011). The higher proportions of LPC in reflex and flush tears in the current study suggests either an up-regulation of $sPLA_2$ which may be an active mechanism to degrade and remove phospholipid from the tear film, or may be caused by a reduction of sequestration of LPC by lipocalin in reflex and flushed tears. However, if this was the case there should be a decrease in the mole% of PC, but the current study found that relative level of PC actually increased in flush and reflex tears. This further raises the issue of the origin of PC in the tear lipidome.

The lower mole% of PC observed in basal tears in the current study might be due to the partitioning of PC into the bulk lipid phase in the tear lipid layer (Butovich 2013). This is facilitated either by the thick lipid layer acting as a shield, preventing PC from binding to tear lipocalin or, PC forming homo or hetero dimers with anionic lipids, making it less hydrophilic (Butovich 2013). However, the effect of partitioning is not

evident in reflex and flush tear samples. The higher mole% of PC in flush and reflex tears observed in the current study could be from the cells of lacrimal glands while reflexing or from corneal/conjunctival cells dislodged during flushing.

OAHFA, an amphiphilic lipid component in the tear lipidome were detected in every basal tear sample whereas in 40% of reflex and flush samples OAHFA were undetected, likely due to the dilution effect. OAHFAs have been found in reduced concentrations with increased dry eye disease severity and potentially could be used as indicators for dry eye disease progression (Lam *et al.* 2011).

4.5 CONCLUSION

Flush or reflex tear samples could not be used as an alternative for basal tears as the concentration of several lipid classes was below the limit of detection in reflex and flush tears and the mole% of various lipid species in the total lipidome significantly varied with each collection technique. The lipid profile obtained from basal tears closely aligns with the meibum profile and is appropriate for tear lipid analysis. The issue of obtaining less tear volume at a slower rate remains a concern with basal tear collection using microcapillary tubes. However, with the nano-electro spray ionization mass spectrometry, a tear volume of 2-5 µl is sufficient to detect a range of tear lipid species (Brown *et al.* 2013). Therefore, basal tears will be collected using micro capillary tubes and will be used to characterise tear lipidome in the future studies. Approaches to maximise the tear volume collected using this method are considered in Chapter 5.

CHAPTER 5. EFFECT OF EXOGENOUS LIPID SUPPLEMENTS IN HABITUAL CONTACT LENS WEARERS

5.1 INTRODUCTION

Based on the preliminary studies described in CHAPTER 2 and CHAPTER 3, contact lens wear alters the clinical, functional and biochemical aspects of tear lipid layer. The associations between non-invasive surface drying time and biochemical components after 6-8 hours of contact lens wear (CHAPTER 3) indicate an important role of the lipid layer in modulating contact lens wear comfort. In addition, with an exogenous phospholipid spray, the stability of the tear film was less compromised at the end of 6-8 hours of contact lens wear when compared to a saline-based placebo spray. To investigate these associations further, chapter 5 describes a randomised crossover, placebo-controlled study of habitual contact lens wearers that aims to establish the effect of exogenous lipid supplements on the tear lipid layer and their influence on lens wear comfort. The study tests the hypotheses that improved contact lens comfort is associated with a thicker tear lipid layer and alterations in biochemical composition that result in reduced tear evaporation. Use of an exogenous tear lipid supplement increases lipid layer thickness, reduces lipid degradation, increases tear film stability, reduces tear evaporation and subsequently improves contact lens comfort.

5.2 METHODS

5.2.1 STUDY PARTICIPANTS

Participants with a history of soft contact lens wear of at least 5-6 hours per day for a minimum of 5 days per week were recruited through email and poster advertisements. The study protocol was approved by the Human Research Ethics Advisory panel at the

University of New South Wales and was conducted in accordance with the Declaration of Helsinki (HREA approval no: HC12584). All study participants gave informed consent prior to study commencement. Forty participants (28 females and 12 males) with a mean age of 26±9 years were enrolled. Table 5-1 shows the demographic data of the study participants. The sample size (n=40) was based on an outcome variable of tear evaporation rate (CHAPTER 2) with an expected standard deviation of 53 $g/m^2/h$, to detect a clinically significant difference of 44 $g/m^2/h$ (coefficient of repeatability×1.98×SD), assuming an estimate of type 1 error α=0.05 and power of 80%. The sample size of 40 was including a 10% possible drop out factor.

Table 5-1 Subject demographic data

Patient Factors		Number
Gender	Female	28
	Male	12
Ethnicity	Asian	23
	Caucasian	17
Symptomatology	Asymptomatic	24
	Symptomatic	16

5.2.2 STUDY PROTOCOL

The study was designed as a double-masked, randomised crossover, placebo-controlled intervention study. Prior to the intervention phase, the study included two baseline visits at the end of the day, 1) without lens wear and 2) with lens wear. Two types of exogenous lipid supplement were used as interventions along with a saline-based placebo for each. The interventions included an emulsion drop containing phosphatidylglycerol (PG) (Systane Balance, Alcon, Frenches Forest, NSW, Australia) and a liposomal spray containing phosphatidylcholine (PC) (Tears again, BioRevive,

Burnley, Victoria, Australia). The placebo used was non-preserved saline solution (sodium chloride injection 0.9%, AstraZeneca, North Ryde, Australia) delivered in a spray or a drop bottle. Each participant used two interventions and two placebo interventions three times a day for two weeks in a random order with 48 hours washout period between the intervention stages. Participants were advised not to wear contact lenses during each washout period. The intervention visits occurred at the end of day 1 and day 14 of each intervention type (4x2=8 visits). All participants were fitted with new lenses (Ciba Vision, Air Optix®) prior to the commencement of each intervention type. Participants were instructed to wear their contact lenses for 6-8 hours on the day of measurements. Each participant followed the same care regimen throughout the study period that involved a hydrogen peroxide disinfection (AOSEPT Plus® Ciba Vision, NSW, Australia). Each visit was scheduled strictly within 30-45 minutes following the last use of treatment. At each visit, including the baseline visits, the following tests were conducted in an order from least invasive to the most invasive technique.

5.2.2.1 Ocular comfort

The Contact Lens Dry Eye Questionnaire (CLDEQ-12) was used at the baseline visit only to categorise habitual contact lens wearers into symptomatic and asymptomatic wearers. The Ocular Comfort Index (OCI) was administered at the end of each intervention stage to measure a change in comfort following the intervention. A numerical rating scale ranging from 0 to 10 in 0.50 steps was used to record the overall ocular comfort at every visit. The rationale for the use of these instruments is described in Chapter 3, section 3.2.2.1.

5.2.2.2 Tear Osmolarity

Tear osmolarity is considered key indicator for dry eye diagnosis (Farris 1994, Khanal *et al.* 2008, Sullivan *et al.* 2010, Versura *et al.* 2010). Tear osmolarity was measured using an osmometer which collects 50 nanoliters of tears and displays tear osmolarity in mOsms/l (TearLab osmometer, San Diego, CA) (Benelli *et al.* 2010, Eperjesi *et al.* 2012). One eye was randomly selected for osmolarity testing. Due to the large scale of the study, the osmolarity for one eye only rather than worse eye is reported.

5.2.2.3 Tear evaporation rate

Tear evaporation rate was measured using a modified dermatologic instrument as described in CHAPTER 2.

5.2.2.4 Lipid layer grade and non-invasive surface drying time (NISDT)

The appearance of the lipid layer pattern and the non-invasive surface drying time (NISDT) were observed using the Tearscope as described in Chapter 3, section 3.2.2.2.1 and 3.2.2.2.2. As mentioned in section 3.2.2.2.2, in this thesis, NISDT refers to the tear film stability on the contact lens surface.

5.2.2.5 Tear collection and biochemical analyses

Basal tears (up to 15 µl of tears from each eye) were collected using a glass microcapillary tube to detect the concentration and activity of $sPLA_2$, the concentration of a lipid aldehyde malondialdehyde (MDA), and the concentration of various lipid classes. To obtain sufficient tear volume for performing all analyses, 45 minutes was allocated for tear collection with regular breaks. The concentration of phospholipase A_2 in tear samples was quantified using a double antibody sandwich ELISA (Cayman Chemical Company, MI USA). The catalytic activity of $sPLA_2$ was measured by the

hydrolysis of 1, 2-dithio analogue of diheptanoyl phosphatidylcholine to detect free thiols (Cayman Chemical Company, MI USA). The concentration of MDA was measured using a carbocyanine dye yielded from the reaction between N-methyl-2-phenylindole and MDA at 45°C (Biokits.com Bioxytech^{R} MDA-586^{TM}, Dublin, USA). Tear lipid extraction was performed using a methyl-tert-butyl-ether/methanol/ammonium acetate two-phase extraction. A nanoelectrospray tandem mass spectrometry (ESI/MS) was used to characterise the tear lipidome and to detect the active ingredients present in the lipid supplements used in the current study (Brown *et al.* 2013). The assay protocol, tear lipid extraction and procedures are described in Chapter 3, section 3.2.2.3.

5.2.3 STATISTICAL ANALYSIS

All statistical tests were performed using Statistical Package for Social Sciences (IBM SPSS Statistics 19, New York). A linear mixed model was developed to compare the clinical, functional and biochemical variables of the contact lens wearers with the lipid and placebo treatments. Non-parametric tests were conducted to compare any categorical variables. To determine associations between the variables, Pearson or Spearman correlation coefficient was used where appropriate. Prior to the main analysis, the influence of demographic factors such as age, gender and ethnicity in ocular comfort was assessed. A p value of <0.05 was considered statistically significant and those values significant at 10% were reported as a slight but not statistically significant change. A multivariate analysis was used to determine the tear lipid predictors of ocular comfort using a linear mixed model constructed using backward elimination of variables initially followed by forward entry. The final model included only the factors that were significant at the 5% level and factors significant at the 10% level if the R^2 value increased at least by 2%.

Since the study included multiple visits, the results were analysed in two stages. A linear mixed model was obtained for i) treatment visits including lipid and saline placebo with baseline contact lens wearing data and ii) treatment visits with type (lipid or placebo), method (spray or drop) and duration (day 1 or 14) of treatment as factors. A linear mixed model was derived to compare each treatment (lipid spray, lipid drop, placebo spray and placebo drop) at day 1 and day 14 to that of baseline to test the efficacy of repeated use of treatments. In each stage the results are reported for changes in i) ocular comfort, ii) clinical and functional variables and iii) biochemical variables including concentration of lipid classes.

5.3 RESULTS

Based on the scores obtained from the CLDEQ-12, there were 16 symptomatic and 24 asymptomatic contact lens wearers among the 40 participants recruited. A paired *t* test comparing right and left eye measurements for ocular comfort scales, NISDT and tear evaporation rate showed no significant difference between eyes ($p>0.05$). Similarly, there was no significant difference between the right and left eye grades of lipid layer pattern when analysed using Wilcoxon sign rank test ($p>0.05$). Tear osmolarity was either recorded for the right or left eye at each visit in a random order. Hence, for all the clinical variables such as tear osmolarity, NISDT, lipid layer grade and tear evaporation rate, only one eye measurement was considered for statistical analysis. Since basal tears were collected from both eyes, all biochemical variables analysed in this study were considered as an average measurement of right and left eye. Age, gender and ethnicity did not show a significant influence on ocular comfort and hence were not included in the multivariable analysis.

Since there was no baseline prior to each treatment stage, an analysis that determined the effect of treatment order was carried out. There were four treatment stages such as lipid spray, placebo spray, lipid drop and placebo drop with order number assigned as 1, 2, 3, and 4 respectively. In total, there were 16 order combinations. With so many combinations of orders, it is expected that order will have no significant effect. However, it was added into the model for one of the major outcome variable NISDT, and the p value was 0.68 at 95% significance level.

5.3.1 BASELINE COMPARISONS

Prior to the mixed model analysis, the baseline data (i.e. without spray or drop use) with and without lens wear were compared. The baseline comparisons assessed whether 1 day of contact lens wear changed ocular comfort, tear osmolarity, tear film stability, tear evaporation rate, concentration and activity of $sPLA_2$, concentration of MDA and concentration of various lipid classes in tears. Table 5-2 summarises the results of baseline comparisons. A detailed description of each outcome variable then follows.

Table 5-2 The effect of contact lens wear on tear lipid variables of participants

Purpose of analysis		Outcome variables	Effect of contact lens wear
To investigate the effect of 1 day of contact lens wear on each outcome variable measured.		Ocular comfort score	No change
		Osmolarity	Increased (p=0.003)
		Tear/lipid layer stability	Decreased (p<0.001)
		Evaporation rate	Increased (p=0.03)
		Lipid layer thickness	Decreased (p<0.001)
		$sPLA_2$ Concentration	No change
		$sPLA_2$ Activity	
		MDA Concentration	
		Cholesterol esters (CE)	
		Wax esters (WE)	
		Free cholesterol (Chl)	
		Triglycerides (TAG)	
		Phosphatidylcholine (PC)	
		Phosphatidylethanolamine (PE)	Decreased slightly (p=0.08)
		Phosphatidylserine (PS)	Decreased slightly (p=0.07)
		Sphingomyelin (SM)	Decreased slightly (p=0.07)
		Lysophosphatidylcholine (LPC)	No change
		Lysophosphatidylethanolamine (LPE)	
		(O-acyl)-ω-hydroxy fatty acid (OAHFA)	
Summary	**Effect of contact lens wear**	• With contact lens wear, tear osmolarity and tear evaporation rate increased, tear film stability and lipid layer thickness reduced, and the concentration of some phospholipid classes slightly reduced.	
	Symptomatic wearers vs. asymptomatic wearers at baseline	• Symptomatic wearers showed slightly (p=0.07) lower ocular comfort, reduced tear film stability (p=0.002), higher concentration of Chl and SM (p=0.001 and p=0.01 respectively) and slightly (p=0.09) higher levels of LPC compared with asymptomatic wearers.	

5.3.1.1 Ocular comfort

Ocular comfort scores using the numerical rating scale showed no significant difference (p=0.99) between lens wear and no lens wear (Table 5-3). However, symptomatic subjects had slightly (p=0.07) reduced ocular comfort compared with the asymptomatic group without lens wear whereas there was no significant differences between these groups (p=0.26) during contact lens wear. A scatter plot analysis showed that symptomatic wearers had a significant association between ocular comfort with and without lens wear (r=0.58, p=0.01) (Figure 5-1) but the asymptomatic group did not (p=0.78). Ocular comfort score using the Ocular Comfort Index was recorded only during lens wear visits and not at baseline.

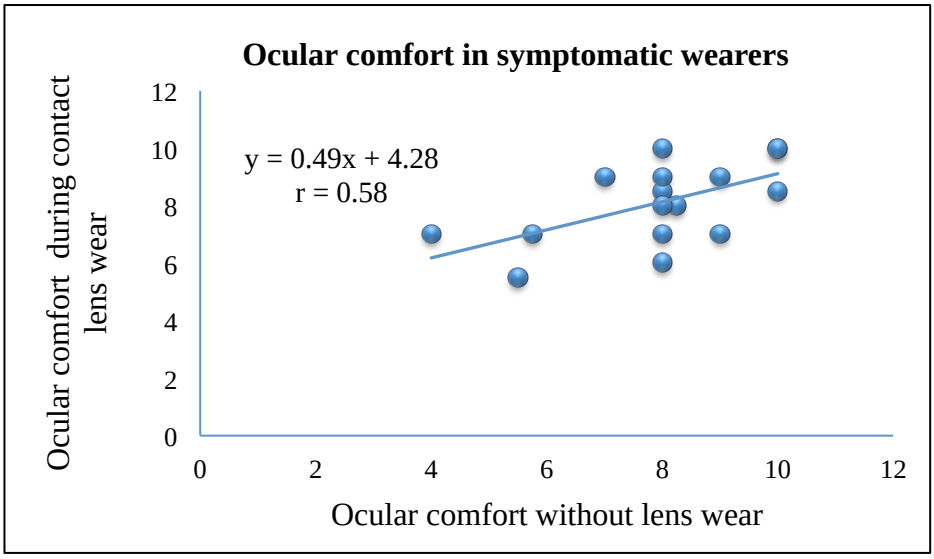


Figure 5-1 Ocular comfort with and without lens wear in symptomatic wearers

5.3.1.2 Clinical and functional variables

Clinical and functional variables included tear osmolarity, NISDT, lipid layer grade and tear evaporation rate. Since NISDT and tear evaporation rate did not exhibit a normal distribution, the data were log transformed and the p values for the respective variables

are reported based on the log-transformed data. With contact lens wear, the tear osmolarity increased (p=0.003) and NISDT reduced (p<0.001) compared to without contact lens wear (Table 5-3). In symptomatic wearers, NISDT was substantially reduced compared to asymptomatic wearers during lens wear (6.8±2.7 vs. 4.2±3.3 seconds, p=0.002). Without lens wear, symptomatic and asymptomatic wearers showed similar pre-corneal tear breakup times (17.8±6.3 vs. 16.1±6.1 seconds, p=0.15). Tear evaporation rate significantly increased (p=0.03) with lens wear (Table 5-3) but was not significantly different between symptomatic and asymptomatic wearers.

The lipid layer grades were significantly different (p<0.001) during lens wear compared to no lens wear (Table 5-3). Wave (45%), amorphous (32.5%) and coloured fringes (22.5%) were the common lipid layer patterns (grades 3-5 respectively) observed when there was no contact lens in the eye. With contact lens wear, the lipid layer ranged from grades 0-5 with closed meshwork (20%) and wave (47%) patterns being the most common (Figure 5-2). However, there was no significant difference between symptomatic and asymptomatic wearers in their lipid layer distribution.

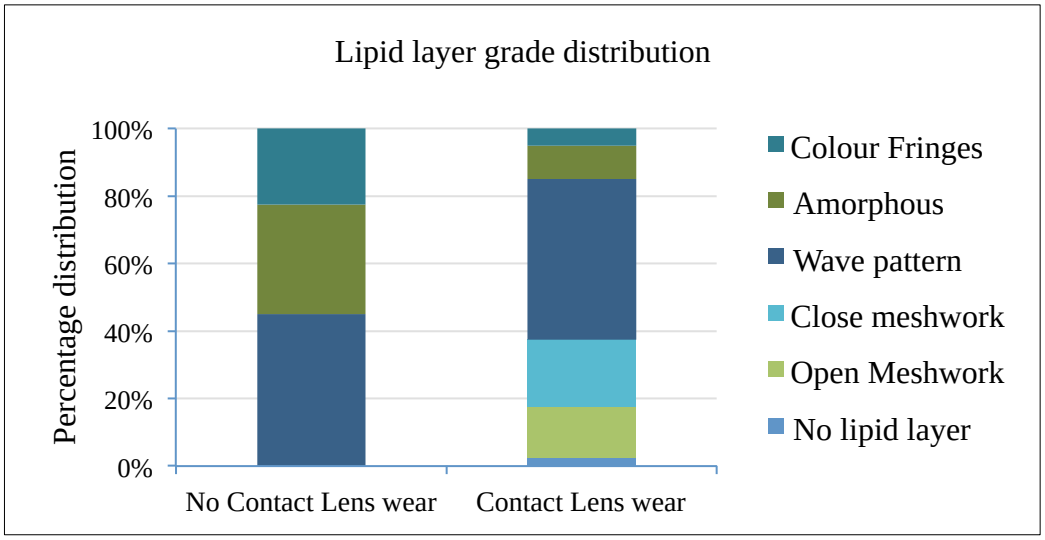


Figure 5-2 Distribution of lipid layer pattern with and without contact lens wear

5.3.1.3 Biochemical variables

Due to the high inter-subject variability in biochemical tear data, log transformation was performed for all variables prior to analysis. Neither contact lens wear nor the patient's symptomatology had a significant effect on the concentration and activity of $sPLA_2$, or the concentration of lipid aldehyde MDA. Similarly, non-polar lipid classes such as CE, WE, Chl and TAG did not show a significant difference with and without lens wear. The concentration of phospholipid classes such as PE (p=0.08), PS (p=0.07) and SM (p=0.06) slightly reduced with lens wear (Table 5-3). The concentrations of other polar lipid classes such as PC, LPC, LPE and OAHFA did not change with lens wear. Symptomatic wearers showed significantly higher concentrations of Chl (p=0.001), SM (p=0.01) and slightly higher concentration of LPC (p=0.09) in tears than asymptomatic wearers with lens wear (Figure 5-3). Lipid class ratios were compared between symptomatic and asymptomatic wearers with and without lens wear but were not significantly different.

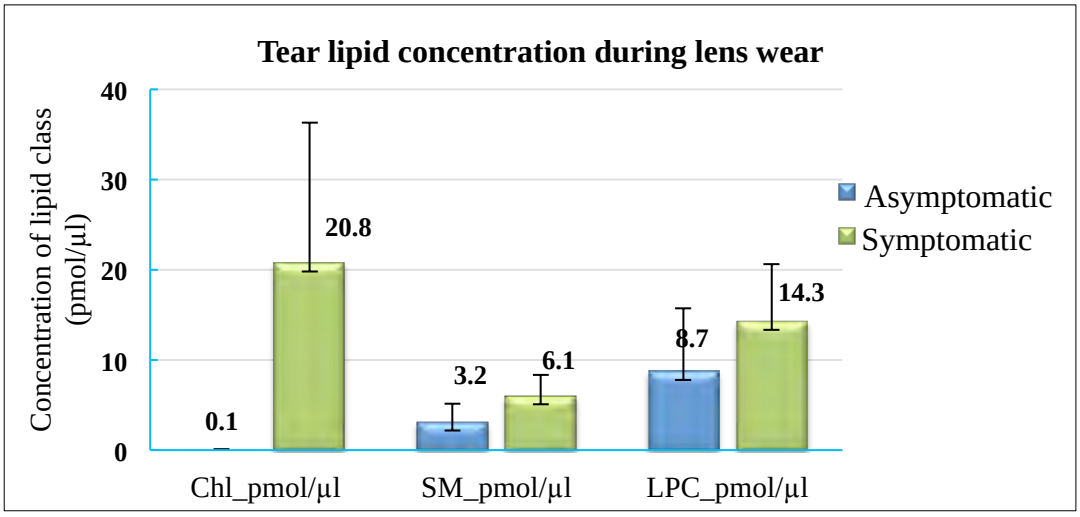


Figure 5-3 Concentration (pmol/µl) of free cholesterol (Chl), sphingomyelin (SM) and lysophosphatidylcholine (LPC) in the tears of symptomatic and asymptomatic contact lens wearers during lens wear. Error bars indicate standard deviation.

Table 5-3 shows the results from the baseline comparison of tear lipid variables with and without contact lens wear.

