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Lipid composition of human meibum: a review*

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Abstract

The structure and function of meibomian gland lipids in the tear film are highly complex. Evidence shows that the precorneal tear film consists of discrete layers: the inner mucin layer, the middle aqueous layer and the outer lipid layer. In this review we focus on the outer, biphasic lipid layer of the tear film which consists of a 'thick' outer, non-polar layer and a 'thin' inner, polar layer. We discuss the main composition of the polar and non-polar lipids within meibum (wax esters, cholesteryl esters, mono-, di- and tri-acylglycerols, ceramides, phospholipids *et cetera*). We address the composition of meibomian lipids in subjects suffering from various ocular diseases in comparison with the composition in healthy individuals. Further analysis is needed to determine whether a correlation exists between the etiology of various ocular diseases and the fluctuation on the lipids as well as to establish whether or not tear lipid analysis can be used as a diagnostic tool.

Key words: Meibum, tear lipids, polar lipids, non-polar lipids, dry eye disease

Introduction

The cornea does not have a blood supply to sustain the corneal epithelium. Therefore, tears are needed to nourish and maintain the ocular surface[1]. The human tear film or precorneal tear film (PTF) consists of a mixture of secretions of the lacrimal gland, accessory lacrimal glands, meibomian glands, goblet cells and other endocrine systems. The PTF covers the ocular surface in a more or less continuous structure, approximately 20 µm thick[2]. Exact details of this structure have not been confirmed experimentally but there is strong evidence supporting the existence of discrete layers within the PTF. The layer closest to the surface of the eye is the carbohydrate rich, polar glycocalyx (sometimes referred to as the mucin layer). Current models suggest that the polar glycocalyx directly anchors a continuous aqueous-mucin layer in which salts and hydrophilic substances are dissolved[3]. The middle layer of the PTF is referred to as the core aqueous stratum in which proteins, oxygen and nutrients are predominantly found[4, 5]. The aqueous layer protects the ocular surface by creating a suitable environment for the cornea[5]. The outermost layer of the tear film is the biphasic tear film lipid layer (TFLL), consisting of a 'thick' outer non-polar

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layer (NPL) and 'thin' inner polar layer (PL)[3, 6-10]. The polar lipids form two intermediate layers between the aqueous layer and the outer non-polar lipid layer[10, 11].

Lipids closely coupled with tears are found in various regions: the TFLL, the meibomian secretions on both the upper and lower eyelids[12-15], the meibomian gland itself, the lacrimal glands and lastly in corneal epithelial secretions[13]. The blinking mechanism is responsible for spreading the meibum lipids over the ocular surface thereby reforming the tear film with every blink cycle[16, 17]. The TFLL is a key component of the PTF. TFLL functions include protecting the ocular surface by forming a protective hydrophobic barrier against microorganisms, preventing ocular surface dehydration and lubricating the eyelids during blinking[18, 19].

The purpose of this review is to provide an overview of the main composition of the lipid layer/meibomian lipids and relate changes of lipid structures to dry eye disease.

Lipidomics

Lipidomics is the study of the pathways and networks of cellular lipids in biological systems[20]. It is a promising and quickly escalating research field constituting a specialized component of the general field of metabolomics, the study of all metabolites in a cell[20-22]. Lipidomic studies involve the identification, quantification and structural analysis of lipids within a cell, tissue or organism. Data obtained from lipidomic studies give more insight into the functions of lipids and how lipids interact with other molecules such as proteins and other cell components[20, 22]. Lipidomic studies can thus help to clarify the biochemical mechanisms[20] of lipid-related disease processes such as chronic blepharitis[23] or dry eye disease[24]. By using methods such as ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry, nuclear magnetic resonance spectroscopy or fluorescence spectroscopy, one can identify changes in cellular lipid metabolism, interactions and homeostasis[14, 20].

The basic biochemistry of lipids

The organization and function of meibomian gland lipids in the PTF are not simple. The composition of the TFLL, that is, the amounts and various types of lipids present, determine the flow properties of the TFLL (and hence also PTF) as well as the rate of diffusion of water, oxygen and carbon dioxide across it. In order to understand how lipid composition affects the properties of the PTF, it is useful to examine the basic biochemistry of lipids.

