Androgen Influence on the Meibomian Gland

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PURPOSE. The hypothesis in the study was that androgens control meibomian gland function, regulate the quality and/or quantity of lipids produced by this tissue, and promote the formation of the tear film’s lipid layer. To test this hypothesis, a study was conducted to determine whether androgen receptor protein exists in the epithelial cell nuclei of rat meibomian glands and, in addition, whether androgen deficiency and/or treatment influences the gross morphology, neutral lipid content, and fatty acid profile of the rabbit meibomian gland, as well as the appearance of the tear film lipid layer.

METHODS. Rat lids were obtained and processed for immunohistochemistry. Meibomian glands from intact, androgen- and/or placebo-treated rabbits were analyzed by histology, and glandular lipids were evaluated by gas chromatography, high-performance liquid chromatography (HPLC), and mass spectrometry. The rabbit tear film lipid layer was assessed by interferometry.

RESULTS. In the current study androgen receptor protein existed within acinar epithelial cell nuclei of rat meibomian glands; androgen deficiency was associated with alterations in the lipid content of the rabbit meibomian gland; 19-nortestosterone treatment modulated the fatty acid profile in the total and neutral lipid fractions of the rabbit meibomian gland; and androgens did not appear to influence the gross morphology of meibomian tissue or to exert a demonstrable effect on the rabbit tear film lipid layer.

CONCLUSIONS. The findings show that the meibomian gland is an androgen target organ and that androgens influence the lipid profile within this tissue. However, the extent to which androgens regulate the production of these lipids and whether this action may impact tear film stability remain to be determined. (Invest Ophthalmol Vis Sci. 2000;41:3732–3742)

Meibomian gland function is critically important in maintaining the health and integrity of the ocular surface.1–6 This gland, through its production and secretion of lipids, promotes the stability and prevents the evaporation of the tear film.3,6 Conversely, meibomian gland dysfunction, and the resultant lipid insufficiency, leads to a decreased stability and an increased evaporation of the tear film.3,6 Indeed, meibomian gland dysfunction, which is often underdiagnosed,7 is believed to be the major cause of dry eye syndromes throughout the world.8 Consistent with this belief is the observation that the majority of tear loss in dry eye is due to evaporation.6,9 However, despite the importance of the meibomian gland in preserving the well-being of the eye, almost no research has been published concerning the physiological regulation of this tissue.

It is quite possible, though, that meibomian gland function is controlled by androgens. The meibomian gland is a large sebaceous gland, and androgens are known to regulate the development, differentiation, and lipid production of sebaceous glands throughout the body.10–13 More specifically, androgens appear to act primarily on acinar epithelial cells in sebaceous glands, and these cells contain both androgen receptor mRNA and protein (in their nuclei). These acinar cells respond to androgens by producing proteins that augment both the synthesis and secretion of lipids. Interestingly, sebaceous gland activity and secretion decrease with age, and this aging-associated dysfunction has been correlated with both an atrophy of acinar cells and a reduction in serum androgen levels.14 In fact, the age-related cellular shrinkage in certain sebaceous glands has been directly correlated with a decline in androgen content in the surrounding skin.14

Given this background, we hypothesize that androgens control meibomian gland function, regulate the quality and/or quantity of lipids produced by this tissue, and promote the formation of the tear film’s lipid layer. To test this hypothesis, we sought to determine in the present study whether androgen receptor protein exists in the epithelial cell nuclei of rat meibomian glands and whether androgen deficiency and/or treatment influences the gross morphology, neutral lipid content, and fatty acid profile of the rabbit meibomian gland and the appearance of the tear film lipid layer.


**MATERIALS AND METHODS**

**Animals, Surgical Procedures, and Hormone Treatment**

Young adult male and/or female Sprague-Dawley rats (6 weeks old) and New Zealand White rabbits (3–4 months old) were obtained from Zivic–Miller Laboratories (Allison Park, PA) or Pine Acre Rabbitry (Norton, MA). All studies with these animals adhered to The Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research. Animals were housed in constant-temperature rooms with light-dark intervals of 12 hours. When indicated, rabbits were castrated or underwent a sham operation by veterinary surgeons at Pine Acre Rabbitry and allowed to recover for at least 11 days before further experimentation. Depending on the study, castrated rabbits were administered either Flocillin or Baytril (both from JA Webster, Sterling, MA) after surgery.