Table 5-3 Comparison of tear lipid variables with and without contact lens wear

Variables	Contact lens (Mean±SD)		*p* value
	NO	YES	
Ocular comfort scores (1–10) (Higher score indicates better comfort)	8.4±1.3	8.3±1.2	0.999
Tear osmolarity (mOsms/l)	298±8.4	304±9.7	**0.003**
***Tear film stability(seconds)**	17.1±6.4	5.8±3.2	**<0.001**
***Evaporation rate ($g/m^2/h$)**	109±70	137±74	**0.032**
#Lipid layer grade	4.0±1	3.0±1	**<0.001**
***$sPLA_2$ Concentration (µg/ml)**	43.3±23.8	51.1±27.8	0.517
***$sPLA_2$ Activity (mmol/min/ml)**	0.41±0.3	0.53±0.4	0.108
***MDA Concentration (mM)**	1.14±0.8	1.16±1.0	0.898
***Cholesterol ester (CE) (pmol/µl)**	127±70	175±210	0.432
***Wax ester (WE) (pmol/µl)**	47±37	111±139	0.694
***Free cholesterol (Chl) (pmol/µl)**	33.4±8.5	9.7±14.8	0.229
***Triglyceride (TAG) (pmol/µl)**	2.4±1.4	2.9±4.3	0.338
***Phosphatidylcholine (PC) (pmol/µl)**	4.2±1.7	8.8±7.5	0.380
***Phosphatidylethanolamine (PE) (pmol/µl)**	1.0±0.2	0.5±0.6	0.080
***Phosphatidylserine (PS) (pmol/µl)**	1.7±0.8	1.8±1.6	0.072
***Sphingomyelin (SM) (pmol/µl)**	7.3±1.7	4.5±2.5	0.066
***Lysophosphatidylcholine (LPC) (pmol/µl)**	13.4±3.1	11.4±7.0	0.177
***Lysophosphatidylethanolamine (LPE) (pmol/µl)**	8.4±3.4	7.4±6.8	0.594
***(O-acyl)-ω-hydroxy fatty acid (OAHFA) (pmol/µl)**	9.8±7.6	6.2±3.6	0.118

**p* value based on the log-transformed data. # Median±interquartile range is shown, SD: standard deviation.

5.3.2 EFFECT OF LIPID AND PLACEBO SUPPLEMENTS COMPARED TO BASELINE

The two types of lipid supplements used were a liposomal spray and a lipid emulsion drop with their respective placebo (saline spray and saline drop). A linear mixed model assessed whether ocular comfort, tear osmolarity, NISDT, tear evaporation rate,

concentration and activity of $sPLA_2$, concentration of MDA and concentration of various lipid classes in tears were affected by the lipid spray or the lipid drop with respect to baseline. Since the visits were conducted at the end of day 1 and day 14 following the administration of treatments, the analysis was performed for day 1 and day 14 separately. Table 5-4 summarises the results from the current analysis. A detailed description on the effect of the lipid supplements and placebo on each tear lipid variable follows.

Table 5-4 Summary of results where the effect of treatment on each outcome variable compared to the baseline data was investigated

Purpose of analysis	Outcome variables	Effect of treatment compared to baseline			
		Spray		Drop	
		Day 1	Day 14	Day 1	Day 14
To investigate whether the lipid or placebo treatment changed the outcome variable compared to baseline at day 1 and day 14 following the treatment	Ocular comfort score	No effect		Reduced with the lipid only (p=0.007)	
	Ocular comfort Index score			No effect	
	Osmolarity				
	Tear/lipid layer stability	Reduced with the placebo only (p=0.001)		No effect	Reduced with the lipid and placebo (p<0.001)
	Evaporation rate	No effect		No effect	
	Lipid layer thickness			No effect	Reduced with the placebo only (p=0.03)
	$sPLA_2$ Concentration			Increased with the lipid and placebo (p=0.01)	No effect
	$sPLA_2$ Activity	No effect		No effect	
	MDA Concentration				
	Cholesterol esters (CE)			Increased with the lipid and placebo (p=0.02)	Increased with the placebo only (p=0.02)
	Wax esters (WE)			No effect	

<table>
<tr><th rowspan="3">Purpose of analysis</th><th rowspan="3">Outcome variables</th><th colspan="4">Effect of treatment compared to baseline</th></tr>
<tr><th colspan="2">Spray</th><th colspan="2">Drop</th></tr>
<tr><th>Day 1</th><th>Day 14</th><th>Day 1</th><th>Day 14</th></tr>
<tr><td>Free cholesterol (Chl)</td><td rowspan="2">Increased with the lipid only (p=0.02, p=0.01 respectively)</td><td rowspan="2">No effect</td><td colspan="2">Increased with the lipid and placebo (p<0.001, p=0.001 respectively)</td></tr>
<tr><td>Triglycerides (TAG)</td><td>Increased with the lipid and placebo (p=0.001)</td><td>No effect</td></tr>
<tr><td>Phosphatidylcholine (PC)</td><td colspan="2" rowspan="6">No effect</td><td colspan="2">No effect</td></tr>
<tr><td>Phosphatidylethanolamine (PE)</td><td>Increased with the lipid and placebo (p<0.001)</td><td>No effect</td></tr>
<tr><td>Phosphatidylserine (PS)</td><td>No effect</td><td>Increased with the lipid</td></tr>
<tr><td>Sphingomyelin (SM)</td><td>Increased with the lipid and placebo (p=0.001)</td><td>No effect</td></tr>
<tr><td>Lysophosphatidylcholine (LPC)</td><td colspan="2" rowspan="2">No effect</td></tr>
<tr><td>Lysophosphatidylethanolamine (LPE)</td></tr>
<tr><td>(O-acyl)-ω-hydroxy fatty acid (OAHFA)</td><td>No effect</td><td>Increased with the lipid only (p=0.03)</td><td>Increased with the lipid and placebo (p=0.003)</td><td>Increased with the placebo only (p=0.001)</td></tr>
</table>

<table>
<tr><th colspan="2" rowspan="3">Purpose of analysis</th><th rowspan="3">Outcome variables</th><th colspan="4">Effect of treatment compared to baseline</th></tr>
<tr><th colspan="2">Spray</th><th colspan="2">Drop</th></tr>
<tr><th>Day 1</th><th>Day 14</th><th>Day 1</th><th>Day 14</th></tr>
<tr><td rowspan="2">Summary</td><td>Effect of treatment compared to baseline</td><td colspan="4">The drop treatment significantly changed some variables from baseline compared to the spray.
The lipid drop reduced ocular comfort at day 1 and reduced tear film stability at day 14. The placebo drop reduced tear film stability and lipid layer thickness at day 14. The lipid and placebo drop increased the concentration of $sPLA_2$, CE, Chl, TAG, PE, SM and OAHFA at day 1 only whereas, at day 14, the placebo drop increased the concentration of CE and OAHFA.
The lipid spray only increased the concentration of Chl and TAG at day 1 and OAHFA at day 14, and the placebo spray reduced tear film stability at day 1 and 14.</td></tr>
<tr><td>Effect of treatment in symptomatic wearers vs. asymptomatic wearers compared to baseline</td><td colspan="4">Drop treatment
Symptomatic wearers had higher concentration of LPE at day 1 and 14 compared to asymptomatic wearers with the lipid drop. A reduced concentration of OAHFA and WE was observed in symptomatic wearers compared to asymptomatic wearers at day 1 with the placebo drop.
Spray treatment
Symptomatic wearers showed lower ocular comfort and reduced tear film stability at day 1 and 14 and, increased $sPLA_2$ activity and LPE at day 14 compared to asymptomatic wearers with the lipid spray.</td></tr>
</table>

5.3.2.1 Lipid spray

5.3.2.1.1 Ocular comfort

The ocular comfort measured using the numerical rating scale remained unaffected with the lipid spray and the placebo spray compared to the baseline comfort at day 1 (p=0.37, p=0.99) and at day 14 (p=0.11, p=0.43) (Table 5-5). However, symptomatic wearers showed reduced ocular comfort scores compared to asymptomatic wearers at day 1 (p=0.02) and day 14 (p=0.005) with the lipid spray. Similarly, the comfort scores were significantly reduced in symptomatic wearers compared to asymptomatic wearers with placebo spray at day 1 (p=0.02) but not at day 14 (p>0.05) (Figure 5-4). The ocular comfort index (OCI) measured at day 14 showed no change in ocular comfort either with the lipid spray (p=0.99) or with the placebo (p=0.13) compared to baseline (Table 5-5).

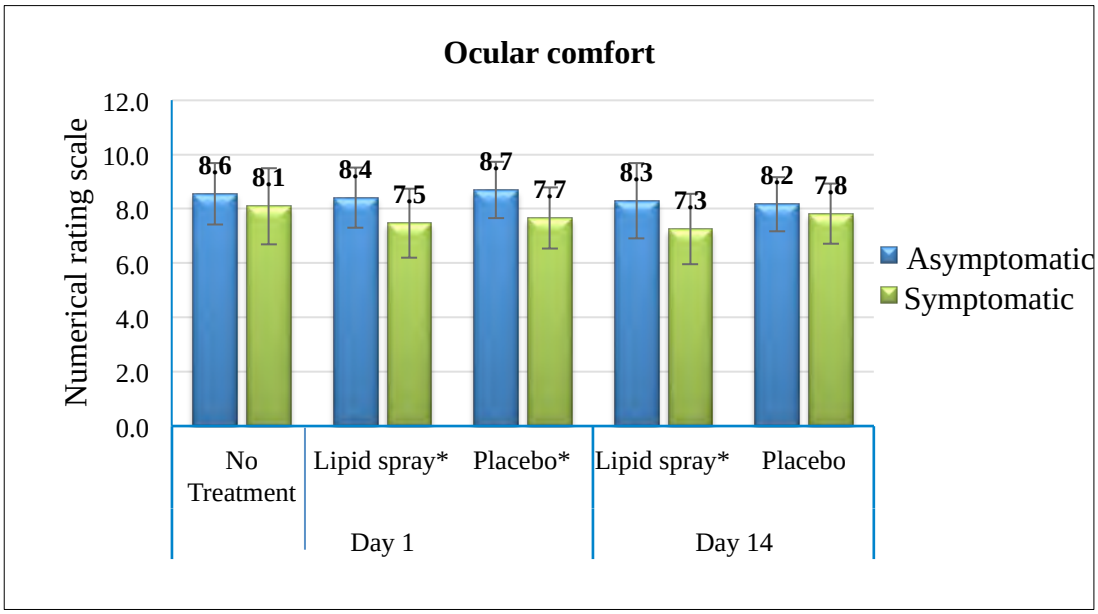


Figure 5-4 Ocular comfort scores of symptomatic and asymptomatic wearers following the use of the lipid spray and the placebo at day 1 and day 14. Error bars indicate standard deviation. * indicates groups are statistically different

5.3.2.1.2 Clinical and functional variables

The NISDT significantly reduced with the placebo spray from baseline to day 1 (p=0.002) and day 14 (p=0.01) whereas the lipid spray had no effect on the NISDT either at day 1 (p=0.99) or at day 14 (p=0.11) compared to baseline (Table 5-5). Symptomatic wearers had shorter NISDT compared to asymptomatic wearers at baseline (p=0.002), with the lipid spray (p=0.03, p=0.03) and with the placebo (p=0.04, p=0.02) at day 1 and day 14 respectively (Figure 5-5).

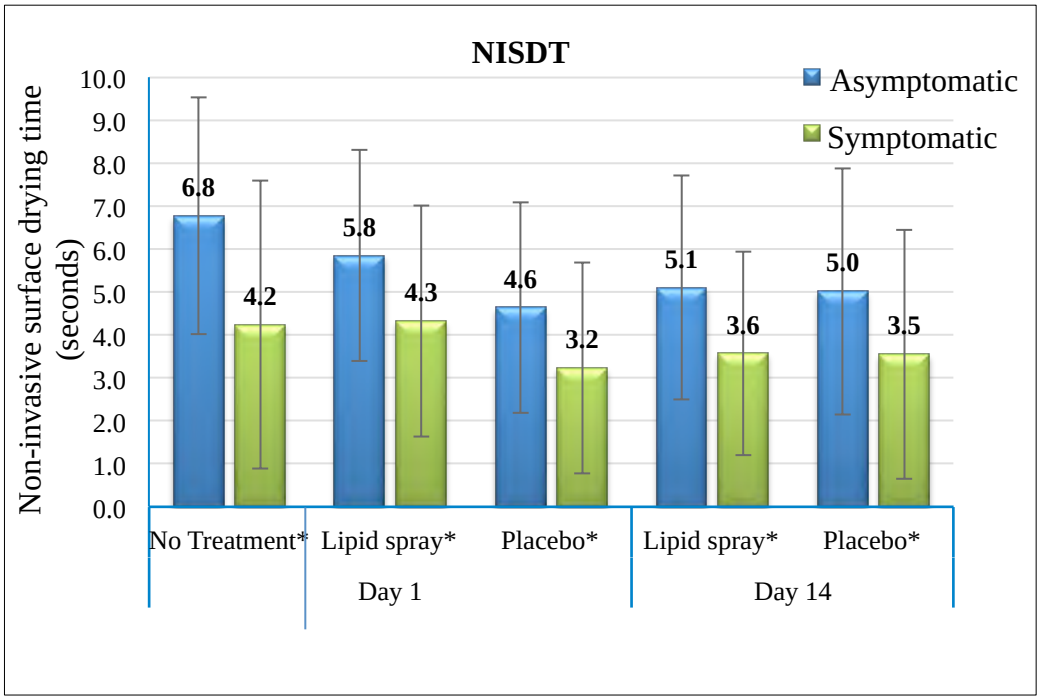


Figure 5-5 Non-invasive surface drying time of symptomatic and asymptomatic wearers following the use of the lipid spray and the placebo at day 1 and day 14. Error bars indicate standard deviation. * indicates groups are statistically different

Tear osmolarity and tear evaporation rate did not change from baseline either with the lipid spray or with the placebo spray at day 1 (p=0.92, p=0.11) or at day 14 (p=0.36, p=0.48) (Table 5-5). A Freidman's ANOVA compared the effect of treatment on lipid

layer patterns. Neither lipid spray nor placebo affected the lipid layer pattern from baseline at day 1 (p=0.12) or at day 14 (p=0.29) (Table 5-5).

5.3.2.1.3 Biochemical variables

The concentration and activity of $sPLA_2$ and the concentration of MDA in tears remained unaffected with the lipid spray or the placebo spray at day 1 (p=0.29, p=0.65, p=0.99) and at day 14 (p=0.14, p=0.96, p=0.96) compared with baseline (Table 5-5). Similarly, symptomatic and asymptomatic wearers showed no significant difference in the concentration (p=0.12) and activity of $sPLA_2$ (p=0.15) and the concentration of MDA (p=0.65) in tears with the lipid or placebo spray at day 1. However, at day 14, the activity of $sPLA_2$ in tears of symptomatic wearers was higher than asymptomatic group irrespective of the treatment type (lipid or placebo spray) (p<0.05) (Figure 5-6).

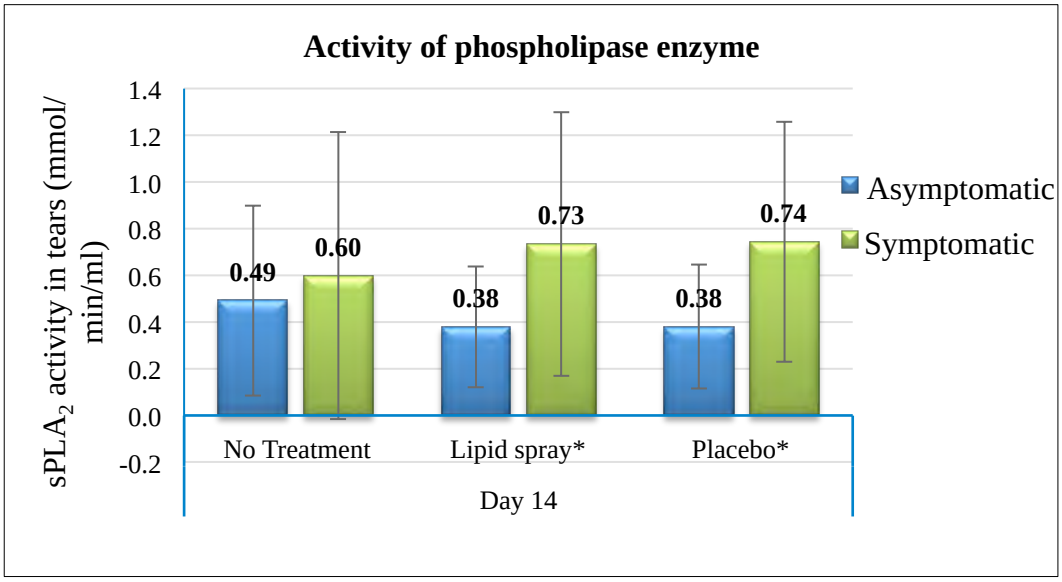


Figure 5-6 Activity of $sPLA_2$ in tears of symptomatic and asymptomatic wearers following the use of the lipid spray and the placebo at day 14. Error bars indicate standard deviation. * indicates groups are statistically different

The active ingredient present in the lipid spray (phosphatidylcholine (34:2)) was not detected in any of the tear samples analysed. Either at day 1 or at day 14, the majority of lipid classes such as CE, WE, PC, PE, PS, SM, LPC and LPE did not significantly change ($p>0.05$) in their concentration with the lipid or placebo spray compared to the baseline concentration (Table 5-5). A higher concentration of Chl ($p=0.03$) and TAG ($p=0.05$) compared to baseline was observed with the lipid spray day 1. There was a slight ($p=0.06$) increase in the concentration of TAG with the placebo spray compared to the baseline concentration at day 1 only. With the use of the lipid spray, the concentration of OAHFA increased ($p=0.03$) from baseline at day 14 but did not significantly change with the placebo spray ($p=0.78$) (Table 5-5).

Patient's symptomatic status had no effect on the concentration of any lipid classes at day 1. However, symptomatic wearers showed higher concentrations of LPE compared to asymptomatic wearers at day 14. The concentration of LPE was higher in symptomatic wearers compared to asymptomatic wearers with the use of the lipid spray ($p=0.02$) but not with the use of placebo spray at day 14 (Figure 5-7). The lipid class ratios between symptomatic and asymptomatic wearers were not significantly different with the spray treatment.

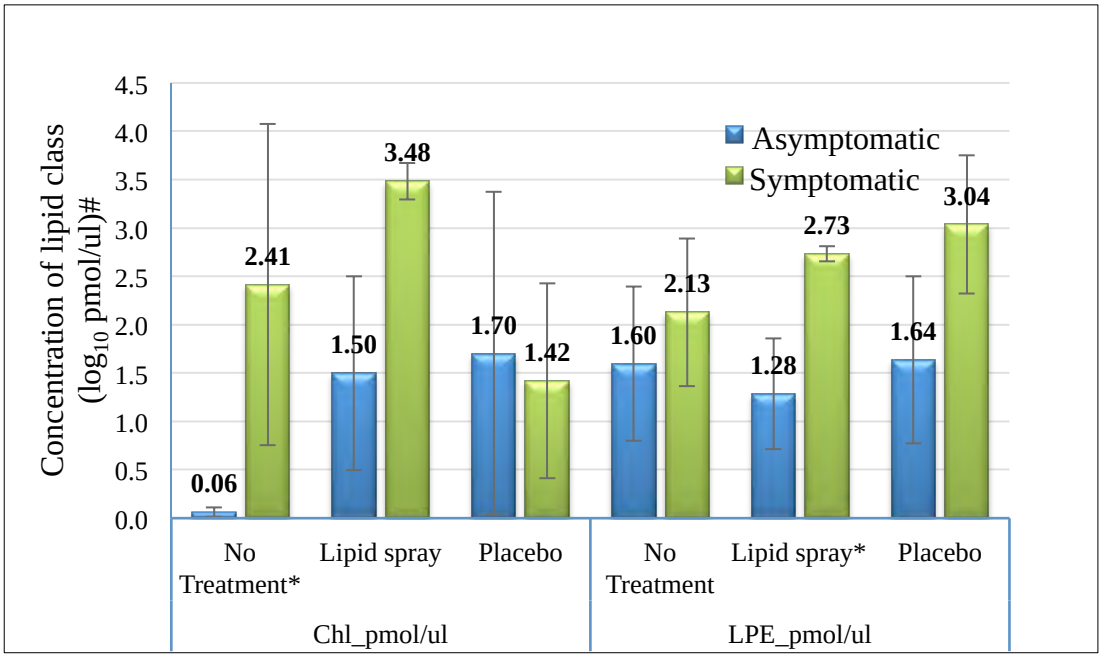


Figure 5-7 Concentration of free cholesterol (Chl) and lysophosphatidylcholine (LPE) in symptomatic and asymptomatic wearers at day 14 following lipid spray and placebo spray treatment. Error bars indicate standard deviation. * indicates groups are statistically different. # indicates concentration of each lipid class is shown as the log-transformed values.

Table 5-5 shows the linear mixed model results for the effect of the lipid spray and the placebo spray on ocular comfort and tear lipid variables with respect to the baseline data (with lens wear and no intervention) at day 1 and day 14 of the treatment stage.

Table 5-5 Effect of spray supplements at day 1 and day 14 compared with no intervention

Variables	Baseline with lens wear (No Treatment)	Spray supplement (Mean±standard deviation)					
		Day 1			Day 14		
		Lipid spray	Placebo spray	*p* value	Lipid spray	Placebo spray	*p* value
Ocular comfort scores (1-10) (higher score indicates better comfort)	8.4±1.2	8.0±1.3	8.3±1.2	0.278	7.9±1.4	8.0±1.0	0.103
Ocular comfort index (1-100) (higher score indicates lower comfort)	29.7±5.7	NR	NR	NR	29.5±5.7	27.4±7.3	0.091
Osmolarity (mOsms/l)	304±9.7	304±13.7	304±13.6	0.927	301±13.5	301±10.5	0.362
***NISDT (seconds)**	5.7±3.2	5.2±2.6	4.1±2.5	**0.001**	4.5±2.6	4.4±3.4	**0.015**
***Evaporation rate ($g/m^2/h$)**	137±74	173±104	156±93	0.112	151±74	151±96	0.479
#Lipid layer grade	3.0±1.0	3.0±1.0	2.0±1.0	0.122	3.0±1.0	2.0±1.0	0.298
***$sPLA_2$ Concentration (µg/ml)**	51.2±28	48.3±24	49.6±24	0.293	49.5±26	40.5±22	0.139
***$sPLA_2$ Activity (mmol/min/ml)**	0.53±0.5	0.59±0.4	0.56±0.4	0.653	0.53±0.5	0.50±0.4	0.961
***MDA Concentration (mM)**	5.9±12.3	4.6±6.1	4.5±6.6	0.998	3.3±4.3	3.8±5.6	0.964
***Cholesterol esters (CE) (pmol/µl)**	175±210	906±996	1059±900	0.130	308±269	121±132	0.299
***Wax esters (WE) (pmol/µl)**	111±139	416±501	364±165	0.205	172±152	66±72	0.394
***Free cholesterol (Chl) (pmol/µl)**	9.7±15	60.6±25.0	37.1±12.0	**0.017**	20.9±21.7	12.5±15.0	0.304
***Triglycerides (TAG) (pmol/µl)**	2.9±4.3	18.6±22.0	29.2±9.2	**0.010**	7.3±8.3	1.4±2.3	0.032
***Phosphatidylcholine (PC) (pmol/µl)**	8.8±7.5	12.1±6.9	6.4±5.2	0.607	15.3±15	7.3±6.3	0.686
***Phosphatidylethanolamine (PE) (pmol/µl)**	0.52±0.6	1.4±0.7	1.2±0.5	0.132	0.6±0.6	0.3±0.3	0.686

Variables	Baseline with lens wear (No Treatment)	Spray supplement (Mean±standard deviation)					
		Day 1			Day 14		
		Lipid spray	Placebo spray	*p* value	Lipid spray	Placebo spray	*p* value
***Phosphatidylserine (PS) (pmol/µl)**	1.8 ±1.6	3.0±2.1	2.4±1.8	0.489	2.5±3.5	1.2±0.6	0.800
***Sphingomyelin (SM) (pmol/µl)**	4.5±2.5	10.2±3.3	6.2±3.7	0.173	6.5±2.8	3.2±2.8	0.083
***Lysophosphatidylcholine (LPC) (pmol/µl)**	11.3±7.0	9.4±7.1	0.0±0.0	0.078	16.1±11.0	13.4±7.9	0.235
***Lysophosphatidylethanolamine (LPE) (pmol/µl)**	7.4±6.8	5.8±3.2	2.5±1.3	0.904	6.3±5.8	11.6±12.4	0.682
***(O-acyl)-ω-hydroxy fatty acid (OAHFA) (pmol/µl)**	6.2±3.6	31.2±24	25.6±20	0.999	20.9±9.8	10.0±6.1	**0.026**

*Log transformed data was used for analysis. # Median±interquartile range is shown, NR: not recorded, ocular comfort index was administered only at the end of each intervention stage (day 14).