Lipids are loosely defined as a group of biological compounds that are insoluble in water, but are relatively soluble in several non-polar organic solvents such as benzene, chloroform and ether. They therefore constitute a heterogeneous class of more or less non-polar molecules with a variety of physicochemical properties. As a result several classification schemes have been used in the description of lipids. For example, lipids can be divided into two subgroups based on chemical composition: simple lipids and complex lipids. Simple lipids are known to be the elementary building blocks of complex lipids but can also play inimitable roles as individual molecules[15]. Simple lipids contain C, H, and O[25]. They are molecules such as fatty acids (FA) and sterols[26]. Complex lipids have more than two types of constituents[27] and may contain elements such as phosphoric acid, amine, or carbohydrates in addition to the lipid component[25]. Wax esters (WE), cholesteryl esters (CE), mono-, di-, and tri-acylglycerols (MAG, DAG, and TAG), ceramides (Cer) and phospholipids (PL) are some examples of complex lipids found in almost any living cell and tissue[15]. Secondly lipids can also be classified according to their polarity: nonpolar or polar (Figure 1). Nonpolar lipids, primarily TAG and CE, have very low aqueous solubility and do not mix at the air-water or oil-water interface to form a monomolecular layer (monolayer)[28-30]. The main polar lipids are FA, glycerophosphatides, oxidized polyunsaturated FA (oxy-PUFA), MAG; hydroxy-ceramides (HO-Cer) and glycosphingolipids[15, 29]. Polar lipids contain charged or uncharged polar groups, giving these lipids an amphiphilic character (molecules that have both hydrophobic and hydrophilic groups)[30]. These lipids have the ability to ionize and/or form hydrogen bonds with water molecules which cause an increase in their aqueous solubility[15]. O-acyl-ω-hydroxy fatty acids (OAHFAs) are good examples of polar lipids found in meibum[31].

Thirdly, lipids can be classified as non-ionic (or non-charged) and ionic (ionizable or charged). Lastly,

