Association Between Serum Levels of Testosterone and Estradiol With Meibomian Gland Assessments in Postmenopausal Women

Anna F. Ablamowicz, Jason J. Nichols, and Kelly K. Nichols

School of Optometry, University of Alabama at Birmingham, Birmingham, Alabama, United States

Correspondence: Kelly K. Nichols, University of Alabama at Birmingham, 1716 University Boulevard, HPB 112, Birmingham, AL 35294-0010, USA; nicholsk@uab.edu.

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Purpose. The aims of this analysis were to determine if there is an association between serum levels of testosterone and estradiol with meibomian gland (MG) morphology and lipid layer thickness.

Methods. The data used for this analysis were collected from postmenopausal women with and without dry eye disease. Meibography was used to assess MG dropout on the central two-thirds of the eyelid and biomicroscopy was used for assessing MG expressibility and meibum quality. Venous blood samples were drawn for serum hormone level analysis. The Kruskal-Wallis test and Spearman correlations were used for statistical analysis.

Results. One hundred ninety-eight postmenopausal women with an average age of 61.2 ($\pm$9.1) years were included in this analysis. Testosterone levels showed significant differences between MG dropout grades 1 and 4 ($P = 0.002$) and grades 2 and 4 ($P = 0.01$), whereas estradiol levels were different based on MG dropout ($P = 0.53$). No significant correlations were found between testosterone ($r = 0.10$, $P = 0.17$) or estradiol ($r = 0.05$, $P = 0.45$) and lipid layer thickness.

Conclusions. Testosterone levels were increased with MG dropout, which was significant between the mild and severe dropout groups, whereas no significant differences were found with estradiol and any MG assessment. Although the literature suggests an association of serum hormone levels and pathogenesis of dry eye disease in postmenopausal women, analysis of active sex steroid precursors and local tissue hormone levels may prove more useful.

Keywords: dry eye, postmenopausal women, meibomian gland, testosterone, estradiol

Meibomian glands (MGs) are sebaceous glands located in the tarsal plates of the upper and lower eyelids with orifices found along the lid margins anteriorly to the mucocutaneous junction.[1] Clusters of secretory acini are connected to a central duct and arranged in a circular fashion to make up one MG. Meibocytes within the secretory acini are filled with an oily product known as meibum, which is transported from the acini to the central duct and forced upwards to be secreted from the orifice. This secretion force arises from both a secretory pressure created by meibum production and compression pressure created by the muscular action of the orbicularis and muscle of Riolan that occurs during blinking.[1] Meibum consists primarily of wax and sterol esters and is secreted as a clear oil directly into the tear film creating the lipid layer.[2] This lipid layer on the anterior surface of the tears serves to slow the evaporation of the tear film and stabilize it by lowering surface tension.[3]

The current definition for meibomian gland dysfunction (MGD) states it as a diffuse, chronic abnormality of the MGs characterized by glandular duct obstruction and/or changes in meibum secretion.[4] Meibomian gland dysfunction is considered to be one of the leading causes of evaporative dry eye disease.[5] The prevalence of MGD is estimated to range widely from 3.5% to 69% with a higher prevalence in Asian populations (60%–69%) and lower prevalence in Caucasians (3.5%–19.9%) in adults older than 40 years.[6] Hormonal changes, age, systemic conditions, and environment are all factors in the pathogenesis of MGD.[7]

Meibomian gland dysfunction can onset in a nonobvious form with lack of inflammatory signs in a hyposecretory obstructive phase.[8] Obstructive MGD is caused by hyperkeratinization of the duct and orifice, which may be brought on my aging, hormonal changes, medications, or contact lens wear.[9] It is hypothesized that hyperkeratinization blocks meibum secretion, leading to a buildup of pressure that damages the gland and leads to atrophy and loss of meibocytes.[1] Meibomian gland dysfunction can also cause alterations in meibum composition possibly due to bacterial lipases acting on the lipid and creating free fatty acid molecules that irritate the lid margin and stimulate keratinization.[10] Regardless, hyposecretion and/or alteration in composition of meibum can lead to increased evaporation of the tear film, which may in turn cause evaporative dry eye disease.[11,12]

