Meibomian Gland Physiology in Pre- and Postmenopausal Women

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Purpose. The purpose of this study was to assess the change of meibomian gland physiology during the menstrual cycle and compare its physiology among pre- and postmenopausal women and men.

Methods. This study involved 18 healthy subjects: 6 premenopausal women with a regular 28- to 30-day menstrual cycle, 6 postmenopausal women, and 6 men who were age-matched to the premenopausal women. All subjects measured basal body temperature every day at home and were seen once weekly for 5 weeks, and the menstrual state of the premenopausal subjects was masked until after the investigation. At each visit, the meibomian gland orifice (MGO) diameter, lid margin meibum level as meibometry value, meibomian gland morphology, and fluorescein breakup time (F-BUT) of tear film were evaluated, and serum samples were obtained for analysis of sex steroid hormones.

Results. The meibomian gland physiology showed cyclic change during the menstrual cycle. The MGO diameter and meibometry value of the meibomian glands decreased in the latter half of the luteal phase until menstruation, correlating well with a shortening of F-BUT. The MGO diameter and meibometry value were significantly higher in postmenopausal women and men than in premenopausal women; however, F-BUT was significantly longer in men than in pre/postmenopausal women.

Conclusions. The findings of this study show that meibomian glands exhibit a cyclic change in premenopausal women, as well as sex- and age-related physiologic differences.

Keywords: meibomian gland, meibomian gland dysfunction, estrogen, progesterone, testosterone, menstrual cycle

Meibomian glands are large, modified sebaceous glands that synthesize and secrete a mixture of lipids, known as "meibum," that play the important role of stabilizing the tear film.1-5 Meibomian gland dysfunction (MGD) that alters the quality and quantity of meibum often leads to tear film instability and evaporative dry eye6-8 and is believed to be the leading cause of dry eye.9 In fact, MGD is reportedly observed in more than two-thirds of dry eye patients.10,11 It is widely known that the majority of patients with dry eye syndrome are women.6 The prevalence of Sjögren’s syndrome, which is considered a primary cause of aqueous deficient dry eye, is reportedly significantly higher in females,6 whereas the prevalence of MGD, which is considered a primary cause of evaporative dry eye, is somewhat controversial (i.e., some studies have reported a higher prevalence in males,12,13 whereas other studies have reported no sex-related difference and that it is actually associated with aging).14 Furthermore, in younger patients, the ocular surface inflammation associated with meibomitis, such as meibomitis-related keratoconjunctivitis, ocular rosacea, and phlyctenular keratoconjunctivitis, is predominantly observed in women.15-17 However, the underlying mechanism involved in this sex-associated difference in these diseases has yet to be elucidated.

Meibomian glands are hormone target tissues. In mice and humans, meibomian glands reportedly contain mRNA and proteins of sex steroid hormone receptors such as androgen,18-20 estrogen,20-22 and progesterone.20,22 These sex steroid hormones regulate the gene expressions in the meibomian glands.23-25 Testosterone reportedly regulates more than 1500 genes in male mouse meibomian glands, in which the genes related to lipid metabolism, lipid transport, and sterol biosynthesis are up-regulated; however, the genes related to keratinization are down-regulated.25,26 In contrast, 17β-estradiol reportedly regulates nearly 200 genes in female mouse meibomian glands, in which the genes related to lipid catabolism are up-regulated and the genes related to lipid synthesis are down-regulated.25 It has been reported that the meibomian glands of mice apparently change their morphology in response to estrogen and progesterone.27 Thus, it is reasonable to posit that the effects of sex steroid hormones would have an impact on the meibomian gland physiology in both pre- and postmenopausal women, as well as men.

In the sebaceous gland, androgens reportedly increase the secretion of sebum,28 whereas estrogens have an opposite effect.29,30 In fact, estrogens have been used for the treatment of acne due to its ability to reduce sebaceous gland function and sebum secretion.30,31 It has been recognized that acne gets worse prior to menstruation, commonly referred to as “premenstrual flare,” and studies have shown that the pilosebaceous glands undergo physiologic changes during the
Menstrual cycle. Hence, we theorized that the meibomian gland, as a modified sebaceous gland, also undergoes physiologic changes during the menstrual cycle.

Thus, the purpose of this present study was to assess the cyclic change of meibomian gland physiology during the menstrual cycle and to compare its physiology among pre-and postmenopausal women and men.

