Expression Profiling of Nonpolar Lipids in Meibum From Patients With Dry Eye: A Pilot Study

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PURPOSE. The purpose of this investigation was to characterize differentially expressed lipids in meibum samples from patients with dry eye disease (DED) in order to better understand the underlying pathologic mechanisms.

METHODS. Meibum samples were collected from postmenopausal women with DED (PW-DED; n = 5) and a control group of postmenopausal women without DED (n = 4). Lipid profiles were analyzed by direct infusion full-scan electrospray ionization mass spectrometry (ESI-MS). An initial analysis of 1-45 representative peaks from four classes of lipids in PW-DED samples revealed that additional manual corrections for peak overlap and isotope only slightly affected the statistical analysis. Therefore, analysis of uncorrected data, which can be applied to a greater number of peaks, was used to compare more than 500 lipid peaks common to PW-DED and control samples. Statistical analysis of peak intensities identified several lipid species that differed significantly between the two groups. Data from contact lens wearers with DED (CL-DED; n = 5) were also analyzed.

RESULTS. Many species of the two types of diesters (DE) and very long chain wax esters (WE) were decreased by ~20% in PW-DED, whereas levels of triacylglycerols were increased by an average of 39% ± 3% in meibum from PW-DED compared to that in the control group. Approximately the same reduction (20%) of similar DE and WE was observed for CL-DED.

CONCLUSIONS. Statistical analysis of peak intensities from direct infusion ESI-MS results identified differentially expressed lipids in meibum from dry eye patients. Further studies are warranted to support these findings.

Keywords: dry eyes, lipids, mass spectrometry, meibum, triacylglycerols

Dry eye disease (DED) has been estimated to affect 5% to 35% of people worldwide and is present in patients encompassing a wide age range.1 Postmenopausal women are one of the most common classes of patients with DED.1 DED is thought to arise primarily from dysfunction of the meibomian glands, resulting in decreased quality and quantity of meibomian gland secretions (meibum).2 However, the precise molecular mechanisms that underlie DED are still unclear.3 Identification of quantitative and qualitative differences in the composition of meibum between patients with DED and control subjects will contribute significantly to our understanding of DED. A few studies have reported differential expression of lipid species between control and DED samples in meibum and tear film. These earlier studies mainly used liquid chromatography coupled with tandem mass spectrometry (MS/MS) for lipid analyses.4,5 However, more recent “shotgun lipidomics” approaches using direct infusion MS and MS/MS analysis offer distinct advantages for both identification and quantification of meibum lipids.6,7 For example, continuous direct infusion provides sufficient time for acquisition of high-quality MS and MS/MS spectra, facilitating accurate peak identification. Additionally, using constant solution composition allows for simple correction of ionization efficiencies, facilitating accurate quantification.8,9 Therefore, in the present study, we used a direct infusion approach to investigate meibum lipid composition in two groups of subjects: postmenopausal women with DED (PW-DED) and a control group of postmenopausal women without DED. In addition, out of curiosity and convenience, we examined meibum samples from contact lens wearers with DED (CL-DED) and also found some interesting differences that may warrant further studies.

One of the major difficulties in quantifying lipid molecules in meibum results from the large variation observed between samples due to several underlying causes. These causes include imprecise collection methods,10,11 minute sample sizes that can result in significant contributions from impurities12 acquired during sample collection and preparation,13 and varying ionization efficiency and thus detection sensitivity for numerous lipid species of interest (particularly lipids of weak polarity) that differ from the limited number of internal standards used.8 To combat these issues, we recently developed a protocol that minimizes these sources of variation. Using this protocol, we were able to accurately quantify 145 lipid species belonging to four major classes of neutral lipids in human meibum. Quantitative profiling of these lipids was made possible by minimizing the influence of impurities through direct collection of meibum samples from the orifices of the meibomian glands and thorough cleaning of the instrumental analysis system. This novel method allowed analysis of as little as 8 nL (7 μg) of meibum by direct infusion electrospray ionization mass spectrometry (ESI-MS).7 We successfully achieved absolute...
quantification of lipids by spiking the meibum samples with synthesized lipid standards along by making manual corrections for peak overlaps and isotopic distribution (for wax esters [WE], cholesteryl esters [CE], type I α-st diester, composition fatty acid [FA]/hydroxy FA/cholesterol [DE-I], and α, type II diester, composition FA/diol/FA [DE-II]), as well as ionization efficiencies (for WE and CE only, due to the availability of commercial lipid standards).6

