Complete Androgen Insensitivity Syndrome

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Effect on Human Meibomian Gland Secretions

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Objective: To determine whether androgen receptors affect the fatty acid profiles of neutral and polar lipids in human meibomian gland secretions.

Methods: Meibomian gland secretion samples were obtained from both eyes of (1) women with complete androgen insensitivity syndrome, a condition characterized by dysfunctional androgen receptors, and (2) age-matched female and male controls. Samples were processed for high-performance liquid chromatography, mass spectrometry, or both and for analysis of the mass spectra of neutral and polar lipid fatty acid fragment ions by 3 different methods.

Results: Androgen receptor dysfunction is associated with significant alterations in the appearance of nu-merous molecular species in the neutral and polar lipid fractions of meibomian gland secretions. The ability to detect these differences, and to assess their nature and extent, was facilitated by the use of several analytic approaches. Sex-related differences exist in the expression of a variety of neutral and, especially, polar fatty acid products in meibomian gland secretions.

Conclusions: Androgens exert a significant effect on neutral and polar lipids in human meibomian gland secretions, and these hormonal effects may be mediated through androgen receptors.

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RECENTLY, RESEARCHERS have discovered that the meibo-mian gland is an androgen target organ.[1] Androgens seem to regulate meibo-mian gland function, improve the quality or quantity of lipids produced by this tissue, and stimulate formation of the tear film's lipid layer (M. A. Zeligs, K. Gordon, Dehydroepiandrosterone therapy for the treatment of dry eye disorders, Int Patent Application WO 94/04155, 1994).[2,3] Conversely, researchers have also discovered that androgen deficiency may be a critical etiologic factor in the pathogenesis of meibo-mian gland dysfunction, altered lipid profiles in meibomian gland secretions, tear film instability, and evaporative dry eye (J.M.C., K.L.K., R. M. Sullivan, RN, M.R.D., D.A.S., unpublished data, 2002).[4-6]

These findings have significant clinical importance given that the meibomian gland plays an essential role in maintaining the health and integrity of the ocular surface.[7-12] This tissue, through its synthesis and secretion of neutral and polar lipids, promotes the stability of and prevents evaporation from the preocular tear film.[7,9-12] Moreover, meibomian gland dysfunction, and the resulting lipid insufficiency, is believed to be an important cause of dry eye syndromes throughout the world.[13] These syndromes lead to significant corneal damage, visual impairment, and quality of life changes[7,9,10,14-16] and are estimated to afflict more than 10 million people in the United States alone.[17] Consequently, the androgen deficiency that occurs during menopause, aging, and Sjögren syndrome[18-20] may contribute substantially to the meibomian gland dysfunction and evaporative dry eye often observed in these conditions.[21-23]

We hypothesize that androgen action on the meibomian gland, and its impact on lipid production, is mediated primarily through hormone binding to intranuclear androgen receptors. In support of this hypothesis, we (1) identified androgen receptor protein within acinar epithelial cell nuclei of human meibo-mian glands[24]; (2) found that antiandrogen therapy is associated with meibo-mian gland dysfunction, altered neutral lipid profiles in meibomian gland secretions, diminished tear film stability, and

functional dry eye[4]; and (3) discovered that complete androgen insensitivity syndrome (CAIS), due to completely dysfunctional androgen receptors,[25,26] is associated with an increase in the signs and symptoms of dry eye (J.M.C., K.L.K., R. M. Sullivan, RN, M.R.D., D.A.S., unpublished data, 2002).

However, it is unclear whether androgen receptors, per se, mediate the androgen effect on neutral lipid patterns in meibomian gland secretions. In addition, it is unknown whether androgens, in the presence or absence of receptors, have any effect on polar lipids in these secretions. Polar lipids, such as phosphatidylethanolamine and phosphatidylcholine, are critical components of the tear film and serve to maintain the oil-aqueous interface and provide functional stability for the neutral lipids.[11,14,27] Therefore, to further test our hypothesis, we endeavored in this study to determine whether androgen receptors affect fatty acid (FA) profiles of neutral and polar lipids in human meibomian gland secretions. To achieve this objective, we compared the lipid patterns in secretion samples from individuals with CAIS with those from age-matched female and male controls.

METHODS

PARTICIPANTS

Women with CAIS (n=9; mean±SE age, 40.4±2.3 years) were recruited nationally from the Androgen Insensitivity Syndrome Support Group (San Diego, Calif). Age-matched female (n=9; mean±SE age, 40.2±3.0 years) and male (n=10; mean±SE age, 37.1±1.5 years) controls without a clinical history of androgen insensitivity were recruited from the Boston environs. After individuals provided informed consent, meibomian gland secretion samples were obtained according to previously described procedures.[4,5] In brief, secretions were collected from the left and right eyes by gently applying pressure against the lower eyelid with a cotton-tipped applicator and collecting the expelled fluid using a chalazion curette (surgical stainless steel). These secretions were obtained while directly visualizing the meibomian gland orifices with a biomicroscope, thereby minimizing any possibility of contamination with other lid margin lipids. The samples were placed in glass tubes containing a 2:1 mixture of chloroform-methanol, and the tubes were then capped and stored at –70°C until experimental analysis. These studies were approved by the human studies committees of the Brigham and Women's Hospital and the University of Chicago Medical Center (Chicago, Ill) and were performed in accordance with guidelines established by the Declaration of Helsinki.

BIOCHEMICAL AND ANALYTIC METHODS

Meibomian gland secretions were analyzed for FA molecular species in neutral and polar lipids by high-performance liquid chromatography (HPLC) (Spectra-Physics Model 8700; Thermo Separation Products, San Jose, Calif) coupled with mass spectrometry (MS) (Finnigan 4500; Thermo Finnigan LLC, San Jose) or by direct-injection electrospray MS. For neutral lipid assessment by HPLC-MS, samples were suspended by sonication in n-heptane for injection and then separated on a silica column (Inertsil; Keystone Scientific Inc, Bellefonte, Pa) measuring 10 cm × 2 mm and having a complex, multistep gradient. The gradient combined mobile phases of isooctane-tetrahydrofuran (99:1, volume per volume), isopropanol-chloroform (4:1, vol-

ume per volume), and isopropanol-water (1:1, volume per volume) and had a linear flow velocity of 0.4 mL/min.[4,5] The vaporizer temperature in the moving-belt interface of the HPLC-MS was 310°C. Mass spectrometry was performed in positive-ion, chemical-ionization mode using ammonia reagent gas, and data were acquired using a data acquisition system (Teknivent Vector/Two; Teknivent Corp, Maryland Heights, Mo).