METHODS

Subjects

This study involved 18 healthy subjects: 6 premenopausal women (mean age: 26.4 ± 2.3 [SD] years, range: 24–30 years) with a regular 28- to 30-day menstrual cycle and duration of 6 to 7 days, 6 postmenopausal women (mean age: 56.3 ± 3.1 [SD] years, range: 53–60 years), and 6 men who were age-matched with the premenopausal women (mean age: 28.5 ± 3.7 [SD] years, range: 25–34 years). The study protocols were approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, and in accordance with the tenets set forth in the Declaration of Helsinki, written informed consent was obtained from all subjects prior to their participation. All subjects were seen once weekly for 5 weeks, and the menstrual state of the premenopausal subjects was masked until after the investigation. Moreover, the premenopausal women were seen once weekly for an additional 5 weeks to confirm their menstrual status. Subjects excluded from the study included smokers, contact lens wearers, and those with eye and/or general disease or currently taking medications.

Menstrual Cycle and Basal Body Temperature

As per the previously described method, the menstrual cycle was divided into six phases: the first 2 days of menstruation (phase I), 2 days before menstruation (phase VI), and the remaining time being divided into four equal periods (typically, 6-day periods; phases II–V). Basal body temperature (BBT), which is measured immediately after awakening and prior to any physical activity, was measured daily to confirm the menstrual state in the premenopausal women, as well as postmenopausal women and men as a comparison.

Serum Sex Steroid Hormones

Blood specimens were obtained from the subjects between the hours of 8 and 9 AM, and serum was prepared and then stored at −20°C until assayed. Serum levels of estradiol (E2), progesterone (P), and total testosterone (T) were determined by electro-chemiluminescence immunoassay (ECLIJA), whereas those of free testosterone (fT) were determined by radioimmunoassay (RIA) at SRL, Inc. (Tokyo, Japan).

Meibomian Gland Orifice Diameter

The left lower lid margin was photographed in each subject using a slit-lamp microscope at a magnification of ×40. As shown in Figure 1, the location of each meibomian gland orifice is seen as a series of concentric rings of varying reflectivity, at the center of which the opening of the duct is presumed to lie, a slightly darker region within the pale, central, disc-like zone. We refer to this pale zone here as the meibomian gland orifice (MGO) zone and measured its inner transverse diameter in the central eight MGO zones using computer software (Adobe Photoshop; Adobe Systems, Inc., San Jose, CA, USA). We take this to be representative of the orifice size.

The repeatability and reliability of the measurement was confirmed using six photographs taken of the same subject via the same photographic procedure and measured on the computer software. The best quality of photograph was used for the actual measurement. The mean value of the eight measurements was calculated at each visit. After the six visits, each phase of menstrual cycle was confirmed by BBT and serum concentrations of E2 and P. Then, the average number of each phase of all premenopausal women was calculated.

Meibometry Value

Meibum was harvested from the central third of the left lower lid margin, and the volume was analyzed by use of a laser meibometer, as per the previously described methods. Briefly, the meibometry technique involved lipid sampling using a loop of 8-mm-wide translucent plastic tape (Courage + Khazaka Electronic GmbH, Cologne, Germany). The loop was formed by heat-sealing the tape at a predetermined point for a loop length of 20 mm. The plastic loop was then mounted in an ultrasonography probe holder (NIDEK Co., Ltd., Gamagori, Japan) and applied to the central third of the lower lid margin at a pressure of 0 mm Hg with the eye being held in an upward gaze. Contact was then maintained for 3 seconds. The tape was then air-dried for 3 minutes to allow for evaporation of any contaminating tear fluid. The optical density of the strip of increased transparency on the tape produced by the retained blot of oil was then read in the laser meibometer (window size: 2.5 × 5 mm). The results were expressed as means ODU (arbitrary optical density units) ± SE.
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Meibomian Gland Morphology

Video meibography was used to obtain a transilluminated meibomian gland image of the left lower eyelid, and the technique involves exposing the tarsal conjunctiva to an infrared beam emitted from the 16-window T-shaped head of the probe. The transilluminated structure of the meibomian gland was then conducted to a highly sensitive, infrared, charge-coupled-device video camera and digitally recorded. The full length (from the orifice to the opposite end of the gland) and width (at 0.5 mm from the orifice) of the central eight meibomian glands was then measured from the still images via the use of computer software (Adobe Photoshop).

Fluorescein BreakUp Time of Tear Film

At each visit, the fluorescein breakup time (F-BUT) of the tear film in each patient was measured three times with a metronome 15 minutes after meibometry and photography and then averaged.

Statistical Analyses

The statistical relationships between the accumulated data were analyzed by use of the Spearman’s rank correlation coefficient.

Results

Cyclic Change of BBT

Examples of the BBTs of the pre- and postmenopausal women measured in this study are shown in Figure 2. Typically, in premenopausal women, the BBT pattern is biphasic (Fig. 2A); that is, lower temperatures before ovulation and higher temperatures thereafter. Higher levels of estrogen present during the preovulatory (follicular) phase of the menstrual cycle lower BBTs, whereas higher levels of progesterone released by the corpus luteum after ovulation raise BBTs. Therefore, we confirmed in which phase a patient was at each visit. In the postmenopausal women, the BBT pattern showed no cyclical change (Fig. 2B). The same result was found in the men.

