scientific reports

OPEN

Sex-based differences in conjunctival goblet cell responses to pro-inflammatory and pro-resolving mediators

Menglu Yang[1], Haakon K. Fjærvoll[1,2], Ketil A. Fjærvoll[1,2], Nicholas H. Wang[1], Tor P. Utheim[3,4], Charles N. Serhan [5] & Darlene A. Dartt[1✉]

Many conjunctival inflammatory diseases differ between the sexes and altered conjunctival goblet cells (CGCs) response is often involved. Inflammation is initiated by the release of pro-inflammatory mediators and terminated by the biosynthesis of specialized pro-resolution mediators (SPMs). Herein, we determined the sex-based difference in the responses of CGCs to inflammatory stimuli or pro-resolving lipid SPMs and their interaction with sex hormones. GCs were cultured from pieces of human conjunctiva in RPMI media. CGCs were transferred 24 h before the start of experiments to phenol red-free and FBS-free media to minimize exogenous hormones. RT-PCR, immunofluorescence microscopy (IF), and Western Blot (WB) were performed to determine the presence of sex hormone receptors. Cellular response to pro-inflammatory stimuli or SPMs was studied by measuring the increase in intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) using fura 2/AM microscopy. Use of RT-PCR demonstrated estrogen receptor (ER) α in 4/5 males and 3/3 females; ERβ in 2/4 males and 2/3 females; and androgen receptors (AR) in 3/3 male and 3/3 female CGCs. Positive immunoreactivity by IF and protein expression by WB was detected using antibodies for the ERα and ERβ in 3/3 males and 3/3 females, while AR were only present in males. Significantly different Ca^{2+} responses between sexes were found with carbachol only at 10^{-3} M, but not with histamine or leukotriene (LT) B_4 at any concentration used. Incubation with dihydrotestosterone (DHT), estrone (E1), or estradiol (E2) at 10^{-7} M for 30 min significantly inhibited the LTB_4-stimulated $[Ca^{2+}]_i$ increase in male and female CGCs. Incubation with DHT, E1, and E2 overnight significantly inhibited the LTB_4 response in females, while DHT and E2 significantly inhibited the LTB_4 response in males. The SPM lipoxin A_4 (LXA_4) (10^{-9}–10^{-8} M), but not the resolvins D1 or D2, induced an $[Ca^{2+}]_i$ increase that was significantly higher in males compared to females. We conclude that male and female CGCs showed differences in the expression of sex hormone receptors. Treatment with sex hormones altered pro-inflammatory mediator LTB_4-induced response. Males compared to females have a higher response to the ω-6-fatty acid derived SPM LXA_4, indicating males may terminate inflammation in conjunctival goblet cells faster than females.

The prevalence of many ocular surface diseases differs between men and women. In the US, women have an age-adjusted 70% increase compared to men in the risk of dry eye disease[1,2], and more antihistamine medicines were prescribed to women[3]. The differences in biological characteristics are one of the major probable causes of this phenomenon[3]. Sex affects health at multiple biological levels, from chromosomes to sex hormones. In leukocytes, X and Y chromosomes have different profiles of immune system-related genes[4]; while sex hormones also moderate immunity. For example, estrogens at the level of the ovulatory phase or pregnancy suppress cytotoxicity of natural killer (NK) cells, interferon-γ production, and T-cell subpopulation and their effect on the innate immune response[5]. Several autoimmune diseases such as systemic lupus erythematosus (SLE), Sjogren's disease (SJD), Grave's disease (GD), rosacea, and rheumatoid arthritis (RA) have a higher prevalence in women

[1]Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114, USA. [2]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway. [3]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway. [4]Department of Ophthalmology, Oslo University Hospital, Oslo, Norway. [5]Department of Anesthesia, Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. ✉email: Darlene_dartt@meei.harvard.edu

than men[6]. These findings suggest that sex affects the immune and inflammatory processes, and that sex-based differences are present at both cellular and systemic levels.

In the eye, sex-based differences are observed in multiple components of the eye[7–11] and impact multiple processes[12–14]. Sex hormones modulate tear film stability and homeostasis of the ocular surface[15]. Sex-based differences were also demonstrated in the resolution of inflammation in dry eye by an action in females to decrease regulation of the immune response of the ocular surface by the pro-resolving mediator lipoxin(LX) A$_4$ that exacerbates dry eye[13]. Disturbances of the tear film are often found in patients with alteration of hormone levels such as menopause or pregnancy[15]. Furthermore, androgens stimulate meibomian gland secretion by transcriptome regulation, while estrogens induce proinflammatory gene expression in corneal epithelial cells and meibomian glands[16]. In the conjunctiva, the number of goblet cells is higher in men than women, and in women, the goblet cell count decreases around ovulation[17], suggesting additional sex differences in goblet cells. However, little additional work has been published on the sex-based differences in the conjunctiva and its goblet cells, a main component of the ocular surface.

The conjunctiva surrounds the cornea, overspreads the sclera, and lines the eyelids. The conjunctival goblet cell, a major cell type of the conjunctival epithelium, synthesizes, stores, and secretes MUC5AC, a large, secretory gel-forming mucin. This mucin forms the inner layer of the tear film and protects the ocular surface from exogenous agents. Physiologically conjunctival goblet cell secretion is induced by stimulating a complex reflex arc consisting of the afferent sensory nerves in the cornea and conjunctiva that activate efferent parasympathetic and sympathetic nerves with efferent nerve endings surrounding the goblet cells[18]. The mucin secretion function is closely mediated by the intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$)[19].

In ocular surface inflammation, evidence suggests that the goblet cell is one of the targeted cell types[20]. In inflamed eyes, goblet cells can be stimulated by infiltrating pro-inflammatory mediators, including prostaglandins (PGs), leukotrienes (LTs), and allergic mediators such as histamine[21]. The excessive mucin secretion causes catarrhous and discomfort[20,22], which happens in chronic inflammatory diseases such as allergic conjunctivitis and early dry eye. To actively terminate inflammation and restore homeostasis, specialized pro-resolving mediators (SPMs) are biosynthesized and released[23]. There are multiple families of SPMs identified, including LXs, resolvins (Rv), maresins (Mar), and protectins, biosynthesized from omega 6 and omega 3 fatty acids. Members of the D- and E series resolvins, as well as lipoxins and maresins can stimulate conjunctival goblet cell secretion to maintain ocular surface homeostasis and terminate the excessive secretion induced by proinflammatory mediators[24–27]. Furthermore, multiple SPMs are present in human emotional tears with different concentrations in men and women[17].

Herein, we studied the presence of the sex hormone receptors and their regulatory action on normal function of conjunctival goblet cells (CGC). We also investigated the sex-based differences in cellular response of goblet cells in homeostasis by using a cholinergic agonist (carbachol, Cch); in acute inflammation by using LTB$_4$; in allergy by using histamine, and in resolution of inflammation by treating with two of the D-series resolvins and LXA$_4$. We hypothesize that conjunctival goblet cells respond differently to pro-inflammatory and pro-resolving stimuli in men than women and that the goblet cell response can be altered by exogenous sex hormones.

Results

Differential expression of sex hormone receptors in goblet cells cultured from male compared to female human conjunctiva.
To determine the expression of the estrogen and androgen receptors in conjunctival goblet cells, RT-PCR and WB were performed using cultured human goblet cells. IF was used to determine the cellular location of the sex hormone receptors on conjunctival goblet cells grown on coverslips. $Er\alpha$ mRNA was expressed in 4 out of 5 males and 3 out of 3 females at the expected size of 100 bp (Fig. 1A). ERα protein was present in 3 out of 3 males and 3 out of 3 females by WB analysis (Fig. 1B). Helix pomatia lectin (HPA), a marker of goblet cell secretory product, was present in both male and female goblet cells as a rough granular pattern indicating secretory granules, consistent with previous publications[27]. ERα immunoreactivity was present as a fine punctate pattern concentrated in the cytoplasm in males (Fig. 1C) and the same punctate pattern in both the cytoplasm and nucleus females (Fig. 1D).

