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The Role of Cholesterol in Chronic Blepharitis

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Chronic blepharitis has been a difficult disease to define either microbiologically or biochemically. Sterols from meibomian secretions of normal subjects and patients were analyzed, and important differences were observed.

Based on analyses of these secretions, two significantly different ($P < 0.001$) types of normal subjects were found, those with and those without cholesterol esters [Norm(CP) and Norm(CA), respectively]. All patients' secretions contained cholesterol esters. Evidence was obtained which suggests that oxysterols may control the ester cholesterol accumulation. Furthermore, only when cholesterol esters were present did wax and sterol esters containing unsaturated fatty acids accumulate. Over 90% of these unsaturated fatty acids were normal (unbranched); the rest were iso-fatty acids. Preliminary results also suggest that the ester fatty alcohols are much more complex than previously reported; seven alcohols were common to all samples analyzed. Additionally, highly oxygenated alcohols were detected, especially in the meibomian keratoconjunctivitis (MKC) disease group. The MKC samples also contained an alcohol (mass, M/Z 378) not present in any of the other samples analyzed. Based on analysis of variance and linear-regression models, it was determined that the long-chain (C20–28) fatty acids were more important in determining disease signs. Furthermore, in the MKC group, the ratio of unsaturated C18 fatty acids to cholesterol in the wax and sterol esters was significantly different ($P < 0.05$) from the Norm(CP) group. The authors discuss the fact that rabbit meibomian secretions are stable, despite containing a very high percentage of ester sterols, and relate this to their high percentage of branched-chain fatty acids and low percentage of unsaturated fatty acids. These results, together with previous work, further suggest that lipid abnormalities result in the many signs and symptoms of chronic blepharitis. Invest Ophthalmol Vis Sci 32:2272–2280, 1991

Chronic blepharitis is one of the most common conditions seen in the ophthalmologist's office.[1] We currently recognize six clinically distinguishable groups of chronic blepharitis: (1) staphylococcal (STAPH); (2) seborrheic, alone (SBBL); (3) seborrheic with a clinical appearance of staphylococcal infection (MIX); (4) seborrheic with meibomian seborrhea (MBSB); (5) seborrheic with spotty inflammation of the meibomian glands (secondary meibomianitis, 2 MEIB); and (6) severe inflammation of the meibomian glands (meibomian keratoconjunctivitis, MKC).[3] Keratoconjunctivitis sicca (KCS) frequently is associated with the disease groups but not with normal patients[3] and may be related to chronic blepharitis; both conditions have characteristic lipid abnormalities.[1,4]

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We investigated the effects of patient physiology, microbial flora, and drug therapy on the lipid component of the meibomian secretion (meibum) to understand better the etiology of chronic blepharitis.[1,5,6] Comparative studies of the lipid component of meibum done in our laboratory led to the discovery of specific and significant differences between disease groups and normal subjects with regard to: (1) the content of free fatty acids;[1] (2) the composition of the intact wax and sterol esters;[7] and (3) the fatty acids from the sterol esters (fatty acid methyl esters, or FAMEs).[8] Our results suggest that many of the signs associated with chronic blepharitis are primarily related to lipid abnormalities that are physiologic or microbial[3,5] in origin. Thus the disease signs associated with chronic blepharitis appear to be related, at least in part, to abnormal lipid components.

There have been numerous reports on the importance of the lipid composition of meibum. A very detailed compositional study was done[9] and reported the composition of human meibum as follows: hydrocarbons (7%), sterol esters (27%), wax esters (32%), triglycerides (4%), polar lipids (15%), free sterols (2%), and free fatty acids (2%). The importance of the tear film lipid layer, derived primarily from meibomian

gland secretions, in tear film stability has been discussed.[10] Others[11,12] considered the importance of branched-chain fatty acids and alcohols in determining the melting points of waxes and the importance of unsaturated fatty acids in determining the melting points of cholesterol esters. They concluded that both branching and unsaturation lower the melting point of wax and sterol esters.