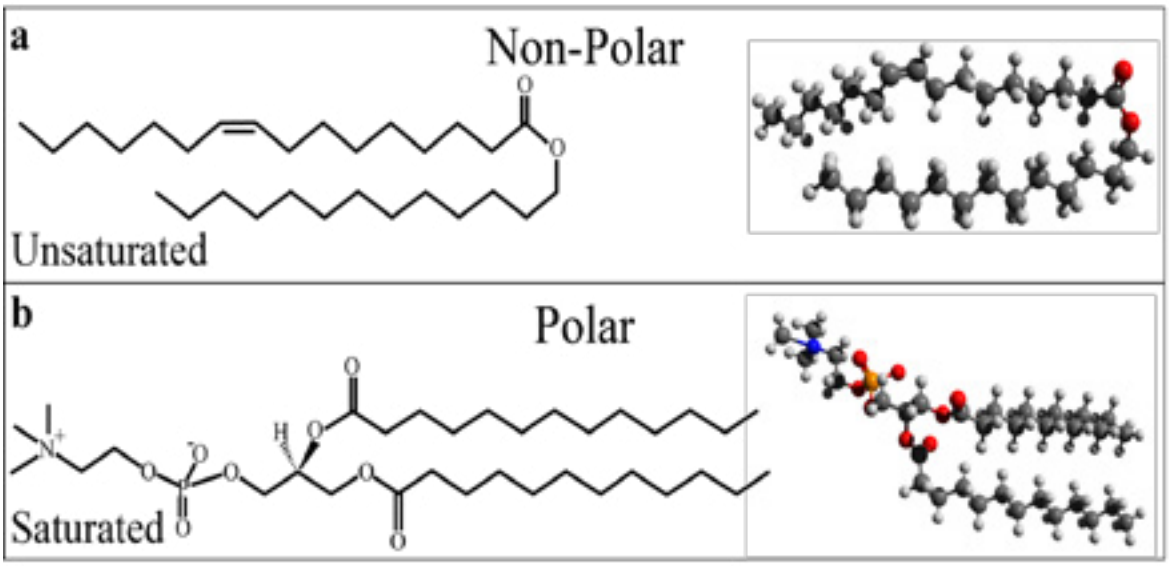

Figure 1: Non-polar and polar lipid structures which represent lipids found within meibum. **a**. Non-polar, unsaturated wax monoester. **b**. Polar, saturated phosphatidylcholine. Line-bond structures are shown on the left whereas the ball-and-stick model is shown on the right. (The Line-bond structures were obtained from Lipid Metabolites and Pathway Strategy (LIPID MAPS) and the ball-and-stick structures were rendered with Avogadro 3D molecular structure editor)**.**

lipid classification is often based on the function of the lipid in biosynthetic pathways[15]. For the purpose of this review, in line with the biphasic model of the TFLL, the scheme that classifies lipids according to their polarity will be followed to distinguish between non-polar lipids in the outermost layer of the TFLL and polar-lipids in the inner layer.

A previous study has shown that human meibomian glands do not secrete polar lipids[32]. Various additional studies, however, indicate that polar lipids do indeed form part of human meibomian lipids[10, 14, 18, 33-41].

Polar lipids

Polar lipids provide structural stability to the tear film via their short-chain saturated fatty acids (C12–C18) where the chain lengths do not vary to a great extent[36]. The main polar lipids found in the meibum are phospholipids (PL), sphingolipids (SL)[9, 36] and OAHFAs, recently discovered amphiphilic compounds confirmed to be present by Butovich *et al.*[31] The major PL species include phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylglycerol (PG). The polar SL lipids in the tear film are sphingomyelin (SM), ceramides (Cer), glucosylceramide (GluCer), dihexosylceramide (DihexCer) and cerebrosides. PL and SL initiate segregation of the phases of the TFLL. Hydroxylated sphingolipids such as cerebrosides play an especially important role in this segregation process[36]. Thus, polar lipids form the amphiphilic sublayer between the aqueous layer and the thick nonpolar lipid layer in the tear film[10, 36].

Structural integrity of the polar layer is necessary for the tear film to have the right flow characteristics. The integrity of the TFLL depends on the components having a particular polarity (not too polar or unpolar), the degree of saturation of hydrocarbon chains and chain length and on the pH of the underlying layer[14, 36]. According to McCulley and Shine[36], 3-5 % of TAGs are located in the polar monolayer. Specific TAGs (if the carbon chain length and degree of saturation allow this) and short-chain WE play a role in bridging the polar and non- polar lipid layers. As an aside, proteins like lipocalin (a major tear protein) play a role in scavenging lipids and stabilize the lipid layer by means of intercalation[42].

Non-polar lipids

The thick, non-polar outer lipid layer functions in decreasing water evaporation and controlling the rate of diffusion of carbon dioxide, oxygen as well as ions into the TFLL[36].The main non-polar lipids found in the TFLL are CE[43, 44], DAG[11, 45], diesters (DE)[43], fatty acids alcohols (FAl)[43], free cholesterol (free Chl)[4],

free sterols (FS)[36, 43, 45], hydrocarbons (HC)[35], sterol esters (SE)[44-46], TAG[35-36, 43-46] and WE[35, 36, 43-46].

Limited water evaporation is achieved through the structural integrity of the lipid layer. Structural integrity of the non-polar lipid layer (NPLL) is dependent on a properly structured underlying polar phase[47] as well as packing of its long-chain FA (C20-31), FAl and long-chain HC which increase cohesiveness allowing the sustainability of the structural integrity during a blink[36].

How does lipid composition affect properties of the TFLL?

It is important to understand why chain length, degree of saturation and polarity affect the properties of the TFLL as well as the role that hydrophobic interaction, *cis*- and *trans*-double bonds, fluidity, ionization and pH play in shaping the TFLL[13]. pH determines the charge state of polar lipids and therefore affects the properties of the polar lipid layer (PLL). Aqueous components of the tear film have a pH of about[48] 7.6-8; the free carboxyl groups of the OAHFA should therefore be ionized in the tear film, thus increasing the amphiphilicity of the tear film structures[15].