Polar lipids in meibomian gland secretions were evaluated using 2 different procedures. One method involved analysis of samples by electrospray HPLC-MS. Secretion samples were dissolved in 50 µL of HPLC mobile phase (methylene chloride–[aqueous 0.1M ammonium acetate, pH 3.9]) (68:28.4:3.6, volume per volume), and a 20-µL aliquot was separated by HPLC on an APS-Hypersil-2 amino column (Keystone Scientific Inc) measuring 10 cm × 1 mm. The mobile phase was delivered at 50 µL/min, and the total flow from the column was directed to the electrospray ion source of a LCQ quadrupole ion trap MS system (Thermo Finnigan LLC). The heated capillary was maintained at 200°C, and the source voltage was kept at 4.5 kV. Full positive-ion spectra were acquired from mass-charge (m/z) ratios 150 to 950 as the sum of 5 microscans, with the automatic gain control target set at 2×10^7. The other method used for polar lipid evaluation was direct-injection electrospray MS. Samples were dissolved in 100 µL of methanol, and a 10-µL aliquot was injected into a stream of methanol flowing at 20 µL/min directly into the electrospray ion source of the LCQ quadrupole ion trap MS system. The heated capillary was again maintained at 200°C, with a source voltage at 4.5 kV. Full positive-ion spectra were acquired from m/z ratios 150 to 950 as the sum of 3 microscans, with the automatic gain control target set at 5×10^7. Spectra were averaged over the elution profile, and background was subtracted using baseline spectra before and after the sample elution. All solvents were Optima grade (Fisher Scientific International, Medford, Mass).

To analyze HPLC and MS data, and to determine whether significant differences exist between the neutral and polar lipid mass spectra of women with CAIS vs controls, 2 different analytic systems were used. In the first system, as previously reported,[5] a discrete, integer-valued distribution of m/z ratios (neutral, m/z ratios 100-900; polar, m/z ratios 150-950) was gathered for each participant in designated lipid fractions. Each distribution represented the time average of a gaussian HPLC peak defined by characteristic ions falling within specific points of neutral lipid elution (cholesterol and cholesterol esters, m/z ratio 369 over ~1.8 minutes; wax esters, m/z ratios 636, 650, 664, and 678 over ~1.8 minutes) and polar lipid elution (phosphatidylethanolamine, m/z ratios 746-748.5[+], 774-777.5[+], and 798-806 over ~2.85-3.10 minutes; phosphatidylcholine, m/z ratios 756-762[+], 806-817, and 836-839 over ~4.07-4.72 minutes). The precise HPLC regions for phosphatidylethanolamine and phosphatidylcholine elution were preidentified by the use of L-α-phosphatidylethanolamine, distearoyl and DL-α-phosphatidylcholine, dipalmitoyl standards (Sigma-Aldrich Corp, St Louis, Mo), respectively. Resulting MS profiles were normalized by their individual sums to represent each m/z ratio as a percentage composition of the sample. For statistical analysis, an unpaired *t* test was performed between women with CAIS and control data by treating each m/z ratio as a separate variable and each individual in a group as an observation. For m/z units with significant (*P*<.05) alterations between groups, differences in sum-normalized mean values greater than 0.01% were tabulated. Significant differences in random noise were found at levels on the order of 0.001%.

The second system used to evaluate data was created to analyze and compare the entire mass spectra (ie, encompassing ~15 000 data points per sample) of neutral (m/z ratios 100-900) and polar (m/z ratios 150-950) lipids. Development of this system required the writing of dynamic computer programs and

algorithms using a scientific and engineering software tool (Matlab 4.2c and 5.0; The MathWorks Inc, Natick, Mass) to permit exportation and translation of neutral (Teknivent Vector/Two machine format) and polar (ASCII text format) data and conversion of continuously distributed m/z ratio data into discrete integer-valued m/z ratio units. Statistical analysis of the sum-normalized data (ie, percentage composition) was conducted using the unpaired, 2-tailed *t* test. The HPLC-MS data were integrated from 0 to 16 minutes (ie, the period of the complete HPLC elution) before comparison. At every m/z ratio, the ratio of the mean difference between groups and the *P* value from the *t* test (Δ/P) was calculated using a custom database structure in Matlab 5.0. Values greater than 2.0 ($\approx\Delta>0.02$ and $P<.01$) were considered very significant.

Correlation coefficients of m/z ratio distributions within and between groups were also calculated using Matlab. Iterative calculations within groups produced $[n(n-1)/2]-1$ values for each internal comparison (*n* being the number of samples per group) and $n \times m$ values for comparisons between groups (*n* being the number of samples in first group and *m* the number of samples in second group). Average correlation coefficients are reported for each comparison.

RESULTS

To determine whether androgen receptors affect the FA patterns of neutral and polar lipids in human meibomian gland secretions, samples were collected from the left and right lower eyelids of women with CAIS (n=9) and age-matched female (n=9) and male (n=10) controls. Samples were then processed for HPLC, MS, or both and for analysis of the mass spectra of neutral and polar lipid FA fragment ions by 3 different methods. The first method involved evaluation of the m/z ratios of wax and cholesterol ester FA, as well as phosphatidylethanolamine and phosphatidylcholine species, and was facilitated through a time-based decomposition of HPLC-MS elution plots. This approach targeted specific, elution time–dependent HPLC regions that were associated with these molecular species. However, this technique permitted examination of only a small percentage of the total data. The second method involved analysis of all neutral and polar lipid HPLC-MS data, which were integrated from 0 to 16 minutes (ie, the duration of the HPLC elution) before comparison. In effect, this approach was time independent and allowed evaluation of entire mass spectra. The third method was used solely for polar lipid assessment and involved analysis of all m/z ratio data after direct-injection electrospray MS. This last approach, which did not use HPLC, was completely time independent.

Our findings using all 3 analytic methods demonstrate that androgen receptor dysfunction is associated with substantial alterations in the appearance of numerous molecular species in the neutral and polar lipid fractions of meibomian gland secretions. However, the ability to detect these differences, and to assess their nature and extent, depended on the analytic approach.

The first regional method of data analysis showed that substantial differences exist between women with CAIS and controls in specific m/z ratios of wax and cholesterol ester FA (**Figure 1** and **Figure 2**), phosphatidylethanolamine species (**Figure 3** and **Figure 4**), and phosphatidylcholine species (**Figure 5** and **Figure 6**). These differences involved an increase or decrease in the

expression of a variety of FA-containing ions in secretions of women with CAIS compared with male and female controls, and some of these changes seemed to be almost "all" or "none" (eg, the ion at m/z ratio 654 in Figures 3 and 4). In contrast, no significant differences were evident between the neutral and polar lipid profiles of female and male controls.

The second method of analysis, which evaluated all HPLC-MS data, also identified considerable differences in lipid patterns of meibomian gland secretions between women with CAIS and female and male controls. However, these differences were most pronounced in the polar lipid fraction (**Table 1**) and were either very small (ie, individuals with CAIS vs female controls) or undetectable (ie, individuals with CAIS vs male controls) in neutral lipids (Table 1). This analytic approach identified significant differences between male and female controls in the expression of certain FA products. The degree of these sex-related variations was greater in polar than in neutral lipids. Moreover, the identity and magnitude of polar ion differences between women with CAIS and female controls vs women with CAIS and male controls were not typically the same (Table 1).