5.3.2.2 Lipid drop

5.3.2.2.1 Ocular comfort

The ocular comfort measured using the numerical rating scale reduced with the lipid drop (p=0.05, p=0.01) but was unchanged with the placebo (p>0.05) compared to the baseline comfort at day 1 and day 14. The ocular comfort index (OCI) measured at day 14 showed that there was no change in ocular comfort either with the lipid drop or with the placebo (p>0.05) compared to baseline (Table 5-6). Symptomatic wearers showed slightly lower ocular comfort scores using the numerical rating scale (p=0.08) and the ocular comfort index (p=0.09) compared to asymptomatic wearers at day 14.

5.3.2.2.2 Clinical and functional variables

At day 1, the NISDT was unchanged with the lipid drop or the placebo compared to baseline (p>0.05) whereas by day 14, both the lipid drop (p=0.02) and placebo drop (p<0.001) reduced NISDT from baseline (Table 5-6). Symptomatic and asymptomatic wearers did not significantly differ in their NISDT at day 1 following the drop treatment (p>0.05). However, symptomatic wearers had shorter NISDT compared to asymptomatic wearers at baseline (p=0.002), with the placebo drop (p=0.03) but not with the use of the lipid drop (p>0.05) at day 14 (Figure 5-8).

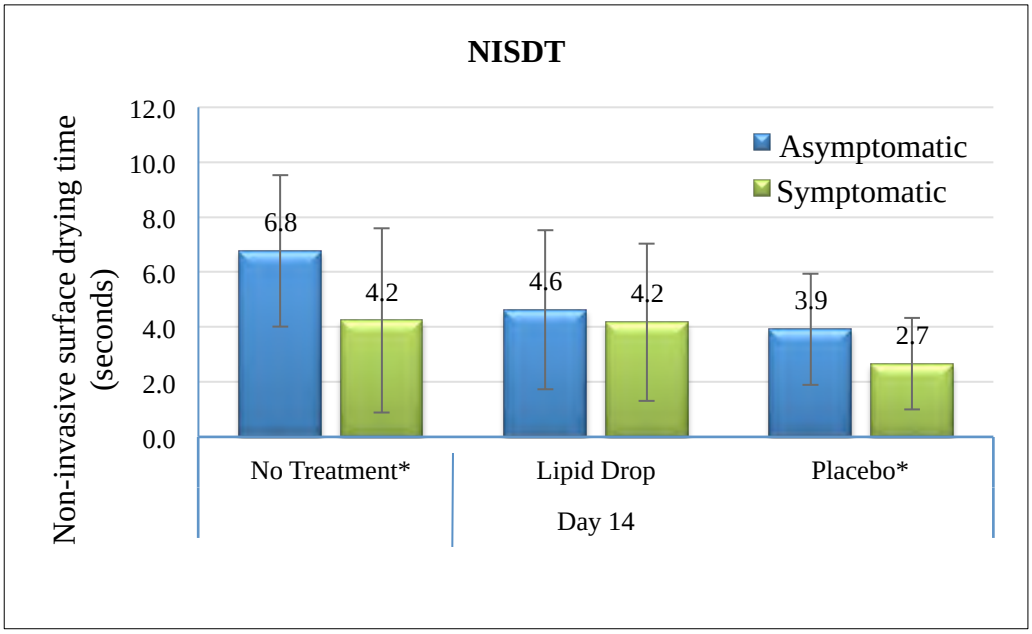


Figure 5-8 Non-invasive surface drying time of symptomatic and asymptomatic wearers following the use of lipid drop and placebo at day 14. Error bars indicate standard deviation. * indicates groups are statistically different

A Freidman's ANOVA was used to compare the effect of drop treatment on lipid layer distribution. The placebo drop significantly changed the lipid layer distribution (p=0.03) with a higher percentage of open and close meshwork patterns compared to the baseline distribution (27.5 vs 15%, 32.5 vs 20%) at day 14, whereas the lipid drop did not significantly change the patterns from baseline (p=0.20) (Figure 5-9). Patient's symptomatic status did not have an effect on the lipid layer distribution following the drop treatment. Tear osmolarity and tear evaporation rate did not change from baseline either with the lipid drop or with the placebo at day 1 or at day 14 (p>0.05) (Table 5-6).

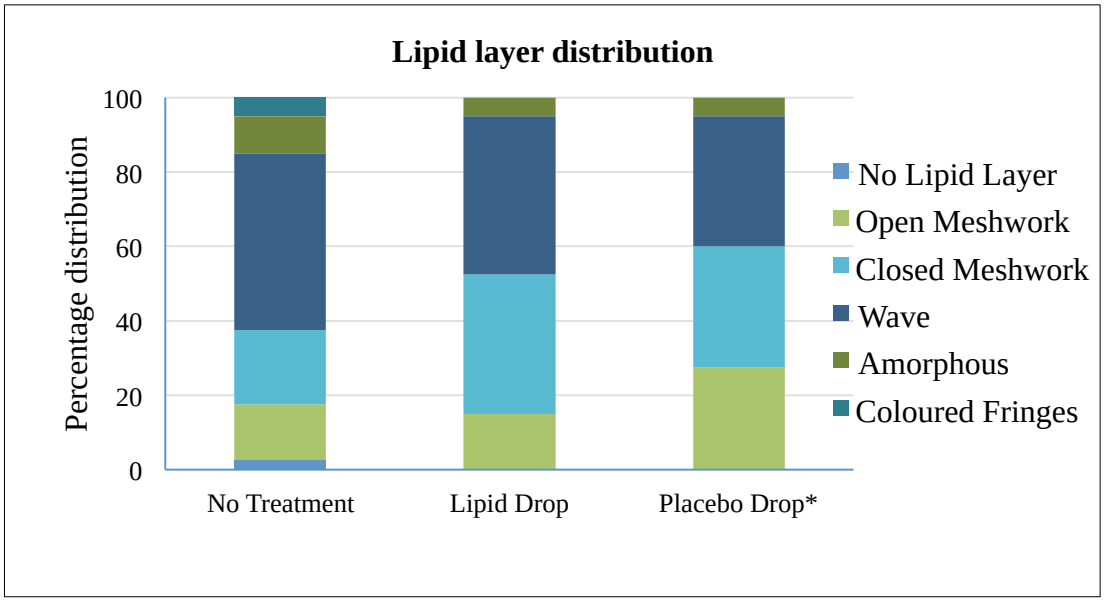


Figure 5-9 Lipid layer distribution of contact lens wearers following lipid drop and placebo drop treatment at day 14. * indicates placebo drop significantly changed the lipid layer distribution from baseline

5.3.2.2.3 Biochemical variables

The concentration of $sPLA_2$ in tears at day 1 increased with the lipid drop (p=0.02) and the placebo (p=0.04) compared to the baseline concentration. However, by day 14, the concentration of $sPLA_2$ was similar to baseline (p=0.57). The activity of $sPLA_2$ and the concentration of MDA in tears remained unaffected with the lipid drop and the placebo at day 1 (p=0.46, p=0.90) and at day 14 (p=0.24, p=0.96) compared with baseline (Table 5-6). Symptomatic and asymptomatic wearers showed no significant difference in the concentration and activity of $sPLA_2$ and the concentration of MDA in tears with the lipid or placebo drop at day 1 or at day 14 (p>0.05).

The phospholipid ingredient present in the lipid drop (phosphatidylglycerol) was not detected in any of the tear samples analysed. However, several lipid classes exhibited changes in their concentration while using the drop supplements (Table 5-6). Lipid classes such as CE (p=0.04, p=0.05), Chl (p<0.001, p<0.001), TAG (p=0.001, p=0.01),

PE (p=0.001, p=0.002), SM (p=0.005, p=0.003), and OAHFA (p=0.004, p=0.01) increased in concentration with the lipid drop and the placebo drop compared to the baseline concentration at day 1. Lipid classes such as WE, PC, PS, LPC and LPE did not significantly change their concentration in tears from baseline at day 1 (p >0.05). By day 14, the concentration of all lipid classes except CE, Chl, PS and OAHFA returned to similar levels to that of the baseline concentration. At day 14, both lipid drop and the placebo drop increased the concentration of Chl (p=0.004, p=0.004) whereas only the placebo drop increased the concentration of CE (p=0.02) and OAHFA (p=0.001) from baseline. The lipid drop increased the concentration of PS in tears (p=0.03) compared to baseline but not the placebo drop (p=0.20) at day 14.

At day 1, the placebo drop increased the concentrations of OAHFA and WE in asymptomatic wearers compared to symptomatic wearers (p=0.02, p=0.01) (Figure 5-10). Conversely, symptomatic wearers had higher concentrations of LPE compared to asymptomatic wearers with the lipid drop at day 1 (p=0.01) and at day 14 (p=0.04) (Figure 5-10). Similar to the spray treatment, neither the lipid drop nor the placebo drop had an effect on the lipid class ratios between symptomatic and asymptomatic wearers.

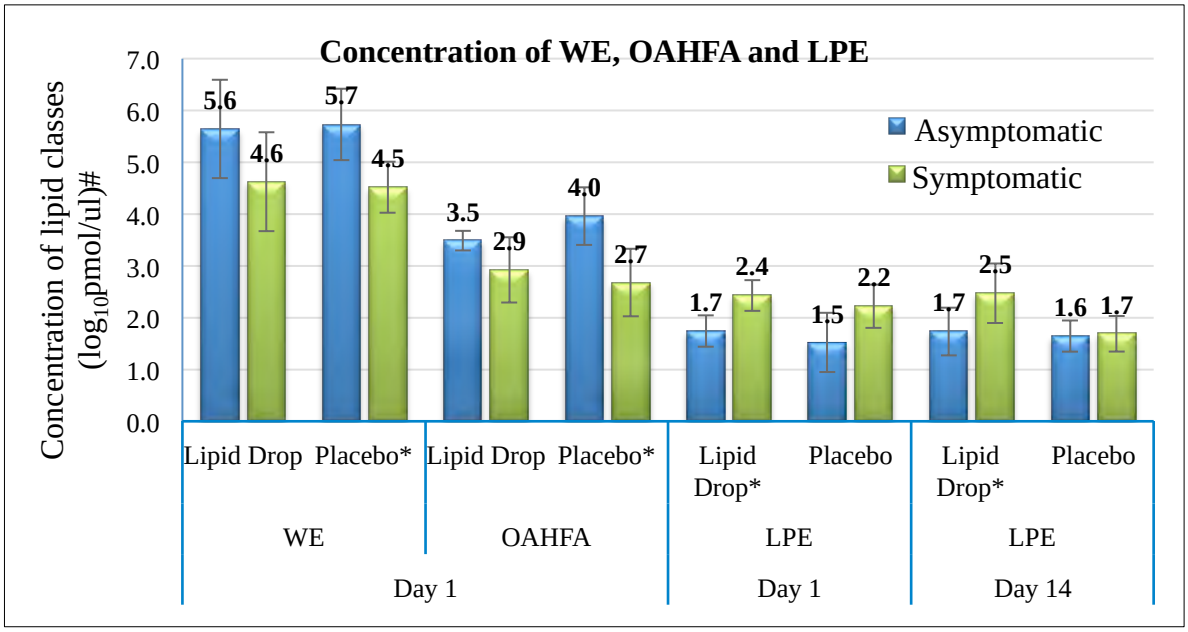


Figure 5-10 Concentration of wax esters, (O-acyl)-ω-hydroxy fatty acids and lysophosphatidylcholine in symptomatic and asymptomatic wearers following the drop treatment. Error bars indicate standard deviation. * indicates groups are statistically different. # indicates concentration of each lipid class is shown as the log-transformed values

Table 5-6 shows the linear mixed model results on the effect of the lipid drop and the placebo drop in ocular comfort and tear lipid variables with respect to the baseline data (with lens wear and no intervention) at day 1 and day 14 of the treatment stage.

Table 5-6 Effect of the drop supplements at day 1 and day 14 compared with no intervention

Variables	Baseline with lens wear (No Treatment)	Drop supplement (Mean±standard deviation)					
		Day 1			Day 14		
		Lipid drop	Placebo drop	*p* value	Lipid drop	Placebo drop	*p* value
Ocular comfort scores (1-10) (higher score indicates better comfort)	8.4±1.2	7.9±1.4	8.5±1.1	**0.007**	7.7±1.2	8.3±1.0	**0.007**
Ocular comfort index (1-100) (higher score indicates lower comfort)	29.7±5.7	NR	NR	NR	29.0±6.1	28.1±5.5	0.191
Osmolarity (mOsms/l)	304±9.7	304±11	305±11	0.937	305±12	301±10	0.262
***NISDT (seconds)**	5.7±3.2	5.2±3.3	4.8±3.3	0.147	4.4±2.9	3.4±2.0	**<0.001**
***Evaporation rate ($g/m^2/h$)**	137±74	143±74	162±105	0.167	142±79	168±108	0.072
#Lipid layer grade	3.0±1.0	3.0±1.0	2.0±1.0	0.302	2.0±1.0	2.0±1.0	**0.026**
***$sPLA_2$ Concentration (µg/ml)**	51.2±28	54.5±25	58.2±29	**0.012**	47.1±25	52.3±28	0.574
***$sPLA_2$ Activity (mmol/min/ml)**	0.53±0.5	0.45±0.5	0.43±0.4	0.464	0.47±0.5	0.40±0.3	0.238
***MDA Concentration (mM)**	5.9±12.3	5.1±6.6	6.4±12.0	0.899	3.8±5.1	3.9±6.3	0.957
***Cholesterol esters (CE) (pmol/µl)**	175±210	219±451	437±459	**0.018**	280±224	479±451	**0.017**
***Wax esters (WE) (pmol/µl)**	111±139	212±184	177±164	0.054	130±130	242±236	0.053
***Free cholesterol (Chl) (pmol/µl)**	9.7±15	44.1±15	44.0±13	**<0.001**	38.1±36	32.3±20	**0.001**
***Triacylglycerols (TAG) (pmol/µl)**	2.9±4.3	10.9±7.8	7.1±6.7	**0.001**	5.6±6.7	7.3±9.9	0.118
***Phosphatidylcholine (PC) (pmol/µl)**	8.8±7.5	12.5±10.3	9.4±5.4	0.522	17.6±29	9.9±6.8	0.349

Variables	Baseline with lens wear (No Treatment)	Drop supplement (Mean±standard deviation)					
		Day 1			Day 14		
		Lipid drop	Placebo drop	*p* value	Lipid drop	Placebo drop	*p* value
***Phosphatidylethanolamine (PE) (pmol/µl)**	0.52±0.6	1.3±0.6	1.3±0.5	**<0.001**	1.5±1.7	1.0±0.6	**0.045**
***Phosphatidylserine (PS) (pmol/µl)**	1.8 ±1.6	2.4±1.3	2.9±1.9	0.103	3.6±3.4	2.6±1.5	**0.027**
***Sphingomylein (SM) (pmol/µl)**	4.5±2.5	10.0±7.0	9.0±3.8	**0.001**	8.2±8.0	7.4±3.8	0.059
***Lysophosphatidylcholine (LPC) (pmol/µl)**	11.3±7.0	13.6±5.0	10.6±6.6	0.221	10.9±6.3	7.2±3.2	0.151
***Lysophosphatidylethanolamine (LPE) (pmol/µl)**	7.4±6.8	8.8±4.2	7.5±4.2	0.564	8.4±5.5	4.5±1.5	0.180
***(O-acyl)-ω-hydroxy fatty acid (OAHFA) (pmol/µl)**	6.2±3.6	23.4±11	28.4±25	**0.003**	16.8±13	30.9±26	**0.001**

*Log transformed data was used for analysis. # Median±interquartile range is shown, NR: not recorded, ocular comfort index was administered only at the end of each intervention stage (day 14).

5.3.3 EFFECT OF EACH TREATMENT ON DAY 1 AND 14 COMPARED TO BASELINE

A linear mixed model was derived to compare each type of treatment on Day 1 and Day 14 to that of the baseline. This analysis tested the effect of repeated use of each treatment type on ocular comfort, tear osmolarity, tear film stability, tear evaporation rate, concentration or activity of $sPLA_2$, concentration of MDA and tear lipid classes. Table 5-7 shows the linear mixed model results. Neither lipid supplements nor placebo improved ocular comfort from baseline. Ocular comfort was further reduced with the lipid drop on day 1 and day 14 (p=0.005). NISDT remained similar to that of baseline at day 1 and day 14 with the lipid supplements however, with the placebo, the NISDT reduced from baseline on day 1 and day 14. The majority of lipid classes such as CE (p=0.01, p=0.02), WE (p=0.04, p=0.04), Chl (p<0.001, p=0.002), PE (p=0.001, p=0.02), SM (p=0.002, p=0.002) and OAHFA (p=0.02, p=0.001) increased their concentration with the placebo drop at day 1 and 14 compared to baseline. Both lipid supplements (spray and drop) increased the concentration of Chl (p=0.03, p<0.001), and TAG (p=0.02, p<0.001) only on day 1 compared to baseline.

Table 5-7 Comparison of each treatment type between day 1 and 14 to that of baseline

Variables	Baseline with lens wear (No Treatment)	Type	Method	Days (Mean ± SD)		*p* value
				1	14	
Ocular comfort scores (1-10) (higher score indicates better comfort)	8.4±1.2	Lipid	Spray	8.0±1.3	7.9±1.4	>0.05
			Drop	7.9±1.4	7.7±1.2	**0.005**
		Placebo	Spray	8.3±1.2	8.0±1.0	>0.05
			Drop	8.5±1.1	8.3±1.0	>0.05
Ocular comfort index (1-100) (higher score indicates lower comfort)	29.7±5.7	Lipid	Spray	NR	29.5±5.7	>0.05
			Drop	NR	28.3±7.6	>0.05
		Placebo	Spray	NR	26.8±8.4	>0.05
			Drop	NR	28.1±5.5	>0.05
Tear osmolarity (mOsms/l)	304±9.7	Lipid	Spray	304±14	302±13	>0.05
			Drop	304±11	305±12	>0.05
		Placebo	Spray	304±14	301±10	>0.05
			Drop	305±11	301±10	>0.05
***NISDT (seconds)**	5.7±3.2	Lipid	Spray	5.2±2.6	4.5±2.6	>0.05
			Drop	5.2±3.3	4.4±2.9	>0.05
		Placebo	Spray	4.1±2.5	4.4±3.3	**0.001**
			Drop	4.8±3.3	3.4±2.0	**<0.001**
#Lipid layer grade	3.0±1.0	Lipid	Spray	3.0±1.0	3.0±1.0	>0.05
			Drop	3.0±1.0	2.0±1.0	>0.05
		Placebo	Spray	2.0±1.0	2.0±1.0	>0.05
			Drop	2.0±1.0	2.0±1.0	**0.03**

Variables	Baseline with lens wear (No Treatment)	Type	Method	Days (Mean ± SD)		*p* value
				1	14	
			Drop	54.6±25	47.1±25	**0.04**
		Placebo	Spray	49.6±24	40.5±22	>0.05
			Drop	58.2±29	52.3±28	>0.05
***sPLA$_2$ Activity (mmol/min/ml)**	0.53±0.5	Lipid	Spray	0.59±0.4	0.52±0.4	>0.05
			Drop	0.45±0.4	0.47±0.4	>0.05
		Placebo	Spray	0.56±0.5	0.50±0.3	>0.05
			Drop	0.43±0.4	0.40±0.2	>0.05
***MDA Concentration (mM)**	5.9±12.3	Lipid	Spray	4.6±6.1	3.3±4.2	>0.05
			Drop	5.1±6.5	3.8±5.1	>0.05
		Placebo	Spray	4.5±6.5	3.6±4.9	>0.05
			Drop	6.4±11.9	3.8±5.6	>0.05
*** CE (pmol/µl)**	175±210	Lipid	Spray	906±996	308±269	>0.05
			Drop	518±451	279±224	**0.05**
		Placebo	Spray	999±900	121±132	>0.05
			Drop	437±458	478±451	**0.005**
***WE (pmol/µl)**	111±139	Lipid	Spray	416±501	172±152	>0.05
			Drop	212±183	130±129	>0.05
		Placebo	Spray	363±300	66±71	>0.05
			Drop	177±164	242±236	**0.01**
***Chl (pmol/µl)**	9.7±15	Lipid	Spray	60.6±25	20.9±22	**0.03**
			Drop	44.1±15	38.0±36	**<0.001**
		Placebo	Spray	37.0±10	12.0±15	>0.05
			Drop	44.0±13	33.0±20	**<0.001**
***TAG (pmol/µl)**	2.9±4.3	Lipid	Spray	18.6±22	7.3±8.0	**0.02**
			Drop	10.9±8.0	5.6±7.0	**<0.001**
		Placebo	Spray	29.2±20	1.4±2.0	**0.02**

Variables	Baseline with lens wear (No Treatment)	Type	Method	Days (Mean ± SD)		*p* value
				1	14	
			Drop	7.1±6.0	7.3±9.0	**0.02**
***PC (pmol/µl)**	8.8±7.5	Lipid	Spray	12.1±7.0	15.3±15	>0.05
			Drop	12.5±10	17.6±28	>0.05
		Placebo	Spray	6.4±6.0	7.3±6.0	>0.05
			Drop	9.4±5.0	9.9±6.0	>0.05
***PE (pmol/µl)**	0.52±0.6	Lipid	Spray	1.4±0.7	0.59±0.6	>0.05
			Drop	1.3±0.6	1.5±1.7	**0.02**
		Placebo	Spray	1.2±0.2	0.29±0.3	>0.05
			Drop	1.3±0.5	1.0±0.6	**0.001**
***PS (pmol/µl)**	1.8 ±1.6	Lipid	Spray	3.0±2.1	2.5±3.5	>0.05
			Drop	2.4±1.3	3.6±3.4	>0.05
		Placebo	Spray	2.4±1.0	1.2±0.6	>0.05
			Drop	2.9±1.9	2.6±1.5	>0.05
***SM (pmol/µl)**	4.5±2.5	Lipid	Spray	10.2±3.3	6.5±2.8	**0.02**
			Drop	10.0±7.0	8.2±8.0	**0.05**
		Placebo	Spray	6.2±3.0	3.2±2.8	>0.05
			Drop	9.0±3.8	7.4±3.7	**<0.001**
***LPC (pmol/µl)**	11.3±7.0	Lipid	Spray	9.4±7.2	16.1±11	>0.05
			Drop	13.6±5.0	10.9±6.3	>0.05
		Placebo	Spray	0.0±0	13.4±7.9	**0.01**
			Drop	10.6±6.6	7.2±3.2	>0.05
***LPE (pmol/µl)**	7.4±6.8	Lipid	Spray	5.8±3.2	6.3±5.8	>0.05
			Drop	8.8±4.2	8.4±5.5	>0.05
		Placebo	Spray	2.5±2.0	11.6±12	>0.05
			Drop	7.5±4.2	4.5±1.5	>0.05
***OAHFA (pmol/µl)**	6.2±3.6	Lipid	Spray	31.1±24	20.9±10	>0.05

Variables	Baseline with lens wear (No Treatment)	Type	Method	Days (Mean ± SD)		*p* value
				1	14	
			Drop	23.4±10	16.8±13	**0.003**
		Placebo	Spray	25.6±11	10±6.1	>0.05
			Drop	28.4±25	31±25	**0.001**

*Log transformed data was used for analysis. # Median±interquartile range is shown, NR: not recorded, ocular comfort index was administered only at the end of each intervention stage (day 14), CE: cholesterol ester, WE: wax ester, Chl: free cholesterol, TAG: triglyceride, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, SM; sphingomyelin, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OAHFA: (O-acyl)-ω-hydroxy fatty acid.