The stability of the tear film lipid layer also is influenced by its lipid composition. Biophysical investigations of meibomian lipid secretions suggest that patients with meibomianitis or older patients with KCS have abnormal tear film lipids.[13] Model studies further suggest that the stability of the lipid layer depends not only on the type of fatty acid esterified to the cholesterol but also the relative amount of sterol ester in the lipid layer.[14] The density of these lipid layers also is influenced by other lipid components present, including small percentages (5%) of triglycerides at normal body temperature (37°C).[15] In addition, an increased thickness of the tear lipid layer has been noted in patients with blepharitis.[16]

As previously discussed, studies in our laboratory revealed significant differences in meibomian lipid composition between patients with chronic blepharitis and normal individuals. Not only were differences observed in wax and sterol esters but also in the fatty acids derived from these esters. These observations are particularly important because wax and sterol esters comprise over 60% of normal meibomian gland secretions. We describe an extension of our previous work to include: (1) the free and ester sterol components of these meibomian secretions; (2) the use of gas chromatography-mass spectrometry (GC-MS) to identify previously unidentified ester fatty acids; and (3) preliminary data concerning ester fatty alcohols. We report a new understanding of the importance of lipid composition in the development of chronic blepharitis and the role of cholesterol esters in this process.

Materials and Methods

Lipid Separations and Identification

Patients for this study (eight normal subjects and six from each disease group), methods used for collecting meibomian secretions, separation of lipid components, gas–liquid chromatographic (GLC) analysis, peak integration, and identification have been previously detailed.[7] Briefly, the meibomian secretions (−70°C) were brought to room temperature, dissolved in chloroform, and spotted on silica gel H thin-layer chromatography (TLC) plates; the plates were developed with hexane, diethyl ether, and glacial acetic acid (75:25:1). The positions of the lipid classes were located by comparison with standards after visualization with dichlorofluorescein.[1] Esters, in sealed nitrogen-flushed tubes, were trans-esterified with a mixture of 14% boron trifluoride–methanol (1.0 ml) and benzene (0.5 ml) for 3 hr at 100°C. Ester components (fatty acid methyl esters, fatty alcohols, and sterols) were separated on silica gel H TLC plates with hexane, diethyl ether, and glacial acetic acid (75:25:1) as the developing solvent. Final elution of the individual lipid components from the silica gel H depended on the chemical characteristics of each individual lipid class. Free sterols from the initial TLC plate were eluted with 2% methanol in chloroform. This eluate was washed with 0.5 N ammonium hydroxide to remove the dichlorofluorescein visualization dye. Sterols derived from the ester fraction after trans-esterification were eluted similarly with chloroform and washed with 0.5 N ammonium hydroxide. The FAMEs and fatty alcohols were eluted with chloroform.

Further characterization of the individual lipid classes was done by GLC or GC-MS. Sterols and fatty alcohols were analyzed without further derivatization. As previously described,[8] GLC analyses were done isothermally on a wide-bore capillary column using a Hewlett Packard 5710A chromatograph (Palo Alto, CA) with flame ionization detector. Peak areas were determined by area normalization with a Hewlett Packard 3390 reporting integrator. Injection ports and detectors were maintained at 250°C. The carrier gas (nitrogen) flow rate was 5 ml/min, and auxiliary gas (nitrogen) flow rate was 25 ml/min. The GLC analyses of FAMEs were done isothermally at 220°C on a 30-m × 0.75-mm Supelcowax 10 (Supelco, Bellefonte, PA) column; the analyses of underivatized sterols were done isothermally on a 15-m × 0.53-mm DB-17 (J & W Scientific, Folsom, CA) column.

The GC-MS was done with a Hewlett Packard 5890 gas chromatograph coupled to a VG 30-250 quadrapole mass spectrometer (VG Masslab Ltd., Cheshire, England). The GC separations were achieved on a 15-m × 0.25-mm DB-17 column (helium carrier-gas, linear velocity 68 cm/min) using a temperature starting at 160°C for 2 min followed by linear programming to 260°C at 4°C/min. The splitless injection temperature was 240°C, and the direct interface temperature was 240°C. The mass spectrometer was operated in full-scan mode from 50–650 atomic mass units at 2 sec/scan with a source temperature of 240°C and an electron impact energy of 70 eV.