$Er\beta$ was found in 2 out of 4 males and 2 out of 3 females at the expected size of 143 bp by RT-PCR (Fig. 2A). In WB, ERβ presented as a double band in 3 out of 3 females, and a trace of ERβ band was found in 3 out of 3 male samples (Fig. 2B). ERβ immunoreactivity was detected as a punctate pattern throughout the cytoplasm and the nuclear area in both males (Fig. 2C) and females (Fig. 2D).

Ar mRNA was identified in 3 out of 3 males and 3 out of 3 females at the expected size of 106 bp (Fig. 3A). AR was detected by WB in 2 out of 2 male samples, however, no protein was detected in 3 out of 3 females (Fig. 3B). AR immunoreactivity appeared as a punctate pattern that was more concentrated in the cytoplasm than the nucleus in males (Fig. 3C), but no AR immunoreactivity was detected in females (Fig. 3D). Incubation with the isotype control antibody in IF showed no apparent immunoreactivity for any of the three receptors (Supplemental Fig. 1) indicating that the antibodies used were specific for each receptor.

In summary, the sex hormone receptors are differentially present in human conjunctival GCs from males and females. Although little difference was found in the expression of mRNA for all three receptors, the pattern of expression was very different at the protein level. The ERβ protein was more abundant in females than males. In contrast, AR protein was only found in males. ERα showed the most consistency between the sexes, but a sex-based difference can still be detected in the subcellular localization.

Differential response of goblet cells cultured from male and female conjunctiva to cholinergic agonists but not allergic mediators.
To determine the rapid, short-term activation of conjunctival goblet cells, we used three different types of known goblet cell stimuli: (1) the cholinergic agonist Cch (10^{-5}–10^{-3} M)
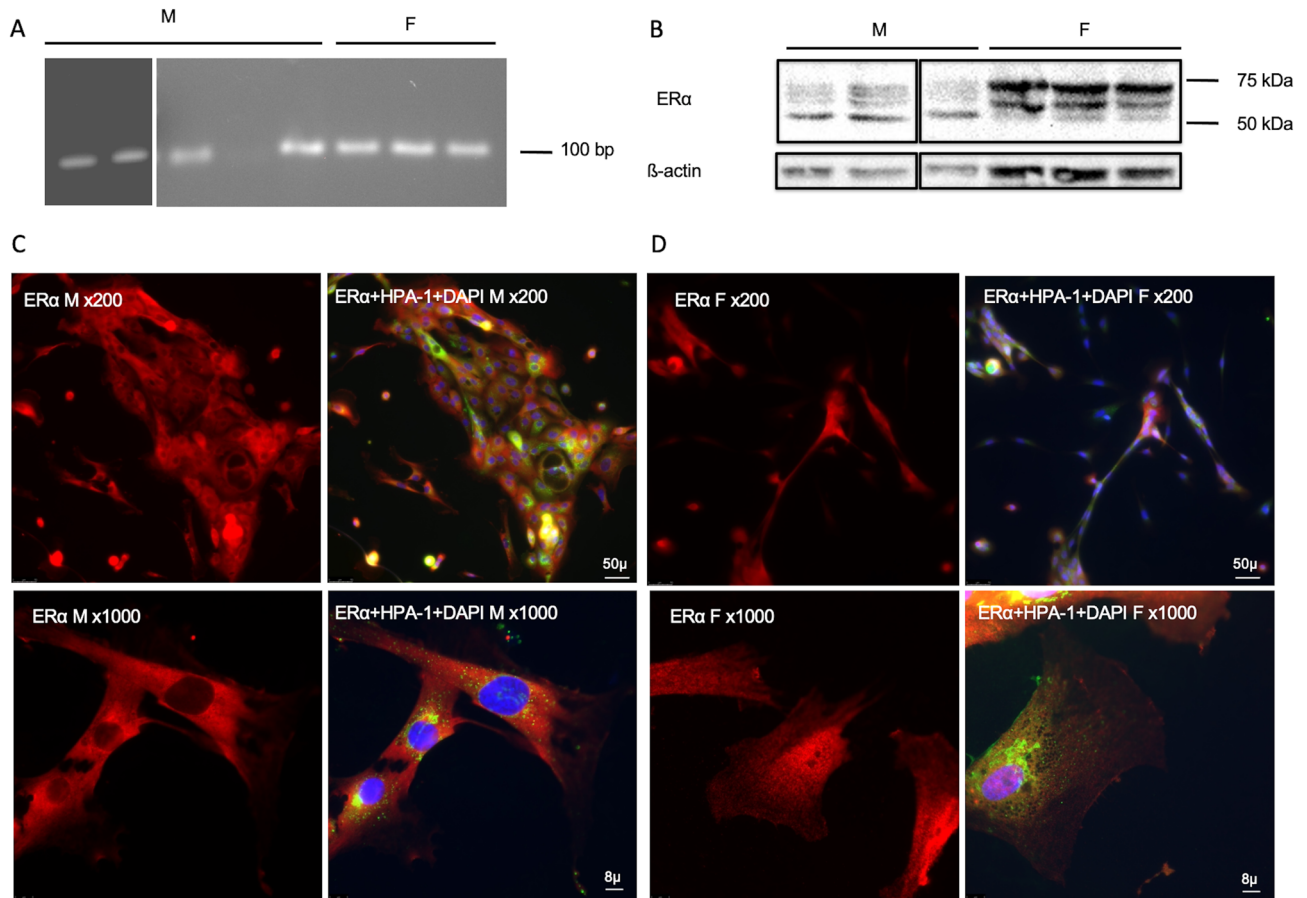
Figure 1. Identification of ERα in human conjunctival goblet cells cultured from males and females. RNA was isolated from cultured goblet cells and RT-PCR performed using primers for human *Erα*. A single band was detected at around 100 bp in samples from 4 out of 5 male (M) and 3 out of 3 females (F) (**A**). Protein samples were collected from cell pellets and Western Blot analysis was performed using antibody against ERα. A major band at 50 kDa was detected in males, while a major band at 75 kDa and a minor band between 75 and 50 kDa were detected in females (**B**). Immunofluorescence microscopy was performed on cultured goblet cells using antibodies to ERα (**C** and **D**). (**C**) indicate immunofluorescence to ERα (red) in male cells; (**D**) indicate immunofluorescence to ERα (red) in female cells; an overlay of anti-ERα, HPA-1 (green, indicates the secretory granules of goblet cells), and DAPI (blue, indicates cell nuclei) is shown next to the single channel image of ERα. Magnification was ×200 in the upper panels; ×1000 in the lower panels. Full gels and blots are included in "Supplementary information". Original blots/gels are presented in Supplementary Figs. 5 and 6.

to mimic physiological stimulation by the parasympathomimetic neurotransmitter acetylcholine, (2) LTB_4 (10^{-11}–10^{-8} M) an eicosanoid mediator of allergic conjunctivitis as well as of other types of inflammation and (3) histamine (10^{-8}–10^{-5} M) a major mediator of allergic conjunctivitis. The stimuli were applied to male and female goblet cells, and the change in $[Ca^{2+}]_i$ was measured.

Cch (10^{-5}–10^{-3} M) compared to basal significantly increased $[Ca^{2+}]_i$ in both male and female cells (Fig. 4A,B). Only at a carbachol concentration of 10^{-3} M was the peak $[Ca^{2+}]_i$ response significantly different between males and females at 112.2 ± 40.54 nM in males and 577.0 ± 213.9 nM in females ($p < 0.05$) (Fig. 4B).

LTB_4 used at 10^{-11} to 10^{-8} M caused an increase in $[Ca^{2+}]_i$ in both male and female goblet cells with a maximum response to LTB_4 at 10^{-8} M with the $[Ca^{2+}]_i$ significantly increased to 230.8 ± 80.44 nM in males and 521.6 ± 161.6 nM in females (Fig. 4C,D). No significant difference in Ca^{2+} response was found at any concentration of LTB_4 when comparing the response of male and female cells (Fig. 4C,D).

When the goblet cells were stimulated by histamine, histamine caused an increase in peak $[Ca^{2+}]_i$ in male but not female goblet cells (Fig. 4E,F). The maximum increase in $[Ca^{2+}]_i$ of 593.3 ± 104.5 nM occurred at 10^{-6} M histamine in males. In contrast, the maximum increase in $[Ca^{2+}]_i$ of 583.3 ± 130.1 nM was detected at 10^{-5} M in females (Fig. 4E). No significant difference in peak $[Ca^{2+}]_i$ was found between the two groups at any histamine concentration (Fig. 4F).