To determine the influence of androgen treatment on the lipid profiles in the meibomian gland, age-matched, castrated rabbits were randomly assigned to groups and treated for 14 days with systemic placebo, systemic 19-nortestosterone, topical placebo, or topical 19-nortestosterone. The systemic exposure was achieved by the implantation of subcutaneous slow-release pellets (Innovative Research of America, Sarasota, FL) into the subcapsular region of rabbits that had been anesthetized with intramuscular ketamine and xylazine. These pellets, which contained either vehicle (cholesterol, lactose, cellulases, phosphates, stearates) or 19-nortestosterone (200 mg in vehicle), were designed to release physiologival amounts of androge over a period of 21 days. The topical exposure involved the pipetting of a drop (40 μl/eye; one sterile pipet tip/eye) of either vehicle (nonbuffered, isotonic formulation; Allergan, Irvine, CA) or 19-nortestosterone (0.1% in vehicle formulation) to both eyes of rabbits twice daily (morning and afternoon). All rabbits administered systemic implants wore collars for at least 12 days to prevent removal of the pellets. To assess whether androgens modulate the histologic expression of neutral lipids in the meibomian gland, rabbits were treated topically with either vehicle (Allergan formulation) or 0.1% testosterone (Allergan formulation) according to the previously described treatment regimen. The ocular surfaces of all rabbits exposed to topical formulations were evaluated daily. In addition, rabbits were weighed before and after hormone treatment.

**General Procedures**

Tears were collected from both eyes of rabbits after Somlethol injection into the marginal ear vein, according to a reported protocol. In brief, the tip of a graded microcapillary pipet was placed at the inner canthus and gently moved along the palpebral conjunctiva. This procedure was repeated twice on each eye and obtained the entire available tear content. Tear volumes were measured accurately to within 0.1 μl. Blood was obtained by aspiration from the heart, permitted to clot at room temperature, then centrifuged at 12,400g for 4 minutes. Serum supernatants were stored at −20°C and later analyzed to determine the levels of testosterone with a radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA). Rabbit lids were removed, and meibomian glands were isolated under a dissecting microscope and processed for histologic or biochemical techniques. After rats were killed by carbon dioxide inhalation, lids and prostates were removed, embedded in optimal cutting temperature compound (OCT; Tissue-Tek; Miles, Naperville, IL) and stored in liquid nitrogen until immunohistochemical examination. Statistical analysis of data were performed by ANOVA or with the unpaired, two-tailed Student’s t-test.

**Histologic Procedures**

Tissues were processed for the evaluation of gross morphology by light microscopy, the glandular content of neutral lipids by image analysis, and the identification of androgen receptor protein by immunohistochemistry. For morphologic assessment, rabbit lids were fixed in 4% paraformaldehyde, dehydrated in increasing ethanol concentrations and chloroform, embedded in paraffin, and sectioned (8 μm). Tissue sections were then transferred to glass slides (Fisher Premium; Fisher Scientific, Medford, MA) deparaffinized, stained with hematoxylin and eosin, and examined under a light microscope (Axioskop; Carl Zeiss, Thornwood, NY).

To evaluate the meibomian gland content of neutral lipids, tissue sections were stained with oil red O, a method that has been used to monitor the effect of androgens on neutral lipid production in human prostatic cells. Lids were frozen in OCT compound and sectioned (14 μm). Sections were then placed on microscope slides (Superfrost/Plus; Fisher Scientific), incubated for 2 days in propylene glycol, stained for 1.5 hours in an 0.5% oil red O-propylene glycol solution, rinsed sequentially in 85% propylene glycol and distilled water and placed in Gill’s hematoxylin solution for 30 seconds. In selected control sections, lipids were extracted with chloroform and methanol before initiating the staining protocol. As a further test, certain sections were stained only with oil red O.