Peak overlap and isotopic distributions are caused by the presence of multiple isotopic peaks for each ion of interest. Elements with isotopes of different natural abundance exist in most ions, particularly, the element carbon includes two major isotopes, carbon-12 and carbon-13, with significantly high natural abundances (98.93% and 1.07%, respectively). Because lipid molecules contain many carbon atoms, the corresponding ions detected in a mass spectrometer are typically composed of multiple isotopic peaks differing by approximately 1 Da. The presence of multiple isotopic peaks for each lipid molecule, on one hand, leads to overlap of lipid species of different unsaturation levels with the difference of 2 Da; on the other hand, it requires the intensities of these isotopic peaks to be summed to achieve more accurate quantitation.14 The overlap and isotopic distribution may be corrected either manually6,14 or with an existing software, based on theoretical prediction.15 However, the correction requires information for the elemental compositions corresponding to these peaks (i.e., prior identification of the lipid species); in addition, the spectra for correction are generally less complicated, with overlap primarily from isotopic peaks of the same class of lipids, whereas overlap from peaks of other lipid classes is negligible. Therefore, these deisotoping software programs are more readily used for targeted or focused lipid analysis such as precursor ion scan or neutral loss scan, usually for common classes of lipids with known elemental compositions, where there is often less overlapping between peaks. The deisotoping software may also be applied to ultra-high resolution mass spectrometry platforms, with which the peak overlap problem is significantly reduced.

However, if the analysis is nontargeted and performed with relatively low-resolution mass spectrometers, peaks in the mass spectra may be of unknown identities and may overlap with peaks of different classes of lipids. As a result, it is difficult to deisotope most of these peaks. When the goal of a study is not to quantify the absolute amount of lipids but to identify lipids with a significant change in the relative amount between two groups, deisotoping processing may be unnecessary for certain types of samples such as meibum due to the specific pattern demonstrated6,16

In the present study, we first evaluated the accuracy and feasibility of performing statistical analysis of peak intensities for two groups of samples, that is, PW-DED and control samples, in the absence of any corrections by using 145 representative lipid peaks accurately quantified in our previous study.6 Next, we developed a method for a systematic comparison of 2500 peaks (all peaks with an intensity greater than 0.5% of the highest intensity peak present in each ESI-MS spectrum) in the spectra of these meibum samples. Implementation of this method resulted in the identification of many more lipid species differentially expressed between PW-DED samples and control samples than the original report.6 Meibum from dry eye patients of contact lens wearers were also studied, and similar results were obtained.

**METHODS**

The experimental procedures for preparing human meibum samples for mass spectrometry were described previously.6 Briefly, human meibum samples were collected in micro-capillary tubes directly from the orifices of several of the meibomian glands. As a pilot study, samples were collected from three groups of subjects: PW-DED (n = 5), CL-DED (n = 5), and postmenopausal women without DED (control, n = 4). Each stock solutions of stock samples were prepared by dissolving each meibum sample in a chloroform-methanol mixture (2:1, v/v) at an approximate concentration of 134 μg/mL. Individual samples were tested separately. The working solutions were prepared by 50-fold dilution of these stock solutions with methanol, along with the addition of an appropriate concentration of sodium iodide dissolved in methanol as the additive. The final composition of each working solution was a chloroform-methanol mixture (1.3:98.7, vol/vol) containing ~3.4 μM total lipids and 5 μg/mL NaI (35 μM). Each working solution was analyzed by direct infusion full-scan mass spectrometry using a quadrupole-time-of-flight mass spectrometer (Q-TOF II; Waters Corp.; Milford, MA, USA) at a flow rate of 40 μL/min. The source temperature was set at 100°C, and the desolvation temperature was set at 250°C. Capillary voltage was 3.2 kV; desolvation gas flow rate was 500 L/h. All MS spectra were acquired on the same day to minimize variation due to instrument response. Samples were analyzed by alternating between the groups to minimize potential running order effects. Blank samples without lipids, containing solvent and additive only, were run between subject samples to ensure negligible carryover.6 The raw data acquired in that study6 were statistically analyzed in this report, as in the following description. The study protocol was approved by the Ohio State University institutional review board in accordance with the Declaration of Helsinki.