The third method of data analysis, which examined all polar lipid MS data, demonstrated again that profound differences exist in the lipid profiles of meibomian gland secretions of women with CAIS relative to those of female and male controls (**Table 2** and **Figure 7**). This approach also permitted identification of significant differences in the expression of many prevalent FA fragment ions (eg, m/z ratios 873, 875, 874, 902, 476, 269, and 761 in Table 2) that were not apparent after using the second HPLC-MS method. One example was the prominent ion at m/z ratio 873, which had a Δ/P ratio of 70.7 comparing the data of male controls and individuals with CAIS (Table 2). This analytic system also showed that (1) significant sex-associated differences exist in the appearance of specific polar lipid species and (2) the nature and extent of differences in polar FA product expression between individuals with CAIS and female or male controls were not usually identical (Table 2). Statistical analysis of these data further demonstrated relatively high average correlation coefficients within and low average correlation coefficients between women with CAIS, female controls, and male controls (**Table 3**).

COMMENT

The present investigation demonstrates that the lack of androgen receptors is associated with significant alterations in the appearance of numerous molecular species in the neutral and polar lipid fractions of human meibomian gland secretions. Thus, women with CAIS, compared with male or female controls, had striking differences in the expression of specific wax and cholesterol ester FA products, certain phosphatidylethanolamine and phosphatidylcholine species, and the overall mass spectra of neutral or polar lipid FA fragment ions. These changes in individuals with CAIS are consistent with our hypothesis that the androgen control of lipid production in the meibomian gland is mediated, at least in part,
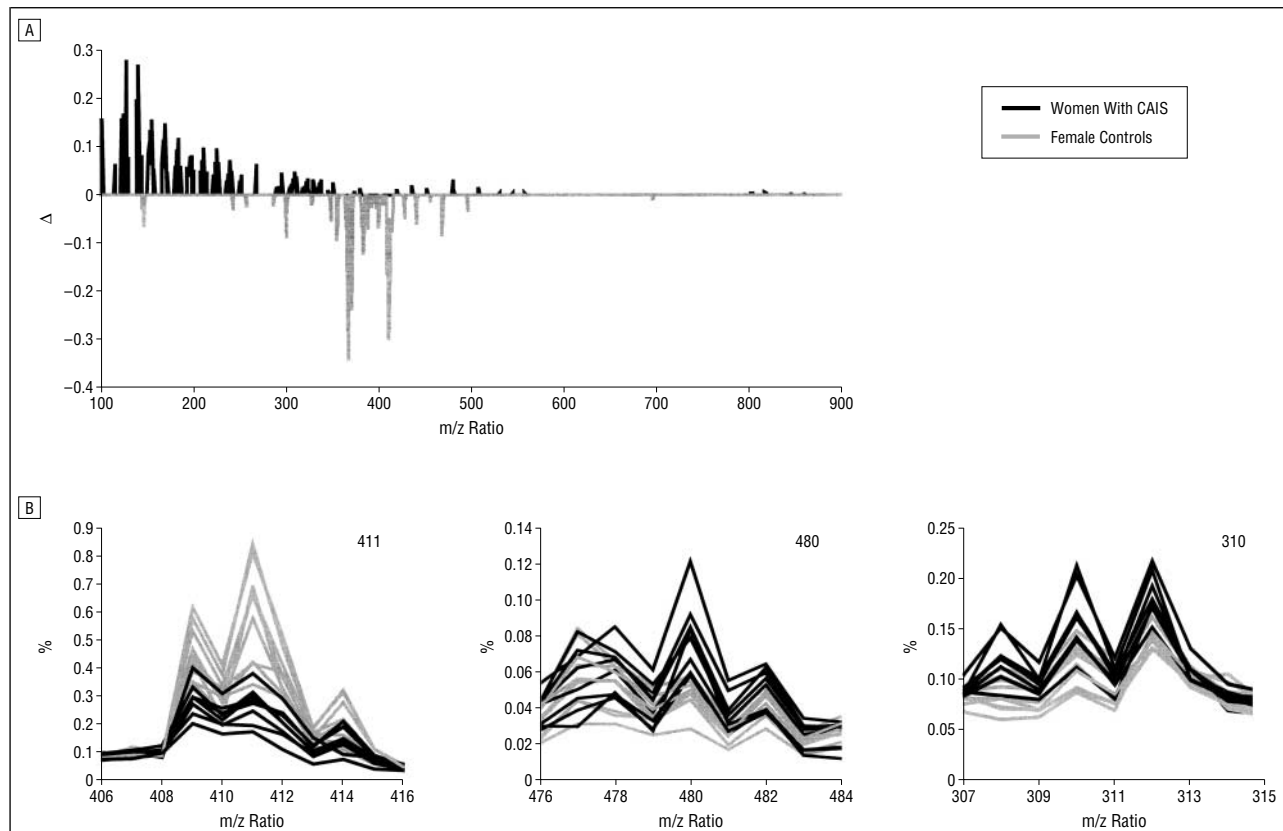
Figure 1. Effect of complete androgen insensitivity syndrome (CAIS) on the neutral lipid mass spectrum of meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related female controls (n=9). A, The significant difference (Δ) between groups in the mean percentage of total neutral lipid fatty acid (FA) fragmentation products is on the y-axis. Positive and negative peaks represent where (and to what extent) the CAIS or control group is larger, respectively. The negative values are for demonstration purposes. B, Patterns of percentage of FA product composition (%) in individual samples for a given mass-charge (m/z) ratio.

through the binding to intranuclear androgen receptors. In addition, given that a disruption in the quality of meibomian gland lipids may promote tear film instability and evaporation,[7,9,11,14,15] these lipid alterations may contribute to the increased signs and symptoms of dry eye in CAIS women (J.M.C., K.L.K., R. M. Sullivan, RN, M.R.D., D.A.S., unpublished data, 2002).

Our finding that androgen receptor dysfunction is paralleled by a change in the lipid patterns of meibomian gland secretions might have been predicted based on our earlier observations and those of others.[1,2,4-6] The meibomian gland is a large sebaceous gland, and androgens have been shown to control the development, differentiation, and lipid synthesis of sebaceous glands throughout the body.[28-32] This hormone action is invariably due to androgen interaction with specific androgen receptors in acinar epithelial cell nuclei and leads to alterations in gene transcription, protein translation, and lipid production.[32-35] Furthermore, androgen effects on sebaceous gland function may be inhibited by exposure to antiandrogens[36,37] or by defects in androgen receptors (eg, CAIS).[38] In like manner, the meibomian gland contains androgen receptor protein within acinar epithelial cell nuclei,[24] and androgens seem to modulate gene expression and lipid production in this tissue.[1-6,39-41] These androgen effects seem to depend on the presence of intact androgen receptors[40,42] and may be attenuated by treatment with antiandrogen compounds.[4,5] Our re-

sults do not rule out the possibility that androgens may also affect meibomian gland lipid composition through nonclassic processes, such as occur after hormone binding to stereospecific plasma membrane receptors[43] or to sex-hormone binding globulin.[44] However, whether these alternative routes for androgen action are operative in the human meibomian gland remains to be elucidated.