After these procedures, slides were rinsed in distilled water and coverslipped with Aqua-Mount (Lerner Labs, Pittsburgh, PA). The extent of staining in sections was then evaluated directly by scanning the coverslipped slides at 2700 dpi with a slide scanner (SprintScan 35 ES; Polaroid, Cambridge, MA) and analyzing the images by computer (Photoshop ver. 4.01; Adobe, San Jose, CA; Power Macintosh 8600/200, 96 Mb RAM; Apple, Cupertino, CA). The rationale for this direct scanning approach was threefold: It provided an effective magnification of approximately ×10 while spatially interpolating colors to give a more uniform description of staining characteristics; it decreased the susceptibility of the resultant colorimetric average to the high-frequency variances visible at greater magnifications; and stained portions of the gland could be objectively identified with a multipoint sample within the Select Color Range command in the image analysis software (Photoshop; Adobe). The fuzziness (variance) of the color-specific samples was set to 17/255 to remove bias incurred during sampling. The raw red, green, and blue values (RGB) of these selections were recorded and plotted in both RGB and the Lab color space, to arrange colors chromatically rather than linearly. Lab color, as used in this model, contains a luminance (L) channel to reflect the relative intensity of a color and two chrominance channels (a and b) grouped by psychophysical definitions of color. The transformation is shown below:
Before applying the Select Color Range command, an approximate rectangular boundary was used to eliminate background staining and, without loss of objectivity, constrain the software program’s analysis to only the staining associated with meibomian gland lipids. Given that lipid staining typically contained a two-tone profile (i.e., two distinct shades of red, with each color focus containing a dark red core surrounded by a lighter, pink exterior), analysis involved selecting and recording average color data for the dark, light, and total areas. Luminosity, RGB, area, and deviation data were extracted from the Image: Histogram menu option (Photoshop; Adobe). Data were collected into Excel where mean, variance, and the t-test analyses were performed. Colorimetric analysis was completed by computer (Matlab, ver. 4.2c; The MathWorks, Natick, MA) by calculation of the distance between weighted centers of mass in RGB and Lab color space. When plotted on three-dimensional RGB axes, each data point carried the red, green, and blue averages of a single stain class of a given section to determine position, and the area of the class as a weight. Taken as a whole, each group’s data points defined a volume in space. The Euclidian distance of centers of mass between groups was calculated by the square root of the sum of the squared differences in each coordinate.

To explore whether androgen receptor protein exists in the rat meibomian gland, tissues were examined according to published immunohistochemical techniques. Briefly, embedded frozen rat lids and prostates were cut at −20°C into 6-μm sections. Sections were transferred to poly-L-lysine-coated glass slides (Sigma, St. Louis, MO), fixed in acetone (JT Baker, Phillipsburg, NJ), and exposed sequentially to 4% paraformaldehyde, dipped in a lithium carbonate (Aldrich Chemical, Milwaukee, WI) solution, and preserved in mounting medium (Crystal Mount; Biomeda, Foster City, CA). During this immunohistochemical protocol, the application of various reagents to sections was interspersed with either air drying or the rinsing of slides with PBS or double-distilled water. To verify antibody reactivity in individual experiments, sections of rat prostate glands were included as positive (i.e., with first antibody) and negative (i.e., with rabbit IgG) control tissues. Photographs demonstrating the immunohistochemical distribution of androgen receptor protein in the meibomian gland were obtained by using a microscope and imaging system (Eclipse E800; Nikon, Garden City, NY; and a Spot ver.1.1; CE Diagnostic Instruments, Inc., Image System; Micro Video Instruments, Avon, MA). Images were imported into the computer image analysis program (Photoshop; Adobe) and printed (XLS 8600 printer, Eastman Kodak, Rochester, NY).

Rabbit tissues were not used in these immunohistochemical studies, because extensive cross reaction with IgG would have occurred after application of the secondary antibody.