A total of 145 representative lipid peaks in spectra from PW-DED and control samples6 were processed in three different ways for subsequent statistical analyses. Peak intensities were processed in three different ways: 1) with corrections for both peak overlap and isotopic distribution; 2) with corrections for peak overlap only; and 3) without corrections. The feasibility of data analysis in the absence of corrections was evaluated by comparing results from statistical analyses for the three types of peak processing.

Subsequently, large datasets containing more than 500 peaks common to PW-DED and control samples or CL-DED and control samples (see Supplementary Data) in the absence of correction were generated to identify differentially expressed lipids. Values are reported as mean ± SD.

Statistical analysis of the data described above was performed using a free online program, MetaboAnalyst (Xia Laboratory, McGill University, Quebec City, Quebec, Canada),17 to conduct Student’s t-tests.18 Data were uploaded using zipped files containing MS peak lists. For data processing, mass tolerance was set to 0.15 m/z, and peaks with missing values in any one of the samples were excluded to avoid the influence of random peaks. Data points were then filtered by the setting “mean intensity value” of the program in order to remove noise and low-intensity signals. After each data point was filtered, it was then normalized by the summed intensity of all remaining data points, with the parameter “normalization by sum” of MetaboAnalyst. As a result, the relative intensity of each peak can be compared across different samples to find differentially expressed peaks.

Statistical analyses resulted in lists of P values and intensity ratios (for dry eye versus normal) for each peak of interest. These lists were used to construct volcano plots in which the x axes represent the base 2 logarithm of the intensity ratio (log2 (DED/ Control)), and the y axes represent the negative base 10 logarithm of the P values (−log10 (P)). The volcano plots combine the information of statistical signifi-
cance (P value) of a change along with the magnitude of the change (intensity ratio), both parameters being important for determining an effective change. With this type of plot, data points with larger magnitude of changes appear far from the center horizontally, whereas changes in intensity ratio in both directions (increase or decrease) appear symmetrically from the center. Therefore, if the corresponding lipids of interest and data points with greater magnitude of changes appear far from the center, resulting in two regions of interest: top right and top left.19

Differences in expression were considered effective for lipid species with ≥1.05-fold changes and P ≤ 0.10. A higher threshold than the typical value of 0.05 was used for the P value due to the relatively small sample size.20 Additionally, other peaks that differed from the differentially expressed lipid peaks by a value that corresponded to the mass of (CH₂)₂ or (CH₂)₃ within ±5 ppm were also considered; these peaks probably correspond to closely related lipids that follow the same synthetic pathway. Therefore, differential expression of these lipids is expected to follow the same trend. The smaller difference (i.e., higher P values) between the two groups of samples for some of these peaks was likely due to factors such as partial overlap or low intensity. Therefore, if the corresponding P values of these lipids were only slightly higher (0.10–0.15), they were also considered significantly different (see Tables 3 and 5).

### Results

**Analysis of 145 Representative Peaks in Spectra From PW-DED and Control Meibum Samples**

Lipids expressed differentially in meibum samples from PW-DED and control subjects were compared using 145 representative peaks analyzed with three different methods of data processing (see Table 1). The three methods yielded similar results (see Table 2).

Correction of peak intensity values for the effects of peak overlap and isotopic distribution most accurately reflects actual responses of the corresponding lipids of interest and subsequently real differences between the groups of samples. With this correction, 22 of the 145 peaks analyzed were differentially expressed in PW-DED samples (P ≤ 0.1) (Table 1).