Minimal assessment for diagnosis of MGD includes observation of MG expressibility and evaluation of meibum quality in addition to noting any abnormalities in the tear film and/or in the appearance of the eyelid margin (e.g., scalloping, hyperemia) that may indicate MG atrophy or dropout.[13] Meibomian gland expressibility is assessed relative to the amount of pressure needed for meibum secretion to occur.[14] Healthy,

clear meibum expresses with relatively low pressure, whereas paste-like meibum in MGD requires more pressure and higher melting temperature.[15] The quality of the meibum expressed is evaluated on clarity and consistency.[16] Assessment of the tear film in patients with MGD may reveal a reduced tear film break-up time and thinner lipid layer.[17] Meibomian gland dropout can be seen by directly observing a scalloped lid margin with divots indicative of gland loss or damage, or through imaging of the glands through an everted eyelid known as meibography.[13]

Regulation of production and secretion of meibum by the MGs is influenced by hormonal, neural, and mechanical factors.[1] Both androgens and estrogens regulate the meibomian glands through hormone binding to receptors.[18,19] Sex steroid receptors for androgens[20] and estrogen[21,22] have been identified on the meibomian glands that may have an influence on synthesis of meibum.[23] Where androgens stimulate synthesis and secretion of lipids from the MGs, estrogens cause a decrease in lipid production.[23,24] Furthermore, testosterone deficiency has been shown to promote meibomian gland dysfunction especially in patients taking antiandrogen therapy.[18,25] These patients showed changes in the appearance of the meibomian glands as well as a reduced quality of meibum secretion.[19] Ocular surface effects of estrogens on the meibomian glands are more controversial, although it is thought that a high level of serum estradiol is a risk factor for dry eye[26] through its suppression of lipid synthesis and promotion of lipid catabolism in the meibomian glands.[23]

Epidemiologic studies indicate a higher prevalence of dry eye in postmenopausal women.[27,28] Menopause induces changes in hormone levels throughout the body, including decreases in testosterone and estrogen.[29] Therefore, it is possible that dry eye in postmenopausal women might be explained by these changes in circulating sex steroids. The purpose of this analysis was to determine if there is an association between serum levels of testosterone and estradiol and meibomian gland assessment of dropout, expressibility, and meibum quality along with tear film lipid layer thickness.

Methods

Subject Selection

Postmenopausal (defined as at least 1 year since last menses and confirmed with serum hormone analysis) women were enrolled in the Dry Eye in Menopause cross-sectional study (National Eye Institute EY015519). This research was conducted in accordance with the Declaration of Helsinki with Institutional Review Board approval. Informed consent was obtained from subjects after explanation of the nature and possible consequences of the study. Subjects currently taking prescription medication for ocular disease were excluded from participation. Subjects who had ocular surgery within the previous year were also excluded. Only subjects with normal lid anatomy and position with no other anterior segment disease, including ocular allergies, significant anterior blepharitis, corneal infections, pterygia, and inflamed pinguecula, were included. Women on hormone replacement therapy (HRT) were included as long as there were no changes in HRT over the previous month. Subjects were classified as having dry eye on the basis of the Schaumberg questionnaire administered at the beginning of the study visit.[27] Subjects were asked the following questions: (1) have you been diagnosed with dry eye, (2) how often do you experience eye dryness, and (3) how often do your eyes feel irritated. Questions 2 and 3 included possible responses of "constantly," "often," "sometimes," or "never." Subjects reporting a previous clinical diagnosis of dry eye or severe symptoms (both symptom question responses as either constantly or often) were categorized into the dry eye group.