### Analysis of the Total Lipid and Fatty Acid Profiles in the Meibomian Gland

To isolate total lipids from rabbit meibomian glands, frozen tissues were either minced on a glass plate or freeze fractured and then homogenized in water (5 ml). Aliquots (100 μl) were extracted with 2 ml of a chloroform-methanol (CM; 2:1) mixture by ultrasonic disruption. Particulate matter was sedimented by centrifugation in a benchtop centrifuge (Marathon 15K, Fisher Scientific) for 5 minutes at 3000 rpm, and the supernatant was transferred to a clean 13 × 100-mm screw-capped test tube. The sediment was re-extracted with another 2 ml CM (2:1), and particulate matter was again sedimented. After combining the supernatants with the first one, the sediment was extracted with 2 ml CM (1:2). Particulate matter was then again sedimented by centrifugation, and the supernatant was combined with the others. The combined supernatants were then dried under a stream of nitrogen and stored at −70°C until experimental analysis.

To determine the relative amounts of total saponifiable fatty acids, samples of total gland lipids were subjected to alkaline hydrolysis, solvent extraction, acidification, and ether extraction, and fatty acids were measured by gas chromatography using a flame ionization detector (5890 Series II; Hewlett-Packard, Palo Alto, CA). The free fatty acids were separated with a capillary column (HP-FFAP; Agilent Technologies, Palo Alto, CA; length = 50 m, internal diameter = 0.53 mm). The initial oven temperature was 180°C. After sample injection, the initial column temperature was maintained for 10 minutes. Fatty acid separation was effected with a linear temperature gradient to 240°C at a rate of 10°C per minute. Helium (carrier gas), hydrogen, and air pressures equalled 20, 15, and 30 psi, respectively. Peak areas of gas chromatography graphic data were measured by triangulation.
To evaluate trimethylsilyl (TMS) derivatives of fatty acid methyl esters and long-chain alcohols by gas chromatography-mass spectrometry, the following procedures were conducted. Methyl ester derivatives were formed by heating the samples at 60°C for 15 minutes with 100 μl of 14% BF₃ (Alltech, Deerfield, IL) in methanol in a sealed vial, and then evaporating the reagents under a stream of nitrogen at 40°C. TMS ethers were prepared by adding 100 μl of a N,O-bis(trimethylsilyl)trifluoroacetamide–hexamethyldisiloxane–trimethylchlorosilane–pyridine (5:2:1:2, vol/vol) mixture (Alltech) and heating at 60°C for 30 minutes. Two-microliter injections were made directly from the trimethylsilylation reagent. The derivatives were separated on a 30 m × 0.25 mm column (DB-5; J & W Scientific, Folsom, CA). The temperature gradient for separation of fatty acid methyl esters, long-chain alcohols, and their TMS derivatives was from 100°C to 300°C at a temperature increase of 10°C/min. The injector temperature was maintained at 300°C, and the hydrogen (carrier gas) pressure was kept at 10 psi. The transfer lines from the gas chromatograph to the mass spectrometer were maintained at 300°C. Mass spectrometric (model 4500; Finnigan, Madison, WI) analysis of the analytes was performed with ammonia chemical ionization (0.7 torr), with the ionizer maintained at 180°C. The mass range mass-charge (m/z) ratio 175 to 410 was scanned with a 0.26-second cycle time. Data acquisition (Vector/Two; Teknivent, Maryland Heights, MO) began 5 minutes into the analytical run.

To analyze fatty acid molecular species, total glandular lipids were separated by HPLC (Spectra-Physics Model 8700; Thermo Separation Products, San Jose, CA) with a silica column (Inertsil; Keystone Scientific, Bellefonte, PA). Samples were suspended by sonication in n-heptane for injection. Neutral lipids were separated with a complex, multistep gradient with combined mobile phases of isooctane-tetrahydrofuran (99:1, vol/vol), isopropanol-chloroform (4:1, vol/vol), and isopropanol-water (1:1, vol/vol) and a linear flow velocity of 0.4 ml/min. The temperature of the vaporizer in the moving-belt interface of the HPLC mass spectrometer (Finnigan) was 310°C. Mass spectrometry was performed in the positive ion, chemical ionization mode with ammonia reagent gas.