Results were very similar when peak intensities were corrected only for the effects of peak overlap. In this case, 23 peaks were significantly different in PW-DED samples compared to control samples (Table 2). The change in the number of significantly different peaks was due to a slight decrease in the P value for DE-II 66:2 from 0.102 to 0.100 (Table 1). Therefore, comparison of the two analyses reveals...
that differences due to corrections for isotopic distribution were negligible.

In the absence of any corrections, 25 of the 145 peaks were significantly different between PW-DED and control samples (Table 2). The increase in the number of significantly different peaks was because the P values for the two peaks (DE-Ch 50:1 and DE-Ch 52:1) decreased to 0.074 and 0.087, respectively, with no corrections, from 0.143 and 0.141 with both corrections, and from 0.138 and 0.137 with peak overlapping correction alone, respectively (Table 1). The decrease in P values that led to an increase in the number of peaks with statistically significant differences is most likely due to the relatively high level of overlap of the M+2 isotopic peaks of the corresponding more unsaturated species with the monoisotopic peaks of the less unsaturated species. However, generally, the difference was negligible.

Systematic Statistical Analysis of ≥500 Peaks in Spectra of Meibum Samples From PW-DED, CL-DED, and Control Subjects

The PW-DED and CL-DED groups shared a total of 541 and 533 common peaks with the control group, respectively. Statistical analysis of each comparison identified a list of peaks that were significantly differentially expressed (Tables 3, 4, 5; Figs. 1, 2). Some of the significantly downregulated peaks correspond to isotopic peaks from the same lipid species (Figs. 1, 2). These peaks were assigned as isotopic based on differences in m/z values, and these assignments were supported by consistent intensity ratios and P values. For instance, four type II diester peaks, m/z 1003.9517, m/z 1004.9544, m/z 1005.9642, and m/z 1006.9670, were found to be down-regulated in dry eye meibum samples. The m/z differences between the first and last two peaks were 0.0027 and 0.0028, respectively, which is close to that expected for neighboring isotopic peaks due to the differences between 13C and 13C (1.0035). The m/z difference between the first and third peaks was 2.0125, which is close to that expected for the difference due to one double bond (i.e., two hydrogen atoms [2.0141]). As a result, these four peaks corresponded to isotopic peaks of two molecular species, DE-II 66:3 and DE-II 66:2, respectively. The P values for the two isotopic peaks (m/z 1003.9517 and m/z 1004.9544) of DE-II 66:3 were 0.007 and 0.005, respectively, whereas the corresponding intensity ratios were 0.71 and 0.72, respectively, both sets of values being almost identical. Similarly, for the two isotopic peaks (m/z 1005.9642 and m/z 1006.9670) of DE-II 66:2, the P values were 0.031 and 0.036, respectively, and the intensity ratios were 0.72 and 0.73, respectively, both sets of values being almost the same. However, the P values for DE-II 66:3 were different from those for DE-II 66:2. Therefore generally, the P values and intensity ratio for isotopic peaks of the same molecular species were typically quite close, although there could be exceptions due to the variation in signal-to-noise ratios.

DISCUSSION

Systematic Statistical Analysis of Lipid Peaks Without Corrections for Peak Intensities

Manual correction of peak intensities increases the accuracy of quantitative analysis of lipids. However, this process is labor-intensive and requires prior identification of all lipids of interest, therefore, is not feasible for analysis of datasets with many more peaks including unknown species. Fortunately, this study suggests that most differentially expressed lipids can be characterized in the absence of these corrections (Tables 1, 2). The main reason for this was probably the particular lipid pattern of meibum samples, where the intensity of more unsaturated lipids is usually lower than the corresponding less unsaturated lipids of the same class. This intensity pattern of meibum lipids, combined with the fact that the M+2 isotope peak has much lower intensity than its corresponding monoisotopic peak, results in peak overlap being essentially negligible. However, for samples that do not follow a pattern of lipid peaks similar to meibum specimens, the method described in the reported study may not work and needs to be tested.