Ocular Examination/Procedures

All subjects were administered a reproductive history questionnaire including a medical history survey. Slit-lamp examination included MG evaluation and imaging of the tear film and MGs. Meibomian gland evaluation was performed by trained examiners using a Haag-Streit BX 900 slit-lamp biomicroscope (Koeniz, Switzerland) according to the method suggested by Foulks and Bron.[9] Meibomian gland expressibility was determined by using digital pressure on the tarsus of the lower lid. Pressure was applied for at least 5 seconds with a gradual increase of force until meibum was secreted from the glands in the central lower lid. The central 10 glands in the lower lid were assessed for both expressibility and meibum quality. The expressibility scale was based on the amount of pressure needed to express the glands and was scored as follows: 0 = minimal pressure, 1 = mild pressure, 2 = moderate pressure, and 3 = heavy pressure needed for expression. Meibum quality based on color and viscosity was scored as follows: 0 = clear, 1 = cloudy, 2 = granular, 3 = solid and paste-like, and 4 = no meibum expressed.

Meibomian Gland Imaging

Images of the meibomian glands in the lower eyelids were taken by using an AVT Stingray camera (Allied Vision Technologies, Newburyport, MA, USA) attached to a Haag-Streit BX 900 slit lamp. The lower eyelid was everted over a transillumination light guide consisting of a 20-gauge disposable fiber optic light allowing for visualization of MG ducts and acini.[30,31] The focus was set on the central 10 glands in the lower lid, using ×10 magnification. Images were recorded and saved for subsequent MG dropout analysis. Meibomian gland dropout is total or partial loss of acinar tissue that can be detected by meibography.[13] Dropout was assessed on the central two-thirds of the lower lid and was graded by the portion of the lid containing partial or missing glands. The scale used was as follows: 1 = no partial or missing glands were visible, 2 = less than 25% of the image contained partial meibomian glands, 3 = between 25% and 75% of the image contained partial meibomian glands, and 4 = more than 75% of the image contained partial meibomian glands.[32]

Lipid Layer Thickness

Spectral interferometry was used to measure lipid layer thickness as described previously.[33] An optical system designed by using a white light point source was used to focus a 33-μm diameter spot onto the tear film. Lipid layer thickness was assumed to be uniform within the measurement spot. The light reflected from the tear film and layers was captured by using a spectrophotometer where the intensity of reflectance was recorded. Based on the interference theory of thin films, reflectance captured increases as a function of lipid layer thickness and wave number and so Fourier analysis of the reflectance spectra was used to determine the thickness of the lipid layer.

Blood Samples

Two tubes of venous blood (10 mL) were drawn and were analyzed for serum hormone levels including testosterone (with sex hormone-binding globulin [SHBG] and albumin for free testosterone) and estradiol (E2; with E2/SHBG to estimate bioavailable E2).

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Table 1. Subject Demographics

Parameters	Dry Eye, $n = 74$	Non–Dry Eye, $n = 124$
Demographics		
Age, y	61.4 ± 9.2	61.0 ± 9.2
Years since last regular menstrual bleeding, y	15.1 ± 15.1	14.5 ± 12.3
% Caucasian	68.9	78.2
% African American	31.1	20.2
% Asian	–	1.6
Meibomian gland assessments		
MG dropout, median	2	2
MG expressibility, median	2	1
Meibum quality, median	1	1

Statistical Analysis

Analysis of associations between serum hormone levels, MG assessments, and lipid layer thicknesses was performed. Within-group differences in skewness and normality testing with the Kolmogorov-Smirnov test revealed nonnormally distributed data. Nonparametric testing included the Kruskal-Wallis test and Spearman rank coefficients were applied by using SAS 9.4 (Cary, NC, USA).