In all, the cellular response of goblet cells to different stimuli that mimic physiological nerve stimulation, inflammation, and allergy was not different between males and females except for the highest concentration of the cholinergic agonist, in which females demonstrated a greater response than males.
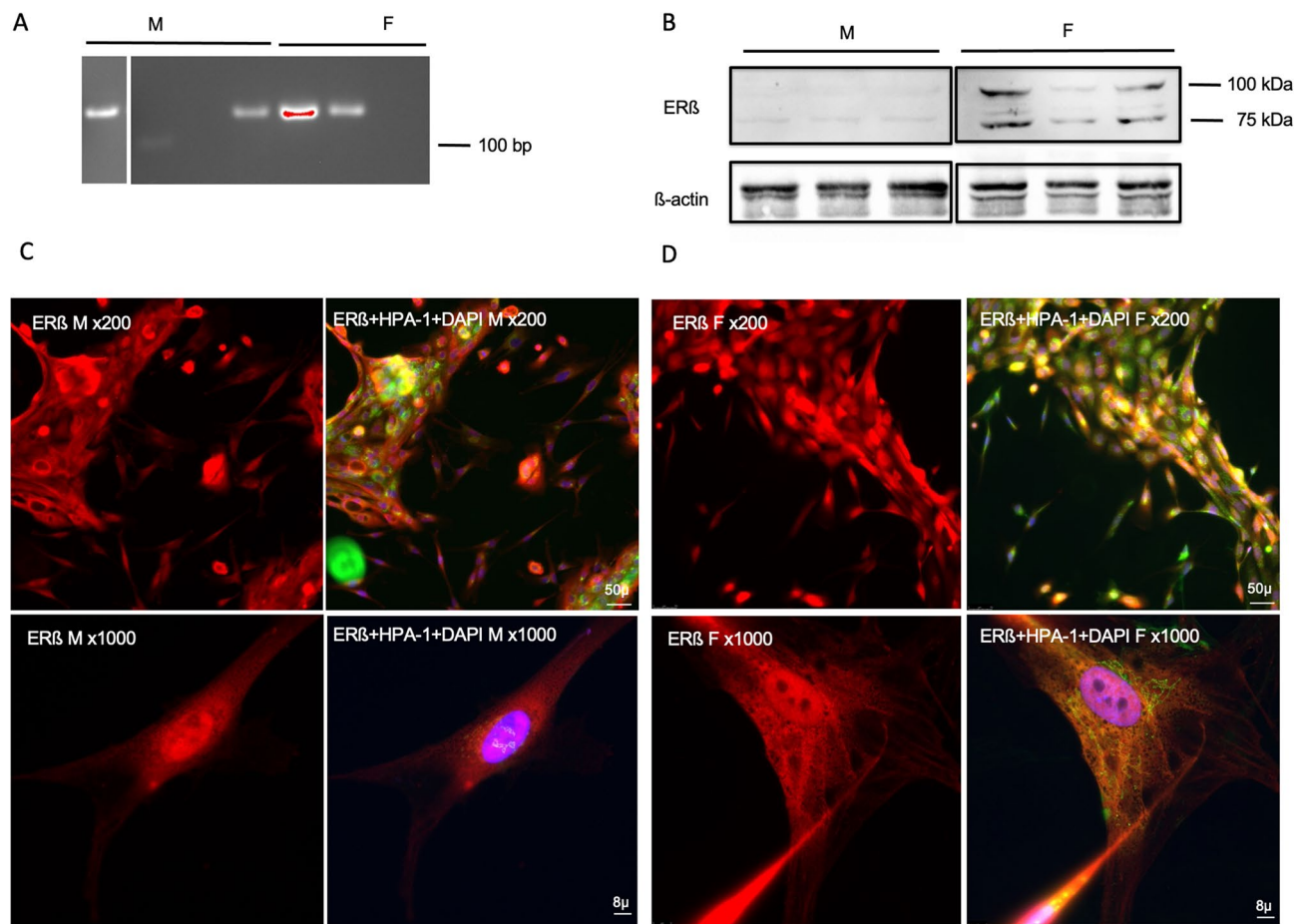
Figure 2. Identification of ERβ in human conjunctival goblet cells cultured from males and females. RNA was isolated from cultured goblet cells and RT-PCR performed using primers for human *Erβ*. A single band was detected at around 100 bp in 2 out of 4 samples from males (M) and 2 out of 3 samples from females (F) (**A**). Protein samples were collected from cell pellets and Western Blot analysis was performed using antibody against ERβ. Two bands at 100 and 75 kDa respectively were detected in female samples (**B**). Trace immunoreactivity of the same size as in females was present in male samples (**B**). Immunofluorescence microscopy was performed on cultured goblet cells using antibodies to ERβ (**C** and **D**). (**C**) indicate immunofluorescence to ERβ (red) in male cells; (**D**) indicate immunofluorescence to ERα (red) in female cells; an overlay of anti-ERβ, HPA-1 (green, indicates the secretory granules of goblet cells), and DAPI (blue, indicates cell nuclei) is shown next to the single channel image of ERβ. Magnification was ×200 in the upper panels; ×1000 in the lower panels. Original blots/gels are presented in Supplementary Figs. 5 and 6.

Sex hormones inhibit conjunctival goblet cell response to the eicosanoid inflammatory mediator LTB$_4$, but not cholinergic agonist carbachol nor the autacoid histamine.

To study the action of sex hormones on cellular responses to inflammatory mediators, we treated goblet cells from male and female conjunctiva with a sex hormone alone or for varying times followed by the addition of LTB$_4$. The sex hormones used were dihydrotestosterone (DHT), estrone (E1), and 17-β-estradiol (E2). DHT is a highly potent form of testosterone, estrone a low potency estrogen synthesized in peripheral tissues, and estradiol a high potency estrogen mainly synthesized in the ovary. The addition of 10^{-7} M DHT, E1, or E2 alone did not significantly increase the $[Ca^{2+}]_i$ compared to basal in conjunctival goblet cells of a mixed series of both sexes (Supplemental Data 2). Histamine at 10^{-5} M, the positive control significantly increases the $[Ca^{2+}]_i$ from basal.

To determine the short-term action of sex hormones on physiological, inflammatory, and allergic agonists, 10^{-7} M DHT, E1, or E2 or vehicle were administered to goblet cells 30 min prior to addition of LTB$_4$ (10^{-8} M). In male cells, LTB$_4$ at 10^{-8} M significantly increased the $[Ca^{2+}]_i$ to 540.90 ± 171.20 nM ($p < 0.05$) (Fig. 5A,B). A 30 min treatment with DHT significantly decreased the $[Ca^{2+}]_i$ to 70.02 ± 8.70 nM ($p < 0.05$); with E1 significantly decreased the $[Ca^{2+}]_i$ to 76.70 ± 13.04 nM ($p < 0.05$); and with E2 significantly decreased the $[Ca^{2+}]_i$ to 74.32 ± 7.4 nM ($p = 0.04$) (Fig. 5A,B). In female cells, LTB$_4$ 10^{-8} M alone significantly increased the $[Ca^{2+}]_i$ to 559.7 ± 209.2 nM from basal (Fig. 5C,D). A 30 min treatment with DHT significantly decreased the $[Ca^{2+}]_i$ to 97.60 ± 35.37 nM ($p < 0.05$) (Fig. 5C,D); with E1 significantly decreased the $[Ca^{2+}]_i$ to 62.95 ± 5.11 nM ($p < 0.05$) (Fig. 5D); and with E2 significantly decreased the $[Ca^{2+}]_i$ to 65.15 ± 10.58 nM (Fig. 5D).