5.3.4 COMPARISON BETWEEN LIPID SUPPLEMENTS AND PLACEBO

A linear mixed model was used to assess whether the type (lipid or placebo), the method (spray or drop) or the duration (day 1 and day 14) of treatment affected the ocular comfort, tear osmolarity, tear film stability, tear evaporation rate, concentration or activity of $sPLA_2$, concentration of MDA or tear lipids. Table 5-8 shows the summary of the linear mixed model results from the current analysis.

Table 5-8 Summary of results on the effect of the type, method and duration of the treatment on outcome variables

Purpose of analysis	Outcome variables	Effect of the treatment based on		
		Type (Lipid vs. placebo)	Method (Drop vs. Spray)	Duration (Day 1 vs. 14)
To investigate the effect of the type (lipid or placebo, the method (spray or drop) or the duration (day 1 and day 14) of treatment on each outcome measured	Ocular comfort score	Reduced with the lipid compared to the placebo (p<0.001)	Reduced with the lipid drop compared to the placebo drop (p<0.001)	Reduced at day 14 compared to day 1 (p=0.05)
	Ocular comfort Index	No effect		
	Osmolarity			
	Tear/lipid layer stability	Reduced with the placebo compared to the lipid (p<0.001)	Improved with the lipid drop and lipid spray compared to the placebos (p<0.001)	Reduced at day 14 compared to day 1 (p=0.007)
	Evaporation rate	No effect	Reduced with the lipid drop compared to the placebo drop (p=0.01)	No effect
	Lipid layer thickness	No effect		
	$sPLA_2$ Concentration	No effect	Increased with the lipid drop compared to the lipid spray (p=0.02)	Reduced at day 14 compared to day 1 (p=0.001)
	$sPLA_2$ Activity		Increased with the lipid spray compared to the lipid drop (p=0.03)	No effect

<table>
<tr><th colspan="2" rowspan="2">Purpose of analysis</th><th rowspan="2">Outcome variables</th><th colspan="3">Effect of the treatment based on</th></tr>
<tr><th>Type (Lipid vs. placebo)</th><th>Method (Drop vs. Spray)</th><th>Duration (Day 1 vs. 14)</th></tr>
<tr><td colspan="2" rowspan="11"></td><td></td><td></td><td>(p=0.001, p=0.002 respectively)</td><td></td></tr>
<tr><td>Free cholesterol (Chl)</td><td colspan="2" rowspan="2">No effect</td><td rowspan="2">Reduced at day 14 compared to day 1 (p=0.002)</td></tr>
<tr><td>Triglycerides (TAG)</td></tr>
<tr><td>Phosphatidylcholine (PC)</td><td colspan="3">No effect</td></tr>
<tr><td>Phosphatidylethanolamine (PE)</td><td colspan="2">No effect</td><td>Reduced at day 14 compared to day 1 (p=0.01)</td></tr>
<tr><td>Phosphatidylserine (PS)</td><td colspan="3">No effect</td></tr>
<tr><td>Sphingomyelin (SM)</td><td rowspan="2">Increased with the lipid compared to the placebo (p=0.001)</td><td colspan="2" rowspan="2">No effect</td></tr>
<tr><td>Lysophosphatidylcholine (LPC)</td></tr>
<tr><td>Lysophosphatidylethanolamine (LPE)</td><td colspan="3">No effect</td></tr>
<tr><td>(O-acyl)-ω-hydroxy fatty acid (OAHFA)</td><td>Increased slightly (p=0.06) with the placebo compared to the lipid</td><td>Increased with the placebo drop compared to the placebo spray (p=0.005)</td><td>No effect</td></tr>
<tr><td>Summary</td><td>Effect of the method and duration of the treatment</td><td colspan="4">• The lipid spray improved tear film stability however; the spray also increased the activity of phospholipase enzyme. The lipid drop improved tear film stability and tear evaporation rate however, ocular comfort was reduced and the concentration of phospholipase enzyme was increased.
• Ocular comfort, tear film stability, concentration of $sPLA_2$, Chl, TAG, PC, SM and LPC reduced at day 14 compared to day 1.</td></tr>
</table>

Purpose of analysis	Outcome variables	Effect of the treatment based on		
		Type (Lipid vs. placebo)	Method (Drop vs. Spray)	Duration (Day 1 vs. 14)
Effect of the treatment in symptomatic wearers vs. asymptomatic wearers	• The lipid spray reduced the ocular comfort and increased the activity of the $sPLA_2$and the concentration of LPE in symptomatic wearers compared to asymptomatic wearers. • The lipid drop improved the lipid layer distribution in symptomatic wearers however; the concentration of LPE was higher in symptomatic wearers compared to asymptomatic wearers.			

5.3.4.1 Ocular comfort

Using the numerical rating scale, ocular comfort reduced at day 14 compared to day 1 (p=0.05), reduced with the lipid drop when compared to the placebo drop (p<0.001) but there was no change in comfort between the lipid spray and the placebo spray (p=0.10) (Table 5-9). Using the OCI, comfort improved slightly (p=0.06) with the placebo when compared to either of the lipid supplements.

Using the numerical rating scale, asymptomatic wearers had higher ocular comfort compared to symptomatic wearers with the lipid spray at day 1 (8.4±1.1 vs. 7.5±1.3, p=0.02) and day 14 (8.3±1.4 vs. 7.3±1.3, p=0.02) whereas with the lipid drop, symptomatic and asymptomatic wearers showed similar comfort scores. With the placebo, only the spray showed a significant (7.7±1.1 vs. 8.7±1.0, p=0.005) decrease in ocular comfort in symptomatic wearers compared to asymptomatic wearers.

5.3.4.2 Clinical and functional variables

Tear osmolarity was unaffected by intervention. NISDT improved with the lipid spray or the lipid drop compared to their placebos (p<0.001) but was reduced at day 14 (p=0.007) compared to day 1 (Table 5-9). Symptomatic wearers showed a shorter NISDT compared to asymptomatic wearers with the placebo at day 1 (3.7±3.1 vs. 4.9±2.8, p=0.04) and day 14 (3.1±2.9 vs. 4.5±2.5, p=0.02) whereas with both lipid supplements, symptomatic and asymptomatic wearers showed similar NISDT. Tear evaporation rate decreased with the lipid drop compared to the placebo drop (p=0.01). Conversely, evaporation rates were higher with the lipid spray compared to the drop and were not significantly different between the lipid and placebo spray (Table 5-9). Patient symptomatology did not affect the tear evaporation rate with any treatment visit (p=0.35).

The overall treatment had a significant effect in the lipid layer distribution (p=0.04), but the effect became insignificant when the type, method and duration of the treatment were included as factors in the analysis. Asymptomatic wearers showed a similar distribution of lipid layer patterns with each treatment whereas symptomatic wearers showed a significant change in the distribution with the lipid drop (p=0.02). Symptomatic wearers had a higher percentage of closed meshwork (50%) and wave pattern (31.3%) with the lipid drop compared to the placebo drop (31.3% and 25% respectively) at day 14 (Figure 5-11).

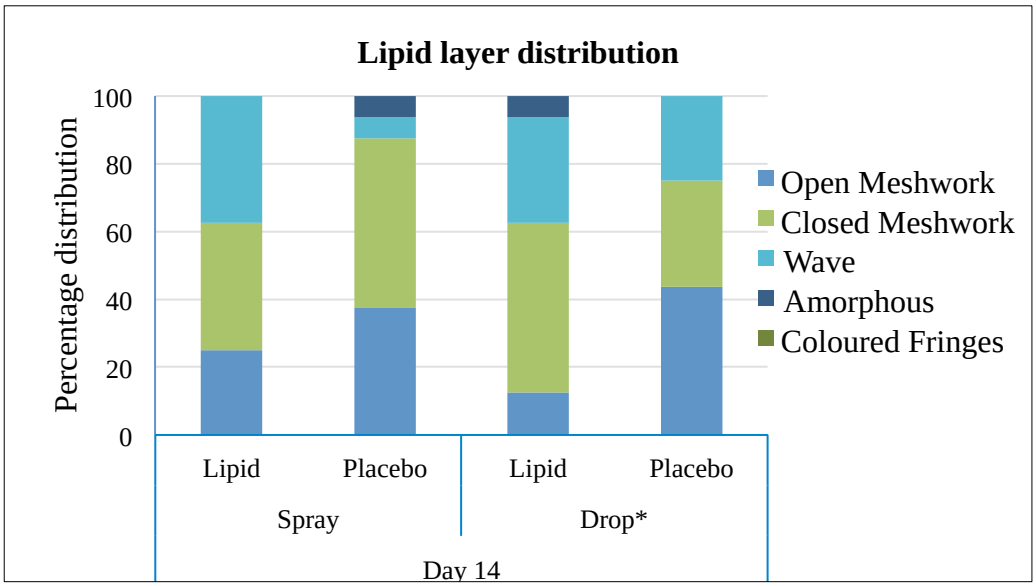


Figure 5-11 Lipid layer distribution in symptomatic wearers following the use of lipid and placebo supplements at day 14. *indicates groups are statistically different

5.3.4.3 Biochemical variables

The concentration of $sPLA_2$ in tears was higher with the lipid drop compared to the lipid spray (p=0.02) and was lower at day 14 compared to day 1 (p=0.001) (Table 5-9). Patient's symptomatic status had no effect on the concentration of $sPLA_2$ in tears at any treatment visit (p=0.64). The activity of $sPLA_2$ in tears however, increased with the

lipid spray compared to the lipid drop (p=0.03) irrespective of the type and duration of the treatment (Table 5-9). Symptomatic wearers had higher $sPLA_2$ activity compared to asymptomatic wearers with the lipid (p=0.03) or placebo spray (p=0.02) at day 14. The concentration of MDA in tears remained unaffected with intervention or with the patient's symptomatic status.

The concentration of CE (p=0.001), OAHFA (p=0.005) and WE (p=0.002) was increased with the placebo drop compared to the placebo spray but did not differ between the lipid drop and lipid spray (p>0.05) (Table 5-9). The concentration of Chl (p=0.002), TAG (p=0.002) and PE (p=0.01) in tears significantly reduced by day 14 compared to day 1 irrespective of the type and method of treatment (Table 5-9). The concentration of SM in tears increased (p=0.02) with the lipid drop compared to the placebo drop and reduced (p=0.05) at day 14 compared to day 1. Similarly, the concentration of LPC in tears slightly but not significantly increased with the lipid spray compared to the placebo spray (p=0.09) and reduced (p=0.03) at day 14 compared to day 1. Phospholipid classes such as PC, PS and LPE did not change based on the type, method or duration of the treatment (Table 5-9).

There were differences in lipid classes between symptomatic and asymptomatic wearers. The concentration of lysophospholipid classes such as LPC (p=0.04) and LPE (p=0.003) and OAHFA (p=0.05). LPC and LPE were higher in symptomatic wearers whereas OAHFAs were higher in asymptomatic wearers. There was a significant difference between the groups for the concentration of LPE in tears when using the lipid spray (7.2±2.8 vs. 2.8±0.8, 14.4±1.2 vs. 3.1±2.2) and the lipid drop (10.8±3.5 vs. 4.9±1.9, 12.2±5.6 vs. 5.2±3.0) both at day 1 (p=0.01, p=0.02) and day 14 (p=0.01, p=0.04) respectively. Table 5-9 shows the linear mixed model results.

Table 5-9 Effect of the type, method and duration of the treatment among habitual contact lens wearers

Variables	Type	Method	Days (Mean ± SD)		*p* value					
			1	14	Type	Method	Days	Type _Method	Type _Days	Method_ Days
Ocular comfort scores (1-10) (higher score indicates better comfort)	Lipid	Spray	8.0±1.3	7.9±1.4	**<0.001**	0.706	**0.052**	**0.036**	0.637	0.925
		Drop	7.9±1.4	7.7±1.2						
	Placebo	Spray	8.3±1.2	8.0±1.0						
		Drop	8.5±1.1	8.3±1.0						
Ocular comfort index (1-100) (higher score indicates lower comfort)	Lipid	Spray	NR	29.5±5.7	0.068	0.897	NR	0.117	NR	NR
		Drop	NR	28.3±7.6						
	Placebo	Spray	NR	26.8±8.4						
		Drop	NR	28.1±5.5						
Tear osmolarity (mOsms/l)	Lipid	Spray	304±14	302±13	0.380	0.298	0.107	0.637	0.318	0.456
		Drop	304±11	305±12						
	Placebo	Spray	304±14	301±10						
		Drop	305±11	301±10						
***NISDT (seconds)**	Lipid	Spray	5.2±2.6	4.5±2.6	**<0.001**	0.417	**0.007**	0.993	0.607	0.155
		Drop	5.2±3.3	4.4±2.9						
	Placebo	Spray	4.1±2.5	4.4±3.3						
		Drop	4.8±3.3	3.4±2.0						

Variables	Type	Method	Days (Mean ± SD)		*p* value					
			1	14	Type	Method	Days	Type _Method	Type _Days	Method_ Days
***Evaporation rate ($g/m^2/h$)**	Lipid	Spray	173±104	151±78	0.409	0.720	0.433	**0.024**	0.771	0.242
		Drop	143±74	142±79						
	Placebo	Spray	155±93	151±96						
		Drop	162±105	168±107						
***$sPLA_2$ Concentration (µg/ml)**	Lipid	Spray	48.3±24	49.5±26	0.291	**0.018**	**0.001**	0.106	0.551	0.977
		Drop	54.6±25	47.1±25						
	Placebo	Spray	49.6±24	40.5±22						
		Drop	58.2±29	52.3±28						
***$sPLA_2$ Activity (mmol/min/ml)**	Lipid	Spray	0.59±0.4	0.52±0.4	0.435	**0.032**	0.408	0.930	0.989	0.591
		Drop	0.45±0.4	0.47±0.4						
	Placebo	Spray	0.56±0.5	0.50±0.3						
		Drop	0.43±0.4	0.40±0.2						
***MDA Concentration (mM)**	Lipid	Spray	4.6±6.1	3.3±4.2	0.926	0.747	0.402	0.954	0.852	0.762
		Drop	5.1±6.5	3.8±5.1						
	Placebo	Spray	4.5±6.5	3.6±4.9						
		Drop	6.4±11.9	3.8±5.6						
*** CE (pmol/µl)**	Lipid	Spray	906±996	308±269	0.378	**0.026**	0.073	**0.032**	0.165	0.127

Variables	Type	Method	Days (Mean ± SD)		*p* value					
			1	14	Type	Method	Days	Type _Method	Type _Days	Method_ Days
		Drop	518±451	279±224						
	Placebo	Spray	999±900	121±132						
		Drop	437±458	478±451						
***WE (pmol/µl)**	Lipid	Spray	416±501	172±152	0.315	0.096	0.288	**0.022**	0.169	0.279
		Drop	212±183	130±129						
	Placebo	Spray	363±300	66±71						
		Drop	177±164	242±236						
***Chl (pmol/µl)**	Lipid	Spray	60.6±25	20.9±22	0.588	0.218	**0.002**	0.555	0.994	0.152
		Drop	44.1±15	38.0±36						
	Placebo	Spray	37.0±10	12.0±15						
		Drop	44.0±13	33.0±20						
***TAG (pmol/µl)**	Lipid	Spray	18.6±22	7.3±8.0	0.166	0.695	**0.002**	0.215	0.806	0.239
		Drop	10.9±8.0	5.6±7.0						
	Placebo	Spray	29.2±20	1.4±2.0						
		Drop	7.1±6.0	7.3±9.0						
***PC (pmol/µl)**	Lipid	Spray	12.1±7.0	15.3±15	0.214	0.363	0.916	0.487	0.641	0.856
		Drop	12.5±10	17.6±28						

Variables	Type	Method	Days (Mean ± SD)		*p* value					
			1	14	Type	Method	Days	Type _Method	Type _Days	Method_ Days
	Placebo	Spray	6.4±6.0	7.3±6.0						
		Drop	9.4±5.0	9.9±6.0						
***PE (pmol/µl)**	Lipid	Spray	1.4±0.7	0.59±0.6	0.460	0.177	**0.011**	0.700	0.763	0.115
		Drop	1.3±0.6	1.5±1.7						
	Placebo	Spray	1.2±0.2	0.29±0.3						
		Drop	1.3±0.5	1.0±0.6						
***PS (pmol/µl)**	Lipid	Spray	3.0±2.1	2.5±3.5	0.906	0.350	0.208	0.730	0.718	0.129
		Drop	2.4±1.3	3.6±3.4						
	Placebo	Spray	2.4±1.0	1.2±0.6						
		Drop	2.9±1.9	2.6±1.5						
***SM (pmol/µl)**	Lipid	Spray	10.2±3.3	6.5±2.8	**0.036**	0.209	**0.051**	**0.007**	0.719	0.283
		Drop	10.0±7.0	8.2±8.0						
	Placebo	Spray	6.2±3.0	3.2±2.8						
		Drop	9.0±3.8	7.4±3.7						
***LPC (pmol/µl)**	Lipid	Spray	9.4±7.2	16.1±11	**0.001**	0.163	**0.037**	0.220	0.192	**0.001**
		Drop	13.6±5.0	10.9±6.3						
	Placebo	Spray	0.0±0	13.4±7.9						

Variables	Type	Method	Days (Mean ± SD)		*p* value					
			1	14	Type	Method	Days	Type _Method	Type _Days	Method_ Days
		Drop	10.6±6.6	7.2±3.2						
***LPE (pmol/µl)**	Lipid	Spray	5.8±3.2	6.3±5.8	0.912	0.473	0.636	**0.057**	0.709	0.595
		Drop	8.8±4.2	8.4±5.5						
	Placebo	Spray	2.5±2.0	11.6±12						
		Drop	7.5±4.2	4.5±1.5						
***OAHFA (pmol/µl)**	Lipid	Spray	31.1±24	20.9±10	**0.055**	**0.005**	0.543	**0.001**	**0.054**	0.493
		Drop	23.4±10	16.8±13						
	Placebo	Spray	25.6±11	10±6.1						
		Drop	28.4±25	31±25						

*Log transformed data was used for analysis, SD: standard deviation, CE: cholesterol ester, WE: wax ester, Chl: free cholesterol, TAG: triglyceride, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, SM; sphingomyelin, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OAHFA: (O-acyl)-ω-hydroxy fatty acid. Type: lipid or placebo, Method: spray or drop, Days: duration of treatment, Type_Method: interaction between type and method of treatment, Type_Days: interaction between type and duration of treatment, Method_Days: interaction between method and duration of treatment.

5.3.5 ASSOCIATIONS

5.3.5.1 Ocular comfort

Ocular comfort scores obtained using the numerical rating scale and the ocular comfort index questionnaire showed a moderate agreement in their scores (r=-0.34, p <0.001). As the NISDT increased (r=0.21, p=0.003) and tear evaporation rate decreased (r=-0.19, p=0.008), the ocular comfort improved significantly. Ocular comfort slightly increased with thicker lipid layer patterns (r=0.13, p=0.06). With higher $sPLA_2$ concentration and activity in tears, the ocular comfort reduced (r=-0.21, p=0.007 and r=-0.20, p=0.01 respectively).

5.3.5.2 Clinical and functional variables

Tear osmolarity increased with thinner lipid layer patterns, with shorter NISDT and with a lower concentration of OAHFA in tears (r=-0.14, p=0.01, r=-0.11, p=0.05 and r=-0.30, p=0.03 respectively). As the lipid layer thickness increased, the NISDT increased (r=0.81, p<0.001) whereas the tear evaporation rate (r=-0.12, p=0.02), and the concentration and activity of $sPLA_2$, (r=-0.20, p<0.001, r=-0.15, p=0.01) reduced. With longer duration of NISDT, the tear evaporation rate decreased (r=-0.21, p<0.001) and the concentration and activity of $sPLA_2$ reduced (r=-0.20, p<0.001 and r=-0.15, p=0.01). As the tear evaporation rate increased, there were higher levels and increased activity of $sPLA_2$ in tears (r=0.14, p=0.01 and r=0.13, p=0.02). The concentration of MDA slightly increased with an increased rate of tear evaporation (r=0.12, p=0.08) but only reached statistical significance when the treatment visits were excluded from the analysis (r=0.39, p=0.007). Similar treatment effect was not observed with any other variables.

5.3.5.3 Biochemical variables

The concentration of $sPLA_2$ in tears was associated with the activity of $sPLA_2$ and the concentrations of Chl, SM, LPC and OAHFA in tears. A higher concentration of the enzyme was associated with higher enzyme activity, LPC, LPE and lower concentration of OAHFAs (r=0.50, p<0.001, r=0.41, p<0.001, r=0.40, p=0.001 and r=-0.30, p=0.03).

Table 5-10 shows the overall association of ocular comfort with the clinical, functional and biochemical variables of tear lipid layer.

Table 5-10 Associations between ocular comfort and clinical, functional and biochemical aspects of the lipid layer

Variables		OCI	Osmolarity	LLG	NISDT	Evaporation rate	$sPLA_2$ concentration	$sPLA_2$ activity	MDA
OCI	**Correlation (r)**		-.017	-.133	-.210	.188	.209	.203	.093
	***p* value**		.806	.060	**.003**	**.008**	**.007**	**.010**	.360
Osmolarity	**Correlation (r)**	-.017		-.136	-.098	.018	-.203	-.036	.085
	***p* value**	.806		**.006**	**.049**	.717	**.000**	.517	.237
LLG	**Correlation (r)**	-.133	-.136		.814	-.115	-.200	-.149	-.050
	***p* value**	.060	**.006**		**.000**	**.021**	**.000**	**.007**	.490
NISDT	**Correlation (r)**	-.210	-.098	.814		-.212	-.202	-.153	-.024
	***p* value**	**.003**	.049	**.000**		**.000**	**.000**	**.006**	.740
TER	**Correlation (r)**	.188	.018	-.115	-.212		.140	.125	.123
	***p* value**	**.008**	.717	**.021**	**.000**		**.010**	**.024**	.086
$sPLA_2$	**Correlation (r)**	.209	-.203	-.200	-.202	.140		.402	.081
	***p* value**	**.007**	**.000**	**.000**	**.000**	**.010**		**.000**	.270
$sPLA_2$ Activity	**Correlation (r)**	.203	-.036	-.149	-.153	.125	.402		.120
	***p* value**	**.010**	.517	**.007**	**.006**	**.024**	**.000**		.106
MDA	**Correlation (r)**	.093	.085	-.050	-.024	.123	.081	.120	
	***p* value**	.360	.237	.490	.740	.086	.270	.106	
CE	**Correlation (r)**	.107	.010	.038	.098	.113	-.153	.020	.215
	***p* value**	.454	.932	.741	.391	.323	.207	.871	.183
WE	**Correlation (r)**	.107	-.026	.024	.033	.080	-.180	-.022	.202
	***p* value**	.456	.823	.834	.770	.481	.136	.859	.212
Chl	**Correlation (r)**	.046	-.149	-.191	-.057	.017	.267	.143	.182

Variables		OCI	Osmolarity	LLG	NISDT	Evaporation rate	$sPLA_2$ concentration	$sPLA_2$ activity	MDA
	***p* value**	.750	.191	.092	.616	.883	**.026**	.251	.262
TAG	**Correlation (r)**	.156	-.184	-.098	.023	.038	.020	-.075	.233
	***p* value**	.275	.105	.388	.842	.742	.867	.549	.148
PC	**Correlation (r)**	.138	-.045	.093	-.094	-.185	-.201	-.181	.020
	***p* value**	.335	.695	.414	.411	.103	.095	.146	.904
PE	**Correlation (r)**	.109	-.131	.012	.041	-.038	.118	.006	.242
	***p* value**	.447	.249	.914	.723	.737	.333	.962	.132
PS	**Correlation (r)**	-.122	-.038	.224	.166	-.148	-.188	-.026	.156
	***p* value**	.395	.737	**.047**	.143	.192	.118	.835	.337
SM	**Correlation (r)**	-.027	-.199	.060	.047	-.175	.290	.125	.135
	***p* value**	.853	.079	.598	.681	.123	**.015**	.319	.408
LPC	**Correlation (r)**	.187	-.136	-.144	-.046	-.113	.267	.025	.046
	***p* value**	.189	.232	.207	.688	.320	**.026**	.840	.777
LPE	**Correlation (r)**	.054	-.065	-.100	-.135	-.127	.170	.049	.078
	***p* value**	.705	.569	.380	.235	.267	.159	.697	.633
OAHFA	**Correlation (r)**	.057	-.295	-.111	-.102	-.085	-.300	-.227	.373
	***p* value**	.760	**.025**	.407	.445	.526	**.031**	.113	.061

OCI: ocular comfort index, LLG: lipid layer grade, NISDT: non-invasive surface drying time, $sPLA_2$: secretory phospholipase enzyme, MDA: malondialdehyde, CE: cholesterol ester, WE: wax ester, Chl: free cholesterol, TAG: triglyceride, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, SM; sphingomyelin, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OAHFA: (O-acyl)-ω-hydroxy fatty acid.