Chain length affects how well lipids pack together by hydrophobic interaction and therefore determines structural characteristics of the TFLL. Degree of saturation, that is how many C to C double (C=C) bonds are present, determines the fluidity of the TFLL. For example, more fluidity will require shorter chains and more C=C bonds, which might lead to increased tear film instability[36]. In addition, the degree of oxidation also determines polarity.

In order for water to evaporate from the tears the interactions between lipids in the TFLL must be broken (the stronger the interactions between the lipids in the TFLL the lower the evaporation rate of water will be). Borchman *et al.*[13] remarked that the *trans* conformation of C=C bonds is much more stable than the *cis* conformation. When lipids are in *trans* conformation the HC chains will be extended, thus van der Waals interactions will be maximal and the distance between the hydrocarbon chains minimal causing a decrease in lipid mobility[13]. Interactions also affect viscosity and therefore the flow properties of the meibum secretions onto the eye[36].

Tear lipids vs. meibum lipids

Meibomian lipids have been studied widely but little research has been done on the tear film lipids themselves. Lipids in the TFLL differ and are far more complex than meibum lipids [13, 37, 41]. A study done by Wollensak *et al.*[49] showed that WE and CE comprise 45% of the tear lipids which is similar to their abundance in meibum. However, tear lipids differ from meibomian lipids in that non-esterified cholesterol and PL each comprise 15% of the lipids in tears, whereas meibum contains only 0-7% of these lipids[49]. More recently, Rantamaki *et al.*[41] showed that PC and PE were the two most common polar lipids in tear fluid and that TAG was the only non-polar lipid present. They did not find any CE or WE by *mass spectrometry* analysis. Studies regarding the lipid composition of meibum differ in their conclusions[49]. Mostly because the accuracy and precision of quantifying the meibomian lipid composition is complicated by inter-individual differences[35], the size of the meibum sample and the complexity of lipids[13]. Table 1 summarizes of the abundance of different lipids in human meibum as determined by various researchers.

Lipid-binding proteins and tear film stability

Some proteins in the human tear film were shown to have lipid binding capabilities[50]. Human tear lipocalin and beta-lactoglobulin are examples of proteins shown to have a high affinity for lipids[10]. Tear lipocalin, also known as lipocalin-1, is the main lipid binding protein and is one of the most abundant tear proteins which comprise 15-33% of the proteins in the aqueous phase of the tear film[51-53]. Tear lipocalin plays a role in the viscosity of the tear film as well as stabilising the TFLL by binding to a variety of lipids such as cholesterol, fatty acids, glycolipids and glycerophospholipids[13, 42, 54].

Meibum composition in Dry Eye Disease

Dry eye disease is a multifactorial disease of the tears and ocular surface[55]. Tear break-up time (TBUT) measured in seconds, is a common diagnostic tool used by ophthalmologists and optometrists to diagnose dry eye disease. TBUT can be defined as the

interval between a full blink and the breakup of the tear film[56- 57]. A healthy TBUT lasts 10 s or more but patients suffering with dry eye disease[10, 15, 57] have a TBUT of around 6 s. A short TBUT usually leads to evaporation of the aqueous phase leaving the ocular surface exposed to the air and prone to dehydration. This might lead to irritation, inflammation and eventually infection. One of the main causes of tear film instability is meibomian gland dysfunction, also known as posterior blepharitis, in which the glands produce less meibum[15, 58]. Hypo-production may result in the PTF becoming unstable due to the altered composition and/or consistency of the polar lipids[58]. Chronic meibomian gland dysfunction and androgen receptor dysfunction (as well other types of blepharitis) are some of many causes of dry eye disease [57, 59]. According to Butovich and coworkers[60] environmental factors such as low temperature can cause the tear film to become inflexible, resulting in inability to form a uniform tear film.