The short-term action of sex hormones was also determined for carbachol at 10^{-3} M and histamine at 10^{-5} M using the same experimental design used for LTB$_4$. Neither DHT, E1, nor E2 incubated for 30 min blocked the
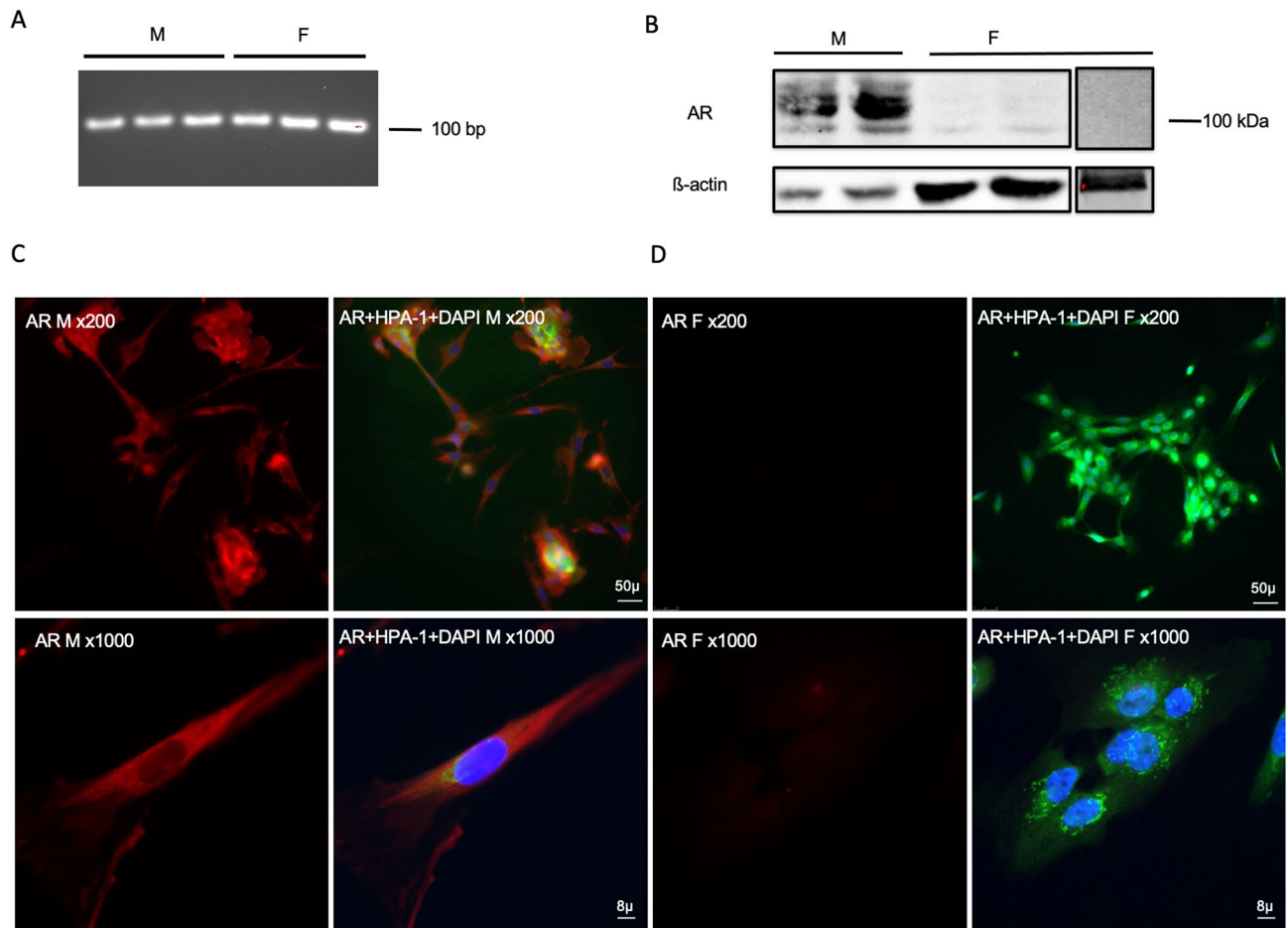
Figure 3. Identification of AR in human conjunctival goblet cells cultured from males and females. RNA was isolated from cultured goblet cells and RT-PCR performed using primers for human *Ar*. A single band was detected at around 100 bp in samples from both males (M) and females (F) (**A**). Protein samples were collected from cell pellets and Western Blot analysis was performed using antibody against AR. A major band at 100 kDa was detected in males while no immunoreactivity was detected in females (**B**). Immunofluorescence microscopy was performed on cultured goblet cells using antibodies to AR (**C** and **D**). (**C**) Indicate immunofluorescence to AR (red) in male cells; (**D**) indicate immunofluorescence to AR (red) in female cells; an overlay of anti-AR, HPA-1 (green, indicates the secretory granules of goblet cells), and DAPI (blue, indicates cell nuclei) is shown next to the single channel image of AR. Magnification was ×200 in the upper panels; ×1000 in the lower panels. Original blots/gels are presented in Supplementary Figs. 5 and 6.

carbachol-induced Ca^{2+} response in either males or females (Supplemental Data 3A,B,E). Similarly, neither DHT, E1, nor E2 incubated for 30 min blocked the histamine-induced Ca^{2+} response (Supplementary Fig. 3C,D,F).

To determine the action of long-term stimulation with sex hormones, goblet cells were incubated with DHT, E1, E2 or buffer for 24 h before adding LTB_4. In male cells, LTB_4 10^{-8} M significantly increased the $[Ca^{2+}]_i$ to 404.70 ± 135.90 nM from basal (Fig. 6A,B). Treatment for 24 h with DHT or E2 significantly decreased the $[Ca^{2+}]_i$ to 63.21 ± 11.24 nM ($p < 0.05$); and 55.78 ± 9.49 nM ($p < 0.05$), respectively. In contrast, 24 h treatment with E1 dampened but did not significantly decrease the $[Ca^{2+}]_i$ to 127.60 ± 41.24 nM (Fig. 6B). In cells from females, LTB_4 10^{-8} M significantly increased the $[Ca^{2+}]_i$ to 463.90 ± 122.60 nM (Fig. 6C,D) and to 458.6 ± 118.3 (Fig. 6E,F) from basal. Incubation for 24 h with DHT, E1, or E2 each significantly decreased the LTB_4-triggered $[Ca^{2+}]_i$ increase to 58.52 ± 6.54 nM ($p < 0.05$), 85.18 ± 21.16 nM ($p < 0.05$) (Fig. 6C,D), and 94.17 ± 44.96 nM ($p < 0.05$) (Fig. 6E,F), respectively.

The long-term action of sex hormones was determined for carbachol and histamine using the same experimental protocol as for LTB_4. Neither DHT, E1, nor E2 incubated for 24 h blocked the carbachol- or histamine-induced Ca^{2+} response in either males or females (Supplemental Data 4).

In summary, sex hormones incubated for both the short-term and long-term inhibited the cellular Ca^{2+} response induced by the eicosanoid inflammatory mediator LTB_4, but not the cholinergic agonist carbachol nor the autacoid histamine.

Differential response of goblet cells from male compared to female conjunctiva to LXA_4, but not RvD1 or RvD2.