Our finding that the ability to detect, and to determine the nature and extent of, lipid changes in meibomian gland secretions of women with CAIS depends on the analytic method used might also have been predicted. Our first approach focused on selecting specific areas of HPLC elution curves that were associated with neutral wax and cholesterol ester FA, as well as polar phosphatidylethanolamine and phosphatidylcholine species. Data from these windowed regions were then integrated and compared. An advantage of this system was that targeted molecular species, with potentially high signal-to-noise ratios, could be evaluated. Using this approach, we identified many significant and consistent differences in the expression of FA products in secretions of women with CAIS compared with both control groups. However, this method also had limitations, including (1) the time variance inherent to HPLC, which disallowed the precise alignment of windowed regions for comparative analyses; (2) an inability to consistently identify peaks related to certain neutral
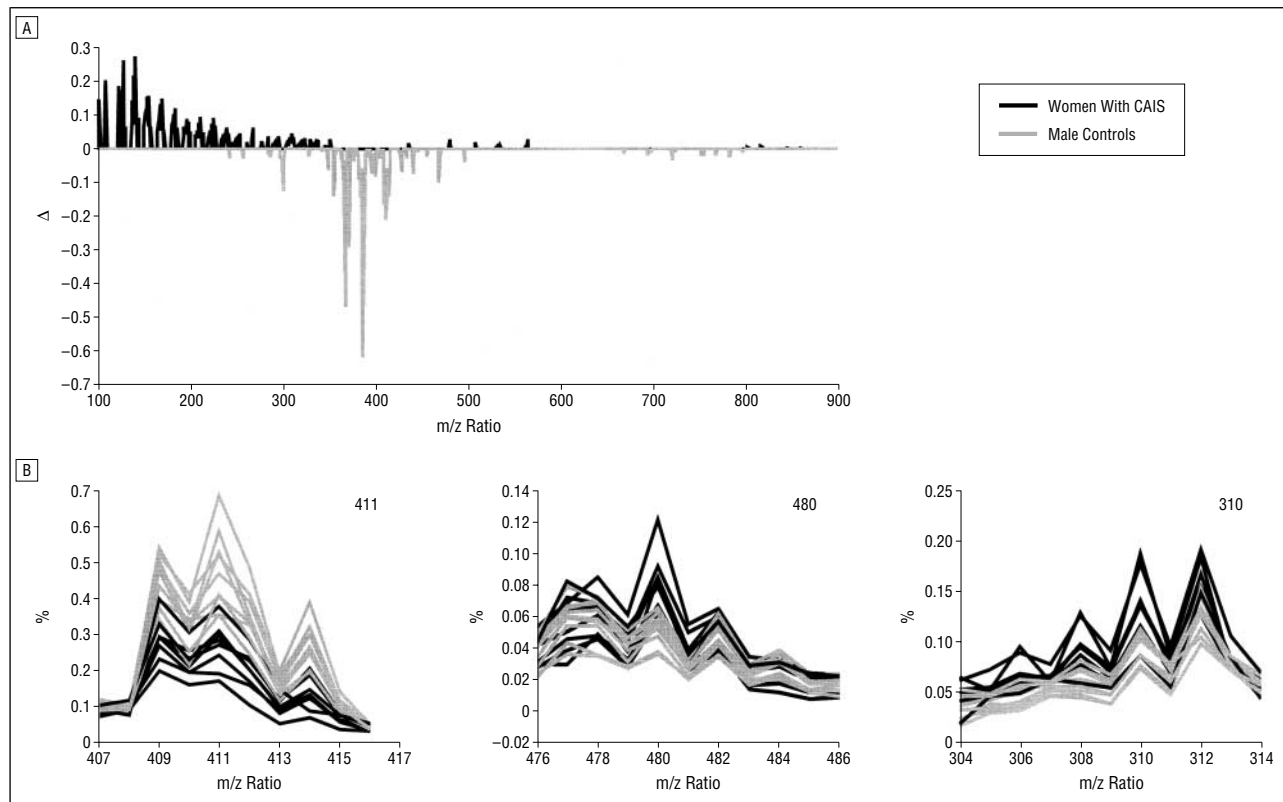
Figure 2. Effect of complete androgen insensitivity syndrome (CAIS) on the neutral lipid mass spectrum of meibomian gland secretions. Secretion samples were collected from the left and right eyes of women with CAIS (n=9) and age-related male controls (n=10) and were analyzed and compared as described in Figure 1 (legend).
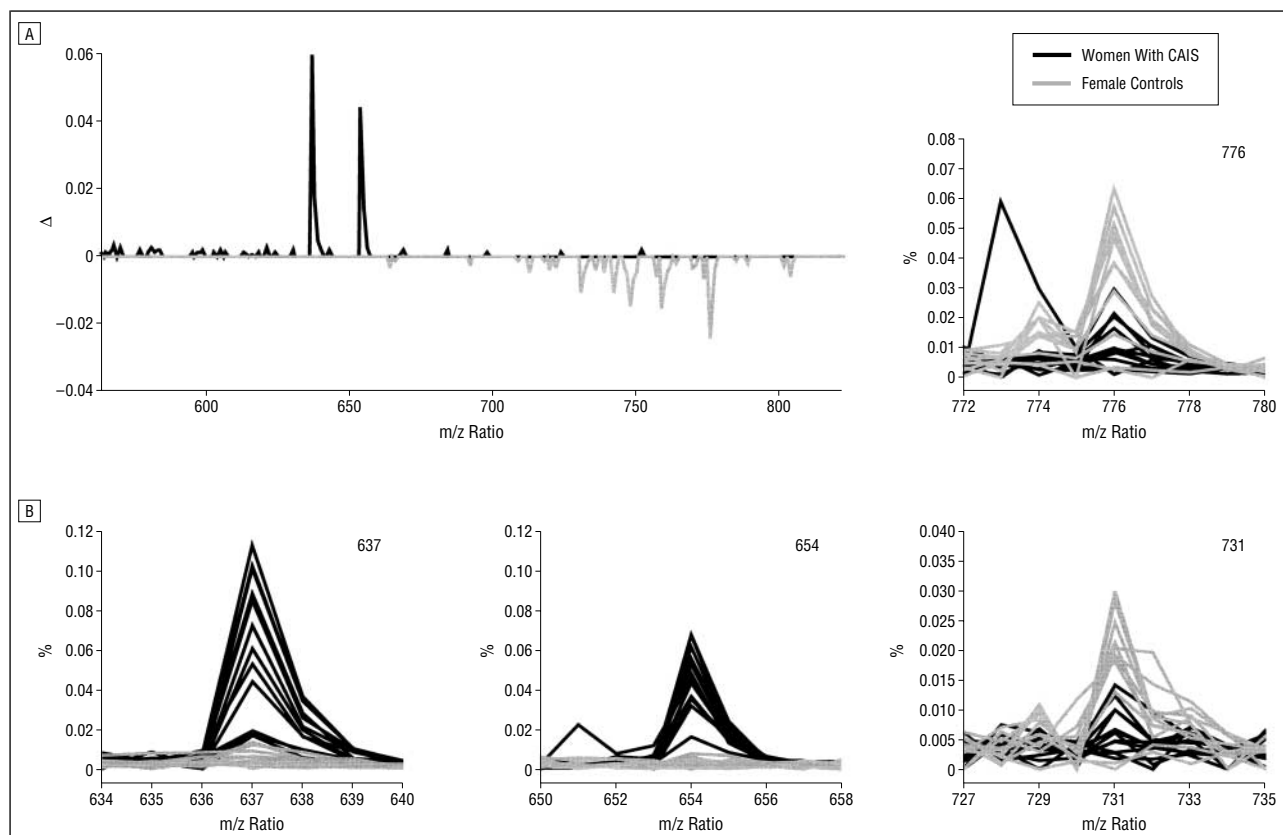