Results

There were 198 women included in this study analysis with an average age of 61.2 ± 9.1 years. These women were identified as postmenopausal on the basis of subject-reported 12 months amenorrhea and confirmed with serum hormone levels as follicle-stimulating hormone < 30 IU/L and estradiol < 73 pmol/L based on the definition by Sherman.[34] Of these subjects, 74 were classified as dry eye and 124 were classified as normal (Table 1). As shown in Table 2, the median serum testosterone levels were 22.94 ng/dL for the dry eye group and 20.53 ng/dL for the normal group ($KW = 1.67$, $P = 0.20$). Likewise, the median serum estradiol levels were 10.34 pg/mL for the dry eye group and 8.38 pg/mL for the normal group ($KW = 0.41$, $P = 0.52$). Analysis of subgroups was performed to consider whether subjects had received HRT in the 3 months before being seen for the study to account for the possibility of synthetically controlled hormone levels skewing the results. As shown in Table 3, the median serum testosterone levels were 21.86 ng/dL for the dry eye group and 20.24 ng/dL for the control group within the no-HRT subgroup ($KW = 1.79$, $P = 0.18$), while the levels were 24.67 ng/dL for the dry eye group and 27.27 ng/dL for the normal group in those who had received HRT ($KW = 0.0014$, $P = 0.97$). Similarly, the median estradiol levels were 10.33 pg/mL for the dry eye group and 8.43 pg/mL for the control group within the no-HRT subgroup ($KW = 0.34$, $P = 0.56$), while the levels were 14.00 pg/mL for the dry eye group and 8.13 pg/mL for the normal group in those who had received HRT ($KW = 0.32$, $P = 0.57$).

The average serum levels for both testosterone and estradiol by MG dropout severity are shown in Table 4. The median testosterone levels for MG dropout gradings of 1, 2, 3, and 4 were 21.06, 19.36, 21.70, and 20.06 ng/dL, respectively ($KW = 9.23$, $df = 3$, $P = 0.03$). Post hoc pairwise comparisons showed significant differences in testosterone levels between the MG dropout scores of 1 and 4 ($KW = 9.22$, $P = 0.002$) and between scores 2 and 4 ($KW = 6.31$, $P = 0.01$). The median estradiol levels for MG dropout gradings of 1, 2, 3, and 4 were 8.15, 10.4, 8.51, and 9.35 pg/mL, respectively ($KW = 2.24$, $df = 3$, $P = 0.53$). As shown in Table 5, the median testosterone levels for MG expressibility scores of 0, 1, 2, and 3 were 20.94, 18.78, 27.12, and 24.01 ng/dL, respectively ($KW = 5.25$, $df = 3$, $P = 0.15$). Likewise in Table 5, the median estradiol levels for MG expressibility scores of 0, 1, 2, and 3 were 9.73, 8.51, 9.05, and 9.46 pg/mL, respectively ($KW = 1.37$, $df = 3$, $P = 0.71$). As shown in Table 6, the median testosterone levels for meibum quality gradings of 0, 1, 2, 3, and 4 were 20.73, 22.70, 18.35, 26.99, and 26.38 ng/dL, respectively ($KW = 7.21$, $df = 4$, $P = 0.13$). Also in Table 6, the median estradiol levels for meibum quality gradings of 0, 1, 2, 3, and 4 were 9.64, 8.03, 11.28, 14.54, and 8.08 pg/mL, respectively ($KW = 8.23$, $df = 4$, $P = 0.08$).

Spearman correlation coefficients for all variables are shown in Table 7. A positive correlation was found between meibum quality and MG expressibility ($r = 0.4$, $P < 0.0001$) and between meibum quality and MG dropout ($r = 0.28$, $P < 0.0001$). A positive correlation was also found between testosterone and MG dropout ($r = 0.2$, $P = 0.005$) and between testosterone and estradiol ($r = 0.27$, $P < 0.0001$). Dry eye status was found to be statistically correlated with expressibility ($r = 0.14$, $P = 0.04$) and lipid layer thickness ($r = 0.17$, $P = 0.01$). Although positive correlations were found between testosterone ($r = 0.10$, $P = 0.17$) and estradiol ($r = 0.05$, $P = 0.45$) with lipid layer thickness, these correlations were not statistically significant. Median (M) values for lipid layer thicknesses for the dry eye ($M = 35.32$ nm) and normal ($M = 31.58$ nm) groups are shown in Table 8.

Discussion

Recent studies in the literature suggest that hormones may influence the meibomian glands, and hormonal changes during and after menopause may impact the development of dry eye disease in women.[35] The results of this study showed that although average values of both testosterone and estradiol were higher in the dry eye group, the differences were not statistically significant even when accounting for whether the subjects had received HRT in the 3 months before the study visit. Testosterone levels increased with increased MG dropout, which was significant between the mild and severe dropout groups. No significant differences in testosterone or estradiol levels between MG expressibility and meibum quality gradings were found.