Identification of specific lipids was achieved by comparison with standards. The FAMEs were identified from equivalent chain lengths obtained from standards;[8] cholesterol and cholestanol were identi-

fied by comparison of retention time with standards. Identities of these sterols and previously unidentified FAMEs were confirmed by GC-MS. Where identification was not achieved, such as with the oxysterols and fatty alcohols, use of GC-MS permitted identification of important molecular characteristics.

Statistical Analyses

Simple statistical analyses were done.[17] Where appropriate, levels of significance given are directionally significant (two-tailed test) and include standard deviation, analysis of variance (ANOVA), Dunnett's test (multirange), and linear correlation.

Discriminate analysis (linear multiple regression) was also done with some data sets. Using discriminant analysis, the lipid composition was matched to the clinical group diagnosis (normal, MBSB, 2 MEIB, MKC, SBBL, MIX, and STAPH). For a data set containing several quantitative variables and a classification variable defining groups of observations, discriminant analysis develops a discriminant criterion to classify the observations into the groups. This discriminant criterion, which is linear in nature, may be used to classify future observations. We previously showed the power of this statistical technique.[8] In light of the new data we obtained in this study, we extended these previous statistical analyses to include groupings of lipids with related biochemical and biophysical properties.

Criteria used in selecting lipid groupings were based on known biochemical and biophysical properties of the lipids quantified thus far. For example, it is known[9] that in normal individuals most fatty acids longer than 19 carbons are esterified to sterols rather than fatty alcohols. Furthermore, there are large biophysical differences between unsaturated and saturated fatty acids[14] and between normal (unbranched) and branched-chain fatty acids.[12] Therefore, lipid groupings were selected as follows: (1) cholesterol; (2) normal C14–18 saturated FAMEs; (3) normal C20–28 saturated FAMEs; (4) normal C14–18 unsaturated FAMEs; (5) normal C20–28 unsaturated FAMEs; (6) iso-C14–18 saturated FAMEs; (7) iso-C20–28 saturated FAMEs; (8) iso-C14–18 unsaturated FAMEs; (9) anteiso-C15–19 saturated FAMEs; and (10) anteiso-C21–27 saturated FAMEs. Certain lipid ratios also were determined because they could be an indication of specific biochemical abnormalities, such as peroxisomal disorders.[18]

Results

Free (Nonesterified) Sterols

Free sterols in all groups were composed primarily of cholesterol and the cholesterol dehydration product, cholestadiene. The sum of these two sterols was taken as the amount of original free cholesterol; no cholestanol was detected in these fractions. As will be explained shortly, the normal group of individuals was separated into two groups of four individuals each. When the free cholesterol (μg) from the two normal groups and the six disease groups was analyzed statistically (ANOVA), no significant group differences were noted ($F = 1.32$, $P > 0.50$). When the amount of cholesterol (μg) was correlated with the original amount of secretion taken for analysis (Table 1), however, the correlation coefficient was highly significant ($r = +0.870$, $P < 0.001$); ester cholesterol is shown for comparative purposes.

Ester Sterols

As observed with the free sterols, cholesterol was the predominant ester sterol in all six disease groups; this was not true for the normal individuals. Samples from four of the eight normal subjects contained no cholesterol but did contain small amounts of cholestanol. Sterols of these normal subjects were dominated by oxysterols—cholesterol metabolites containing more than one oxygen function. By contrast, samples from the second set of the normal individuals contained predominantly cholesterol in amounts similar to those found in the disease groups. These two groups are designated Norm(CA) and Norm(CP) to indicate that cholesterol was absent or present, respectively. Because GLC analysis indicated that the oxysterols were detectable only in the two normal groups

Table 1. The relationship between free cholesterol (Ch) meibomian secretions to sample weight and ester cholesterol

	Clinical Group							
	NORM(CA)	*NORM(CP)*	*MKC*	*2 MEIB*	*MBSB*	*SBBL*	*MIX*	*STAPH*
Sample wt. (μg)	790	790	1428	740	805	657	552	828
Free Ch (μg)	2.12	2.21	3.56	2.31	2.07	1.87	1.82	2.31
Ester Ch (μg)*	6†	31	65	203	208	88	147	138

* Norm(CA) contained only cholestanol.

† Significantly different ($P < 0.01$).