Figure 3. Effect of complete androgen insensitivity syndrome (CAIS) on the mass spectrum of phosphatidylethanolamine species in meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related female controls (n=9) and were analyzed and compared as described in Figure 1 (legend).
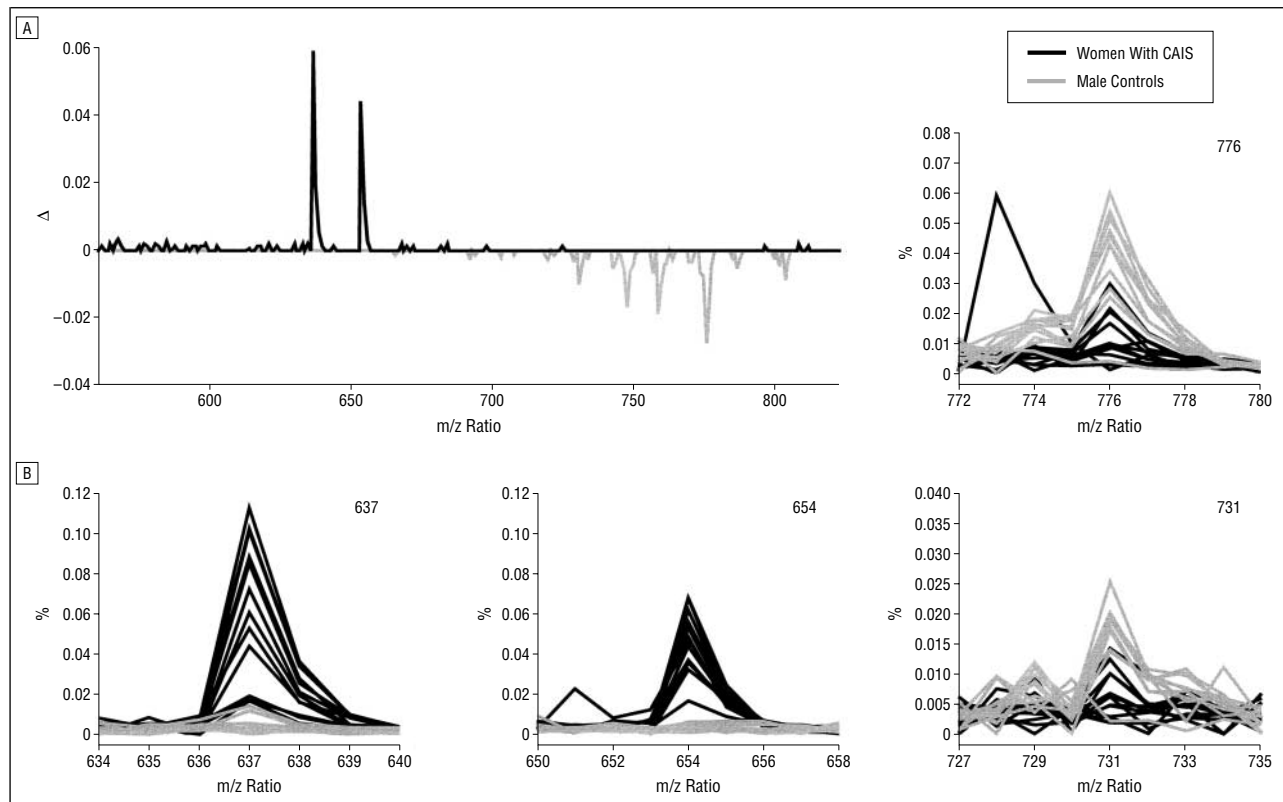
Figure 4. Effect of complete androgen insensitivity syndrome (CAIS) on the mass spectrum of phosphatidylethanolamine species in meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related male controls (n=10) and were analyzed and compared as described in Figure 1 (legend).
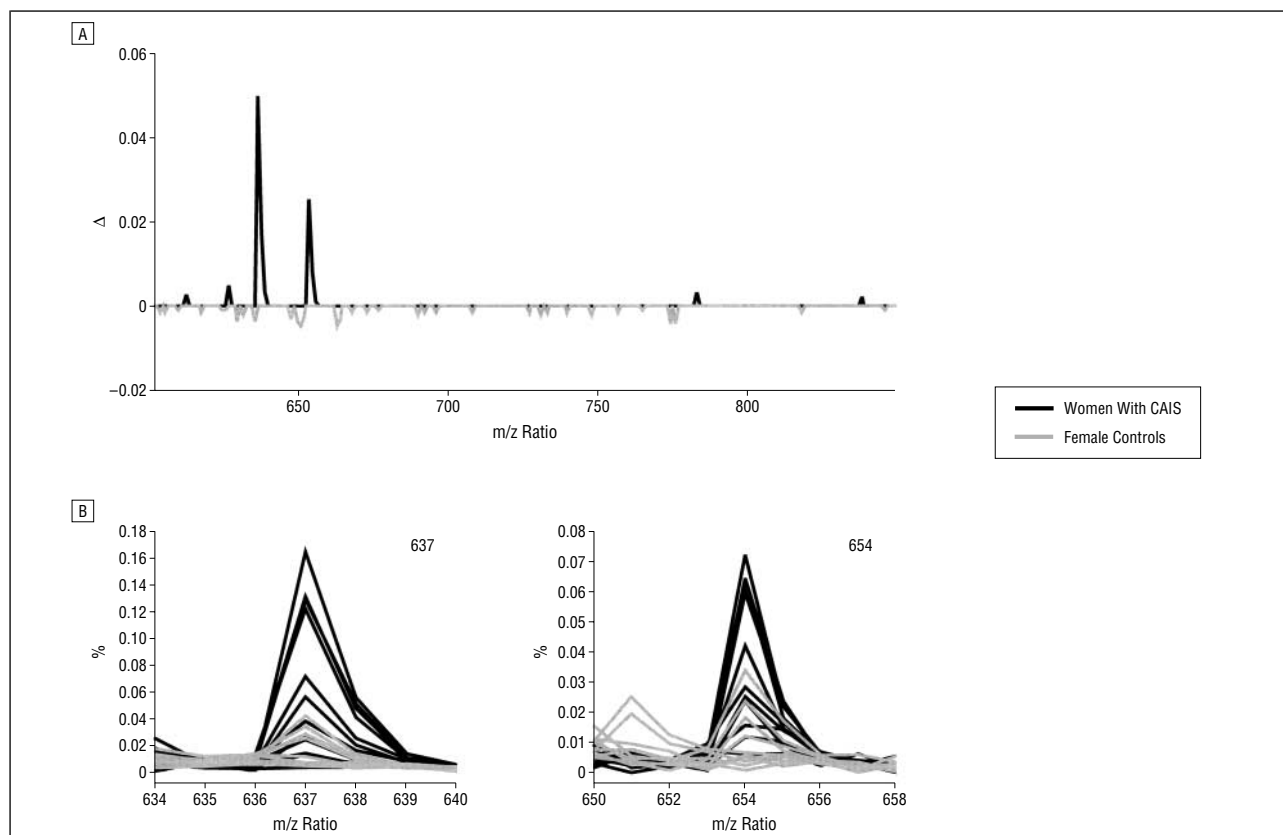