5.3.6 PREDICTORS OF OCULAR COMFORT

Based on the multivariate analysis, tear evaporation rate (p=0.08) and the activity of phospholipase enzyme in tears (p=0.01) were the independent variables that predicted ocular comfort during contact lens wear (Table 5-11). However, the strength of association between ocular comfort and the predicted value (evaporation rate and $sPLA_2$ activity) was weak (r=-0.34, p<0.001). In other words, only 11.6% ($R^2 = (-0.34)^2$) of the variability in ocular comfort was explained by tear evaporation rate and $sPLA_2$ activity. The remaining 88% is due to other factors.

Table 5-11 Multivariate analysis of ocular comfort in contact lens wearers

Parameter	Estimate	SE	df	*p* value	95% CI
Intercept	20.71	3.66	152.7	<0.001	13.4 – 27.9
Evaporation rate	1.31	0.73	152.5	0.077	-0.14 – 2.75
$sPLA_2$ activity	4.50	1.72	151.1	0.010	1.10 – 7.90

SE: standard error, df: degree of freedom

5.4 DISCUSSION

This chapter investigated the effect of two exogenous lipid supplements on various aspects of the tear lipid layer in habitual soft contact lens wearers. Initial analysis was a baseline comparison of effect of the lens wear on the clinical, functional and biochemical variables of the tear film lipids along with ocular comfort without any use of supplements. This was followed by assessing the effect of the exogenous lipid supplements and placebos on tear lipid variables and ocular comfort relative to the contact lens wear baseline. In addition, the lipid supplements were compared with their

placebos with the type (lipid or placebo), method (spray or drop) and duration (day 1 or 14) of the treatment as factors.

5.4.1 OCULAR COMFORT

The ocular comfort with and without contact lenses was identical. This was an unexpected finding, as it would be anticipated that the comfort without contact lenses would be significantly better than with contact lenses. Given that all participants were habitual contact lens wearers and the comfort scores were obtained at the end of the day, the identical comfort scores might be due to a 'fatigue response' or stimulation of ocular surface nociceptors even in the absence of lens wear (Papas *et al.* 2014). Symptomatic wearers had a lower ocular comfort with and without lens wear. The reduced ocular comfort even without lens wear of symptomatic wearers might be an indication of poor tear film characteristics in this population in the current study. Contact lens wearers with lower tear volume and reduced tear break up time have been shown to be more intolerant to contact lens wear (Fanti *et al.* 1980, Glasson *et al.* 2006).

Contrary to the findings from the pilot study (CHAPTER 3), the lipid supplements did not improve ocular comfort from baseline. Ocular comfort reduced with the lipid drop compared to the placebo drop and the spray supplement had no effect on ocular comfort. The comfort score reduced between day 1 and day 14 particularly in symptomatic wearers. Approximately, 40% of participants complained of visual disturbance on instillation of the lipid drop. Though the blurred vision was only momentary, it might have influenced their ocular comfort scores (Papas *et al.* 2014). The reduced ocular comfort at day 14 compared with the beginning of treatment could be due to the dose effect of the 2-week long lens wear, which the lipid or placebo supplements failed to mitigate. Possibly there could be an adaptive effect to the

intervention explained by Yen *et al*. (2001) as an 'autoregulatory mechanism' of the tear film where the tear film parameters return to its normal levels after a period of intervention. This effect has been also shown in a study using punctual plugs (Stahl 2009) and in subjects with nasolacrimal duct obstruction (Stahl *et al.* 2006). Reduced tear film stability, down regulated mucin production and elevated inflammatory cytokines have been observed in healthy populations following 2 weeks of lens wear (Dogru *et al.* 2011) and have been reported to be associated with dry eye disease and contact lens wear (Schultz *et al.* 2000, Yasueda *et al.* 2005, Yoon *et al.* 2007, Ramamoorthy *et al.* 2008, Sengor *et al.* 2011a).

The participants were unable to differentiate between the lipid or placebo spray on ocular comfort. This might be due to the small volume of active and vehicle delivered to the ocular surface. According to the manufacturer's instructions, participants were asked to spray the lipid supplement on their upper eyelid by either closing their eyes or with a down gaze. It is then claimed that the liposomes present in the spray migrate to the ocular surface while blinking. The phospholipid ingredient in the spray was phosphatidylcholine (PC). In the current study, the concentration of PC in tears did not change from baseline with the lipid spray, which might indicate that the active ingredient in the spray was not reaching the ocular surface at measurable levels. A lower volume of lipid spray would be expected to be delivered to the ocular surface compared with the lipid drop.

The lipid drop improved the ocular comfort of symptomatic wearers to the level of the asymptomatic wearers while the lipid spray failed to influence comfort in this population. The phospholipid in the lipid spray is a zwitterionic PC whereas the lipid drop contains an anionic phosphatidylglycerol (PG). Superior effects of anionic based

lipid formulation over a zwitterionic lipid formulation in a dry eye population have been claimed (Korb *et al.* 2012a) however, there is a potential conflict of interest for these authors.

Lipid supplements have been reported to improve ocular comfort in dry eye populations (Shimazaki *et al.* 1998, Di Pascuale *et al.* 2004) and one preliminary study showed evidence of superior ocular comfort with a lipid spray in contact lens wearers compared to a contralateral instillation of a placebo spray (Craig 2010). The findings in the present study suggest that the effect of lipid supplements (if any) might be masked due to i) the effect of prolonged lens wear, ii) adaptation effect of the intervention, and iii) the differences in lipid formulation, mode of delivery and/or the volume of supplement delivered to the ocular surface.

5.4.2 CLINICAL AND FUNCTIONAL VARIABLES

Tear osmolarity and tear evaporation rate increased with lens wear and thinner lipid layer patterns were more frequently observed with lens wear compared to without lens wear. Tear osmolarity is considered to be a significant predictor of dry eye (Farris 1994, Khanal *et al.* 2008, Sullivan *et al.* 2010, Versura *et al.* 2010) and a link between hyperosmolarity and increased tear evaporation rate has been established (Gilbard *et al.* 1982, Iskeleli *et al.* 2002). Several investigators therefore have studied changes in tear osmolarity during contact lens wear in relation to lens related dryness and ocular discomfort (Farris *et al.* 1986, Martin 1987, Iskeleli *et al.* 2002, Miller *et al.* 2004, Nichols *et al.* 2006, Stahl *et al.* 2009, Kojima *et al.* 2011, Sarac *et al.* 2012, Iskeleli *et al.* 2013, Muselier-Mathieu *et al.* 2014). Some of these studies showed no change in tear osmolarity (Dabney *et al.* 2000, Stahl 2009, Iskeleli *et al.* 2013, Muselier-Mathieu *et al.* 2014) and similar tear evaporation rates during lens wear compared to no lens

wear (Hamano *et al.* 1981). However, there is evidence of hyperosmolarity of tears following 60 minutes (Martin 1987), 4 hours (Sarac *et al.* 2012) and even up to 3 months of contact lens wear (Iskeleli *et al.* 2002). Increased tear osmolarity was observed in symptomatic contact lens wearers compared to asymptomatic wearers with lens wear (Nichols *et al.* 2006) and also among hydrogel and silicone hydrogel contact lens wearers compared to non-lens wearers (Miller *et al.* 2004). Similarly, increased tear evaporation rates (Tomlinson *et al.* 1982, Thai *et al.* 2004, Guillon *et al.* 2008, Kojima *et al.* 2011) were observed in hydrogel and silicone hydrogel contact lens wearers compared to non-lens wearers. Differences in the lipid layer distribution with and without lens wear concurred with the previous studies that have shown that contact lens wear leads to lipid layer thinning (Guillon *et al.* 1994, Nichols *et al.* 2003b, Guillon *et al.* 2007).

Neither the lipid supplements nor the placebo showed any effect on tear osmolarity. However, this study used a single eye tear osmolarity measurement rather than the worst eye as recommended for dry eye diagnosis, which may have confounded the findings. Tear evaporation rates decreased in all study participants and a higher percentage of closed meshwork and wave patterns in symptomatic wearers were observed with the lipid drop compared to the placebo drop. The lipid spray did not have an effect on the evaporation rate and lipid layer distribution compared to the placebo spray. An emulsion drop with the same formulation as the lipid drop has shown a similar improvement in tear evaporation rate in a healthy non-contact lens wear population (Pearce *et al.* 2002) and an increased lipid layer thickness in a dry eye population (Korb *et al.* 2002a). An improvement in the lipid layer structure and function with the use of the drop supplement confirms its superiority over the spray supplement,

which might be due to the difference in volume and/or formulation delivered by each supplement.

Tear film stability was significantly lower during lens wear than without lens wear which is consistent with previous findings (Guillon *et al.* 1989a, Guillon *et al.* 1990, Faber *et al.* 1991, Thai *et al.* 2004). Symptomatic wearers showed lower tear film stability than asymptomatic wearers during lens wear, that supports the hypothesis that contact lens wearers with reduced tear film stability are susceptible to lens related dryness and ocular discomfort (Fonn *et al.* 1999, Glasson *et al.* 1999, Hom *et al.* 2009, Pult *et al.* 2009, Wolffsohn *et al.* 2010, Dogru *et al.* 2011, Sengor *et al.* 2011a). With the lipid supplements, tear film stability remained similar to that of baseline at day 1. However, by day 14, the stability reduced from baseline with the lipid drop and placebo. Symptomatic wearers showed reduced tear film stability compared to asymptomatic wearers with placebo whereas they had similar tear film stability with both lipid supplements. The improvement however occurred only at the beginning of the treatment as the tear film stability significantly reduced with lipid and placebo supplements by day 14. The current observations would suggest that the lipid supplements have a transient effect on tear film stability in symptomatic wearers and the effects of long-term lens wear on tear film overrides that improvement. This is consistent with other tear film interventions such as punctual occlusion for increased tear production and reduced contact lens osmolality (Yen *et al.* 2001, Stahl 2009). The frequency and retention time of each supplement are other factors that need to be considered for further exploration.

5.4.3 BIOCHEMICAL VARIABLES

The concentrations of the majority of the biochemical analytes remained unaffected with lens wear except for PE, PS and SM, which were slightly reduced in their concentration with lens wear. Although the reduced concentrations of phospholipid classes during contact lens wear were marginal in the current study, it is consistent with a previous finding by Yamada and colleagues where they observed reduced levels of phospholipids with lens wear and suggested that this was due to the increased activity of secretory phospholipase A_2 ($sPLA_2$) during lens wear (Yamada *et al.* 2006). However, in the current study, $sPLA_2$ activity did not differ between lens and no lens wear but was higher in symptomatic wearers, as has been published previously (Glasson *et al.* 2002).

The current study compared two different commercial products and not merely two individual phospholipid classes. The differences between the two products are attributed to the mode of delivery as well as the formulation including components such as preservatives, and demulcents present in each product, which are discussed further below. The tear lipidome analysis did not detect the phospholipid ingredients (phosphatidylcholine, 34:2 and phosphatidylglycerol) present in either of the supplements. This might be due to the disappearance of lipid supplement between instillation and time of tear collection, which is consistent with the findings in the pilot study suggesting the effect of the liposomal spray, may have disappeared. It would be helpful to analyse the retention of the supplements post insertion, as has been published for a castor oil emulsion drop (Maissa *et al.* 2010).

Though the exogenous ingredients were not detected in the tear lipidome, the lipid drop and the placebo drop appeared to have a transient effect in some of the lipid classes compared to the lipid or placebo spray. Lipid classes such as CE, Chl, TAG, PE, SM,

and OAHFA increased their concentration with the lipid drop and the placebo drop at day 1 compared to baseline. At day 14, the concentration of CE and OAHFA remained higher compared to baseline with the placebo drop. In comparison, the lipid spray increased the concentration of Chl and TAG at day 1 and OAHFA at day 14 compared to the baseline. Studies that investigated the effect of several lubricants on ocular comfort, tear film stability and lens dehydration during contact lens wear showed no advantage of lubricants over the saline solution used as a control (Caffery *et al.* 1990, Golding *et al.* 1990, Efron *et al.* 1991). To date, the current study is the first to assess the effect of lipid supplements on tear biochemistry of contact lens wearers. Though the biochemistry of tear lipids was altered, there is no evidence that the lipid supplements caused the changes observed. The majority of the biochemical changes in tears occurred at the beginning of treatment rather than at day 14. In addition, lipid classes that were less abundant in tears (Chl, TAG, PE, SM and LPC) were found in reduced concentrations at day 14 compared to day 1. It can be speculated that though the supplements alter the lipid biochemistry to a small extent, the effect of long-term lens wear is a more important factor.

Although, the ratios of lipid classes did not significantly differ between symptomatic and asymptomatic wearers, there were some obvious associations between some of the lipid classes (Chl, SM, LPC and LPE) with symptomatic status. The symptomatic group had significantly increased concentrations of Chl and SM and a slight increase in LPC in their tear lipids during lens wear. It has been postulated that bacterial lipases catalyse the hydrolysis of CE resulting higher levels of Chl in patients with meibomian gland dysfunction and chronic blepharitis (Dougherty *et al.* 1986b, Shine *et al.* 2003b). Some *in-vitro* studies have shown disintegration of tear lipid layer with increased concentration of Chl (Arciniega *et al.* 2011a, Arciniega *et al.* 2013). SMs are thought to

have an important role in maintaining the structural stability between the aqueous and lipid interface of tears (Efron *et al.* 1991, Greiner *et al.* 1996). However, higher levels of SM were observed in tears of rabbits with dry eye (Caffery *et al.* 1990) and an *in-vitro* study revealed that excess levels of SM can lead to repulsion of lipid molecules at the aqueous-lipid interface (Georgiev *et al.* 2010). In the current study, higher levels of Chl and SM in symptomatic wearers were observed only at baseline with lens wear. Since Chl and SM are present only in trace amounts in tears, any intervention could have diluted Chl and SM and limited the ability of the study to detect changes in symptomatic wearers.

Lysophospholipids are likely to be degraded from tear phospholipids by the activity of $sPLA_2$ (Brown *et al.* 2013). In the current study, LPE was significantly higher in symptomatic wearers compared to asymptomatic wearers with the use of both lipid supplements at day 14 but not at baseline or day 1. Higher levels of degraded phospholipids at day 14 might be yet another consequence of long-term lens wear. Symptomatic wearers showed higher $sPLA_2$ activity at day 14 with the lipid and the placebo spray but not with the drop supplements. A higher activity of $sPLA_2$ has been found in tears of symptomatic contact lens wearers (Glasson *et al.* 2002). The current finding in which symptomatic wearers exhibited higher $sPLA_2$ activity with the spray supplements further strengthen the argument for the relevance of the volume and the formulation of each supplement delivered to the tear film.

The placebo drop increased the concentrations of OAHFA and WE from baseline in asymptomatic wearers compared to symptomatic wearers at the beginning of treatment. WE form the bulk of lipid layer and OAHFA is the abundant polar lipid detected in meibum and tears. An altered composition of OAHFA and WE have been observed in

the meibum of patients with chronic blepharitis, severe meibomian gland dysfunction and dry eyes when compared to the meibum of healthy individuals (Dougherty *et al.* 1991, Shine *et al.* 1991, Mathers *et al.* 1998, Joffre *et al.* 2008, Lam *et al.* 2011). The higher concentrations of OAHFA and WE in asymptomatic lens wearers might suggest the potential role of supplements based on these abundant lipid classes in reducing lens wear symptomatology.

5.4.4 ASSOCIATIONS BETWEEN OCULAR COMFORT AND TEAR LIPID VARIABLES

Ocular comfort during contact lens wear improved with increased tear film stability and lipid layer thickness, and a reduced tear evaporation rate, and $sPLA_2$ concentration and activity in tears. These results are consistent with earlier findings that linked tear film stability, lipid layer thickness, tear evaporation rate and phospholipase enzyme concentration and activity with contact lens wear related dryness and discomfort (Fonn *et al.* 1999, Glasson *et al.* 1999, Glasson *et al.* 2002, Guillon *et al.* 2007, Lemp *et al.* 2007, Pult *et al.* 2009, Wolffsohn *et al.* 2010, Ali *et al.* 2011, Kojima *et al.* 2011, Sengor *et al.* 2011b). Aho *et al.* (2003) observed a reduced concentration of $sPLA_2$ in tears following 6 hours of lens wear compared to a group of non-contact lens wearers, whereas Yamada *et al.* (2006) did not find a difference in $sPLA_2$ concentration in tears with and without lens wear. An association between an increased concentration of phospholipase enzyme and contact lens intolerance has been reported (Glasson *et al.* 2002).

Increased osmolarity in tears was associated with reduced tear film stability and thin lipid layer patterns. These results support previous observations made by King-Smith *et al.* (2009) that tear film thinning by a factor of three could raise tear

osmolarity which can further lead to tear film instability. In addition, Liu *et al.* (2009) rated similar ocular discomfort scores with induced tear film instability and hyperosmolarity in a dry eye population suggesting a link between the two.

In the current study, a reduced concentration and activity of $sPLA_2$ in tears was observed with thicker lipid layer patterns, higher tear film stability and lower tear evaporation rates. Increased concentration of $sPLA_2$ in tears was also associated with higher concentrations of degraded phospholipids and reduced levels of OAHFA in tears. An association between reduced levels of OAHFA with higher tear osmolarity was observed. In addition, a slight reduction in Chl and MDA with thicker lipid layer patterns and decreased tear evaporation rate respectively was observed.

Increased levels of degraded lipids have been associated with higher phospholipase enzyme activity (Glasson *et al.* 2002, Yamada *et al.* 2006, Chen *et al.* 2009, Wei *et al.* 2011). Recent evidence for the surfactant and bridging properties of OAHFA and an observation of reduced OAHFA levels in a moderate dry eye population signify the potential role of this amphiphilic lipid class in modulating tear film dynamics (Lam *et al.* 2011, Butovich 2013, Schuett *et al.* 2013). The literature shows that Chl levels increase in meibum in pathological conditions such as chronic blepharitis, meibomian gland dysfunction and dry eye disease due to increased keratinisation of glands or due to increased activity of bacterial lipases (Dougherty *et al.* 1986b, Jester *et al.* 1989, Nicolaides *et al.* 1989). In addition, *in-vitro* studies using Langmuir troughs have shown that increased amounts of Chl have the potential to collapse lipid films (Arciniega *et al.* 2013).

Several lipid classes were associated with clinical and functional aspects of lipid layer but were not directly associated with ocular comfort. However, $sPLA_2$ concentration

and activity, along with clinical and functional aspects of lipid layer were associated with ocular comfort. This could mean that although there were alterations in lipid biochemistry during contact lens wear, ocular discomfort was experienced when those changes affected tear film dynamics. Increasing concentrations of lysophospholipids and lipid aldehydes such as MDA may appear subsequent to elevated $sPLA_2$. Degraded lipids might lead to tear film thinning and instability, and reduced tear volume due to increased tear evaporation. Based on these results, it is reasonable to hypothesise that lysophospholipids and OAHFAs are those lipid classes that can act as potential biomarkers for ocular discomfort.

5.5 CONCLUSION

This study set out to perform the effect of exogenous lipid supplements on the tear lipid layer and comfort of habitual contact lens wearers. The findings confirm the current understanding of the link between clinical and functional aspects of tear lipid layer and contact lens related discomfort. The study results concur with the previous observations of poor tear film characteristics leading to contact lens wear discomfort. In other words, an individual's tear film characteristics rather than a lens might be the overriding influence on lens wear symptomatology. To minimise the effect of the lens aging on the tear film lipid layer, the use of a daily disposable lens is recommended for future studies. The anionic phospholipid emulsion drop showed positive effects such as thicker lipid layer appearance and decreased tear evaporation rate in symptomatic wearers compared to the placebo drop. Although, the study did not find a direct association between tear lipid biochemistry and ocular comfort, it did imply that lipid biochemistry influenced tear dynamics leading to lens wear discomfort. To substantiate this finding, a future study that follows neophytes for the causation and intervention of

symptomatology is recommended. In addition, an exploration of tear lipidome after contact lens wear, particularly at the 14-day time point would help to get a comprehensive picture of the lipid profile in contact lens wear. The results also suggests the potential role of lysophospholipids and OAHFAs in modulating symptoms during contact lens wear and suggests further examination of the dose, formulation and volume of lipid formulations based on those specific lipid classes over time.

CHAPTER 6. SUMMARY AND CONCLUSIONS

This thesis investigated the role of the tear lipid layer in contact lens wear comfort. The current chapter summarises the key findings of the thesis relative to the initial aims and objectives, discusses the potential limitations of this work and suggests next steps.

6.1 SUMMARY

Based on the literature review, the thesis tested the hypothesis that the use of exogenous lipid supplements modulate contact lens wear comfort by altering the clinical, functional and biochemical aspects of the tear lipid layer. The methodology chapters (Chapter 2, 3 and 4) developed tools to explore the thesis aims. An instrument to measure the tear evaporation rate was modified, calibrated and validated. A prospective study that assessed the tear lipid layer in symptomatic and asymptomatic contact lens wearers determined appropriate methods for measuring the clinical and biochemical aspects of the tear lipid layer. A randomised crossover trial with a liposomal spray explored the effect of an exogenous lipid supplement on tear lipid layer during short-term contact lens wear. The results suggested that contact lens wear causes clinical and biochemical changes to the tear lipid layer and is associated with an individual's symptomatology. A transient effect of the liposomal spray in clinical and biochemical aspects of the lipid layer in symptomatic wearers was also observed. Tear collection methods were optimised to characterise the tear lipidome. Basal tears using electro spray tandem mass spectrometry was used to analyse individual lipid components.