Table 2. Group averages for the major oxysterols detected in ester sterol samples

		*Lipid average group composition (%)**		
Sterol	*Mass†*	*Norm(CA)*	*Norm(CP)*	*MKC*
Cholesterol	(386)	1.87%	14.42%	14.42%
Oxysterol	(416)	3.38%	0.18%	0.08%
Oxysterol	(432)	0.06%	0.03%	0.17%
Sum trioxysterols		3.44%	0.22%	0.25%
Oxysterol	(402)	0.00%	0.00%	5.02%
Oxysterol	(416)	0.00%	0.07%	0.00%
Sum dioxysterols		0.00%	0.07%	5.02%

* All percentages are relative to total FAMEs.
† Mass is as analyzed and includes permethyl derivatives, which may not have been present in the original secretion.
In Norm(CA) the cholesterol value given is cholestanol.

and the MKC group, these were analyzed further by GC-MS to characterize better the oxysterols present. Four oxysterols were detected and characterized by analysis of their mass spectra. The first, with the shortest GC retention time, contained one hydroxy group and one methoxy (permethyl) group; the mass was 416. The oxysterol with the next longer retention time contained two hydroxy groups and one methoxy group (mass, M/Z 432). This was followed by an oxysterol which contained two hydroxy groups (mass, M/Z 402). The oxysterol with the longest retention time appeared to contain two hydroxy groups and one keto group (mass, M/Z 416). This oxysterol dominated the ester sterols from Norm(CA) as indicated in Table 2. Inspection of the fragmentation patterns, as indicated by the mass spectra of these four oxysterols, did not indicate that any of the oxygen functions were on the sterol side chain.

For comparative purposes, group averages for ester cholesterol are shown in Tables 2 and 3. Samples from all individuals in the disease and Norm(CP) groups contained at least 8% cholesterol relative to total FAMEs. In contrast, samples from Norm(CA) individuals contained a maximum of 3% cholestanol and no cholesterol. Furthermore, there was a highly significant negative correlation between the trioxysterols and ester cholesterol ($r = -0.894$, $P < 0.001$). A similar correlation, using the sum of the dioxysterols, was not significant ($r = +0.381$, $P = 0.20$).

Ester cholesterol, unlike the oxysterols, was found in two lipid fractions—the ester sterol fraction and the FAME fraction. The reason for this relates to the number of sterol hydroxyl groups, their location in the molecule, and the method used for trans-esterification of the original wax and sterol esters. Others[19] reported the formation of both permethyl cholesterol and cholestadiene on treatment of cholesterol esters with 14% boron trifluoride. We observed both permethylcholesterol and cholestadiene in the FAME fractions (as determined by GC-MS), but only underivatized cholesterol was present in the ester sterol fractions. This partitioning is related to the changes in cholesterol polarity on loss of the free hydroxyl group. The formation of FAMEs and permethylcholesterol has been proposed as a method for simultaneous analysis of sterol and fatty acid components of sterol esters.[20]

Unlike the free cholesterol, the ester cholesterol was not correlated with sample weight ($r = -0.205$, $P < 0.10$). However, ANOVA indicated a significant difference among groups, and by Dunnett's test, it was determined that ester cholesterol in the

Table 3. Meibomian secretion ester composition: lipid groupings (FAMEs and cholesterol)