More than 500 peak intensities expressed in different groups of dry eye patients were analyzed without corrections.
Lipid Class | Number of Species | Ratio | P
|-----------|-----------------|-------|-------
| Wax ester | 5               | 0.80 ± 0.08 | 0.07 ± 0.04 |
| Type II diester | 14          | 0.77 ± 0.07 | 0.04 ± 0.02 |
| Type I diester | 5            | 0.78 ± 0.07 | 0.02 ± 0.02 |
| Cholesteryl ester | 6            | 1.22 ± 0.07 | 0.09 ± 0.04 |
| Cholesteryl ester† | 5            | 1.17 ± 0.04 | 0.04 ± 0.02 |
| Triacylglycerol | 9            | 1.39 ± 0.03 | 0.06 ± 0.02 |

* See details in Table 3.
† The assignments were mainly based on accurate mass and characteristic product ion m/z 369.4 (if available), but it is not excluded that the corresponding peaks may be a mixture of CE molecule and some other species.

Differentially Expressed Lipids in Meibum From Postmenopausal Women With DED

Significant differences were found between the lipid expression in meibum samples from PW-DED and that from controls. (Table 3). Previous reports have shown that patients with DED have a profile of lipids present in meibum and tear film that is different from that in the control group. Similar changes in lipid composition are to be expected between meibum and tear film, as lipids in tear film are predominantly derived from meibum.

In the present study, the lipids with the most significant change in expression were a series of triacylglycerols (TG) that...
Lipid Species$^*$ & Ratio & $P$
\hline
**Decreased**

WE & 41:3 & 0.76 & 0.054 \\
   & 42:4 & 0.74 & 0.042 \\
   & 42:3 & 0.85 & 0.121 \\
   & 43:3 & 0.76 & 0.145 \\
   & 44:4 & 0.76 & 0.038 \\
   & 44:5 & 0.79 & 0.025 \\
   & 44:2 & 0.91 & 0.094 \\
   & 45:3 & 0.77 & 0.070 \\
   & 45:2 & 0.82 & 0.045 \\
   & 46:3 & 0.80 & 0.027 \\
   & 46:2 & 0.89 & 0.069 \\
   & 47:2 & 0.87 & 0.031 \\
   & 48:4 & 0.88 & 0.079 \\
   & 48:3 & 0.83 & 0.023 \\

DE-II & 62:3 & 0.86 & 0.080 \\
   & 64:4 & 0.75 & 0.008 \\
   & 64:3 & 0.82 & 0.003 \\
   & 65:3 & 0.79 & 0.002 \\
   & 66:4 & 0.72 & 0.0002 \\
   & 66:5 & 0.84 & 0.059 \\
   & 67:3 & 0.87 & 0.045 \\
   & 68:5 & 0.74 & 0.002 \\
   & 68:4 & 0.80 & 0.041 \\
   & 70:5 & 0.83 & 0.033 \\

**DE-I** & 46:2 & 0.75 & 0.078 \\
   & 47:2 & 0.77 & 0.092 \\
   & 48:3 & 0.68 & 0.014 \\
   & 48:2 & 0.78 & 0.004 \\
   & 48:1 & 0.91 & 0.001 \\
   & 49:3 & 0.80 & 0.058 \\
   & 49:2 & 0.80 & 0.015 \\
   & 50:3 & 0.74 & 0.004 \\
   & 50:2 & 0.80 & 0.107 \\
   & 52:3 & 0.81 & 0.059 \\

**Increased**

WE & 59:0 & 1.21 & 0.111 \\
   & 40:1 & 1.16 & 0.139 \\
   & 40:0 & 1.25 & 0.029 \\

CE & 20:0 & 1.16 & 0.007 \\
   & 22:0 & 1.13 & 0.034 \\
   & 24:0 & 1.09 & 0.126 \\


* From systematic statistical analysis of 533 peaks using meibum from postmenopausal women without dry eye disease as the control. The two numbers for each species of WE and DE-II were the total number of carbon atoms and the number of double bonds, respectively, and for CE and DE-I were the total number of carbon atoms and the number of double bonds in the components excluding the cholesterol moiety; for TG were the total number of carbon atoms and the number of double bonds in the components excluding the glycerol moiety.