On average, normal premenopausal testosterone levels range from 15 to 70 ng/dL and estradiol levels range from 30 to 400 pg/mL.[36,37] In the postmenopausal period, testosterone levels decrease to between 20 to 30 ng/dL and estradiol levels decrease to between 0 to 30 pg/mL.[36,38,39] Median serum testosterone and estradiol levels in both groups in this analysis, although reduced from premenopausal levels, were in the middle of the normal ranges for postmenopausal women. Thus, it is possible that significant differences between the dry eye and normal groups may have been found if the hormone levels had been abnormal. Also, classification of the subjects into dry eye and normal groups did not necessarily take into account clinical signs of dry eye. A case-control study by Gagliano et al.[40] in postmenopausal women with and without severe evaporative dry eye, based on clinical signs, has found lower levels of estradiol and testosterone in the dry eye group than the control group. However, a review by Labrie et al.[41] suggests that because androgens and estrogens are produced in the target tissues from inactive precursors during the postmenopausal period (as opposed to in the adrenal or reproductive organs pre menopause), these steroids exert activity directly in the tissue they are synthesized in with little diffusion into the blood circulation. Thus, interpretations of

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Table 2. Average Testosterone and Estradiol Levels for Both Groups

Group	*N*	Mean	SD	Median	Kruskal-Wallis	
Testosterone, ng/dL						
Dry eye	74	27.48	16.70	22.94	$KW = 1.67$	$P = 0.20$
Normal	124	25.37	15.70	20.53		
Estradiol, pg/mL						
Dry eye	74	14.81	20.45	10.34	$KW = 0.41$	$P = 0.52$
Normal	124	11.64	9.35	8.38		

Table 3. Average Testosterone and Estradiol Levels for Both Groups According to Whether Subjects Had Received HRT in the Past 3 Months

	Dry Eye		Normal			
	N	*M*	*N*	*M*	Kruskal-Wallis	
Testosterone, ng/dL						
No HRT	65	21.86	113	20.24	$KW = 1.79$	$P = 0.18$
HRT in past 3 mo	9	24.67	11	27.27	$KW = 0.0014$	$P = 0.97$
Estradiol, pg/mL						
No HRT	65	10.33	113	8.43	$KW = 0.34$	$P = 0.56$
HRT in past 3 mo	9	14.00	11	8.13	$KW = 0.32$	$P = 0.57$

Table 4. Testosterone and Estradiol Levels According to MG Dropout Severity

	Meibomian Gland Dropout			
	1, $n = 45$	2, $n = 67$	3, $n = 47$	4, $n = 39$
Testosterone, ng/dL				
Mean ± SD	23.34 ± 15.80	25.05 ± 16.37	25.99 ± 14.79	31.50 ± 16.70
Median	21.06*	19.36†	21.70	30.06*†
Estradiol, pg/mL				
Mean ± SD	11.09 ± 9.74	12.82 ± 10.38	11.47 ± 8.22	16.47 ± 26.46
Median	8.15	10.4	8.51	9.35

*† $P < 0.05$.

Table 5. Testosterone and Estradiol Levels According to MG Expressibility

	Meibomian Gland Expressibility			
	0, $n = 50$	1, $n = 53$	2, $n = 56$	3, $n = 39$
Testosterone, ng/dL				
Mean ± SD	26.73 ± 19.18	22.15 ± 12.15	28.26 ± 16.62	27.83 ± 15.19
Median	20.94	18.76	27.12	24.01
Estradiol, pg/mL				
Mean ± SD	13.04 ± 12.17	13.51 ± 11.17	12.61 ± 19.08	11.92 ± 14.35
Median	9.73	8.51	9.05	9.46