	Clinical group							
Lipid group FAMEs (chain type)	*NORM(CA)*	*NORM(CP)*	*MKC*	*2 MEIB*	*MBSB*	*SBBL*	*MIX*	*STAPH*
sum n (all)	28.95%*	50.21%	41.93%	49.70%	51.59%	50.14%	50.27%	51.53%
sum i (all)	32.45%	24.95%	27.79%	24.66%	24.16%	23.26%	24.19%	23.82%
sum ai (all)	35.78%*	24.05%	29.26%	24.14%	22.90%	25.49%	23.56%	23.18%
i(all)/ai(all)	0.91	1.04	0.95	1.03	1.06	0.91	1.03	1.03
Subgroupings								
(1) Cholesterol†	1.87%*	14.40%	14.42%	16.91%	17.70%	15.54%	14.60%	15.00%
(2) n14–18 sat	16.17%*	6.70%	5.30%	4.30%	3.33%	4.78%	4.59%	5.45%
(3) n20–28 sat	12.59%*	1.71%	1.14%	1.07%	0.96%	1.53%	1.51%	1.54%
(4) n14–18 unsat	0.20%*	36.41%	29.96%	37.14%	40.49%	35.82%	37.25%	37.70%
(5) n20–28 unsat	0.20%*	4.66%	4.72%	6.26%	5.76%	6.66%	6.00%	5.72%
(6) i14–18 sat	7.60%	4.82%	6.40%	5.09%	4.95%	4.37%	4.61%	4.75%
(7) i20–28 sat	24.84%	19.17%	20.02%	18.14%	17.71%	17.71%	18.19%	17.99%
(8) i14–18 unsat	0.00%*	0.96%	1.37%	1.44%	1.49%	1.17%	1.39%	1.07%
(9) ai15–19 sat	15.13%*	9.92%	11.34%	10.53%	10.40%	10.78%	10.11%	9.73%
(10) ai21–27 sat	20.65%	14.14%	17.91%	13.61%	12.49%	14.71%	13.45%	13.45%

* Significantly different from Norm(CP), $P < 0.01$.
† The cholesterol designation also includes cholestanol; only cholestenol was detected in the Norm(CA) samples.

Norm(CA) group was significantly different ($q' = -4.09$, $P < 0.01$) from all other groups.

Ester Cholesterol and Fatty Acid Relationships

On comparison of the cholesterol and FAME differences among individual samples, it became obvious that important correlations were present. Most striking was the correlation between ester cholesterol and ester unsaturated fatty acids; this correlation was highly significant and positive ($r = +0.880$, $P < 0.001$). Also, in the Norm(CA) group, the percentage of normal FAMEs was significantly less ($P < 0.01$), and the percentage of anteiso-FAMEs was significantly greater ($P < 0.01$) than in all other groups. However, the ratio of iso- and anteiso-FAMEs was relatively constant among all groups (Table 3).

To understand better the importance of these lipid changes among normal and disease groups, the FAMEs were grouped together based on their biochemical and biophysical characteristics and correlated with disease signs. We previously showed that discriminate analysis based on individual FAME data correctly classified normal (as one group) and disease groups with a 73% probability of correct classification.[8] The current discriminate analysis used nine FAME groupings and cholesterol (Table 3). Initially, all eight normal subjects were classified into two groups based on the ten lipid groupings; discriminate analysis selected the same segregation of individuals as had been suggested previously by observation. Next, the two normal groups, together with the six disease groups, were analyzed by discriminate analysis.

Four lipid groupings were much more important for correct classification of individuals than the other six groupings. These four lipid groupings were (3) normal C20–28 saturated ($F = 15.62$, $P = 0.0001$), (8) iso-C14–18 unsaturated ($F = 6.403$, $P = 0.0001$), (5) normal C20–28 unsaturated ($F = 2.891$, $P = 0.0180$), and (10) anteiso-C21–27 saturated ($F = 1.358$, $P = 0.2566$). Noteworthy is the fact that three of these groupings were very long-chain FAMEs, and two groupings were unsaturated FAMEs. Discriminate analysis using these four FAME groupings resulted in the correct classification of at least one half of the individuals in the groups Norm(CA), 100%; Norm(CP), 75%; SBBL, 67%; MBSB, 50%; and MKC, 50%. These results suggest that where microflora are not a major consideration, broad categories of lipids may be important in determining disease signs. We also can map these differences between normal and disease groups based on the results of discriminate analysis (Fig. 1). This map shows the relative difference between groups based, not on clinical signs, but on the four lipid groupings. We should note that the disease groups are much closer to Norm(CP) than to Norm(CA).

Significant differences were found among disease groups and normal subjects with respect to the ratio of unsaturated normal C18 FAMEs to cholesterol. The MKC group was significantly different ($P < 0.05$) from Norm(CP).