**Interferometry**

Evaluation of the lipid layer of the rabbit tear film was performed by optical interferometry, according to previously described procedures. This noninvasive technique allowed visualization of the structure of the tear film in vivo, and enabled observation of the thickness and spreading characteristics of the superficial lipid layer. In brief, the interferometric method consists of directing a beam of light on the tear film, with observation of the portions of this beam specularly reflected from the air–lipid and the lipid–aqueous boundaries. The original ray is thus split into two specularly reflected rays,
which are optically recombined and brought to a common focus. The optical phase relationship between the two reflected rays is determined by the distance between the two reflecting layers and the refractive indices of the media involved. A series of interference fringes are seen that represent the thickness distribution of the reflecting layer, which, in this case, is a topographical map of the lipid layer over the underlying aqueous phase of the tear film. This procedure’s lower limit of lipid thickness detectability, when using visible light, is approximately 0.1 \( \mu \text{m} \).

Images of the interference patterns of the rabbit tear film were recorded continuously during manually induced, blink-interblink periods by using a high-resolution video camera. Approximately 25-sq mm of tear film area over the central cornea was observed at one time.

**RESULTS**

**Identification of Androgen Receptor Protein in the Rat Meibomian Gland**

To determine whether the rat meibomian gland contains androgen receptor protein, the following experiments were conducted. Lid tissues (1 lid/animal) were obtained fromagematched male and female rats (10 weeks old; 5 per gender) and processed for immunohistochemistry to permit identification of the cellular distribution and the intracellular location of androgen receptor protein. For control purposes, rat prostates were also evaluated for androgen receptor staining.

Our results showed that androgen receptor protein is present in the rat meibomian gland. As demonstrated in Figure 1, androgen receptor protein was located almost exclusively within the nuclei of acinar epithelial cells. This pattern of staining was found in the meibomian glands of all male and female rats. In rat prostatic tissue androgen receptor protein was evident solely within epithelial cell nuclei.

The specificity of androgen receptor staining was verified by using a series of controls. First, preincubation of the anti-androgen receptor antibody with excess amounts of the androgen receptor peptide AR 1–21, which had served to induce the antibody, completely prevented immunohistochemical staining in the meibomian glands (Fig. 1), as well as in the rat prostate. Second, pre-exposure of the first antibody to the nonreactive androgen receptor peptide AR 462-478 did not inhibit the expression of androgen receptor protein in meibomian (Fig. 1) or prostatic tissues. Third, replacement of the first antibody with an irrelevant rabbit IgG antibody preparation led to the absence of specific staining in all tissue samples.

![RGB Sham (blue), Cast (yellow) distance = 21.979](image)

**FIGURE 3.** Influence of androgen deficiency on the oil red O staining patterns of rabbit meibomian glands. Lids were obtained 18 days after sham surgery or orchietomy and processed for histologic evaluation and image analysis. Blue and yellow points represent the values in sham-treated and castrated (cast) rabbits, respectively. The color-coded dotted lines connect the center of mass of each group with the origin for perspective. The Euclidian distance between the centers of mass are reported above each graph. The data for these graphs are reported in Table 1.