Table 6. Testosterone and Estradiol Levels According to Meibum Quality

	Meibum Quality				
	0, $n = 67$	1, $n = 79$	2, $n = 28$	3, $n = 11$	4, $n = 13$
Testosterone, ng/dL					
Mean ± SD	23.74 ± 14.72	26.87 ± 16.0	23.84 ± 14.74	37.32 ± 20.89	29.82 ± 18.85
Median	20.73	22.70	18.35	26.99	26.38
Estradiol, pg/mL					
Mean ± SD	14.13 ± 19.01	11.26 ± 9.89	13.13 ± 9.54	20.37 ± 25.22	8.54 ± 3.29
Median	9.64	8.03	11.28	14.54	8.08

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Table 7. Spearman Rank Correlations for MG Expressibility, Meibum Quality, Dropout, Dry Eye Status (0 = Control, 1 = Dry Eye), Testosterone, Estradiol, and Lipid Layer Thickness

Variable	Expressibility	Meibum Quality	Dropout	Status	Testosterone	Estradiol
Expressibility	–	–	–	–	–	–
Meibum quality	0.40*	–	–	–	–	–
Dropout	0.42*	0.28*	–	–	–	–
Status	0.14*	0.07	0.10	–	–	–
Testosterone	0.11	0.11	0.20*	0.09	–	–
Estradiol	−0.07	0.02	0.04	0.05	0.27*	–
Lipid layer thickness	−0.01	−0.02	0.06	0.17*	0.10	0.05

* $P < 0.05$.

correlations of serum estrogen and androgen levels with target tissue effects seen clinically may be limited.

In this study, meibography images of the lower eyelids were used in assessing the extent of MG dropout. There have been studies that demonstrate a difference in the morphology of the MGs when comparing the upper and lower eyelids, namely, that the MGs in the lower lid are typically wider than the upper lid and the number of MGs is greater in the upper lid.[1,42] However, statistically significant and clinically meaningful correlations between MG dropout in either upper or lower eyelid with dry eye status can still be made.[42–44] Moreover, while examining both eyelids for MGD is desired, it is common practice clinically to examine the lower eyelid for MG atrophy and meibum expression owing to the ease in lid eversion and less patient discomfort. From this knowledge and previous studies that used meibography focused on the lower lid only to investigate alterations to the MGs, we used the lower eyelids for this analysis.[14,32,45,46]

Our analysis shows that testosterone levels were increased with increased MG dropout, which was significant between the mild and severe as well as between the moderate and severe MG dropout groups. While no statistically significant differences were found between estradiol levels and MG dropout severity, there was an apparent increase in average estradiol level for the severe MG dropout group; however, the standard deviation for that value was significantly higher than for the other groups, indicative of outlying and possibly erroneous values confounding the statistical significance. Testosterone is able to downregulate keratinization genes in the MG, which was shown in a mouse model by Schirra et al.[47] Thus, testosterone may have a protective effect of slowing or preventing MG damage and dropout.

Although the pathophysiology behind MG expression and meibum quality is not well elucidated, it is reasonable to assume that if the quality of the meibum is such that it is more viscous and paste-like, the expressibility of the gland may require more pressure. Indeed, this analysis seems to suggest that as gland expressibility score increased, meibum quality score also increased, which does indicate that the more viscous meibum required more pressure for secretion. Subsequently, the force necessary to express the viscous meibum may be greater than what the eyelid is able to exert from normal blinking, which may contribute to hyposecretion of lipid into the tear film.[48] Surprisingly, subjects in the dry eye group in this analysis had a thicker lipid layer, which may be a result of testosterone promotion of lipid synthesis.[24]

Table 8. Lipid Layer Thicknesses for the Dry Eye and Normal Groups

	Lipid Layer Thickness, nm			
	N	Mean	SD	Median
Dry eye	74	46.81	28.22	35.32
Normal	124	37.10	20.79	31.58

In conclusion, this study showed the variability in serum hormone levels in postmenopausal women with and without dry eye and clinical signs of MGD. Further investigation of the hormonal effects on the tear-producing glands and the extent of ocular hormone distribution and bioavailability could elucidate new mechanisms of dry eye in postmenopausal women.

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