Ester Fatty Alcohols

Preliminary GC-MS analyses suggest that the ester fatty alcohols are much more complex than previously reported. The eight individual samples analyzed were as follows: Norm(CA), one; Norm(CP), three; MKC, two; 2 MEIB, one; and MBSB, one. Seven fatty alcohols were found commonly in all samples. These were characterized as follows: (1) mass, 252 and C17 with a combination of two unsaturations or cyclopropane rings; (2) mass, 280 and C19 with a combination of two unsaturations or cyclopropane rings; (3) mass, 308 and C21 with a combination of two unsaturations or cyclopropane rings; (4) mass, 320 and C22 with a combination of three unsaturations or cyclopropane rings; (5) mass, 336 and C23 with a combination of two unsaturations or cyclopropane rings; (6) mass, 348 and C24 with a combination of three unsaturations or cyclopropane rings; and (7) mass, 364 and C25 with a combination of two unsaturations or cyclopropane rings. Among the other alcohols present were highly oxygenated alcohols, which were much more prevalent in the MKC samples than in the others. Also, the MKC samples contained another alcohol (mass, 378) that was not present in any of the other samples; the amount of this compound appeared to increase as the amount of unsaturation increased in the corresponding FAME fraction.

Discussion

Sterols and Sterol Esters

Our results suggest that the presence of esterified cholesterol in meibum may be a necessary precondition that could lead to the development of chronic blepharitis. All meibomian secretions from individuals with disease signs contained at least 8% ester cholesterol (relative to total FAMEs). By contrast, one half of the secretions from normal individuals contained no ester cholesterol. These differences were not a result of in vivo ester hydrolysis; the free cholesterol was not significantly different among groups but was positively correlated with the original sample weight. This free cholesterol may be derived from membranes present in the holocrine cells of the meibomian glands.

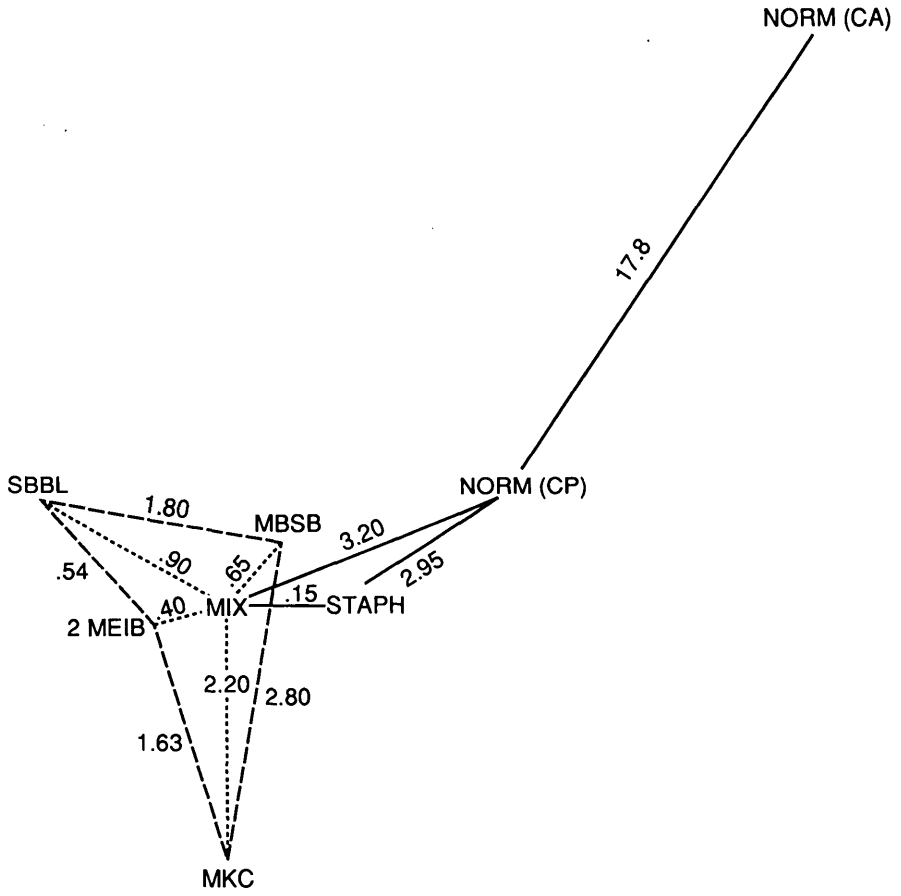

Fig. 1. Map of disease groups and normals showing relative intergroup "distances" based on discriminate analysis of lipid groupings.