A comprehensive analysis on the effect of exogenous lipid supplements on tear lipid layer and ocular comfort in habitual contact lens wearers described the final objective of

the thesis (Chapter 5). The two lipid supplements used in this study were commercially available; a phospholipid based liposomal spray and an emulsion drop. A transient improvement in tear film stability with the two lipid supplements was observed in symptomatic contact lens wearers however, the improvement did not persist for the 14 days of lens wear. A superior effect of the lipid emulsion drop was observed in the structure and function of lipid layer compared to the lipid spray. The lipid spray increased the activity of phospholipase enzyme in tears compared to the lipid drop. However, the concentration of degraded phospholipids remained higher compared to baseline at the end of treatment stage irrespective of the method of treatment (spray or drop). The effect of lipid supplements (if any) might be masked due to prolonged lens wear, insufficient lipid reaching the tear film either due to the mode of delivery and/or the volume of supplement delivered, and the different lipid formulation used in each supplement. The univariate analysis that looked at the associations between ocular comfort and tear lipid variables suggests that changes in tear lipid biochemistry affects the tear film dynamics that might instigate the ocular discomfort experienced by symptomatic lens wearers. The multivariate analysis showed that tear evaporation rate and the activity of phospholipase enzyme in tears were the independent variables that predicted ocular comfort during contact lens wear.

6.2 LIMITATIONS

The detection of MDA in tears was challenging. A minimum volume of 10 µl of basal tear sample was required for the analysis. Due to the insufficient volume of basal tears, MDA was detected only in 60% of the total participants in the current thesis. Recently, the concentration of MDA was measured in an elderly population (Benlloch-Navarro *et al.* 2013) using a high performance liquid chromatography (HPLC). However, the

investigators collected tear samples using Schirmer's strips where the likelihood of samples being contaminated by epithelial cells is high due to its contact with the conjunctiva and the lower lid margin (van Setten *et al.* 1990, Choy *et al.* 2001). Optimising methods to detect MDA by assessing the sensitivity of HPLC technique with tear samples collected using glass microcapillary tubes is a consideration for future studies.

Compliance may have influenced the effectiveness of the supplements. Indeed, in checking compliance, the supplement bottles were collected at the end of each treatment stage and the residual volume was measured. The residual volume varied widely (0.0-4.5 ml). A study design that ensures the compliance by using online diaries or text messages to track the use of supplements is warranted for future studies.

Tear osmolarity was conventionally tested in both eyes and the worse eye's osmolarity is reported. Due to the large scale of the current study (400 visits), tear osmolarity was measured only in one eye in a random order. However, osmolarity measurement was not used to diagnose dry eye in the current study but to ensure that the osmolarity remained within the normal range throughout the study period.

Due to logistic reasons, the main study included only one baseline visit (with no treatment) prior to the start of any treatment stage. A study design that consists of baseline visits prior to each treatment visit is recommended to prevent any carry-over effect of treatments. An additional analysis that included the 'order of treatments stages' showed a non-significant effect on the outcome variables measured, thus minimising the issue due to lack of true baseline visits. While this approach does not replace having a baseline visit immediately prior to each treatment it does add confidence to the study findings.

Lens wear comfort was the main outcome variable measured. Ocular comfort itself is dynamic and hence measuring this parameter is challenging. Although the current study used a validated questionnaire (the Ocular Comfort Index) to detect a change in comfort, the questionnaire does not directly measures lens related comfort. A different outcome measure such as 'a just noticeable difference' (Papas *et al.* 2011) following the intervention might have yielded a different result. In addition, instead of the cross over study design that was implemented in the current thesis, a longitudinal study design that follows either neophytes or a cohort that have discontinued lens wear might provide a different conclusion on lens wear discomfort and is recommended for future work.

6.3 CONCLUSIONS

The thesis has significantly contributed to the body of knowledge of contact lens discomfort by providing a comprehensive assessment of tear lipid layer and its influence in lens wear discomfort. It successfully established the repeatability and validity of a modified dermatologic instrument and determined appropriate methods to assess the function and, clinical and biochemical aspects of the tear lipid layer respectively. There is a clear link between aspects of tear lipid layer and lens wear discomfort. The study findings imply that the changes in lipid biochemistry modulated lens wear discomfort by regulating tear film stability, lipid layer thickness and tear evaporation rate.

6.4 FUTURE RESEARCH

This thesis used two commercially available lipid supplements, i) an anionic phospholipid emulsion drop and ii) a zwitterionic phospholipid spray. The tear lipidome analysis could not detect a peak in those phospholipids (PC and PG) present in the

supplements. However, a superior effect of the anionic phospholipid emulsion drop in tear lipid layer was observed. It is reasonable to speculate that due to the differences in the mode of delivery, the volume delivered by each supplement might have an effect on tear lipid layer. A tear lipidome analysis following the use of both supplements delivered at the same volume to the ocular surface to confirm the presence of the active is recommended. This would also be useful to draw further conclusions on the benefit of those supplements on lipid components and ocular comfort.

The current study did not find a direct association between tear lipid biochemistry and ocular comfort but did suggest that lipid biochemistry influenced tear dynamics leading to lens wear discomfort. To substantiate this finding, a future study that follows neophytes that includes the time course, volume and formulation of the supplement is recommended. The detection of the active in tear film and the time course of decay would be useful to assess the causation and intervention in neophytes. In addition, an exploration of tear lipidome after contact lens wear, particularly at the 14-day time point would help to obtain a comprehensive picture of the lipid profile during contact lens wear.

The results also indicated the potential role of lysophospholipids (LPCs) and (O-acyl)-ω-hydroxy fatty acids (OAHFAs) in modulating symptoms during contact lens wear. Future studies need to be designed to specifically investigate the role of OAHFAs and LPCs as biomarkers for ocular discomfort. Exogenous lipid formulations based on those lipid classes need to be further explored and their role in improving lens wear discomfort investigated.

Furthermore, in this study, all participants wore silicone hydrogel lenses for each intervention stage. Lipid deposition on contact lens surface has been reported to be

associated with lens wear discomfort (Tripathi *et al.* 1991, Zhao *et al.* 2010) and silicone hydrogel lenses are prone to higher amounts of lipid deposition (Panaser *et al.* 2012). An evaluation of lipid deposition on worn contact lenses was not within the scope of this thesis. An extension of the current study where, worn contact lenses analysed for lipid deposits would allow a better understanding in the role of lipid deposits in lens wear discomfort.

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APPENDIX 1 MOLE% OF INDIVIDUAL LIPID SPECIES IN TOTAL TEAR LIPIDOME OF SYMPTOMATIC AND ASYMPTOMATIC CONTACT LENS WEARERS

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
CE 15:0+NH4	0.06 ± 0.02	0.07 ± 0.02
CE 16:0+NH4	0.33 ± 0.04	0.24 ± 0.03
CE 16:1+NH4	0.14 ± 0.04	0.17 ± 0.07
CE 17:0+NH4	0.39 ± 0.06	0.34 ± 0.1
CE 17:1+NH4	0.04 ± 0.01	0.04 ± 0.01
CE 18:0+NH4	0.31 ± 0.09	0.21 ± 0.03
CE 18:1+NH4	1.51 ± 0.2	1.51 ± 0.4
CE 18:2+NH4	0.24 ± 0.1	0.28 ± 0.3
CE 19:0+NH4	0.75 ± 0.04	0.53 ± 0.06
CE 19:1+NH4	0.08 ± 0.004	0.08 ± 0.02
CE 19:2+NH4	0.007 ± 0.01	0.006 ± 0.01
CE 20:0+NH4	3.25 ± 0.8	2.31 ± 0.4
CE 20:1+NH4	1.78 ± 0.4	1.13 ± 0.1
CE 20:2+NH4	0.380± 0.04	0.33 ± 0.02
CE 21:0+NH4	2.11 ± 0.4	1.70 ± 0.1
CE 21:1+NH4	0.08 ± 0.01	0.07 ± 0.01
CE 21:2+NH4	0.01 ± 0.01	0.02 ± 0.02
CE 22:0+NH4	2.56 ± 0.7	1.79 ± 0.4
CE 22:1+NH4	1.87 ± 0.3	1.40 ± 0.2
CE 22:2+NH4	0.42 ± 0.1	0.39 ± 0.1
CE 23:0+NH4	2.21 ± 0.6	1.72 ± 0.1
CE 23:1+NH4	0.09 ±0.03	0.09 ± 0.002
CE 23:2+NH4	0.03 ± 0.03	0.03 ± 0.01

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
CE 24:0+NH4	5.55 ± 0.6	4.74 ± 0.7
CE 24:1+NH4	3.41 ± 0.6	3.08 ± 0.3
CE 24:2+NH4	0.35 ± 0.09	0.38 ± 0.05
CE 25:0+NH4	5.29 ± 1.2	5.24 ± 0.3
CE 25:1+NH4	0.15 ± 0.2	0.17 ± 0.03
CE 26:0+NH4	5.23 ± 0.2	5.51 ± 0.7
CE 26:1+NH4	1.91 ± 0.5	2.04 ± 0.1
CE 26:2+NH4	0.22 ± 0.1	0.23 ± 0.02
CE 27:0+NH4	2.90 ± 0.6	3.16 ± 0.2
CE 27:1+NH4	0.09 ± 0.01	0.09 ± 0.01
CE 28:0+NH4	1.07 ± 0.1	1.07 ± 0.3
CE 28:1+NH4	1.99 ± 0.3	2.01 ± 0.2
CE 28:2+NH4	0.10 ± 0.04	0.13 ± 0.01
CE 29:0+NH4	0.95 ± 0.2	0.93 ± 0.1
CE 29:1+NH4	0.06 ± 0.02	0.09 ± 0.04
CE 30:0+NH4	0.19 ± 0.05	0.18 ± 0.05
CE 30:1+NH4	2.77 ± 0.4	2.77 ± 0.3
CE 30:2+NH4	0.19 ± 0.03	0.19 ± 0.03
CE 31:0+NH4	0.19 ± 0.04	0.25 ± 0.02
CE 31:1+NH4	0.07 ± 0.03	0.12 ± 0.05
CE 31:2+NH4	0	0.01 ± 0.02
CE 32:1+NH4	1.13 ± 0.2	1.34 ± 0.2
CE 32:2+NH4	0.23 ± 0.07	0.29 ± 0.2
CE 33:1+NH4	0.19 ± 0.2	0.06 ± 0.04
CE 34:0+NH4	0.01 ± 0.02	0.04 ± 0.01
CE 34:1+NH4	0.25 ± 0.08	0.28 ± 0.05
CE 34:2+NH4	0.14 ± 0.003	0.13 ± 0.02

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
WE_23:0-O/ 16:0+NH4	0.01 ± 0.04	0.01 ± 0.01
WE_23:0-O/ 16:1+NH4	0.15 ± 0.06	0.077 ± 0.02
WE_23:0-O/ 17:0+NH4	0.50 ± 0.05	0.347 ± 0.03
WE_23:0-O/ 18:1+NH4	0.21 ± 0.1	0.17 ± 0.06
WE_24:0-O/ 16:0+NH4	1.18 ± 0.2	1.05 ± 0.1
WE_24:0-O/ 16:1+NH4	0.80 ± 0.3	0.52 ± 0.2
WE_24:0-O/ 17:0+NH4	2.66 ± 0.2	2.22 ± 0.3
WE_24:0-O/ 18:1+NH4	1.20 ± 1.3	1.12 ± 0.7
WE_25:0-O/ 16:0+NH4	6.51 ± 0.2	7.58 ± 0.1
WE_25:0-O/ 16:1+NH4	0.63 ± 0.4	0.44 ± 0.3
WE_25:0-O/ 17:0+NH4	2.20 ± 0.2	2.08 ± 0.1
WE_25:0-O/ 18:1+NH4	0.95 ± 0.3	0.94 ± 0.4
WE_26:0-O/ 16:0+NH4	4.96 ± 0.1	6.62 ± 0.1
WE_26:0-O/ 16:1+NH4	0.83 ± 0.1	0.68 ± 0.5
WE_26:0-O/ 17:0+NH4	3.14 ± 0.02	3.35 ± 0.2
WE_26:0-O/ 18:1+NH4	1.23 ± 1.2	1.39 ± 0.4
WE_27:0-O/ 16:0+NH4	6.76 ± 0.04	10.28 ± 0.02
WE_27:0-O/ 16:1+NH4	0.23 ± 0.1	0.18 ± 0.2
WE_27:0-O/ 17:0+NH4	0.85 ± 0.05	0.94 ± 0.02
WE_27:0-O/ 18:1+NH4	0.33 ± 0.1	0.36 ± 0.4
TAG 46:0+NH4	0.04 ± 0.03	0.02 ± 0.01
TAG 46:1+NH4	0.02 ± 0.02	0.01 ± 0.005
TAG 46:2+NH4	0.02 ± 0.03	0.02 ± 0.01
TAG 47:0+NH4	0.03 ± 0.02	0.02 ± 0.005
TAG 47:1+NH4	0.002 ± 0.05	0.03 ± 0.02
TAG 47:2+NH4	0.01 ± 0.02	0.02 ± 0.01
TAG 47:3+NH4	0.002 ± 0.005	0

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
TAG 48:0+NH4	0.07 ± 0.04	0.02 ± 0.02
TAG 48:1+NH4	0.06 ± 0.06	0.05 ± 0.03
TAG 48:2+NH4	0.05 ± 0.05	0.02 ± 0.02
TAG 48:3+NH4	0.01 ± 0.03	0.01 ± 0.02
TAG 49:0+NH4	0.01 ± 0.01	0.005 ± 0.01
TAG 49:1+NH4	0.02 ± 0.03	0.01 ± 0.02
TAG 49:2+NH4	0.02 ± 0.03	0.02 ± 0.02
TAG 49:3+NH4	0.004 ± 0.01	0.003 ± 0.01
TAG 49:5+NH4	0.03 ± 0.04	0.007 ± 0.01
TAG 50:0+NH4	0.02 ± 0.02	0.01 ± 0.01
TAG 50:1+NH4	0.05 ± 0.02	0.04 ± 0.01
TAG 50:2+NH4	0.06 ± 0.03	0.07 ± 0.04
TAG 50:3+NH4	0.02 ± 0.03	0.03 ± 0.02
TAG 50:4+NH4	0	0
TAG 51:0+NH4	0	0
TAG 51:1+NH4	0.006 ± 0.01	0.002 ± 0.004
TAG 51:2+NH4	0.022 ±0.02	0.03 ± 0.004
TAG 51:3+NH4	0.002 ± 0.003	0
TAG 51:4+NH4	0	0
TAG 52:0+NH4	0.06 ± 0.07	0.01 ± 0.02
TAG 52:1+NH4	0.03 ± 0.02	0.01 ± 0.006
TAG 52:2+NH4	0.16 ± 0.04	0.12 ± 0.02
TAG 52:3+NH4	0.09 ± 0.03	0.09 ± 0.02
TAG 52:4+NH4	0.004 ± 0.005	0.005 ± 0.005
TAG 52:5+NH4	0	0
TAG 53:0+NH4	0	0
TAG 53:1+NH4	0	0

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
TAG 53:2+NH4	0.03 ± 0.001	0.03 ± 0.005
TAG 53:3+NH4	0.01 ± 0.01	0.01 ± 0.007
TAG 53:4+NH4	0	0
TAG 54:0+NH4	0.01 ± 0.02	0.003 ± 0.007
TAG 54:1+NH4	0	0
TAG 54:2+NH4	0.05 ± 0.05	0.06 ± 0.02
TAG 54:3+NH4	0.23 ± 0.1	0.20 ± 0.03
TAG 54:4+NH4	0.01 ± 0.009	0.02 ± 0.01
TAG 54:5+NH4	0	0.01 ± 0.01
TAG 55:1+NH4	0	0
TAG 55:2+NH4	0	0
TAG 55:3+NH4	0	0
TAG 55:4+NH4	0	0
TAG 56:2+NH4	0	0
TAG 56:3+NH4	0.008 ± 0.007	0.007 ± 0.006
PC 28:0	0	0.004 ± 0.008
PC 28:1	0	0.001 ± 0.002
PC 32:0	0.02 ± 0.006	0.04 ± 0.03
PC 32:1	0.001 ± 0.003	0.009 ± 0.008
PC 34:0	0	0.003 ± 0.004
PC 34:1	0.12 ± 0.02	0.33 ± 0.2
PC 34:2	0.17 ± 0.003	0.31 ± 0.1
PC 36:0	0	0.005 ± 0.01
PC 36:1	0.02 ± 0.02	0.09 ± 0.1
PC 36:2	0.10 ± 0.01	0.16 ± 0.06
PC 36:3	0.06 ± 0.004	0.10 ± 0.07
PC 36:4	0.02 ± 0.02	0.05 ± 0.03

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
PC 38:1	0	0.003 ± 0.006
PC 38:2	0	0.001 ± 0.001
PC 38:3	0.004 ± 0.004	0.01 ± 0.007
PC 38:4	0.02 ± 0.01	0.03 ± 0.02
SM 32:0;2	0	0
SM 32:1;2	0.06 ± 0.03	0.12 ± 0.06
SM 34:0;2	0.02 ± 0.02	0.01 ± 0.01
SM 34:1;2	0.38 ± 0.1	0.69 ± 0.3
SM 34:2;2	0.004 ± 0.006	0.05 ± 0.01
SM 36:1;2	0.06 ± 0.02	0.10 ± 0.06
SM 36:2;2	0.001 ± 0.002	0.01 ± 0.01
SM 38:3;2	0	0
SM 40:1;2	0.08 ± 0.01	0.17 ± 0.1
SM 42:1;2	0.16 ± 0.05	0.33 ± 0.2
SM 42:2;2	0.11 ± 0.04	0.28 ± 0.2
SM 42:3;2	0.01 ± 0.004	0.04 ± 0.03
OAHFA_16:0/ 24:1	0.006 ± 0.004	0.01 ± 0.001
OAHFA_16:0/ 24:2	0.0001 ± 0.0001	0.001± 0.001
OAHFA_16:0/ 26:1	0.01 ± 0.006	0.01 ± 0.002
OAHFA_16:0/ 28:1	0.01 ± 0.007	0.01 ± 0.002
OAHFA_16:0/ 28:2	0.002 ± 0.004	0.003 ± 0.01
OAHFA_16:0/ 30:1	0.07 ± 0.004	0.04 ± 0.01
OAHFA_16:0/ 30:2	0.01 ± 0.006	0.004 ± 0.002
OAHFA_16:0/ 32:1	0.71 ± 0.003	0.14 ± 0.06
OAHFA_16:0/ 32:2	0.02 ± 0.001	0.02 ± 0.006
OAHFA_16:0/ 34:1	0.04 ± 0.007	0.03 ± 0.01
OAHFA_16:0/ 34:2	0.01 ± 0.003	0.01 ± 0.005

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
OAHFA_16:1/ 24:1	0.01 ± 0.004	0.01 ± 0.004
OAHFA_16:1/ 24:2	0.0001 ± 0.0002	0.001 ± 0.001
OAHFA_16:1/ 26:1	0.01 ± 0.01	0.01 ± 0.002
OAHFA_16:1/ 28:1	0.02 ± 0.01	0.03 ± 0.03
OAHFA_16:1/ 30:1	0.14 ± 0.03	0.09 ± 0.01
OAHFA_16:1/ 30:2	0.01 ± 0.006	0.01 ± 0.003
OAHFA_16:1/ 32:1	0.25 ± 0.04	0.23 ± 0.08
OAHFA_16:1/ 32:2	0.02 ± 0.01	0.03 ± 0.003
OAHFA_16:1/ 34:1	0.07 ± 0.006	0.07 ± 0.03
OAHFA_16:1/ 34:2	0.01± 0.007	0.02 ± 0.01
OAHFA_18:0/ 24:1	0.46 ± 0.7	0.004 ± 0.002
OAHFA_18:0/ 24:2	0.22 ± 0.3	0.002 ± 0.003
OAHFA_18:0/ 26:1	0.003 ± 0.002	0.003 ± 0.002
OAHFA_18:0/ 28:1	0.002 ± 0.001	0.004 ± 0.001
OAHFA_18:0/ 28:2	0.001 ± 0.001	0.01 ± 0.005
OAHFA_18:0/ 30:1	0.01 ± 0.01	0.02 ± 0.01
OAHFA_18:0/ 30:2	0.0004 ± 0.008	0.001 ± 0.001
OAHFA_18:0/ 32:1	0.02 ± 0.02	0.03 ± 0.01
OAHFA_18:0/ 32:2	0.02 ± 0.02	0.01 ± 0.008
OAHFA_18:0/ 34:1	0.005 ± 0.005	0.001 ± 0.002
OAHFA_18:0/ 34:2	0	0.001 ± 0.001
OAHFA_18:1/ 24:1	0.09 ± 0.01	0.08 ± 0.01
OAHFA_18:1/ 26:1	0.11 ± 0.01	0.11 ± 0.01
OAHFA_18:1/ 28:1	1.32 ± 2.1	0.08 ± 0.01
OAHFA_18:1/ 28:2	0.61 ± 1.0	0.01 ± 0.002
OAHFA_18:1/ 30:1	0.59 ± 0.07	0.53 ± 0.1
OAHFA_18:1/ 30:2	0.05 ± 0.01	0.05 ± 0.005

Appendix 1Mole% of Individual Lipid Species in Total Tear Lipidome of Symptomatic and Asymptomatic Contact Lens Wearers

Lipid species	Symptomatic (Sy)	Asymptomatic (Asy)
	Mean mole % ± SD*	
OAHFA_18:1/ 32:1	1.20 ± 0.09	1.19 ± 0.3
OAHFA_18:1/ 32:2	0.17 ± 0.04	0.18 ± 0.02
OAHFA_18:1/ 34:1	0.29 ± 0.06	0.31 ± 0.12
OAHFA_18:1/ 34:2	0.07 ± 0.01	0.08 ± 0.02
OAHFA_18:2/ 24:1	0.02 ± 0.001	0.01 ± 0.003
OAHFA_18:2/ 24:2	0	0.0002 ± 0.0002
OAHFA_18:2/ 26:1	0.01 ± 0.007	0.02 ± 0.002
OAHFA_18:2/ 28:1	0.01 ± 0.01	0.01 ± 0.003
OAHFA_18:2/ 30:1	0.08 ± 0.008	0.06 ± 0.02
OAHFA_18:2/ 30:2	0.001 ± 0.004	0.01 ± 0.002
OAHFA_18:2/ 32:1	0.17 ± 0.01	0.17 ± 0.05
OAHFA_18:2/ 32:2	0.03 ± 0.004	0.02 ± 0.007
OAHFA_18:2/ 34:1	0.04 ± 0.006	0.05 ± 0.02
OAHFA_18:2/ 34:2	0.01 ± 0.007	0.03 ± 0.02

*SD = Standard Deviation, CE: cholesterol ester, WE: wax ester, TAG: triglyceride, PC: phosphatidylcholine, SM: sphingomylein, OAHFA: (O-acyl)-ώ-hydroxy fatty acid.