### Table 1. Analysis of Oil Red O Staining in the Meibomian Glands of Sham-Treated or Orchiectomized Rabbits

<table>
<thead>
<tr>
<th>Glandular Areas</th>
<th>Sham-Treated</th>
<th>Orchiectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminosity</td>
<td>114 ± 3</td>
<td>129 ± 1*</td>
</tr>
<tr>
<td>Red values</td>
<td>167 ± 3</td>
<td>179 ± 3*</td>
</tr>
<tr>
<td>Green values</td>
<td>91 ± 3</td>
<td>108 ± 2*</td>
</tr>
<tr>
<td>Blue values</td>
<td>95 ± 4</td>
<td>103 ± 4</td>
</tr>
<tr>
<td><strong>Dark</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminosity</td>
<td>105 ± 3</td>
<td>118 ± 2*</td>
</tr>
<tr>
<td>Red values</td>
<td>184 ± 5</td>
<td>197 ± 2</td>
</tr>
<tr>
<td>Green values</td>
<td>72 ± 3</td>
<td>87 ± 2*</td>
</tr>
<tr>
<td>Blue values</td>
<td>65 ± 4</td>
<td>68 ± 1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminosity</td>
<td>113 ± 4</td>
<td>126 ± 1*</td>
</tr>
<tr>
<td>Red values</td>
<td>167 ± 2</td>
<td>181 ± 3*</td>
</tr>
<tr>
<td>Green values</td>
<td>89 ± 5</td>
<td>104 ± 1†</td>
</tr>
<tr>
<td>Blue values</td>
<td>93 ± 7</td>
<td>97 ± 4</td>
</tr>
</tbody>
</table>

Lids were obtained from rabbits 18 days after sham-surgery or orchietomy, processed for Oil Red O and hematoxylin staining, and analyzed for color intensity. Procedures included analysis of the luminosity and red, green, and blue values of the light, dark, and total areas of all meibomian glands in entire lid sections (\( n = 3 \) lids/group; \( n = 6–12 \) sections/lid). Data, which were generated in arbitrary units, were averaged for each individual lid. Numbers equal the mean ± SE of these averages.

* Significantly (\( P < 0.05 \)) greater than value of sham-treated group.
† Comparison between groups yielded \( P = 0.05 \).
Influence of Androgen Deficiency on the Neutral Lipid Content of the Rabbit Meibomian Gland and the Appearance of the Rabbit Tear Film Lipid Layer

To examine whether androgen deficiency influences the neutral lipid content of the meibomian gland, as well as the appearance of the tear film lipid layer, the following studies were performed. Age-matched, young adult male rabbits (three per group) were castrated or subjected to sham surgery and allowed to recover for at least 15 days. For histologic evaluation, lids (one lid/animal) were obtained and processed for oil red O staining and image analysis. For tear film assessment, animals were examined by interferometry.

Our histologic studies demonstrated that oil red O stained lipids in the meibomian glands of castrated and sham-treated rabbits (Fig. 2). This staining, which was performed on lids collected 18 days after surgery, was abolished if sections were preincubated in chloroform and methanol. Our colorimetric analyses of lid sections (n = 6–12 sections per lid) showed that significant differences existed between the staining patterns of meibomian glands from control and androgen-deficient rabbits.

Effect of Androgen Deficiency and Treatment on the Lipid Composition and Gross Morphology of the Rabbit Meibomian Gland

To determine whether androgens modulate the lipid profile within, and the gross morphology of, the rabbit meibomian...
of TMS derivatives of fatty acid methyl esters in the meibomian gland total lipid fraction, were observed only in samples from hormone-treated rabbits, not in placebo-treated rabbits. Topical androgen exposure also elicited a consistent increase in the amount of high-molecular-weight fatty acids in the diglyceride fraction of meibomian gland lipids (Fig. 6). These long-chain fatty acids, which were also present in meibomian glands of intact male rabbits, almost completely disappeared after orchiectomy and topical placebo administration. Of particular interest, topical hormone effects were apparently due to local action, given that no testosterone could be detected in serum after the application of androgens to the ocular surface (Table 2). In these studies, treatment with topical 19-nortestosterone had no significant impact on the relative percentages of cholesterol esters, cholesterol, triglycerides, or diglycerides in the meibomian gland (data not shown).

The effect of androgen treatment on lipid composition was not paralleled by alterations in the gross morphology of
the meibomian gland. As demonstrated in Figure 7, the appearance of meibomian tissue seemed to be similar in all groups, irrespective of whether animals were intact or castrated and treated with topical or systemic placebo or 19-nortestosterone compounds. In addition, androgen administration had no influence on the body weight or tear volume of rabbits, relative to placebo-treated or intact control animals (Table 2).