There are several biologic mechanisms for controlling the ester cholesterol content of meibum. It is well known that a diet rich in cholesterol or saturated fat can cause an elevation of plasma cholesterol levels; drugs such as cholestyramine have the opposite effect.[21,22] Dietary deficiencies, such as protein or energy deficiencies, also have been shown to cause an increase in cholesterol esters in certain tissues.[23,24] One drug that reportedly is effective in treating chronic blepharitis, tetracycline, has been shown to decrease total serum cholesterol.[25] Our results suggest that cholesterol synthesis in the meibomian gland may be inhibited by certain oxysterols—known inhibitors of cholesterol synthesis.[26] Some oxysterols also increase cholesterol ester formation,[27] and recently it has been reported that some oxysterols can affect the relative amounts of triglycerides and phospholipids synthesized.[28] Finally, uptake of cholesterol esters into some cells depends on specific binding sites.[29] Thus there is ample evidence that cholesterol ester accumulation can be controlled physiologically by a number of mechanisms.

There is also some evidence that cholesterol esters or cholesterol can be removed selectively from the meibomian gland. A large variation was reported in the cholesterol ester, free cholesterol, free fatty acid, and hydrocarbon content in meibum samples from different individuals.[30] Some of these hydrocarbons may have been derived from cholesterol, fatty acids, or alcohols. There are reports that other glandular secretions contain both cholestene and cholestadiene.[31] However, accumulation of small amounts of cholesterol in meibum may be detrimental; this has been reported to result in an increase in lipase activity against triglycerides.[32] Our previous observation[5] showed that some disease groups such as MKC have more coagulase-negative staphylococci than normal subjects. These microorganisms are capable of hydrolyzing both wax and sterol esters, but we also observed that low doses of tetracycline can inhibit this lipase activity.[6]

One other consideration should be mentioned with regard to the control of cholesterol ester content of meibum. It is known that meibomian gland fatty alcohols can be formed from the corresponding fatty acids,[33] and there is a possibility that the cholesterol ester content is controlled in part either by the rate of biosynthesis of fatty alcohols or by the ability to esterify them to fatty acids. A high cholesterol ester content may reflect an inability to synthesize sufficient

amounts of wax esters. It therefore appears that the way in which the cholesterol ester content of meibum is physiologically controlled may be important.

Fatty Acids

An important observation we made was that only when meibum contains cholesterol esters are significant amounts of unsaturated fatty acids present. Animal models demonstrated that an increase or decrease in dietary sterols can increase or decrease significantly, respectively, the amount of unsaturated fatty acids present in certain tissues.[34,35] In our studies, discriminate analysis suggested that only the ratio of the FAME grouping normal C14–19 unsaturated to cholesterol was an important indicator of disease. Similarly, the ratio normal C18 unsaturated to cholesterol was an important indicator of disease. This ratio was significantly different only in the MKC group. Furthermore, discriminate analysis using ten lipid groupings—one cholesterol and nine FAME groupings—suggested that it was the biochemical response to the presence of cholesterol esters and not the cholesterol itself that indicated disease. Thus only four FAME groupings (iso-C14–19 unsaturated, normal C20–28 unsaturated, normal 20–28 saturated, and anteiso-C21–27 saturated) correctly classified at least one half of the individuals in the groups Norm(CA), Norm(CP), MKC, MBSB, and SBBL. We previously reported that when all FAMEs are used separately; discriminate analysis correctly classified 73% of all individuals.[8]