Figure 5. Effect of complete androgen insensitivity syndrome (CAIS), compared with age-related female controls, on the mass spectrum of phosphatidylcholine species in meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related female controls (n=9) and were evaluated and compared as described in Figure 1 (legend).
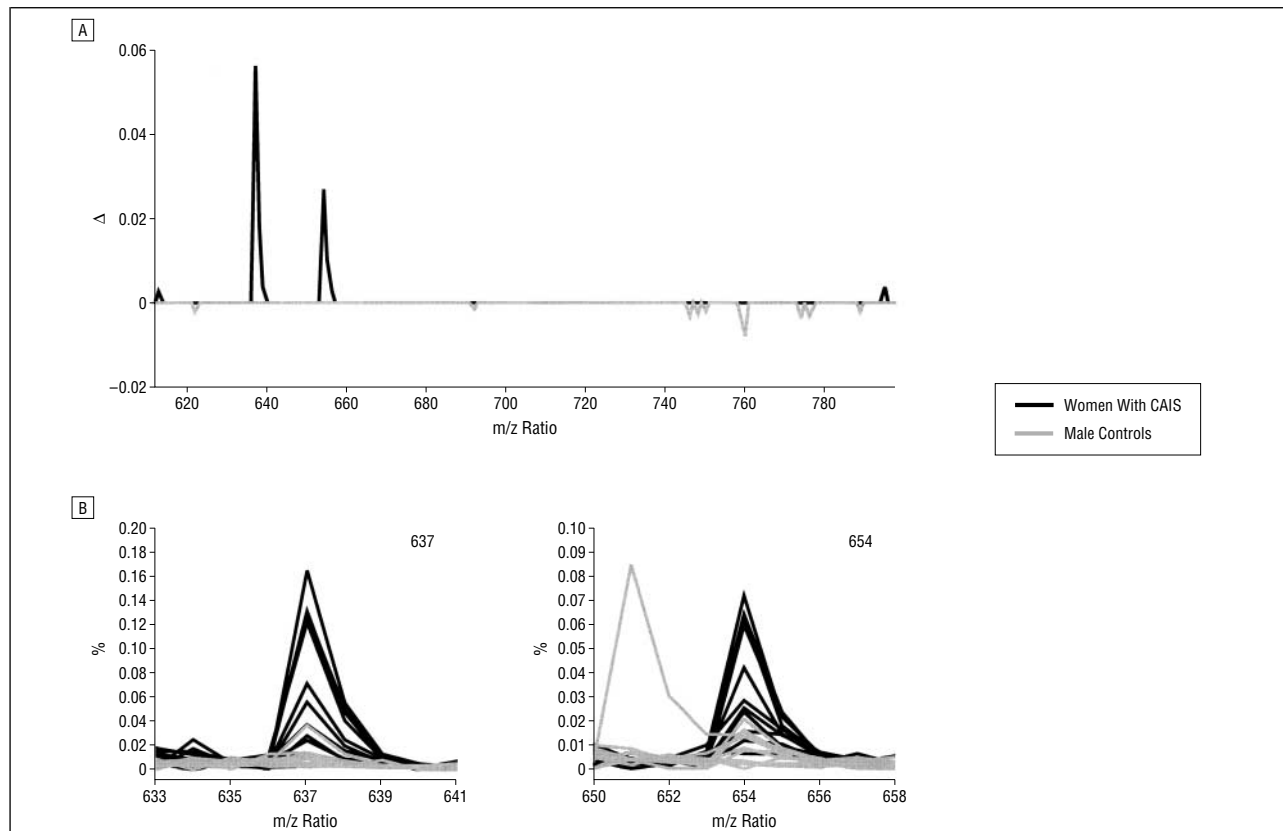
Figure 6. Effect of complete androgen insensitivity syndrome (CAIS) on the mass spectrum of phosphatidylcholine species in meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related male controls (n=10) and were analyzed and compared as described in Figure 1 (legend).

lipid species (eg, diglycerides and triglycerides) that are known to be affected by androgens[5]; and (3) the fact that only a small fraction of total neutral and polar m/z ratio data could be examined. Considering that androgen receptor dysfunction might affect expression of multiple lipids, and not just those related to preselected species, another approach was required to permit identification of possible widespread changes in lipid profiles.

Consequently, we developed the second analytic method, which was time invariant and allowed evaluation of all HPLC-MS data. This approach also identified substantial differences in lipid patterns of meibomian gland secretions between women with CAIS and female and male controls. However, these changes were primarily located in the polar lipid fraction and involved ions that were different from those detected with the first method (ie, windowed regions). Furthermore, the lipid variations between individuals with CAIS and female controls were not necessarily analogous to those between the CAIS and male control groups. These findings suggest that (1) androgen receptor dysfunction has a greater overall impact on polar, compared with neutral, lipids in meibomian gland secretions and (2) other factors, in addition to androgen receptors, affect lipid profiles in men and women.

The reason these 2 analytic methods did not identify changes in the same ions was undoubtedly because of the prevalence of these species in the windowed vs total ion spectra. In effect, the first approach targeted small, specific regions of the HPLC curve, thereby permitting comparison between groups of a small fraction of the fragmentation product data. Given that MS profiles were normalized by their individual sums to represent each m/z ratio as a percentage composition of the sample, this method would allow the analysis of relatively small peaks. In contrast, the second approach involved integration of all HPLC-MS data, and the sum-normalized results would have favored the identification of larger peaks. This latter method sacrificed the ability to detect very small peak differences, particularly given that the integration process summed noise as well. Thus, the ion variations observed with the first method may have occurred in peaks with relatively low prevalence and therefore became undetectable using the second method.