APPENDIX 2 CONCENTRATION AND MOLE% OF INDIVIDUAL SPECIES DETECTED IN BASAL, REFLEX AND FLUSH TEARS

Lipid species	Lipid class	Basal tears		Reflex tears		Flush tears	
		Concentration	Mole%	Concentration	Mole%	Concentration	Mole%
CE 15:0	CE	5.14 ± 1.8	0.06 ± 0.02	0.65 ± 0.4	0.02 ± 0.01	0.27 ± 0.3	0.01 ± 0.01
CE 16:0	CE	23.09 ± 7.2	0.29 ± 0.02	4.11 ± 1.9	0.15 ± 0.05	2.09 ± 0.8	0.21 ± 0.08
CE 16:1	CE	12.87 ± 4.5	0.17 ± 0.03	2.39 ± 1.7	0.06 ± 0.06	2.02 ± 1.0	0.15 ± 0.06
CE 17:0	CE	34.28 ± 10.7	0.43 ± 0.05	5.86 ± 2.8	0.20 ± 0.06	1.81 ± 0.7	0.19 ± 0.06
CE 18:0	CE	25.98 ± 6.8	0.34 ± 0.04	5.18 ± 2.8	0.17 ± 0.06	1.66 ± 0.6	0.15 ± 0.05
CE 18:1	CE	154.75 ± 47.4	1.90 ± 0.17	29.83 ± 12.4	1.23 ± 0.20	10.57 ± 3.8	1.12 ± 0.26
CE 18:2	CE	17.19 ± 3.9	0.27 ± 0.05	6.70 ± 4.1	0.29 ± 0.12	5.66 ± 2.6	0.72 ± 0.41
CE 19:0	CE	70.72 ± 22.6	0.88 ± 0.05	13.54 ± 5.8	0.51 ± 0.12	4.37 ± 1.6	0.47 ± 0.10
CE 19:1	CE	7.75 ± 2.3	0.10 ± 0.02	1.12 ± 0.8	0.02 ± 0.02	0.15 ± 0.1	0.01 ± 0.01
CE 20:0	CE	240.65 ± 69.9	2.99 ± 0.27	50.91 ± 19.8	2.11 ± 0.34	18.13 ± 6.2	2.05 ± 0.43
CE 20:1	CE	144.10 ± 44.5	1.79 ± 0.13	30.03 ± 12.1	1.25 ± 0.23	8.59 ± 2.8	0.94 ± 0.20
CE 20:2	CE	36.74 ± 10.5	0.47 ± 0.03	7.50 ± 3.2	0.28 ± 0.07	3.25 ± 1.8	0.27 ± 0.09
CE 21:0	CE	171.11 ± 59.6	2.01 ± 0.13	32.94 ± 13.0	1.29 ± 0.26	10.88 ± 4.3	1.10 ± 0.22
CE 21:1	CE	7.99 ± 2.8	0.08 ± 0.02	1.67 ± 0.7	0.07 ± 0.04	0.45 ± 0.3	0.06 ± 0.05
CE 22:0	CE	177.77 ± 54.9	2.14 ± 0.16	36.25 ± 14.7	1.41 ± 0.30	12.14 ± 4.8	1.22 ± 0.24
CE 22:1	CE	158.93 ± 52.4	1.93 ± 0.12	30.88 ± 11.4	1.30 ± 0.20	10.12 ± 3.6	1.06 ± 0.21
CE 22:2	CE	39.36 ± 10.7	0.51 ± 0.02	8.73 ± 3.7	0.34 ± 0.08	2.66 ± 0.9	0.28 ± 0.08
CE 23:0	CE	175.03 ± 59.6	2.07 ± 0.13	35.84 ± 14.5	1.36 ± 0.29	13.21 ± 5.4	1.29 ± 0.26
CE 23:1	CE	8.79 ± 2.6	0.10 ± 0.01	1.08 ± 0.8	0.02 ± 0.01	0.27 ± 0.3	0.01 ± 0.01
CE 24:0	CE	388.66 ± 111.2	4.91 ± 0.40	87.70 ± 37.1	3.38 ± 0.65	30.89 ± 11.4	3.19 ± 0.63
CE 24:1	CE	340.73 ± 117.7	4.08 ± 0.40	73.44 ± 25.8	3.14 ± 0.66	28.45 ± 9.6	3.13 ± 0.81
CE 24:2	CE	36.07 ± 9.8	0.48 ± 0.02	7.94 ± 3.3	0.30 ± 0.06	2.23 ± 0.9	0.20 ± 0.06
CE 25:0	CE	474.85 ± 156.1	5.83 ± 0.38	101.31 ± 44.5	3.80 ± 0.77	33.86 ± 13.1	3.40 ±0.68
CE 25:1	CE	14.68 ± 4.2	0.19 ± 0.01	2.39 ± 1.2	0.08 ± 0.03	0.41 ± 0.4	0.02 ± 0.02

Lipid species	Lipid class	Basal tears		Reflex tears		Flush tears	
		Concentration	Mole%	Concentration	Mole%	Concentration	Mole%
CE 26:0	CE	429.43 ± 127.9	5.65 ± 0.69	93.68 ± 41.1	3.57 ± 0.73	31.93 ± 11.0	3.42 ± 0.66
CE 26:1	CE	195.14 ± 64.5	2.36 ± 0.14	37.06 ± 13.9	1.48 ± 0.32	12.22 ± 4.3	1.28 ± 0.25
CE 26:2	CE	20.04 ± 5.4	0.26 ± 0.02	2.69 ± 1.9	0.06 ± 0.04	0.47 ± 0.5	0.02 ± 0.02
CE 27:0	CE	247.15 ± 87.0	3.00 ± 0.33	53.32 ± 25.9	1.89 ± 0.45	17.05 ± 6.1	1.77 ± 0.36
CE 27:1	CE	7.66 ± 2.1	0.10 ± 0.01	1.04 ± 0.7	0.02 ± 0.01	0.15 ± 0.2	0.01 ± 0.01
CE 28:0	CE	86.72 ± 26.7	1.16 ± 0.18	18.71 ± 7.9	0.72 ±0.17	8.00 ± 3.3	0.78 ± 0.16
CE 28:1	CE	187.16 ± 63.3	2.21 ± 0.19	31.47 ± 12.0	1.24 ±0.25	11.16 ± 3.7	1.20 ± 0.24
CE 28:2	CE	10.76 ± 3.2	0.13 ± 0.02	1.24 ± 0.8	0.03 ±0.02	0.00 ± 0.0	0.00 ± 0.0
CE 29:0	CE	55.88 ± 20.3	0.70 ± 0.10	12.30 ± 5.2	0.47 ± 0.10	3.77 ± 1.5	0.35 ± 0.12
CE 29:1	CE	7.07 ± 2.0	0.09 ± 0.02	0.77 ± 0.6	0.02 ± 0.01	0.00 ± 0.0	0.00 ± 0.0
CE 30:0	CE	11.32 ± 4.7	0.14 ± 0.03	2.21 ± 1.1	0.13 ± 0.06	0.78 ± 0.4	0.09 ± 0.05
CE 30:1	CE	221.65 ± 68.9	2.74 ± 0.27	36.52 ± 13.1	1.50 ± 0.32	12.71 ± 4.2	1.41 ± 0.28
CE 30:2	CE	14.69 ± 4.5	0.17 ± 0.03	1.80 ± 1.2	0.04 ± 0.03	0.36 ± 0.4	0.02 ± 0.02
CE 31:0	CE	13.35 ± 5.2	0.17 ± 0.03	2.16 ± 1.3	0.06 ± 0.03	0.45 ± 0.4	0.02 ± 0.02
CE 31:1	CE	6.86 ± 2.2	0.08 ± 0.02	0.67 ± 0.5	0.01 ± 0.01	0.00 ± 0.0	0.00 ± 0.0
CE 32:1	CE	87.42 ± 21.7	1.23 ± 0.16	21.12 ± 4.8	1.45 ± 0.45	9.07 ± 1.9	1.28 ± 0.27
CE 32:2	CE	18.86 ± 4.6	0.25 ± 0.04	3.48 ± 1.6	0.13 ± 0.04	0.51 ± 0.3	0.04 ± 0.03
CE 34:1	CE	15.57 ± 4.4	0.22 ± 0.05	2.04 ± 1.3	0.06 ± 0.03	0.37 ± 0.3	0.02 ± 0.02
CE 34:2	CE	8.25 ± 1.9	0.12 ± 0.02	1.07 ± 0.8	0.02 ± 0.01	0.34 ± 0.3	0.05 ± 0.05
Chl	Chl	450.32 ± 84.1	8.17 ± 1.7	236.90 ± 35.8	17.20 ± 3.5	131.23 ± 24.2	19.89 ± 3.54
LPC 16:0	LPC	75.60 ± 20.1	2.03 ± 0.79	147.70 ± 28.2	12.76 ± 3.6	51.76 ± 12.5	7.19 ± 1.55
LPC 18:0	LPC	17.95 ± 4.6	0.51 ± 0.21	30.21 ± 6.1	2.68 ± 0.84	9.22 ± 2.1	1.34 ± 0.38
LPC 18:1	LPC	17.36 ± 4.1	0.46 ± 0.18	30.77 ± 6.4	2.78 ± 0.87	10.95 ± 2.6	1.52 ± 0.38
LPC 18:2	LPC	2.55 ± 0.6	0.07 ± 0.03	3.95 ± 1.0	0.38 ± 0.13	1.62 ± 0.5	0.22 ± 0.07
LPE 16:0	LPE	8.01 ± 3.6	0.22 ± 0.13	11.27 ± 4.1	1.09 ± 0.43	1.04 ± 0.7	0.20 ± 0.13
LPE 18:0	LPE	9.58 ± 3.3	0.27 ± 0.14	11.39 ± 4.2	1.12 ± 0.49	1.29 ± 0.9	0.20 ± 0.16
LPE 18:1	LPE	13.15 ± 4.2	0.38 ± 0.18	16.12 ± 6.1	1.66 ± 0.71	2.97 ± 1.2	0.48 ± 0.22

Lipid species	Lipid class	Basal tears		Reflex tears		Flush tears	
		Concentration	Mole%	Concentration	Mole%	Concentration	Mole%
OAHFA_16:1/ 30:1	OAHFA	5.14 ± 2.1	0.05 ± 0.01	0.32 ± 0.3	0.01 ± 0.01	0.13 ± 0.1	0.01 ± 0.01
OAHFA_16:1/ 32:1	OAHFA	13.32 ± 5.0	0.15 ± 0.02	1.28 ± 0.9	0.03 ± 0.02	0.27 ± 0.3	0.01 ± 0.01
OAHFA_18:1/ 24:1	OAHFA	5.58 ± 2.4	0.06 ± 0.01	0.35 ± 0.4	0.01 ± 0.01	0.15 ± 0.2	0.01 ± 0.01
OAHFA_18:1/ 26:1	OAHFA	6.45 ± 2.7	0.07 ± 0.02	0.50 ± 0.4	0.02 ± 0.01	0.00 ± 0.0	0.00 ± 0.0
OAHFA_18:1/ 30:1	OAHFA	29.05 ± 11.4	0.32 ± 0.04	3.49 ± 1.4	0.13 ± 0.05	1.42 ± 0.6	0.13 ± 0.06
OAHFA_18:1/ 32:1	OAHFA	63.11 ± 25.1	0.69 ± 0.10	7.53 ± 3.0	0.31 ± 0.09	3.04 ± 1.2	0.29 ± 0.08
OAHFA_18:1/ 32:2	OAHFA	8.44 ± 3.1	0.10 ± 0.02	1.04 ± 0.6	0.03 ± 0.02	0.22 ± 0.2	0.01 ± 0.01
OAHFA_18:1/ 34:1	OAHFA	17.07 ± 6.0	0.20 ± 0.02	1.79 ± 1.1	0.05 ± 0.03	0.46 ± 0.4	0.02 ± 0.02
OAHFA_18:2/ 32:1	OAHFA	9.70 ± 4.0	0.11 ± 0.02	0.84 ± 0.6	0.02 ± 0.01	0.21 ± 0.2	0.01 ± 0.01
PC 32:0	PC	1.34 ± 0.3	0.02 ± 0.01	0.56 ± 0.2	0.06 ± 0.02	0.31 ± 0.1	0.05 ± 0.03
PC 34:1	PC	4.95 ± 1.1	0.09 ± 0.02	3.67 ± 0.4	0.28 ± 0.05	1.98 ± 0.3	0.32 ± 0.07
PC 34:2	PC	10.66 ± 1.7	0.23 ± 0.08	9.91 ± 1.5	0.83 ± 0.2	4.70 ± 0.9	0.81 ± 0.25
PC 36:1	PC	1.02 ± 0.4	0.01 ± 0.00	0.08 ± 0.1	0.01 ± 0.0	0.12 ± 0.1	0.01 ± 0.01
PC 36:2	PC	5.28 ± 0.8	0.11 ± 0.03	3.63 ± 0.5	0.27 ± 0.06	1.94 ± 0.3	0.33 ± 0.1
PC 36:3	PC	4.04 ± 0.7	0.09 ± 0.03	3.78 ± 0.6	0.30 ± 0.07	1.59 ± 0.4	0.26 ± 0.10
PC 36:4	PC	1.99 ± 0.3	0.04 ± 0.01	1.30 ± 0.3	0.11 ± 0.03	0.51 ± 0.2	0.09 ± 0.04
PC 38:4	PC	0.70 ± 0.2	0.01 ± 0.00	0.41 ± 0.2	0.03 ± 0.01	0.06 ± 0.06	0.02 ± 0.02
PC 38:5	PC	0.37 ± 0.2	0.01 ± 0.00	0.20 ± 0.1	0.02 ± 0.01	0.07 ± 0.05	0.01 ± 0.01
PE 36:2	PE	0.94 ± 0.4	0.03 ± 0.02	0.85 ± 0.4	0.11 ± 0.05	0.19 ± 0.2	0.06 ± 0.06
PE 36:3	PE	1.09 ± 0.4	0.03 ± 0.01	1.15 ± 0.4	0.12 ± 0.05	0.53 ± 0.3	0.13 ± 0.08

Lipid species	Lipid class	Basal tears		Reflex tears		Flush tears	
		Concentration	Mole%	Concentration	Mole%	Concentration	Mole%
SM 32:1;2	SM	1.85 ± 0.5	0.03 ± 0.01	0.93 ± 0.3	0.08 ± 0.03	0.60 ± 0.3	0.09 ± 0.06
SM 34:1;2	SM	15.84 ± 1.8	0.32 ± 0.09	12.41 ± 1.2	1.06 ± 0.28	6.57 ± 1.2	1.10 ± 0.29
SM 34:2;2	SM	0.96 ± 0.3	0.02 ± 0.01	0.41 ± 0.2	0.03 ± 0.02	0.11 ± 0.1	0.03 ± 0.03
SM 36:1;2	SM	2.22 ± 0.2	0.05 ± 0.02	1.33 ± 0.4	0.16 ± 0.07	0.43 ± 0.2	0.11 ± 0.06
SM 38:1;2	SM	1.02 ± 0.3	0.03 ± 0.01	0.74 ± 0.3	0.10 ± 0.07	0.50 ± 0.4	0.10 ± 0.09
SM 40:1;2	SM	4.01 ± 0.4	0.09 ± 0.02	4.10 ± 1.1	0.49 ± 0.25	2.01 ± 0.8	0.41 ± 0.22
SM 42:1;2	SM	7.28 ± 0.8	0.16 ± 0.04	6.05 ± 1.2	0.61 ± 0.26	2.38 ± 1.1	0.50 ± 0.31
SM 42:2;2	SM	5.67 ± 0.5	0.11 ± 0.02	3.71 ± 1.0	0.37 ± 0.18	1.37 ± 0.6	0.25 ± 0.15
TAG 48:0	TAG	1.01 ± 0.3	0.02 ± 0.01	0.25 ± 0.1	0.03 ± 0.02	0.88 ± 0.5	0.08 ± 0.04
TAG 48:1	TAG	1.92 ± 0.8	0.04 ± 0.02	0.30 ± 0.3	0.01 ± 0.01	0.51 ± 0.2	0.05 ± 0.03
TAG 48:2	TAG	1.80 ± 0.8	0.04 ± 0.02	0.49 ± 0.4	0.01 ± 0.01	0.41 ± 0.2	0.04 ± 0.02
TAG 48:3	TAG	0.59 ± 0.3	0.01 ± 0.01	0.22 ± 0.2	0.01 ± 0.0	0.12 ± 0.1	0.01 ± 0.01
TAG 50:0	TAG	0.57 ± 0.2	0.01 ± 0.01	0.12 ± 0.1	0.02 ± 0.01	1.08 ± 1.0	0.08 ± 0.07
TAG 50:1	TAG	2.70 ± 0.8	0.04 ± 0.02	0.54 ± 0.4	0.01 ± 0.01	0.45 ± 0.2	0.04 ± 0.02
TAG 50:2	TAG	4.14 ± 1.3	0.07 ± 0.03	0.87 ± 0.6	0.02 ± 0.02	0.44 ± 0.3	0.04 ± 0.02
TAG 50:3	TAG	1.80 ± 0.6	0.03 ± 0.01	0.49 ± 0.3	0.01 ± 0.01	0.07 ± 0.07	0.00 ± 0.0
TAG 52:2	TAG	9.08 ± 2.6	0.13 ± 0.03	1.63 ± 0.7	0.06 ± 0.02	0.74 ± 0.4	0.07 ± 0.03
TAG 52:3	TAG	6.42 ± 1.9	0.09 ± 0.01	1.38 ± 0.9	0.04 ± 0.02	0.38 ± 0.2	0.04 ± 0.02
TAG 54:2	TAG	3.06 ± 1.0	0.04 ± 0.01	0.32 ± 0.2	0.02 ± 0.01	0.03 ± 0.03	0.01 ± 0.01
TAG 54:3	TAG	18.89 ± 5.5	0.26 ± 0.06	3.70 ± 1.5	0.16 ± 0.06	1.18 ± 0.4	0.14 ± 0.05
WE 16:0/22:0	WE	3.65 ± 1.2	0.04 ± 0.01	0.66 ± 0.4	0.02 ± 0.01	0.32 ± 0.2	0.02 ± 0.01
WE 16:0/23:0	WE	6.88 ± 1.6	0.10 ± 0.01	2.37 ± 0.9	0.10 ± 0.03	3.58 ± 0.6	0.69 ± 0.20
WE 16:0/24:0	WE	26.17 ± 6.6	0.35 ± 0.01	7.10 ± 2.6	0.29 ± 0.06	8.99 ± 3.8	1.09 ± 0.28
WE 16:0/25:0	WE	22.00 ± 6.9	0.28 ± 0.01	5.44 ± 2.3	0.20 ± 0.04	1.86 ± 0.7	0.19 ± 0.05
WE 16:0/26:0	WE	29.99 ± 7.4	0.41 ± 0.03	8.10 ± 3.9	0.28 ± 0.06	5.28 ± 2.8	0.49 ± 0.2
WE 16:0/27:0	WE	9.04 ± 2.5	0.12 ± 0.01	2.71 ± 1.3	0.10 ± 0.02	1.04 ± 0.5	0.10 ± 0.04
WE 16:0/28:0	WE	5.26 ± 0.9	0.08 ± 0.01	2.04 ± 0.8	0.09 ± 0.02	4.16 ± 2.1	0.49 ± 0.17
WE 16:1/22:0	WE	15.63 ± 5.0	0.19 ± 0.03	2.78 ± 1.8	0.06 ± 0.04	0.97 ± 0.9	0.04 ± 0.04

Lipid species	Lipid class	Basal tears		Reflex tears		Flush tears	
		Concentration	Mole%	Concentration	Mole%	Concentration	Mole%
WE 16:1/23:0	WE	35.74 ± 7.5	0.56 ± 0.07	15.93 ± 4.6	0.89 ± 0.16	23.89 ± 6.8	5.07 ± 2.0
WE 16:1/24:0	WE	138.97 ± 37.2	1.90 ± 0.08	35.05 ± 11.9	1.49 ± 0.29	36.52 ± 5.8	6.62 ± 1.7
WE 16:1/25:0	WE	124.28 ± 41.3	1.54 ± 0.12	25.31 ± 9.5	1.00 ± 0.22	9.46 ± 3.9	0.94 ± 0.26
WE 16:1/26:0	WE	174.44 ± 47.8	2.28 ± 0.17	41.51 ± 19.4	1.44 ± 0.34	14.25 ± 5.4	1.58 ± 0.37
WE 16:1/27:0	WE	55.19 ± 14.8	0.75 ± 0.07	16.86 ± 7.2	0.65 ± 0.15	6.10 ± 2.1	0.70 ± 0.21
WE 16:1/28:0	WE	29.31 ± 5.5	0.46 ± 0.05	12.05 ± 4.2	0.55 ± 0.14	11.41 ± 2.5	1.74 ± 0.54
WE 16:1/29:0	WE	9.36 ± 2.9	0.12 ± 0.04	2.20 ± 1.7	0.04 ± 0.03	0.65 ± 0.6	0.22 ± 0.22
WE 17:0/21:0	WE	4.74 ± 2.0	0.05 ± 0.01	0.50 ± 0.3	0.01 ± 0.01	0.08 ± 0.08	0.00 ± 0.0
WE 17:0/22:0	WE	6.37 ± 2.2	0.08 ± 0.01	1.07 ± 0.5	0.04 ± 0.01	0.21 ± 0.2	0.01 ± 0.01
WE 17:0/23:0	WE	8.26 ± 2.8	0.11 ± 0.01	2.17 ± 0.7	0.10 ± 0.02	0.78 ± 0.3	0.07 ± 0.02
WE 17:0/24:0	WE	43.01 ± 13.8	0.53 ± 0.03	9.46 ± 3.8	0.36 ± 0.08	3.54 ± 1.5	0.34 ± 0.07
WE 17:0/25:0	WE	38.11 ± 13.4	0.46 ± 0.03	8.33 ± 3.6	0.30 ± 0.07	3.18 ± 1.5	0.30 ± 0.07
WE 17:0/26:0	WE	46.30 ± 12.9	0.60 ± 0.04	11.20 ± 5.4	0.38 ± 0.09	3.92 ± 1.7	0.39 ± 0.09
WE 17:0/27:0	WE	13.12 ± 4.2	0.17 ± 0.01	3.34 ± 1.7	0.11 ± 0.03	1.00 ± 0.4	0.09 ± 0.03
WE 17:0/28:0	WE	4.61 ± 1.0	0.07 ± 0.01	1.62 ± 0.9	0.06 ± 0.02	0.58 ± 0.2	0.05 ± 0.02
WE 18:1/21:0	WE	31.26 ± 11.9	0.35 ± 0.04	4.22 ± 1.9	0.15 ± 0.06	0.83 ± 0.8	0.04 ± 0.04
WE 18:1/22:0	WE	51.25 ± 18.4	0.58 ± 0.07	7.12 ± 3.3	0.25 ± 0.11	1.86 ± 1.5	0.12 ± 0.08
WE 18:1/23:0	WE	62.95 ± 23.8	0.71 ± 0.07	11.64 ± 4.5	0.44 ± 0.11	4.51 ± 2.2	0.40 ± 0.13
WE 18:1/24:0	WE	374.32 ± 129.0	4.30 ± 0.41	63.78 ± 26.1	2.37 ± 0.56	25.36 ± 12.3	2.31 ± 0.55
WE 18:1/25:0	WE	341.04 ± 123.6	3.87 ± 0.37	59.89 ± 25.3	2.16 ± 0.51	22.58 ± 11.4	2.02 ± 0.49
WE 18:1/26:0	WE	470.85 ± 145.9	5.64 ± 0.50	95.60 ± 45.5	3.28 ± 0.77	32.21 ±14.8	3.01 ± 0.68
WE 18:1/27:0	WE	134.87 ± 44.5	1.59 ± 0.15	28.13 ± 13.3	0.98 ± 0.23	9.25 ± 4.3	0.81 ± 0.23
WE 18:1/28:0	WE	42.92 ± 11.5	0.55 ± 0.03	10.34 ± 5.0	0.35 ± 0.09	3.27 ± 1.8	0.23 ± 0.12
WE 18:1/29:0	WE	18.80 ± 5.4	0.24 ± 0.03	2.55 ± 1.8	0.05 ± 0.04	0.68 ± 0.7	0.03 ± 0.03

SE: Standard error, CE: cholesterol esters, WE: wax esters, Chl: free cholesterol, TAG: triglycerides, PC: phosphatidylcholine, PE: phosphatidylethanolamine, SM: sphingomylein, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OAHFA: (O-acyl)-ώ-hydroxy fatty acids.