We next determined the $[Ca^{2+}]_i$ response of goblet cells to the specialized pro-resolving mediators LXA_4, RvD1, and RvD2. LXA_4 is produced earlier in the resolution response than the resolvins as it
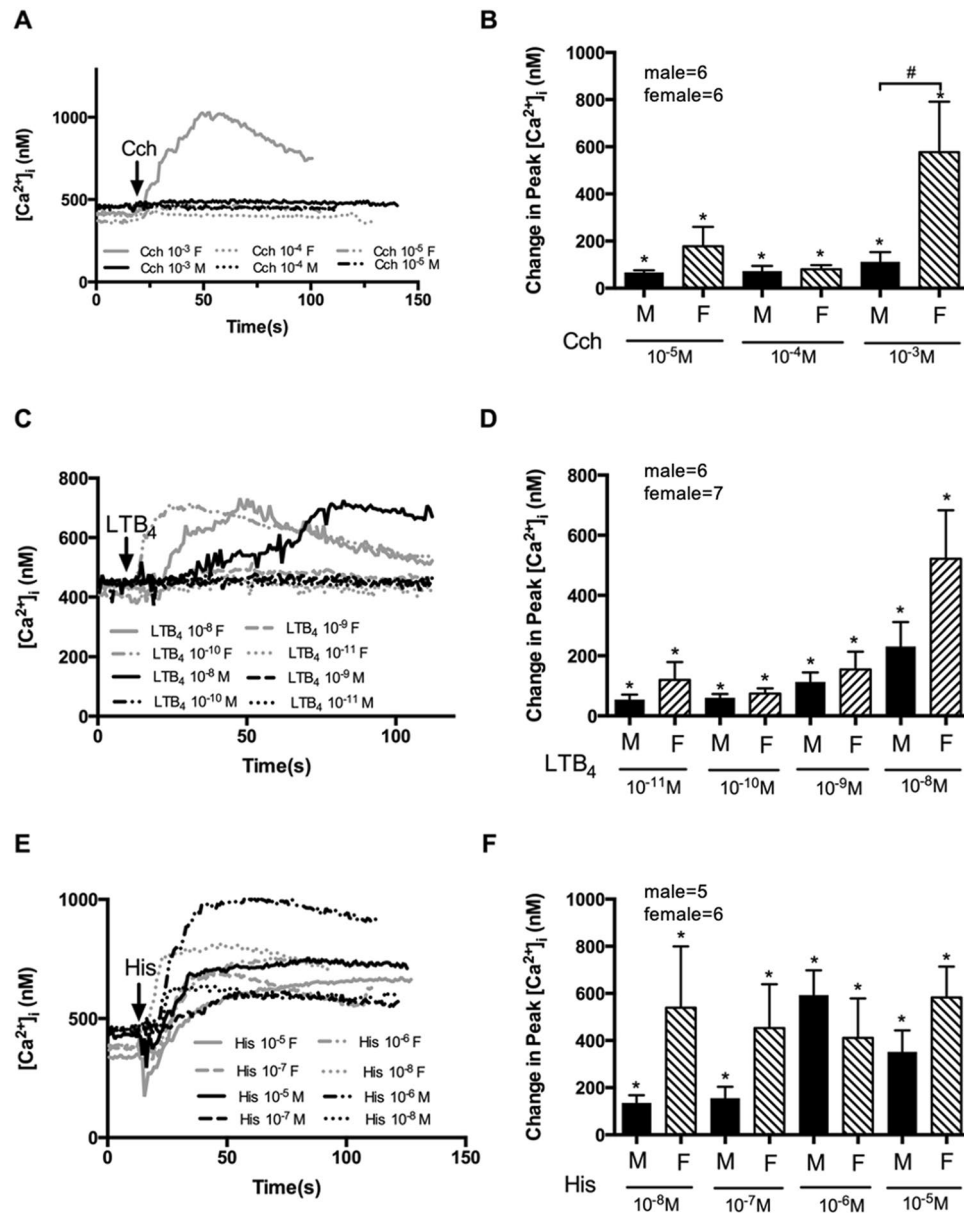
Figure 4. Human conjunctival goblet cells cultured from males and females respond to a neurotransmitter, inflammatory mediator, and allergic mediator. Cultured goblet cells from both sexes were stimulated with carbachol(Cch) 10^{-5}–10^{-3} M (**A** and **B**) or LTB$_4$ 10^{-11}–10^{-8} M (**C** and **D**) or histamine(His) 10^{-8}–10^{-5} M (**E** and **F**). $[Ca^{2+}]_i$ measured by fura2 assay. The average $[Ca^{2+}]_i$ level over time was shown in (**A**, **C** and **E**); Change in peak $[Ca^{2+}]_i$ was calculated and shown in (**B**, **D** and **F**). Number of individuals is indicated on each graph. Data are mean ± SEM; the number of individuals is marked in each graph. Arrow indicates the addition of stimuli. Black lines and solid black bars indicate data from male cells, grey lines and slash-patterned bars indicate data from female cells. *Significance difference from basal. #Significance difference between male and female values.

is biosynthesized from arachidonic acid compared to DHA for the D-series resolvins[23]. When goblet cells cultured from male and female conjunctiva were stimulated with LXA$_4$ (10^{-10}–10^{-8} M) a concentration dependent-response was obtained in male but not female cells (Fig. 7A,B). When peak $[Ca^{2+}]_i$ was determined, a maximum response was obtained with LXA$_4$ used at 10^{-8} M. This concentration significantly increased $[Ca^{2+}]_i$ to 468.6 ± 55.25 nM ($p < 0.05$). In contrast, in female cells, a maximum response was obtained with LXA$_4$ used at 10^{-10} M. This concentration significantly increased $[Ca^{2+}]_i$ to 195.4 ± 60.57 ($p < 0.05$) from basal (Fig. 7A). The change in peak $[Ca^{2+}]_i$ triggered by 10^{-9} M and 10^{-8} M in males was significantly higher than females ($p < 0.05$), implying a significant sex-based difference in Ca^{2+} response to LXA$_4$ (Fig. 7A,B).

RvD1 (10^{-10}–10^{-8} M) caused a concentration dependent-response in male and female cells (Fig. 7C,D). The change in peak $[Ca^{2+}]_i$ in response to RvD1 was maximum at 10^{-8} M for cells from both sexes and was significantly increased to 393.1 ± 148.6 nM in male and 493.7 ± 183.9 nM in female cells (Fig. 7C,D). No significant
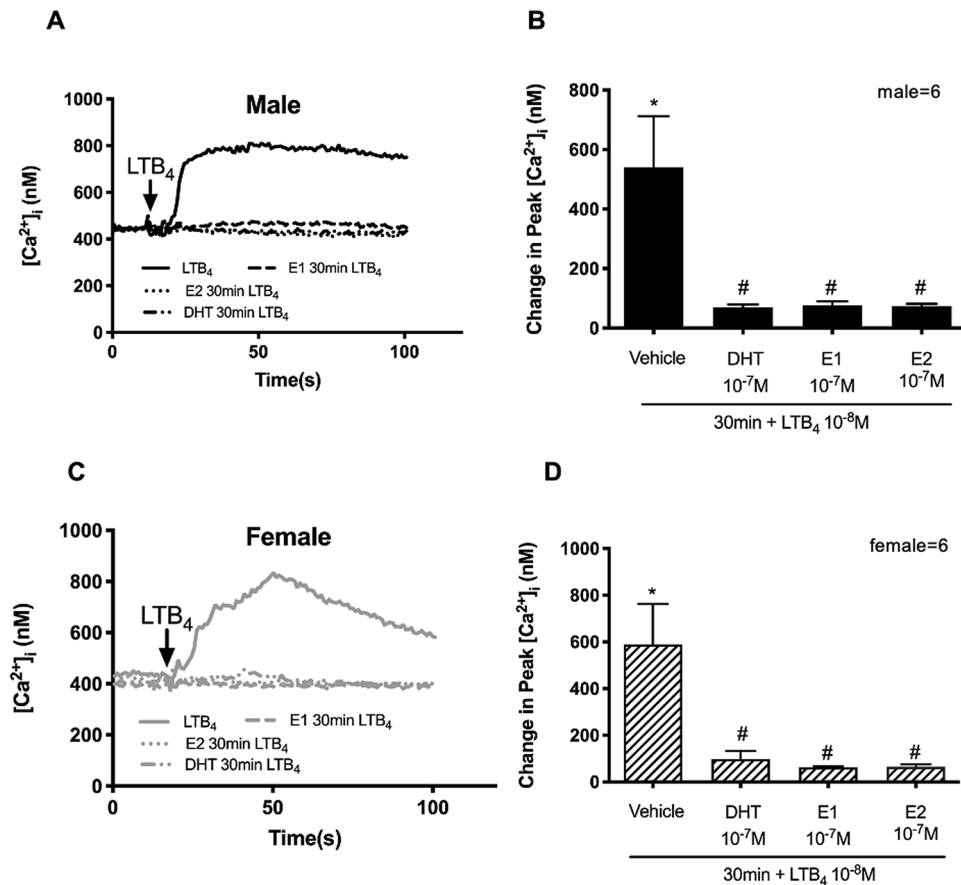
Figure 5. Short-term treatment with sex hormones inhibits the Ca^{2+} response from human conjunctival goblet cells cultured from male and female cells to the inflammatory mediator LTB_4. Cultured goblet cells from male and female conjunctiva were treated with DHT, E1 or E2 (10^{-7} M) or vehicle for 30 min prior to addition LTB_4 (10^{-8} M). $[Ca^{2+}]_i$ was measured by fura2 assay. The average $[Ca^{2+}]_i$ level over time was shown in (**A** and **C**); Change in peak $[Ca^{2+}]_i$ was calculated and shown in (**B** and **D**). Data are mean ± SEM, n = 6 for male cells and n = 6 for female cells. Black lines (**A**) and solid bars (**B**) indicate data from male cells. Grey lines and slash-patterned bars indicate data from female cells. *Significance difference from basal. #Significance difference between male and female cells.

difference was found when comparing the response from male and female cells stimulated with RvD1 at any concentration used (Fig. 7C,D). Similar results were obtained with RvD2 (10^{-10}–10^{-8} M). RvD2-induced $[Ca^{2+}]_i$ increase was concentration-dependent and maximum at 10^{-8} M at which concentration the peak $[Ca^{2+}]_i$ was significantly increased to 573.5 ± 386.4 nM in males and 460.8 ± 144.1 nM in females (Fig. 7E,F). No significant sex-based differences were observed in the Ca^{2+} response to RvD2 at any concentration used.