In a similar fashion, the third approach of evaluation, which was used solely for polar lipid assessment after direct-injection electrospray MS, would also be anticipated to identify new differences in FA product expression between groups. The reason is that this method analyzes the entire meibomian gland lipid secretion and is not subject to the loss of parts of the sample that occurs during HPLC. In addition, the direct-injection approach may ionize more fragments, does not linearize data (ie, as with HPLC), and is accompanied by very low noise. The result is that the rank order of the most prevalent ions would most likely

Table 1. Effect of CAIS on the Polar and Neutral Lipid Profile, as Defined by HPLC-MS, in Meibomian Gland Secretions*

Ion, m/z Ratio†	Mean Rank‡	Male Controls vs Women With CAIS		Female Controls vs Women With CAIS		Male Controls vs Female Controls	
		P Value	Δ/P§	P Value	Δ/P§	P Value	Δ/P§
Polar Lipid Profile							
366	1.04	.001	54.00	.08	0.49	.02	2.50
306	7.14	.001	11.10	.13	0.06	.03	0.23
448	8.82	.087	0.04	...	...	.02	0.33
367	8.93	.001	11.80	...	...	.01	0.77
425	17.07	.012	0.73	.04	0.11	.07	0.10
264	20.82	.030	0.12	.09	0.04	...	...
336	31.39	...	...	.01	1.11	*.02*	*0.84*
263	32.50	...	...	.08	0.09	...	...
394	37.11	.001	2.70	...	...	.009	0.20
262	40.82	...	...	.03	0.23	...	...
449	40.82	...	...	...	...	.02	0.07
570	41.29	*.001*	*0.60*	...	...	*.04*	*0.03*
359	45.32	...	...	.08	0.04	...	...
307	47.57	.001	1.50	...	...	.05	0.02
294	47.86	...	...	.003	1.30	...	...
426	48.82	...	...	...	...	.04	0.03
Neutral Lipid Profile							
112	1.96	...	...	*.08*	*0.01*	*.02*	*0.01*
100	3.27	...	...	...	...	*.04*	*0.02*
126	8.69	...	...	...	...	*.02*	*0.01*
114	9.77	...	...	*.03*	*0.01*	*.005*	*0.06*
447	10.38	...	...	.05	0.00	.009	0.01
123	12.15	...	...	*.03*	*0.01*	*.01*	0.00
138	16.35	...	...	*.06*	0.00	*.02*	*0.01*
128	18.58	...	...	...	...	*.02*	0.00

*CAIS indicates complete androgen insensitivity syndrome; HPLC-MS, high-performance liquid chromatography–mass spectrometry; m/z, mass-charge; ellipses, ions with $P \gg .05$. Meibomian gland secretions were obtained from all participants and processed for the analysis of polar and neutral lipids using HPLC-MS as described in the "Methods" section.

†Major ions that differed significantly ($P < .05$, 1- or 2-tailed) between groups.

‡The relative prevalence of the specified ion.

§The mean difference (Δ) between ion peaks of the 2 groups divided by the significant difference (P value) between the groups. The higher the number, the greater the difference and the smaller the P value. Values greater than 2.0 are generally considered very significant, whereas values less than 2.0 may be close to low-level noise. All ion-related values, except those that are italicized, were expressed to a greater extent in the first group of the comparison set (eg, ion 366, male control>CIAS); the italicized values were present in higher amounts in the second group (eg, ion 570, CAIS>male control).

Table 2. Effect of CAIS on the Polar Lipid Profile, as Defined by MS, in Meibomian Gland Secretions*

Ion, m/z Ratio†	Mean Rank‡	Male Controls vs Women With CAIS		Female Controls vs Women With CAIS		Male Controls vs Female Controls	
		P Value	Δ/P§	P Value	Δ/P§	P Value	Δ/P§
448	2.36	.001	57.30	...	...	.007	6.46
873	5.04	.001	70.70	...	...	.001	68.40
449	9.46	.001	12.80	...	...	.007	1.46
359	11.29	...	...	.003	9.53	*.06*	*0.44*
366	14.00	.003	8.43	...	...	.04	0.65
875	19.82	.001	7.70	...	...	.003	2.47
367	25.50	.002	2.90	...	...	.02	0.31
874	27.11	.001	30.60	...	...	.002	14.20
425	27.36	...	...	.004	1.10	*.04*	*0.10*
306	31.32	.005	1.66	...	...	.06	0.12
902	43.18	.001	5.80	.02	0.11	.001	4.20
476	59.57	.001	3.40	.07	0.02	.01	0.23
269	60.00	...	...	.001	4.10	*.01*	*0.21*
360	63.07	...	...	.03	0.13	*.06*	*0.08*
876	72.04	.001	2.40	.06	0.01	.008	0.23
761	72.07	...	...	.001	5.70	*.04*	*0.16*
762	74.18	...	...	.03	0.05	...	...

*CAIS indicates complete androgen insensitivity syndrome; MS, mass spectrometry; m/z, mass-charge; ellipses, ions with $P \gg .05$. Meibomian gland secretions were obtained from all participants and processed for the analysis of polar lipids using MS as described in the "Methods" section.

†Major ions that differed significantly ($P < .05$, 1- or 2-tailed) between groups.

‡The relative prevalence of the specified ion.

§The mean difference (Δ) between ion peaks of the 2 groups divided by the significant difference (P value) between the groups. The higher the number, the greater the difference and the smaller the P value. Values greater than 2.0 are generally considered very significant, whereas values less than 2.0 may be close to low-level noise. All ion-related values, except those that are italicized, were expressed to a greater extent in the first group of the comparison set; the italicized values were present in higher amounts in the second group.
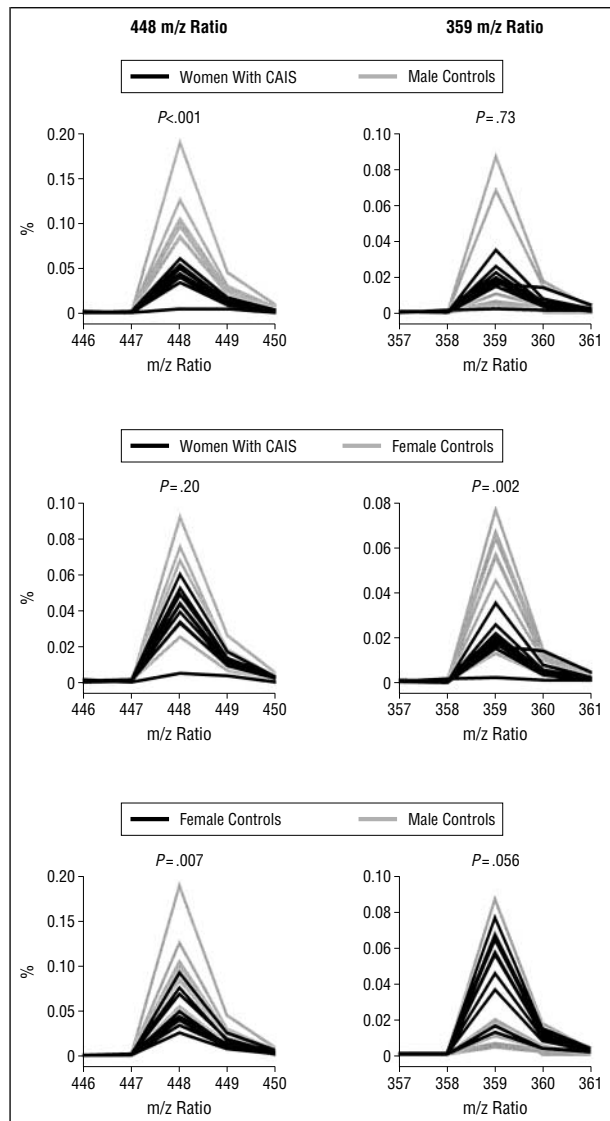
Figure 7. Effect of complete androgen insensitivity syndrome (CAIS) on the appearance of polar lipid fatty acid products in meibomian gland secretions. Secretion samples were obtained from the left and right eyes of women with CAIS (n=9) and age-related female controls (n=9) and male controls (n=10). Samples were analyzed by mass spectrometry and compared as explained in the "Results" section. m/z Indicates mass-charge.