APPENDIX 3 PARTICIPANT INFORMATION SHEET AND CONSENT FORMS

VALIDATING THE VAPOMETER; A NEW MEASURING DEVICE FOR TEAR EVAPORATION RATE

Approval No: 11061

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

You are invited to participate in a study in which the evaporation rate of your tears will be measured using an instrument named the VapoMeter (Delfin Technologies, Kuopio Finland). The measurement will be done in two stages. The first stage will be without contact lens wear and the second stage will be with contact lens wear on your eyes. We would like to investigate the repeatability of this instrument and compare the results between two stages. We would also like to collect tears and look for association between lipid content in your tears and your evaporation rate. You have been selected as a potential participant in this study because your eyes are healthy and have no contraindications to contact lens wear.

Inclusion and Exclusion criteria

If you are between 18 and 35 years of age and not pregnant, do not have a history of allergy, injury or any surgery related to your eyes then you can participate in this study.

Description of the study visits

Baseline visit: If you decide to participate, we will perform a baseline screening examination to determine your suitability for this study. This will involve standard optometric procedures and will take approximately 60 minutes.

Stage 1 (without contact lens wear): If you are currently a contact lens wearer, you will be required to discontinue contact lens wear for at least 24 hours prior to study commencement. Tear evaporation rate will be measured noninvasively using an instrument called VapoMeter (Delfin Technologies, Kuopio Finland). The approximate visit time is 5-10 minutes. An open eye and closed eye measurement will be taken. Vaseline will be applied on to the closed eyelid and lid margins to minimize skin evaporation. This will be removed using soft tissue at the end of the measurement. This measurement will be done three times in a day (morning, noon and evening) and repeated for three days. During the morning visit of the first, second and third day, up to 7 μL (which is less than a drop) of basal tears (non-stimulated tears), reflex tears (tears stimulated by asking you to induce yawning) and flush tears (tears collected after putting 2 drops of sterile saline in your eye) will be collected from each eye separately. You will be asked to tilt your head to one side. A glass microcapillary tube will be then rested gently on the lower lid. Collected tears will be stored for laboratory analysis.

Stage 2 (with contact lens wear): You will be asked to wear contact lenses bilaterally for six hours. A general eye health examination using a slit lamp biomicroscope will be conducted during this visit to ensure the health of the eyes. The procedures will be exactly similar to stage 1.Tear evaporation rate will be measured using VapoMeter and tears will be collected at each day. All contact lenses and products used in this study are currently commercially available and approved by the Therapeutics Goods Administration (TGA) in Australia. At the end of two stages, photos of the front of your eyes may also be taken for documentation.

Risks

We are not aware of any published or anecdotal reports suggesting any damage incurred by the procedures outlined on the previous page. However contact lens wear itself may cause burning, itching, irritation, tearing, light sensitivity, red eye, inflammation, infection and foreign body sensation or discomfort. These symptoms are usually temporary and tend to resolve when contact lenses are removed. Apart from that there is a potential risk of mechanical blunt injury to the eye by the insertion and removal of lenses and collection of tears. The risk will be minimized by appropriate training of the investigators. The most serious adverse effect of contact lens wear is a rare but serious infection called microbial keratitis. This occurs in approximately 1 in 10,000 daily contact lens wearers each year. It may cause significant pain and hospitalisation and may be sight threatening.

If any problems develop during the course of the study, you should contact Ms. Athira Rohit on (02) 9385 7544. If you are unable to get in contact with us, you should go to the Emergency Clinic of the Sydney Eye Hospital at 8 Macquarie Street, Sydney or contact the Sydney Eye Hospital on (02) 9382 7111.

Confidentiality and disclosure of information

Any information that is obtained in connection with this study and that can be identified with you will remain confidential. It will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be provided in such a way that you cannot be identified. Patient data will be kept on computer discs and will be stored in a secure place.

Recompense to participants

Gift vouchers of 15$ will be given at the end of the study visits in recognition of your time in participating this study. We cannot and do not promise any benefits for the participant from this study. Complaints may be directed to the Ethics Secretariat, The University of New South Wales, Sydney 2052 Australia (phone 9385 4234, fax 9385 6648, and email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

Feedback to participants

If you indicate that, you wish for us to do so by providing your email address below, we will email you a summary of research findings on completion of this study.

Your email address (optional) _____

Your consent

Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice. If you have any questions, please feel free to ask us. If you have any additional questions later, contact Prof. Fiona Stapleton on (02) 9385 5287 and she will be happy to answer them. You will be given a copy of this form to keep.

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	……..
Signature of Research Participant	Signature of Investigator
……	……..
Please PRINT name	Please PRINT name
……	……..
Date	Date

REVOCATION OF CONSENT

VALIDATING THE VAPOMETER; A NEW MEASURING DEVICE FOR TEAR EVAPORATION RATE

Approval No: 11061

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardize any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT name

The section for Revocation of Consent should be forwarded to

Prof. Fiona Stapleton

School of Optometry and Vision Science

The University of New South Wales

Sydney 2052

EFFECT OF AN OCULAR SPRAY ON CONTACT LENS COMFORT

Approval No: 11008

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

You are invited to participate in a study examining the effects of tear film components on short-term contact lens wear comfort. The purpose of this study is to investigate whether there are any changes in the tear lipid layer clinically, functionally or biologically associated with short-term contact lens wear and ocular comfort and whether there is a difference in these changes with the use of a liposomal spray (Tears Again, BioRevive). You have been selected as a potential participant in this study because you have no contraindications to contact lens wear, and your vision can be corrected with a contact lens.

Liposomal spray: The discomfort due to contact lens wear is usually due to the disturbance caused to the outer layer of tear film, which is called as the lipid layer. It is responsible in preventing evaporation from the eye surface. Liposomes are bubbles made of lipid, which are used for drug delivery in various conditions. The liposomal spray which is used in this study (Tears Again, BioRevive) contains a major phospholipid; phosphatidylcholine along with other essential fatty acids and vitamin E. These components are delivered in a stable form (liposomes) to the closed eyelid and they move to the lid margin and combine with the natural tear film.

Inclusion & Exclusion criteria

If you are between 18 and 30 years of age and has a refractive error between +4.00 to -8.00 dioptres you can participate in this study. If you are pregnant, have a history of

allergy, injury or any refractive surgery related to your eyes then you cannot participate in this study.

Description of study and risks

If you decide to participate, we will perform a baseline screening examination to determine your suitability for this study. We will fit you with a pair of soft contact lenses and you will be asked to wear the lenses for 6 hours before returning for an examination on the next day. If you are currently a contact lens wearer, you will be required to discontinue contact lens wear for at least 24 hours prior to study commencement. In addition to the baseline visit, you will be required to attend 2 study visits within a week period, each lasting approximately 1 hour. Each study visit will be performed at a similar time of the day, and will be scheduled to allow observations and tests to be made before and after a lens wear period of 6 hours. You may also need to attend another visit, where a liposomal spray will be applied to your closed eye lids and similar observations and tests will be done at first, second and sixth hour following the initial spraying. All contact lenses and products used in this study are currently commercially available and approved by the Therapeutics Goods Administration (TGA) in Australia. At each visit, the following procedures will be performed:

Stage1.

Baseline visit: This will involve standard optometric procedures, which are routinely carried out to determine your suitability for the study. This part of the study will take approximately 60 minutes. Questionnaire: You will be asked a few questions in regards to the level of discomfort in each eye, and the severity and frequency of your symptoms of eye dryness, itchiness, irritation, stinging, burning and sensitivity to light with your

contact lenses. I will then determine the power of the lenses required for your eyes. Tear collection: Up to 5 microliters of tear will be collected from each eye separately. You will be asked to tilt your head to one side. A glass microcapillary tube will be then rested gently on the lower lid. Collected tears will be labelled and stored for laboratory analysis. Tear film assessment: The front of your eyes will be examined behind the slit lamp biomicroscope for any signs of redness, swelling or other relevant signs. This will be followed by tear film assessment using a Tearscope where you will be asked to blink twice before holding your eye open and your lipid layer thickness will be assessed. Tear evaporation rate: Tear evaporation rate will be measured noninvasively using a VapoMeter.

Lens Insertion visit: You will be asked to wear contact lenses bilaterally for six hours. A general eye health examination using a slit lamp biomicroscope will be conducted during this visit to ensure the health of the eyes. This part of the study will take about 15 minutes.

Lens removal visit: All the assessments done at the baseline visit will be repeated including tear sample collection during this visit with your lenses on. When all the observations and tests are done, lenses will be removed from both eyes. This part of the study will take approximately 60 minutes.

Stage2.

With your both eyes closed, a single application of a liposomal spray (Tears Again, BioRevive) and an equal amount of saline spray will be sprayed to both eyes. All the assessments including tear collection which was performed during stage 1 will be repeated at first, second and sixth hour after initial spraying. Hence, after the first,

second and sixth hour of initial spraying you will be required to return to the research clinic for the follow-up assessment.

Risks

We are not aware of any published or anecdotal reports suggesting any damage incurred by the procedures outlined on the previous page. However contact lens wear itself may cause burning, itching, irritation, tearing, light sensitivity, red eye, inflammation, infection and foreign body sensation or discomfort. These symptoms are usually temporary and tend to resolve when contact lenses are removed. Apart from that, there is a potential risk of mechanical blunt injury to the eye by the insertion and removal of lenses and collection of tears. The risk will be minimized by appropriate training of the investigators. The most serious adverse effect of contact lens wear is a rare but serious infection called microbial keratitis. This occurs in approximately 1 to5 in 10,000 people/year. It is sight threatening and may rarely cause significant pain and hospitalisation. If any problems develop during the course of the study, you should contact Prof. Fiona Stapleton on (02) 9385 5287. If you are unable to get in contact with us, you should go to the Emergency Clinic of the Sydney Eye Hospital at 8 Macquarie Street, Sydney or contact the Sydney Eye Hospital on (02) 9382 7111.

Confidentiality and disclosure of information

Any information that is obtained in connection with this study and that can be identified with you will remain confidential. It will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be

provided in such a way that you cannot be identified. Patient data will be kept on computer discs and will be stored in a secure place.

Recompense to participants

We cannot and do not promise any benefits for the participant from this study. Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

Feedback to participants

If you indicate that, you wish for us to do so by providing your email address below, we will email you a summary of research findings on completion of this study.

Your email address (optional) _____

Your consent

Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice. If you have any questions, please feel free to ask us. If you have any additional questions later, contact Prof. Fiona Stapleton on (02) 9385 5287 and she will be happy to answer them. You will be given a copy of this form to keep.

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	……..
Signature of Research Participant	Signature of Investigator
……	……..
Please PRINT name	Please PRINT name
……	……..
Date	Date

REVOCATION OF CONSENT

EFFECT OF AN OCULAR SPRAY ON CONTACT LENS COMFORT

Approval No: 11008

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardize any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT name

The section for Revocation of Consent should be forwarded to

Prof. Fiona Stapleton

School of Optometry and Vision Science

The University of New South Wales

Sydney 2052

COMPARISON OF TEAR LIPID PROFILE AMONG BASAL, REFLEX AND FLUSH TEARS.

Approval No: 13190

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

You are invited to participate in a study examining the effects of different tear collection methods on your tear lipid composition. The purpose of this study is to compare different components in your basal, reflex and flush tears in order to determine if flush method of tear collection can be used as an alternative for basal tear collection. You have been selected as a potential participant in this study because you have healthy eyes and no prior exposure to contact lens wear.

Basal, reflex and flush tears: Basal tears are the non-stimulated tears. Reflex tears are tears produced due to any reflex action; for example while sneezing, crying etc. Flush tears are collected followed by instilling a few drops of sterile saline on to the lower eyelid fold.

Inclusion & Exclusion criteria

If you are between 18-35 years and have not used contact lenses before, you can participate in this study. If you are pregnant, have a history of allergy, injury or any surgery related to your eyes then you cannot participate in this study.

Description of the study

If you decide to participate, we will perform a baseline screening examination to determine your suitability for this study. This will involve standard optometric

procedures, which are routinely carried out to determine your suitability for the study. Each of the basal, reflex, and flush tear samples described above will be collected on three different days in a random order allowing at least 24 hour between each collection method. The baseline examination takes approximately 40 minutes. Tear collection: During the first, second and third day, up to 10 µl (which is less than a drop) of basal tears (non-stimulated tears), reflex tears (tears stimulated by asking you to induce yawning) and flush tears (tears collected after putting 2 drops of sterile saline in your eye) will be collected from your right and left eye separately. You will be asked to tilt your head to one side. A glass microcapillary tube will be then rested gently on the lower lid. Collected tears will be stored for laboratory analysis. The duration of tear collection on each day will be approximately 20 minutes.

Risks

We are not aware of any published or anecdotal reports suggesting any damage incurred by the procedures outlined on the previous section. There is a potential risk of mechanical blunt injury to the eye by collection of tears. The risk will be minimized by appropriate training of the investigators.

If any problems develop during the course of the study, you should contact Ms. Athira Rohit (02) 9385 7420. If you are unable to get in contact with us, you should go to the Red Eye of the UNSW Optometry Clinic at Gate 14 Barker Street, Sydney or contact the clinic on optomclinic@unsw.edu.au or telephone (02) 9385 4624. You can also go to the Emergency Clinic of the Sydney Eye Hospital at 8 Macquarie Street, Sydney or contact the Sydney Eye Hospital on (02) 9382 7111.

Confidentiality and disclosure of information

Any information that is obtained in connection with this study and that can be identified with you will remain confidential. It will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be provided in such a way that you cannot be identified. Patient data will be kept on computer discs and will be stored in a secure place.

Recompense to participants

We cannot and do not promise any benefits for the participant from this study. Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

Your consent

Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice. If you have any questions, please feel free to ask us. If you have any additional questions later, contact Ms. Athira Rohit on (02) 9385 7420 and she will be happy to answer them. You will be given a copy of this form to keep.

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

……	……..
Signature of Research Participant	Signature of Investigator
……	……..
Please PRINT name	Please PRINT name
……	……..
Date	Date

REVOCATION OF CONSENT

COMPARISON OF TEAR LIPID PROFILE AMONG BASAL, REFLEX AND FLUSH TEARS.

Approval No: 13190

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardize any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT name

The section for Revocation of Consent should be forwarded to

Prof. Fiona Stapleton

School of Optometry and Vision Science

The University of New South Wales

Sydney 2052

EFFECT OF LIPID SUPPLEMENTATION IN CONTACT LENS COMFORT

Approval No: 12584

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM

You are invited to participate in a study examining the effects of tear film components on contact lens wear comfort. The purpose of this study is to investigate whether there are any changes in the tear lipid layer clinically, functionally or biologically associated with contact lens wear and ocular comfort and whether there is a difference in these changes with the use of lipid supplements. You have been selected as a potential participant in this study because you have no contraindications to contact lens wear, and your vision can be corrected with a contact lens.

Lipid supplementation: The discomfort due to contact lens wear is usually due to the disturbance caused to the outer layer of the tear film, which is called as the lipid layer. It is responsible for preventing evaporation from the eye surface. The lipid supplements used in this study are commercially available lipid spray (tearsagain® BioRevive) and a lipid drop (Systane Balance®, Alcon). The spray and the drop contains phospholipids. The spray is delivered in a stable form to the closed eyelid and they move to the lid margin and combine with the natural tear film whereas the drop is delivered directly to the open eye where it combines with the natural tear film.

Inclusion & Exclusion criteria

If you are above 18 years and a regular soft contact lens wearer, you can participate in this study. If you are pregnant, have a history of allergy, injury or any refractive surgery related to your eyes then you cannot participate in this study.

Description of the study

If you decide to participate, we will perform a baseline screening examination to determine your suitability for this study. You will be asked not to wear your lenses for at least 24 hours prior to the baseline visit. In addition to the baseline visit, you will be required to attend 8 study visits within an 8-week period, each lasting approximately 1 hour. Each study visit will be performed at a similar time of the day and will be scheduled to allow observations and tests to be made with lens wear. You will be fitted with new lenses (Ciba Vision, Air Optix®) in every two weeks. All contact lenses and products used in this study are currently commercially available and approved by the Therapeutics Goods Administration (TGA) in Australia. The study includes 4 stages and a baseline visit.

Baseline visit: This will involve standard optometric procedures, which are routinely carried out to determine your suitability for this study.

Four stages: At the end of the baseline visit, you will be randomised to four stages. Among the four, two will be treatment stages including a lipid spray and a lipid drop and the other two will be a placebo (control) stage including a saline spray and a saline drop. You will be masked to the intervention type throughout the study. The four stages are each 2 weeks long and the visits will be conducted at the first and last day of each intervention type during evening hours. Each visit including the baseline will take approximately 60 minutes and following tests will be performed. Questionnaire: You will be asked a few questions in regards to the level of discomfort in each eye, and the severity and frequency of your symptoms of eye dryness, itchiness, irritation, stinging, burning and sensitivity to light with your contact lenses. I will then determine the power of the lenses required for your eyes. Tear collection: Up to 15 microliters of tear will be

collected from each eye separately. You will be asked to tilt your head to one side. A glass microcapillary tube will be then rested gently on the lower lid. Collected tears will be labelled and stored for laboratory analysis. Tear film assessment: The front of your eyes will be examined behind the slit lamp biomicroscope for any signs of redness, swelling or other relevant signs. This will be followed by tear film assessment using a Tearscope where you will be asked to blink twice before holding your eye open and your lipid layer thickness will be assessed. Tear evaporation rate: Tear evaporation rate will be measured noninvasively using a VapoMeter. Tear osmolarity: Osmolarity refers to the salt content of your eyes. This will be assessed using TearLab system, which is a small device by collecting less than a drop of tear from your eyes.

Risks

We are not aware of any published or anecdotal reports suggesting any damage incurred by the procedures outlined on the previous page. However contact lens wear itself may cause burning, itching, irritation, tearing, light sensitivity, red eye, inflammation, infection and foreign body sensation or discomfort. These symptoms are usually temporary and tend to resolve when contact lenses are removed. Apart from that, there is a potential risk of mechanical blunt injury to the eye by the insertion and removal of lenses and collection of tears. The risk will be minimized by appropriate training of the investigators. The most serious adverse effect of contact lens wear is a rare but serious infection called microbial keratitis. This occurs in approximately 1 to5 in 10,000 people/year. It is sight threatening and may rarely cause significant pain and hospitalisation.

If any problems develop during the course of the study, you should contact Prof. Fiona Stapleton on (02) 9385 5287. If you are unable to get in contact with us, you should go

to the Emergency Clinic of the Sydney Eye Hospital at 8 Macquarie Street, Sydney or contact the Sydney Eye Hospital on (02) 9382 7111.

Confidentiality and disclosure of information

Any information that is obtained in connection with this study and that can be identified with you will remain confidential. It will be disclosed only with your permission, except as required by law. If you give us your permission by signing this document, we plan to publish the results in the scientific literature. In any publication, information will be provided in such a way that you cannot be identified. Patient data will be kept on computer discs and will be stored in a secure place.

Recompense to participants

Gift vouchers of $100 will be given at the end of the study for your time in participating. We cannot and do not promise any benefits for the participant from this study. Complaints may be directed to the Ethics Secretariat, The University of New South Wales, SYDNEY 2052 AUSTRALIA (phone 9385 4234, fax 9385 6648, email ethics.sec@unsw.edu.au). Any complaint you make will be investigated promptly and you will be informed out the outcome.

Feedback to participants

If you indicate that, you wish for us to do so by providing your email address below, we will email you a summary of research findings on completion of this study.

Your email address (optional) _____

Your consent

Your decision whether or not to participate will not prejudice your future relations with the University of New South Wales. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without prejudice. If you have any questions, please feel free to ask us. If you have any additional questions later, contact Ms. Athira Rohit on (02) 9385 7420 and she will be happy to answer them. You will be given a copy of this form to keep.

You are making a decision whether or not to participate. Your signature indicates that, having read the information provided above, you have decided to participate.

.....	.....
Signature of Research Participant	Signature of Investigator
.....	.....
Please PRINT name	Please PRINT name
.....	.....
Date	Date

REVOCATION OF CONSENT

EFFECT OF LIPID SUPPLEMENTATION IN CONTACT LENS COMFORT

Approval No: 12584

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above and understand that such withdrawal **WILL NOT** jeopardize any treatment or my relationship with The University of New South Wales.

....

Signature Date

.....

Please PRINT name

The section for Revocation of Consent should be forwarded to

Prof. Fiona Stapleton

School of Optometry and Vision Science

The University of New South Wales

Sydney 2052

APPENDIX 4 CONFERENCE PRESENTATIONS

ORAL PRESENTATIONS

- **Athira Rohit**, Mark Willcox, Fiona Stapleton, *Tear film lipids and contact lens comfort*, Ocular Surface Science and Dry eye Conference, Sydney, Australia, July 2014.
- **Athira Rohit**, Mark Willcox, Todd Mitchell, Fiona Stapleton, *The effect of lipid supplementations on the tear lipid layer of habitual soft contact lens wearers; an exploratory study*, Association of Research in Vision and Ophthalmology, Orlando, US, May 2014.

POSTER PRESENTATIONS

- **Athira Rohit**, Mark Willcox, Simon Brown, Fiona Stapleton, *Effect of a Liposomal spray on Symptomatic Contact Lens Wearers*, Australian Collage of Optometry conference, Melbourne, Australia, October 2013.
- **Athira Rohit,** Simon Brown, Fiona Stapleton, Mark Willcox, *Comparison of tear lipid profile among basal, reflex and flush tears.* Tear Film and Ocular Surface conference, Sicily, Italy, September 2013.
- **Athira Rohit**, Mark Willcox, Simon Brown, Fiona Stapleton, *The effect of tear lipid biochemistry on tear evaporation rate during contact lens wear*, Association of Research in Vision and Ophthalmology, Seattle, US, May 2013.
- **Athira Rohit**, Mark Willcox, Fiona Stapleton, *A pilot study of tear film lipids in symptomatic contact lens wearers*, British Contact Lens Association, Birmingham, UK, May 2012.

APPENDIX 5 AWARDS AND SCHOLARSHIPS

- 2014: Teaching Fellowship award by School of Optometry and Vision Science, the University of New South Wales, Australia.
- 2010-13: Tuition Fee Remission scholarship (TFRS) from the University of New South Wales, Australia
- 2010-13: Faculty stipend from Vision CRC, Australia
- 2012: Research scholarship by the Cornea & Contact Lens Society of Australia
- 2012: Maki Shiobara postgraduate research scholarship by the Optometry & Vision Research Foundation, Australia

APPENDIX 6 PUBLICATIONS

- **Rohit A,** Stapleton F, Brown S, Mitchell T, Willcox M, Comparison of tear lipid profile in basal, reflex and flush tear samples, *Optometry and Vision Science*, December 2014, 91: 1391-5.
- **Rohit A,** Willcox M, Brown S, Mitchell T, Stapleton F, Clinical and biochemical tear lipid parameters in contact lens wearers, *Optometry and Vision Scienc*e, December 2014, 91: 1384-90.
- **Rohit A,** Ehrmann K, Naduvilath T, Willcox M, Stapleton F, Validating a new device for measuring tear evaporation rates, *Ophthalmic & Physiological Optics*, 2014, 34: 53-62.
- **Rohit A,** Willcox M, Stapleton F, Tear lipid layer and contact lens comfort; a review, *Eye & Contact Lens*, 2013, 39: 247–253.
- Invited article written for *Australian Optometry Newsletter* on "looking for answers in role of lipid layer" May 2013.