In summary, human GCs cultured from male or female conjunctiva responded differently to LXA_4, but no difference was found with the response to another family of SPMs, the D-series.

Discussion

Several tear-producing ocular adnexa, including the lacrimal gland and the Meibomian glands, display sex-based differences in structure and function, resulting in sex-based differences in diseases such as dry eye and allergy[7–11]. Herein we show that message and location of protein for the receptors for estrogens are differently present in cultured human conjunctival goblet cells from both males and females. No AR protein was detected in female samples even though the mRNA was detected, a possible explanation of this finding is the prolonged lack of testosterone in culture. ARs in females are more dependent on the testosterone-induction, and the same action could have occurred with ERβ in male samples. The presence of ERα immunoreactivity in the nucleus in females suggests that ERα could bind to DNA to regulate the expression of target genes. Although the presence of the three sex hormone receptors in biopsy specimens from both sexes has been reported by other studies[28–30], our results indicate there are differences with the expression of sex hormone receptors with the absence of sex hormones.

Even though the sex hormones themselves when tested did not alter the $[Ca^{2+}]_i$ in cells from either sex, they did affect the response to LTB_4 and to the same extent in both males and females. Sex hormones have a variety of effects on cell function. The protective role of the androgen DHT has been observed[31,32], and is consistent
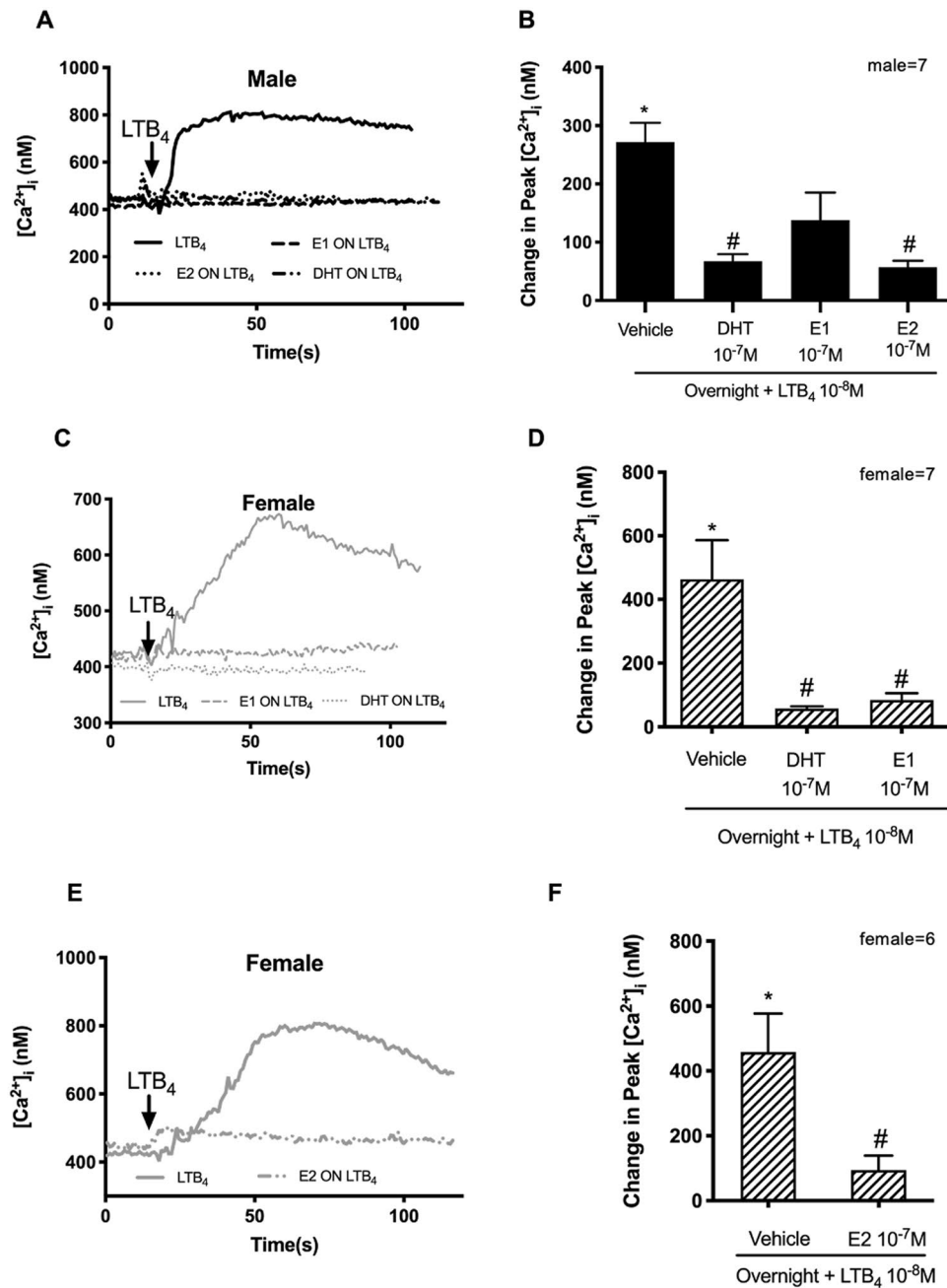
Figure 6. Overnight treatment with sex hormones inhibits the Ca^{2+} response from human conjunctival goblet cells cultured from male and female cells to the inflammatory mediator LTB_4. Cultured goblet cells from male and female conjunctiva were treated with DHT, E1 or E2 (10^{-7} M) or vehicle overnight prior to addition LTB_4 (10^{-8} M). $[Ca^{2+}]_i$ was measured by fura2 assay. The average $[Ca^{2+}]_i$ level over time was shown in (**A**, **C** and **E**); Change in peak $[Ca^{2+}]_i$ was calculated and shown in (**B**, **D** and **F**). Data are mean ± SEM. Number of individuals for each experiment are marked in graphs. Black lines and solid bars indicate data from male cells. Grey lines and slash-patterned bars indicate data from female cells. *Significance difference from basal. #Significance difference between males and females.