Tiffany[35] and McCulley and Shine[36] showed that there is variability in the lipid composition of healthy individuals. Nonetheless, significant differences between normal patients and meibomian gland dysfunction and/or dry eye disease sufferers have been reported[13, 24, 61-63]. Table 2 summarizes studies of differences in the meibum composition of subjects with various ocular surface disorders.

Table 1. Lipid composition of human meibum according to previous studies. CE, cholesteryl esters; DAG, diacylglycerols; DE, diesters; FA, free fatty acids; FAl free fatty acids alcohols; free Chl, cholesterol; FS, free sterols; HC, hydrocarbons; MAG, monoacylglycerols; SE, sterol esters; TAG, triacylglycerols; WE, wax esters. ≠PL, phospholipids refers to sum total of PC, phophatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol. ‡SL, sphingolipids refers to sum total of: SM, sphingomyelin; cerebrosides; CER, ceramides; GluCer, glucosylceramide; dihexcer, dihexosylceramide. † Only ceramides was measured. (**) Not an original study; (+++) Looked at main components of meibum. Numbers are mass percentages of all the compounds investigated in meibum; (-) Not Determined.

Lipid Classes	Tiffany (1978)[35]	Nicholaides (1981)[61]	Ohashi (**)	Shine and McCulley (1997)[36]	Mathers and Lane (1998)[43]	Robosky *et al.*, (2008)[44] +++	Lam *et al.*, (2011)[46] +++
FA	-	1.98	1	-	-	-	-
FAl	-	-	-	-	2.8	-	-
squalene	8-34	-	-	-	-	20	-
free Chl	-	-	-	-	1.2	-	-
WE	13-23	32.32	44	68	45.2	321.1	25.21
SE	-	27.28	33	16	-	-	66.83
CE	-	-	-	-	39	174.5	-
MAG	-	-	-	-	-	-	-
DAG	-	7.74	2	-	-	-	-
TAG	11-43	3.7	5	6	3.1	23.5	4.03
HC	26-38	7.54	2	1	-	-	-
FS	0	1.63	-	15	-	-	-
DE	-	-	-	-	2.3	-	-
OAHFA	-	-	-	-	-	-	3.46
PL≠	-	14.83	-	4	-	-	0.37
SL‡	-	-	-	1.5†	-	-	0.1

Table 2. Overview of relative levels of meibum lipids in tears of subjects suffering from blepharitis, meibomian gland dysfunction and environmental factors that have an effect on dry eye disease found by different investigators. ↔ No change in lipid level compared to control; ↑ Increase in lipid level compared to control; ↓ Decrease in level compared to control. CE, cholesteryl esters; FA, free fatty acids; Free Chl, cholesterol; SE, sterol esters; TAG, triacylglycerols; WE, wax esters.

Lipid molecule	Change in disease state	Lipid molecule	Change in disease state
Branched chain FA	↔[64] ↑[62]	Saturated lipids	↑[66]
Free Chl	↓[43] ↔[23]	SE	↑[67]
CE	↓[13,23] ↑[43]	Total saturated FA	↓[62]
FA	↑[47,62]	TAG	↓[43,46,68]
Linolenic acid	↑[62]	Unsaturated CE	↓[23]
Monounsaturated FA	↓[65] ↑[62]	Unsaturated WE	↓[23]
Non polar lipids	↓[65]	WE	↑[67,43]
OAHFA species	↓[46]		

Concluding remarks

Previous studies have shown that the TFLL is important for maintaining ocular wellbeing. Table 2 highlights the differences in the tear meibum composition of subjects with various ocular surface disorders causing dry eye disease and shows that meibomian lipid analysis might be useful for diagnostic purposes. There are currently gaps in the literature regarding the effects of various ocular diseases on tear lipids and meibum. For example, to our knowledge there have been no studies done on meibomian lipid composition of patients suffering from keratoconus. Further analysis must be conducted to establish whether or not meibum lipid composition fluctuates in patients suffering from ocular disease. These studies are needed to determine if there is any connection between the lipid fingerprint of the meibomian lipids and the etiology of these conditions. This research will contribute to finding the etiology of these diseases as well as filling gaps in the literature.

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