were upregulated in PW-DED samples, with an average increase of 59% ± 3% (Tables 3, 4). These differences in TG levels are in agreement with data from our ongoing analysis of a dataset of dry eye studies of more than 60 samples. The observed increase in levels of TG in PW-DED samples is consistent with a previous report by Lam et al.5 which showed that TG levels were increased in DED patients. However, contrary to expectations, they also reported that TG levels decreased with increasing severity of DED.5 Furthermore, in contrast to our results (Tables 3, 4), Lam et al.5 reported that TG elevated in DED tended to be highly unsaturated, including TG54:7, TG54:5, TG56:7, and TG56:6. The reasons underlying this discrepancy between the two studies are unclear. These differentially expressed TG were not identified from the analysis of the 145 representative peaks as they were not included in the list due to the relatively low intensity and high variability of their peaks, as well as possible peak overlap with CE, leading to difficulty in absolute quantification.5

The lipids with the next largest significant change were a series of diesters. In PW-DED, these DE-II and DE-I decreased by 25% ± 7% and 22% ± 7%, respectively (Table 4). Individual MS peaks associated with intact diesters were first reported by our group6 and later supported by data from other groups22,24,25. However, the presence of these diesters was not mentioned at all in the two previous studies comparing lipids between DED and control samples.4,5 Diesters have been reported to account for approximately 8% of total meibum lipids.5,11 This large quantity of diesters in meibum suggests these lipids may play an important role in the function of the tear film lipid layer, which is predominantly comprised of meibum lipids.22,25 The importance of these diesters is supported by their decrease in PW-DED. Current work is ongoing in our group to better characterize these diesters.26

The lipids with the third greatest significant differences were a series of very long-chain mono- and di-unsaturated WE, which decreased in PW-DED by 20% ± 8% (Table 4). This decrease is consistent with the results from our current dry eye studies with many more samples (Chen J, et al. IOVS 2015;56:ARVO E-Abstract 342). Similarly, WE levels were reported by Lam et al.4 to differ in tear film from DED and control samples. However, the differences in that report were very complicated,4 as both the length of the fatty acid moiety and the molecular weight of the WE were correlated with DED risk in two different directions (either increased or decreased).4 The highly variable pattern in that report makes a direct comparison of these findings difficult.

A series of several short-chain saturated CE, odd-chain monounsaturated CE, and even-chain di-unsaturated CE comprised the group of lipids with the next most significant differences in levels with an average increase of 22% ± 7% in PW-DED samples (Table 4). The observed increase in CE in meibum from PW-DED patients differed from that in a previous report showing that the total levels of CE significantly decreased in DED patients based on nuclear magnetic resonance spectroscopy analysis.27 However, the CE species that increased in our study accounted for only a small percentage of total CE,6 approximately 14% ± 1%. Furthermore, differences in the methodology of the two studies might also have contribute to the discordant results. It is possible that the total CE levels quantified in that study27 might have also included some DE-I. These DE-I lipids are structurally similar to CE and, therefore, might have contributed to the corresponding nuclear magnetic resonance signal used to quantify CE in that paper.27 Consistent with this hypothesis, we found that DE-I levels were decreased in DED samples in this study.
Meibum Lipid Profiles of Dry Eye Disease

FIGURE 1. Volcano plot compares the lipid composition of meibum samples from PW-DED and control subjects. The $y$ axis is negative base 10 logarithm of the $P$ values ($-\log_{10}(P)$) calculated from Student’s $t$ test while the $x$ axis is the base 2 logarithm of the intensity ratios for peaks from PWDED samples and control samples ($\log_2(DED/Ctrl)$). Data points above the horizontal line are statistically significantly different lipid peaks between the two groups of samples ($P < 0.1$). Data points on the left side of the vertical line at ($\log_2(0.95)$) and on the right side of the vertical line at ($\log_2(1.05)$) represent at least 5% differences in relative peak intensity. Data points enclosed within a circle indicate isotopic peaks from the same lipid species.