with the present study in which DHT decreased the action of the inflammatory mediator LTB_4. Interestingly, we found that estrogen also attenuates the action of LTB_4. The role of estrogen is controversial as reports demonstrate protective[33,34], disruptive[35,36], and neutral[37] effects of estrogen in ocular surface diseases. Estrogen also has multiple types of actions. Estrogen regulates expression of a variety of genes involved in dry eye[38]. In addition, estrogen has more rapid non-gene expression action on cells, such as regulation of vascular endothelium by activating mitogen-activated protein (MAP) kinase kinase[39]. On the ocular surface, estradiol downregulates cyclic AMP (cAMP) signaling in human meibomian gland epithelial cells[3]. As the inflammatory mediator LTB_4
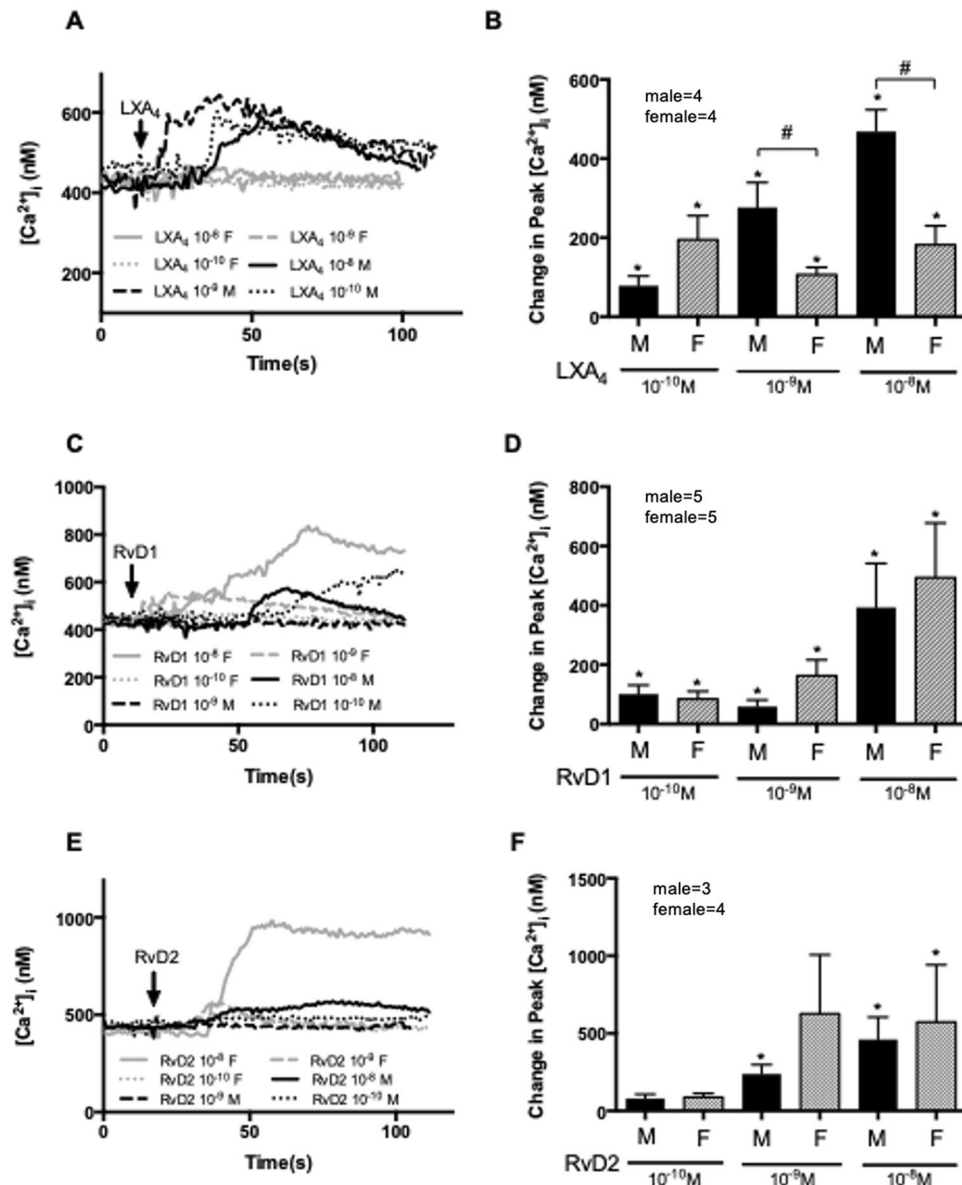
Figure 7. Cultured conjunctival goblet cell Ca^{2+} response to LXA_4 from male is greater than female cells. Goblet cells from both sexes were stimulated with LXA_4 10^{-10}–10^{-8} M (**A** and **B**) RvD1 10^{-10}–10^{-8} M (**C** and **D**) or RvD2 10^{-10}–10^{-8} M (**E** and **F**). $[Ca^{2+}]_i$ was measured by fura2 assay. The average $[Ca^{2+}]_i$ level over time was shown in (**A**, **C** and **E**). Change in peak $[Ca^{2+}]_i$ was calculated and shown in (**B**, **D** and **F**). Data are mean ± SEM, the number of individuals is marked in each graph. Arrow indicates the addition of stimuli. Black lines and solid bars indicate data from male cells. Grey lines and slash-patterned bars and indicate data from female cells. *Significance difference from basal. #Significance difference between males and females.

activates the BLT1 receptor and is coupled to G_i, the BLT1 receptor could work through cAMP in goblet cells. Furthermore, the BLT1 receptor also carries phosphorylation site for MAPK according to Scansite 4.0 https://scansite4.mit.edu/4.0/#home, indicating that it is possible for estrogen to modulate the activity of LTB_4 through the BLT1 receptor. In summary, as one major consequence of goblet cell activation is mucin secretion, the results of our study indicate that sex hormones may protect the ocular surface from the excess tears and itchiness caused by mucin over-secretion during inflammation.

Sex hormones also demonstrate a long-term effect on the LTB_4-Ca^{2+} response, but not on the cholinergic or histaminergic activated Ca^{2+} response. As sex hormones have receptors in the cell nuclei, they could affect the response through the synthesis of proteins. How sex hormones affect the expression of the receptors of the stimuli of each category needs to be further investigated.

We also investigated if neural, histaminergic, eicosanoid, and pro-resolution mediators have differential effects on the Ca^{2+} response in male compared to female human conjunctival goblet cells. We initially hypothesized that the unequal prevalence of conjunctival inflammatory diseases in men and women was due to different cellular responses to allergens or pathogens. However, the results of our experiments indicate that goblet cells

from males and females are equally susceptible to histamine and LTB$_4$ when sex hormones are eliminated from the culture conditions. Carbachol, a parasympathomimetic agonist, has an increased response in females compared to male goblet cells. These results are in contrast to the effect of sex hormones on the cholinergic agonist and LTB$_4$, but not the histamine, responses. Cholinergic agonists have a sex-based difference in their action, but sex hormones do not affect this response. LTB$_4$ stimulation does not have a sex-based difference in cutured goblet cells without sex hormones, but the sex hormones strikingly decrease the LTB$_4$ response both short-term and long-term in cells from both sexes. These results suggest that very different cellular signaling mechanisms regulate sex based differences in agonist stimulated response compared to the effect of exogenously added sex hormones on these responses.

The distinct mechanisms facilitate sex-based differences in conjunctival goblet cells was also observed among pro-resolving mediators. When cultured goblet cells were activated with SPMs, there was a difference between goblet cells from males and females in their response to LXA$_4$ only, with a higher Ca^{2+} response in males compared to females, among the three SPMs tested. LXA$_4$ is one of the earliest discovered SPMs. Together with its receptor ALX/FPR2, LXA$_4$ constitutes an essential protective eicosanoid circuit, regarded as the "jump start" of the resolution of inflammation[40]. LXA$_4$ can be produced by several ocular tissues/cells, including the neutrophils in the corneal limbus, lacrimal glands, and cervical lymph nodes[13], as well as the corneal epithelial cells[41], and is present in the tears of healthy individuals[17]. The beneficial role of LXA$_4$ in reducing chronic inflammation occurs by inhibiting the regulatory T cells in draining lymph nodes, which drive the inflammation towards chronicity[42]. Interestingly, a substantial decrease in lymph node LXA$_4$ biosynthesis was found in an animal model of dry eye specifically in females rather than males[13]. An additional sex-based difference in LXA$_4$ was found when estrogen was demonstrated to down-regulate the LXA$_4$ circuit to induce delayed female-specific wound healing in the cornea[12]. Our current findings add new aspects of the relationship between LXA$_4$ and sex, that is, sex also determines the cellular response to LXA$_4$ in the epithelial conjunctival goblet cells. As LXA$_4$ is biosynthesized from the same precursor as the pro-inflammatory mediator LTB$_4$ and appears at an early stage of resolution of inflammation, we speculate that the active resolution of inflammation begins earlier in males than in females. This finding is consistent with increased disease found in female cells. The sex-based difference in the action of LXA$_4$ on goblet cells also suggests that in future studies oo LXA$_4$, attention should be paid to the sex differences in tissues, as analysis of both sexes together could cover up sex-based differences.