Cyclic Change of MGO Diameter

As shown in Figure 3A, the MGO diameter of premenopausal women was smaller in luteal phase (postovulation) than in follicular phase (preovulation). It was significantly larger at phase II (preovulation, 0.173 ± 0.002 mm) than at phase I (0.166 ± 0.002 mm) (P < 0.05) and significantly smaller at phase V and VI (postovulation and premenstruation, 0.158 ± 0.002 mm and 0.156 ± 0.002 mm, respectively) (P < 0.001). There was no significant difference in the MGO diameter between phases II and III and phases V and VI.

The men, who were age-matched with the premenopausal women, as well as the postmenopausal women, showed a significantly larger MGO diameter (0.201 ± 0.001 mm and 0.188 ± 0.001 mm, respectively) (P < 0.001) compared with that of the premenopausal women at phase II (Fig. 3B).

Cyclic Change of Meibometry Value

Because the meibometry value widely varied among each of the premenopausal women, the results are shown as a percentage of the results at phase I (Fig. 4A). The meibometry value tended to increase up until ovulation and then decrease thereafter. In fact, it was significantly smaller at phase V (74.8 ± 9.6%) than at phase II (106.8 ± 6.2%) (P < 0.05) (Fig. 4A). The mean meibometry value was significantly higher in the men (0.125 ± 0.004 ODU) (P < 0.05) and postmenopausal women (0.155 ± 0.006 ODU) (P < 0.001) than in the phase II premenopausal women (0.062 ± 0.014 ODU) (Fig. 4B).

Cyclic Change of Meibomian Gland Morphology

In the premenopausal women, the length and width of the meibomian gland did not show significant cyclical change during the menstrual cycle (Figs. 5A, 5B). There was no significant difference in the length between the groups (Fig. 5C); however, the widths in the phase II and V pre- and postmenopausal women (0.388 ± 0.012, 0.356 ± 0.017, and 0.376 ± 0.006 mm, respectively) were significantly smaller than those of the men (0.424 ± 0.011 mm) (P < 0.001; Fig. 5D).

FIGURE 2. Cyclic change of BBT. (A) An example of BBTs of a premenopausal subject (a 27-year-old woman). The BBT pattern is biphasic (i.e., lower temperatures before ovulation and higher temperatures afterward). Phases I to III are included in the first half of the cycle and phases IV to VI are included in the last half of the cycle. x, menstruation. (B) An example of BBTs of a postmenopausal subject (a 53-year-old woman). There is only a low temperature phase, but no cyclic change of BBT.
Cyclic Change of F-BUT

Because the F-BUT varied among each of the premenopausal women, the results are shown as a percentage of the result at phase I (Fig. 6A). F-BUT time was significantly shorter at phase V (77.2 ± 5.0%) than at phase II (105.5 ± 3.5%) (P < 0.05; Fig. 6A). The mean F-BUT was not significantly different between the phase II pre-and postmenopausal women (6.08 ± 0.92 and 4.59 ± 0.24 seconds, respectively) (P = 0.15). On the other hand, the men showed significantly longer F-BUT (8.55 ± 0.23 seconds) than that in the premenopausal women (P < 0.05) and postmenopausal women (P = 0.001; Fig. 6B).

Relationship Between Meibomian Gland Physiology and Serum Sex Steroid Hormones

In the premenopausal women, serum concentration of E2 and P increased in the latter half of the menstrual cycle (Fig. 7A); however, tT showed no cyclic change (Fig. 7B). Free testosterone was below the measurable limit in both pre- and postmenopausal women. A significantly higher concentration of both E2 and P was found in phase V (P < 0.01; Figs. 7C, 7D). Averaged tT in premenopausal women (0.504 ± 0.004 ng/mL) decreased to approximately one-third in postmenopausal women (0.147 ± 0.01 ng/mL).

DISCUSSION

To the best of our knowledge, this is the first report regarding the cyclic change of meibomian gland physiology according to the menstrual cycle. In the luteal phase, especially in the latter half of the phase when both E2 and P were high, the meibomian gland orifice became smaller, and the volume of meibum decreased. This presumably accounts for the shorter F-BUT, although some caution must be exercised, given that the F-BUT is a subjective measurement. On the other hand, the MGO size in the postmenopausal women was larger than that in the premenopausal women, thus implying that menopause (i.e., in this study, E2 and P decreased dramatically and tT became approximately one-third of that of premenopausal...
women) has a strong influence on the meibomian gland physiology. Our results regarding MGO size were not the same as those of the sebaceous glands whose duct exit was significantly smaller on days 15 to 20 of the menstrual cycle (phase IV); however, they were comparable with the period of “premenstrual flare,” which is around the 22nd day of the cycle (phase V). Changes in the size of the sebaceous gland duct exit and resistance to sebum outflow were considered helpful in explaining the premenstrual acne. In ovariectomized mouse meibomian glands, the treatment with estrogen

**FIGURE 5.** Cyclic change of the length and width of the meibomian glands. In the premenopausal women, the length (A) and width (B) of the meibomian gland did not show significant cyclical change during the menstrual cycle. There was no significant difference in the meibomian gland length between the groups (C); however, the width in the men was significantly larger than that in the other groups (D). *P < 0.001.