FIGURE 2. Volcano plot compares the lipid composition of meibum samples from CL-DED and control subjects. The $y$ axis is negative base 10 logarithm of the $P$ values ($-\log_{10}(P)$) calculated from Student’s $t$ test whereas the $x$ axis is the base 2 logarithm of the intensity ratios for peaks from CLDED samples and control samples ($\log_2(DED/Ctrl)$). Data points above the horizontal line are statistically significantly different lipid peaks between the two groups of samples ($P < 0.1$). Data points on the left side of the vertical line at ($\log_2(0.95)$) and on the right side of the vertical line at ($\log_2(1.05)$) represent at least 5% differences in relative peak intensity. Data points enclosed within a circle indicate isotopic peaks from the same lipid species.
DED patients (Tables 3, 4). This result appears to be contradictory to our ongoing studies. However, this discrepancy may be due to peak overlap between CE and TG lipid species. These overlapping peaks could not be separated due to the resolution of the mass spectrometer used in this study. Indeed, our ongoing study using a higher resolution tandem mass spectrometer indicated that the peaks assigned here as CE27:2, CE28:2, CE30:2, CE32:3, and CE32:2 are overlapping peaks of these CE and other peaks of unknown identity. Therefore, the fold changes associated with these peaks in the PW-DED samples are likely due to a combination of the differences for both CE and TG, where the TG associated with these peaks increased by approximately 40% (similar to the other TG in Table 3), whereas the polyunsaturated CE decreased by approximately 10% (Chen J, et al. IOVS 2015;56:ARVO E-Abstract 342).

**Differentially Expressed Lipids in Meibum From Contact Lens Wearers With DED**

It is believed that lipids forming the outermost layer of tear film are important in maintaining the stability of the tear film and reducing evaporation; however, which lipids are essential in this role and how these lipids work is still controversial.3 It will help to understand the mechanism by comparing differentially expressed lipids from different groups of dry eye patients relative to their control groups. Using this approach as a pilot study, we compared the lipid composition of CL-DED samples with that of meibum samples from postmenopausal women without DED, serving as controls. Age-matched control samples (i.e., contact lens wearers without DED) were not available because the meibum samples from CL-DED patients were collected for other studies.

Interestingly, in this study, many downregulated lipids in CL-DED samples were similar to those observed for PW-DED samples. In PW-DED, decreases in these WE, DE-II, and D-E species were 20% ± 8%, 23% ± 7%, and 22% ± 7%, respectively, and in CL-DED, the decreases were 18% ± 6%, 20% ± 5%, and 21% ± 7%, respectively. The similar differences in these lipids between the two groups of DED patients suggest that these lipids may be important for maintaining the stability of the tear film. Therefore, these lipids could play roles in some common mechanism. The exact mechanism is unknown at this time. One possibility could be that these differences result from meibomian gland dysfunction (MGD).28–30

Some differences in the downregulated lipids were also observed between samples from these two types of DED patients. For example, the decreased WE and DE species tended to be more unsaturated (with more double bonds) in meibum from CL-DED patients (compare Tables 3 and 5). Interestingly, in contrast to the similarity in downregulated lipids between PW-DED and CL-DED, the upregulated lipids in PW-DED samples, including TG and odd-chain monounsaturated CE (Tables 3, 4), were not found differentially expressed in CL-DED samples. The underlying mechanism explaining these differences remains unclear. The small size of samples and lack of strict control samples for CL-DED make it difficult to draw a conclusion with high confidence. Further studies to understand the link between meibum lipid, MGD, and CL-DED are warranted.

**Future Directions**

Many interesting differences in lipid composition were observed between meibum samples from patients with different types of DED and those of controls. These differences were based on a small number of samples, and this was a pilot in nature. On the other hand, in order not to miss important findings, we compared 145 or ≥500 lipid peaks among different groups of samples without adjustment of the P values.19 Whether P values should be adjusted for multiple comparisons is controversial.31,32 With the multiple comparisons in the absence of adjustment, some false positive discoveries are not excluded. Current work is ongoing in our group to use high-resolution mass spectrometry to identify more differentially expressed lipids using a larger number of meibum samples (Chen J, et al. IOVS 2015;56:ARVO E-Abstract 342).

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