Given the androgen effects on glandular lipid composition, another experiment was performed to determine whether topical testosterone treatment might influence the histologic appearance of neutral lipids in meibomian glands of castrated rabbits. Accordingly, 11 days after castration, age-matched, young adult male New Zealand White rabbits (five to six per group) were treated topically with either testosterone or vehicle for 14 days, and lids were then obtained, stained with oil red O, but not hematoxylin, and processed for image analysis. Our examination of the oil red O staining patterns (four to six sections per lid, 30–34 sections per group) in meibomian glands of placebo- and testosterone-treated rabbits demonstrated that no significant differences existed between these groups. The weighted centers of the RGB and Lab color space transforms were slightly, but not significantly, different (data not shown).

**DISCUSSION**

The present study demonstrates that the meibomian gland is an androgen target organ. This tissue contains androgen receptor protein in acinar epithelial cell nuclei and shows alterations in its lipid content during androgen deficiency. Moreover, 19-nortestosterone treatment modulates the fatty acid profile in the total and neutral lipid fractions of the meibomian gland. However, androgens do not appear to influence the gross morphology of this tissue, or to exert a demonstrable effect on the rabbit tear film lipid layer.

Our identification of androgen receptor protein in acinar epithelial cell nuclei of the rat meibomian gland was not surprising. Sebaceous glands in nonocular tissues are known to express androgen receptor protein in their epithelial cell nuclei, and these receptors are believed to mediate androgen action in these tissues.10–13 In addition, we have recently discovered that the rabbit and human meibomian glands contain androgen receptor mRNA (by using reverse transcription-polimerase chain reaction [RT-PCR], agarose gel electrophoresis, and Southern blot hybridization)23 and that human meibomian tissue harbors androgen receptor protein in epithelial cell nuclei.24 These findings indicate that androgens may act on the meibomian gland through a classic mechanism, involving a receptor-mediated regulation of gene transcription.25 In support of this hypothesis, our preliminary data suggest that androgens may influence the differential expression of at least several mRNAs in the meibomian gland.26 Of note, estrogen and progesterone receptor mRNA and/or protein have also recently been identified in the human meibomian gland.23,27 However, whether these sex steroids affect meibomian gland gene expression or function has yet to be evaluated.

Regarding androgen action on the meibomian gland, our histologic and image analysis studies showed that orchiectomy was associated with a significant change in the RGB staining patterns of meibomian gland neutral lipids. However, this alteration, which was observed after an 18 days of androgen deficiency, was not reversed by a 2-week treatment with topical testosterone. One possible reason for this apparent discrepancy is that the time course of androgen administration was insufficient to restore the overall neutral lipid profile in the meibomian gland. In support of this possibility is the preliminary finding that longer exposure (i.e., 1 month) to topical dehydroepiandrosterone (DHEA), an androgen precursor,28 resulted in an apparent increase (detected by histology) in the lipid content of a rabbit’s meibomian glands.29

A 2-week interval, however, was sufficient for androgen treatment to influence the expression of specific lipids within
meibomian tissue. Thus, topical or systemic administration of 19-nortestosterone to castrated rabbits led to significant changes in the fatty acid profile of meibomian gland neutral lipids, compared with that of placebo-treated controls. In addition, application of 19-nortestosterone to the ocular surface appeared to counter the effect of castration on the $m/z$ ratio of fragmentation products of fatty acids in chemical ionization mass spectrometry and to restore the lipid pattern toward that of meibomian glands in intact male control animals. Our findings are consistent with the previous predictions of several investigators, who speculated that the meibomian gland and its

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial BW (kg)</th>
<th>Final BW (kg)</th>
<th>Tear Volume (μl)</th>
<th>Serum Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>2.82 ± 0.07</td>
<td>3.23 ± 0.12</td>
<td>0.6 ± 0.1</td>
<td>0.53 ± 0.21</td>
</tr>
<tr>
<td>Topical placebo</td>
<td>2.86 ± 0.02</td>
<td>3.33 ± 0.09</td>
<td>0.6 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Topical hormone</td>
<td>2.82 ± 0.13</td>
<td>3.32 ± 0.19</td>
<td>0.6 ± 0.2</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Systemic placebo</td>
<td>2.92 ± 0.09</td>
<td>3.10 ± 0.09</td>
<td>0.5 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Systemic hormone</td>
<td>2.76 ± 0.09</td>
<td>3.02 ± 0.20</td>
<td>0.9 ± 0.4</td>
<td>1.62 ± 0.46</td>
</tr>
</tbody>
</table>