**FIGURE 6.** Cyclic change of F-BUT of tear film. (A) The F-BUT results of the premenopausal women are shown as a percentage of the result at phase I. F-BUT was significantly shorter at phase V than that at phase II. *P < 0.05. (B) The mean F-BUT was not significantly different between the phase II pre- and postmenopausal women (*P = 0.15), but the men showed significantly longer F-BUT than that in the pre- and postmenopausal women. *P < 0.05, **P < 0.01.
alone showed a different morphology compared with the treatment with estrogen plus progesterone. A human epidemiologic study about hormone replacement therapy revealed that women receiving estrogen treatment had a significantly higher prevalence of dry eye than did women who never received the treatment or who received estrogen plus progesterone therapy. The findings of these reports indicate that progesterone should be expected to have different influence against estrogen on meibomian gland physiology. Recently, an experiment using aromatase knockout mice revealed that estrogen does not play a major role in the sex-related differences of the mouse meibomian gland; aromatase is a cytochrome P450 enzyme that catalyzes the formation of estrogens from androgens. However, it has recently been reported that estrogen with calcium treatment induced keratinization genes in a cultivated human meibomian gland epithelial cell line. At least in premenopausal women, estrogen may produce negative effects on MGO diameter by promoting duct keratinization.

The mean meibometry values in the premenopausal women were significantly smaller than those in the postmenopausal women and the men, as expected (i.e., testosterone’s positive effects and estrogens’ negative effects on the meibomian glands). This may help to explain why men who take antiandrogen medicine for prostatic hypertrophy have significant MGD. Chew et al reported that the casual level of meibomian lipid on the lower lid margin shows age- and sex-related differences. In their study, the male levels were higher than those of the females in the age group of 20 to 29 years; however, the male and female levels became indistinguishable above the age of 50. This might be related to the fact that estrogen’s negative effects suddenly decline post menopause. The mean age of the postmenopausal women in this current study was 56 years, and the mean interval after menopause was 6.7 years. Although meibomian gland function is known to decrease with age, it is possible that the female meibomian gland physiology is more strongly influenced by menopause than by subject age, especially during a short-term interval after menopause.

In this study, the meibomian gland physiology in premenopausal women may reflect more associations with estrogen and progesterone than by testosterone. The ocular surface epithelium is known to express sex steroid hormone receptors and their physiology, such as corneal thickness, corneal sensitivity, and conjunctival goblet cell count, reportedly changes according to the menstrual cycle. In addition to those changes of the ocular surface, cyclic change of meibomian glands is hypothesized to have an influence on dry eye during the menstrual cycle, as well as contact lens intolerance. The relationship between serum sex steroid hormones and dry eye and/or MGD in postmenopausal women has been of interest to researchers; however, the results reported previously seemed to be somewhat contradictory. In postmen-
opausal women, 100% of estrogens and androgens are synthesized from adrenal sex steroid precursors, such as dehydroepiandrosterone (DHEA) and DHEA-sulfate, acting intracellularly in peripheral tissues, and are inactivated locally without biologically significant release of active those hormones in the circulation. Even in premenopausal women, only 25% of estrogens originate from ovaries; however, 75% comes from intracrine synthesis in peripheral tissues. Serum testosterone levels represent only a very small fraction of the total androgen pool in humans. Therefore, one cannot simply explain the results by the serum levels of sex steroids. Because meibomian glands have enzymes converting DHEA to androgens and estrogens, it would be more informative to measure local hormone concentration or steroid metabolites using mass spectrometry.

In summary, our findings show that, in premenopausal women, meibomian gland physiology changes according to the menstrual cycle (i.e., in the latter half of the luteal phase, the MGO diameters become smallest, which we equate with the lowest delivery of meibum and the shortest F-BUT). These changes are presumably thought to be influenced by estrogen and progesterone. In postmenopausal women, with estrogen's declining negative effect, MGOs appear to become larger and secrete more meibum; however, F-BUT appears to become shorter. This would suggest that F-BUT is more highly influenced by the quality of meibum after menopause. Our current research is focused on analyzing the fatty acid composition of meibum obtained from pre- and postmenopausal women.

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