Tear Film Characteristics Related to Lipid Composition

Model studies on the physical characteristics of cholesterol ester monolayer films suggest the biophysical significance of the large increase in meibum unsaturated FAMEs noted in the presence of cholesterol esters. For example, the stability of films containing cholesterol esters was much greater if the ester contained *cis*-unsaturated fatty acids rather than saturated or *trans*-fatty acids.[14] In a related observation, it was reported[9] that meibum contains about 4% triglycerides, and model studies show that this level of triglycerides can improve greatly the temperature stability of cholesterol ester monolayers in the vicinity of normal body temperature.[15,36] Our studies using discriminate analysis indicated that the anteiso-branched long-chain fatty acids—anteiso-C21–27 saturated—were important in predicting disease groups, and it may be significant that anteiso-fatty acids have been reported to replace partially the requirement for unsaturated fatty acids in microbial studies.[37] This appears to be the case with meibomian secretions from the rabbit. Thus, even though rabbit meibum contains a high percentage of sterols, it contains only 9% unsaturated fatty acids; the rest of the fatty acids are anteiso-fatty acids.[11,38] We would expect this low level of unsaturated fatty acids to increase the chemical and biochemical stability of rabbit meibum. In Table 4 we can see, that in bovine and rabbit meibum, there appears to be a definite tendency to replace unsaturated fatty acids with saturated anteiso-fatty acids. However, meibum from the rat,[39] mouse,[40] and gerbil[41] contains amounts of unsaturated and anteiso-fatty acids similar to those observed in human meibum.

Monolayer studies using actual meibomian secretions from patients with KCS also were reported and may relate directly to the model studies previously discussed. Lipid monolayers formed from secretions of patients with meibomianitis or of patients (older than 60 of age) with KCS were characteristically different from monolayers formed from normal subjects or from patients younger than 60 years of age.[13] We previously reported a high incidence of KCS in some chronic blepharitis disease groups.[3]

Our results, together our with previous work, sug-

Table 4. Summary of reported wax and sterol ester fatty acid composition of meibomian secretions from various animals

	Animal						
Lipid	*Human*[9]	*Human*[39]	*Rat*[39]	*Mouse*[40]	*Gerbil*[41]	*Bovine*[9]	*Rabbit*[38]
(1) STEROL*	45%	68%	65%	67%	63%	50%	78%
FAME							
(2) n14–18 sat	1.6%	4.8%	14.2%	11.6%	22.5%	3.0%	0
(3) n20–28 sat	1.0%	1.2%	1.9%	1.7%	5.0%	0.7%	0
(4) n14–18 unsat	38.6%	40.6%	45.4%	46.8%	43.1%	21.4%	9.6%
(5) n20–28 unsat	8.6%	11.3%	9.3%	4.0%	0	4.5%	0
(6) i14–18 sat	5.0%	4.2%	8.9%	10.0%	4.8%	4.5%	0
(7) i20–28 sat	21.7%	15.4%	11.2%	9.5%	6.8%	8.0%	0
(8) i14–18 unsat	1.2%	1.6%	0	0	0	0.1%	0
(9) ai15–19 sat	6.2%	8.9%	4.0%	4.7%	9.0%	26.6%	64.9%
(10) ai21–27 sat	14.2%	13.4%	5.0%	11.8%	8.6%	26.6%	25.5%

* Sterol is expressed as a percentage of total FAMEs.

gest that specific changes in meibomian gland lipids either directly or indirectly produce the signs and symptoms associated with chronic blepharitis. They further suggest that it is the accumulation of cholesterol ester in these glands and the consequent lipid changes that result in the symptoms associated with chronic blepharitis. Thus, the ester fraction of meibum contains unsaturated fatty acids only when cholesterol esters are present. The availability of fatty alcohols may play a role in this process of cholesterol accumulation. Additionally, some lipids may be modified by the presence of specific populations of microflora.

Further studies are suggested by our results. For example, why do cholesterol esters accumulate in the meibum of some individuals but not in others? Can this cholesterol accumulation be reversed by diet or drug therapy? Are peroxisomal disorders responsible for the lipid differences between disease and normal groups with cholesterol esters? It is important to remember that human peroxisomes are capable of a wide variety of lipid transformations including cholesterol synthesis, synthesis and beta oxidation of very long-chain fatty acids (>C22), alpha oxidation of fatty acids, and synthesis of ether glycerolipids; peroxisomes also produce significant amounts of hydrogen peroxide.[18]

As discussed previously, it may be important to know specifically if esterification patterns change in disease groups compared with normal subjects who have cholesterol esters. Finally, the role of triglycerides, phospholipids, and especially fatty alcohols in determining the signs associated with chronic blepharitis remains to be clarified.

Key words: meibomianitis, meibum, blepharitis, sterol esters, fatty waxes

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