Table 3. Average Correlation Coefficients (ACCs) Within and Between the Polar Lipid Mass Spectra of Meibomian Gland Secretions From Women With CAIS and Age-Matched Male and Female Controls*

	ACC		
	Internal 1	Internal 2	Between Groups
Male controls vs women with CAIS	0.733	0.650	0.586
Female controls vs women with CAIS	0.673	0.650	0.558
Male vs female controls	0.733	0.673	0.530

*CAIS indicates complete androgen insensitivity syndrome. Meibomian gland secretions were obtained from the left and right eyes of all participants and then processed for mass spectrometry, analysis of mass spectra, and calculation of ACCs within and between groups. An ACC of 1.0 indicates an exact match of lipid patterns across all mass-charge ratios; 0.5, a random distribution of well-correlated and uncorrelated patterns in a single set; and 0.0, a completely uncorrelated set of profiles.

elegant, composition-based model of the tear film lipid layer that highlights the interactions between lipid classes and discusses the importance of FA chain length, unsaturation, and hydroxylation in maintaining the thixotropic and barrier properties of this layer.[11] In addition, this model places particular emphasis on the structural characteristics of specific polar lipids and their critical role in promoting the surfactant nature and stability of the tear film.[11,14] Thus, translation of the m/z ratio data into the identification of specific lipid species would help clarify how androgen action, or the lack thereof, might affect the structure and function of the tear film lipid layer.

The findings of the present study also show that sex-related differences exist in the m/z ratios of a variety of neutral and, especially, polar FA products in meibomian gland secretions. These differences, which were demonstrated when analyzing all HPLC and MS data, may be due to several factors. First, sex or sex steroids (ie, androgens, estrogens, and progestins) seem to modulate the expression of multiple genes in the meibomian gland, including those encoding lipogenic and steroidogenic enzymes, transcription factors involved in the coordinate regulation of lipid production, receptors, and growth factors (Frank Schirra, MD, Tomo Suzuki, MD, PhD, Stephen M. Richards, BS, Meng Liu, MD, PhD, D.A.S., unpublished data, 2002).[39-42,45] These sex and hormone effects could be translated into variations in lipid structure or composition, which could then theoretically lead to differences in fragmentation pathways and resulting ions. Second, some effects of sex steroids (eg, androgens) on the meibomian gland may be sex specific, given that certain actions of these hormones on the accumulation of particular lipid species have been shown to occur only in males or females.[46,47] These sex-associated responses could also lead to differences in ion expression. Third, we[6,48] previously showed that the polar lipid profiles in meibomian gland secretions of women are not uniform and seem to fall into 2 major patterns. This dichotomy, which was not found in secretions of men, also seemed to have a significant impact on the profile of neutral lipids.[7] Fourth, the meibomian gland is innervated,[49-]

be dissimilar between MS and HPLC-MS. This anticipated result was found. Overall, the third method demonstrated that distinct differences exist in the polar lipid patterns of meibomian gland secretions of women with CAIS relative to those of female and male controls, and it allowed the identification of previously undetected variations in ion expression.

A limitation in all of these analytic approaches is that our evaluations focus on the m/z ratios of neutral and polar lipids but do not address their specific identity, possible interrelationships, or potential function in the tear film lipid layer. Many of the different ions may originate from larger FA species, and identification of the various fragmentation products and their precursors might be facilitated by the use of MS-MS technology. If so, this information would be of particular interest given that researchers have recently proposed an

[52] and neurotransmitters are known to affect lipid synthesis in sebaceous glands.[53] Consequently, if sex-related differences exist in the pattern of neural innervation of the meibomian gland, as found with other ocular and exocrine tissues,[54] then this might also contribute to variations in lipid expression between men and women. Of interest, this sex effect on meibomian gland lipids is not limited to the qualitative pattern of molecular species. Studies have also shown that the casual levels of meibomian gland lipids on the lid margin are higher in males than females from puberty until age 50 years.[55]

In summary, our results suggest that androgens act through androgen receptors to modulate lipid production in the meibomian gland. It is possible that this mechanism of action may account for the reported ability of topical androgen administration to increase the synthesis and release of meibomian gland lipids, prolong the tear film breakup time, and alleviate dry eye (M. A. Zeligs, K. Gordon, Dehydroepiandrosterone therapy for the treatment of dry eye disorders, Int Patent Application WO 94/04155, 1994).[3,56]

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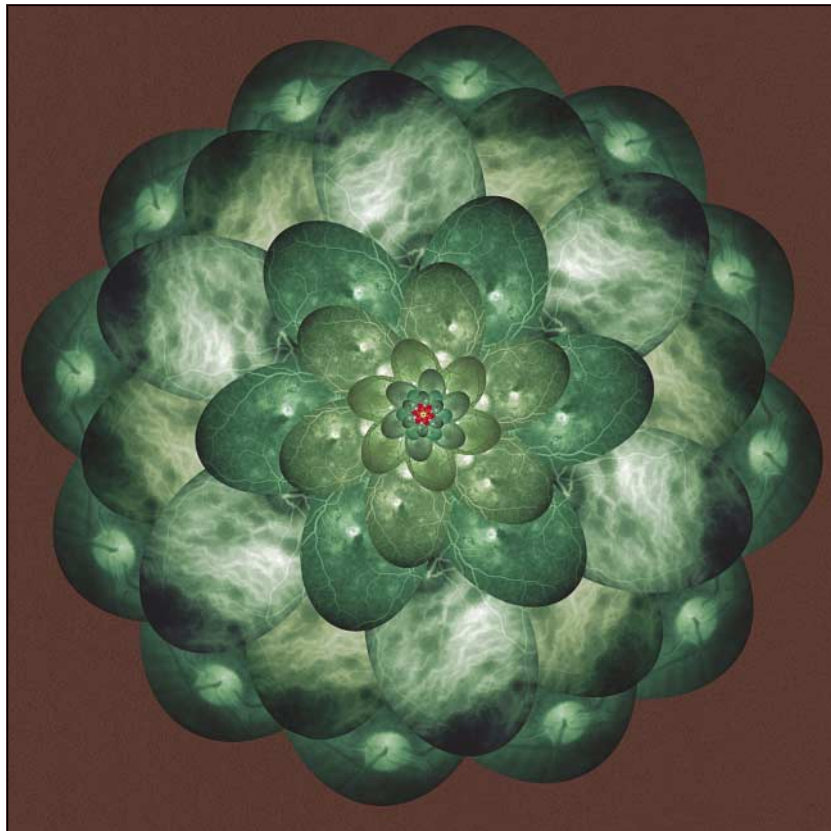
Fundus Flower II. Created by Patrick J. Saine, MEd, CRA, Dartmouth-Hitchcock Medical Center, Lebanon, NH.