Rabbits ($n = 5$/group) were weighed immediately before (Initial), and 18 days after (Final), either no treatment (Intact) or treatment with systemic or topical placebo or 19-nortestosterone (hormone). The mean serum testosterone concentrations in the placebo-treated, as well as the topical 19-nortestosterone-treated, rabbits were below the radioimmunoassay’s level of sensitivity and are therefore recorded as 0. Numbers equal the mean ± SE. BW, body weight.

In our experiments we found no evidence of an androgen effect on the gross morphology of the rabbit meibomian gland. Similarly, other investigators have observed that the size of rat meibomian glands was unaffected by castration or hypophysectomy. This absence of androgen influence appears to be atypical, given that the structure of sebaceous glands throughout the body seems to be exquisitely sensitive to the presence or absence of androgens. However, it is important to note that the nature of the androgen regulation of sebaceous glands is

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**TABLE 2. Influence of 19-Nortestosterone Treatment on Rabbit Body Weight, Tear Volume, and Serum Testosterone Levels**

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**FIGURE 7.** Appearance of meibomian glands in intact, placebo- or 19-nortestosterone-treated rabbits. Lids were collected from animals that had undergone treatment or no treatment, as described in Figure 4, and were processed for histologic evaluation. Hematoxylin and eosin; magnification, ×200.
not uniform. Considerable differences appear to exist in the hormonal control mechanisms of sebaceous glands between species and between different types of sebaceous glands.10

Our studies also showed that no obvious differences existed between the interferometric patterns of the tear film lipid layer of castrated and sham-treated rabbits. One interpretation of these results is that androgens may not influence this layer of the tear film. However, our experience in these studies, when combined with that of others, indicates that this interpretation may be premature, and quite possibly incorrect. Thus, despite our finding that androgen deficiency alters the profile of lipids within the rabbit meibomian gland, this change would not necessarily be reflected on the ocular surface. Apparently, the rabbit meibomian gland contributes very little to the tear film lipid layer: The vast majority of lipids on the rabbit’s ocular surface originate from other adnexal tissues.31 In fact, complete closure of the meibomian gland orifices in rabbits has almost no effect on the thickness of the lipid layer.31 This situation contrasts sharply with that in humans, in which the meibomian gland is the primary source of tear film lipids.31 Another consideration is that the lipid layer of the rabbit tear film is thicker and more stable than that of humans, is nearly stationary after a blink, and shows almost no complex interference patterns or wave formations.32 Indeed, the average interblink period in the rabbits is approximately 45 times longer than that of humans.32 Consequently, the rabbit does not appear to be an ideal model for determining whether androgen–meibomian gland interactions promote the formation of the tear film lipid layer. Other species may be more suitable: For example, investigators have reported that the topical administration of DHEA to dogs (n = 4) and a human (n = 1) with dry eye stimulates the production and release of meibomian gland lipids and prolongs the tear film breakup time.29 Whether this DHEA effect is due to conversion to androgens or other sex steroids is not known.

Lastly, during the course of our experiments, we found that androgen deficiency or treatment had no impact on the tear volume of rabbits. These results are consistent with earlier observations in rabbits,33 but not necessarily in other species. Thus, androgens seem to induce time-, species-, and strain-dependent effects on lacrimal gland secretion. These effects result in a nonuniform increase, decrease, or no influence on the tear volume.33,34 Of particular interest, the nature of androgen action on fluid secretion by the human lacrimal gland appears to be strain dependent.23,24 Thus, androgens seem to induce time-, species-, and strain-dependent effects on lacrimal gland secretion. These effects result in a nonuniform increase, decrease, or no influence on the tear volume.33,34

Whether this DHEA effect is due to conversion to androgens or other sex steroids is not known.

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References