The D series resolvins such as RvD1 and RvD2 used in the present study are biosynthesized from DHA, a process that occurs later in resolution of inflammation than biosynthesis of LXA$_4$. RvD1 and RvD2 have pro-resolution activities as does LXA$_4$ and these D-series resolvins have protective effects on the ocular surface[20,21,43]. In the present study, however, we did not observe any sex-based differences in goblet cell responses to either RvD1 or RvD2. In many cell types RvD1 and LXA$_4$ can use the same receptor, however, in human conjunctival goblet cells RvD1 and LXA$_4$ use different receptors: RvD1 uses GPR32 and LXA$_4$ uses ALX/FPR2[44]. RvD2 uses the GPR18 receptor (unpublished work by Botten, et al.). This could explain the different results for RvD1 and RvD2 compared to LXA$_4$ obtained in the present study. In healthy human tears, we found a significantly higher level of RvD1 and RvD2 in males[17]. Evidence in mice peritonitis model showed elevated key enzymes in the SPMs synthesis, including 12/15-lipoxygenase in males with elimination of sex hormones by gonadectomy, but not in females [45]. Thus we suggest that the sex based differences of LXA$_4$ are mainly in the cellular response to LXA$_4$, while those of RvD1/D2 are mainly the amount of biosynthesis by local tissues. The mechanisms behind the sex-based difference in the amount of biosynthesis of RvD1/D2 needs further investigation.

In conclusion, there are specific mediators that are altered in male versus female conjunctival goblet cell Ca^{2+} responses and whose Ca^{2+} response is affected by exogenous sex hormones using the androgen and estrogen receptors present in goblet cells from both sexes. There is, however, no widespread or generalized effect of sex hormones on goblet cell function. The major effect of the sex hormones is on proinflammatory mediators; the major sex-based difference is in the cellular response to the pro-resolution mediator LXA$_4$. That males have higher response to the ω-6-fatty acid derived SPM LXA$_4$ indicates that males may terminate inflammation in conjunctival goblet cells faster than females. Sex based differences should be taken into consideration when studying inflammation and its resolution in conjunctival inflammatory diseases.

Methods

Human materials.   Human conjunctival tissue was obtained from Eversight Eye Bank (Ann Arbor, MI). All tissue was donated to the eye bank with the prior informed consent and authorization of the donors for use in scientific research. The tissues were placed in Optisol media within 6 h of death. Use of this tissue was reviewed by the Massachusetts Eye and Ear Human Studies Committee and determined to be exempt and does not meet the definition of research with human subjects. The age and sex of each individual donor are listed in Supplementary Table 1.

Goblet cell culture.   Goblet cells from human conjunctiva were grown in organ culture. Pieces of minced conjunctival epithelium were placed on culture dishes with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 2 mM non-essential amino acids (NEAA), 2 mM sodium pyruvate, and 100 μg/ml penicillin–streptomycin. The tissue pieces were removed after nodules of cells were observed. Twenty-four hours before experiments, cells were transferred into FBS-free and phenol red-free media to minimize the potential effect of sex hormones contained in FBS. First passage goblet cells were used in all experiments. Identity of cultured cells was periodically checked by evaluating staining with antibody to cytokeratin 7 (detects goblet cell bodies) and the lectin Helix pomatia agglutinin (HPA) (detects goblet cell secretory product) to ensure that goblet cells predominated.

Immunofluorescence microscopy (IF). First passage goblet cells were grown on glass coverslips and fixed with paraformaldehyde. The coverslips were blocked in 1% bovine serum albumin (BSA) with 0.2% Triton X-100 in PBS for 45 min. Anti-estrogen receptor (ER)α antibody, anti-ERβ antibody or anti-androgen receptor (AR) antibody (Abcam, Cambridge, UK) were used at a 1:100 dilution overnight at 4 °C. HPA conjugated to Alexa Fluor 488 (Invitrogen, Eugine, OR) was used at a 1:200 dilution. Secondary antibodies conjugated either to Cy2 or Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA) were used at 1:150 dilution for 1.5 h at room temperature. Negative control experiments included incubation with the absence of the primary antibody. Antibody specificity was determined by incubation with the primary antibody that was treated overnight with the corresponding immunizing peptide at a 1:5 ratio (Abcam, Cambridge, UK).

Western Blotting Analysis (WB). Cultured goblet cells were were homogenized in radio-immunopre-cipitation assay buffer (RIPA, 10 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1% deoxycholic acid, 1% Triton X-100, 0.1% sodium dodecyl sulfate, and 1 mM EDTA) in the presence of a protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO). The homogenate was centrifuged at 2000g for 30 min at 4 °C. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and processed for western blotting analysis. The same antibodies were used as in IF experiments, with 1:500 dilution. Immunoreactive bands were visualized by the enhanced chemiluminescence method. The specificity of the antibodies was confirmed using the corresponding peptide at a 1:5 ratio (Abcam, Cambridge, UK) as for IF.

RT-PCR. Cultured goblet cells were homogenized in TRIzol (Invitrogen, Carlsbad, CA), and total RNA was isolated. The total RNA was purified using a TURBO DNA-free kit (Thermo Fisher, Waltham, MA). Five micro-grams of purified total RNA were used for complementary DNA (cDNA) synthesis using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). The cDNA was amplified by the polymerase chain reaction (PCR) using primers specific to human *Erα* and *Erβ* and *Ar* receptors using the Jumpstart REDTaq Readymix Reaction Mix (Sigma-Aldrich, St. Louis, MO) in a thermal cycler (Master Cycler, Eppendorf, Hauppauge, NY). The genome sequence was found in the NCBI database (https://www.ncbi.nlm.nih.gov) and primers were designed using primer blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast) (*Erα* F: 5′-GGAGGG CAGGGGTGAA-3′ R: 5′-GGCCAGGCTGTTCTTCTTAG-3′; *Erβ* F: AGAGTCCCTGGTGTGAAGCAAG-3′ R: GACAGCGCAGAAGTGAGCATC-3′; *Ar* F: 5′-AATGGGACCTTGGATGGAGAACTA-3′ R: 5′-TCATAA CATTTCCGGAGACGACAC-3′). The conditions were as follows: 5 min at 95 °C followed by 35 cycles of 1 min at 94 °C, 30 s at an annealing temperature of 62 °C for *Erα* and *Erβ*, 59 °C for *Ar*, and 1 min at 72 °C with a final hold at 72 °C for 10 min. Samples with no cDNA served as the negative control while the presence of *β-actin* was a positive control. Amplification products were separated by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining.

Measurement of $[Ca^{2+}]_i$. First passage human conjunctival goblet cells were plated onto 35-mm glass-bottom culture dishes and incubated at 37 °C overnight. Cells were then incubated for 1 h at 37 °C with Krebs–Ringer bicarbonate buffer containing 119 mM NaCl, 4.8 mM KCl, 1.0 mM CaCl$_2$, 1.2 mM MgSO$_4$, and 25 mM NaHCO$_3$ with 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES) plus 0.5% BSA containing 0.5 μM fura-2/AM (Invitrogen, Grand Island, NY, USA), 8 μM pluronic acid F127 (Sigma-Aldrich, St. Louis, MO, USA) and 250 μM sulfinpyrazone (Sigma-Aldrich) for 1 h. Before calcium measurements were started, cells were washed with KRB-HEPES containing sulfinpyrazone. Ca^{2+} measurements were conducted using a ratio imaging system (In Cyt Im2; Intracellular Imaging, Cincinnati, OH, USA) using wavelengths of 340 and 380 nm and an emission wavelength of 505 nm. Cells were stimulated with agonists, antagonists, and inhibitors. The intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) over time was recorded, and the change in peak $[Ca^{2+}]_i$ was calculated by subtracting the average of the basal value from the peak $[Ca^{2+}]_i$ value.

Statistical analysis. The data are presented as the fold-increase above basal as average ± SEM. Student's t-test was used in two group comparisons, and One-way ANOVA with Dunnett correction was used in multiple group comparisons. $P < 0.05$ was set as statistically significant.

Ethical approval. Institutional Review Board Statement: The use of human tissue was reviewed by the Massachusetts Eye and Ear Human Studies Committee and determined to be exempt and does not meet the definition of research with human subjects.

Data availability
Raw data were generated at Schepens Eye Research Institute. Derived data supporting the findings of this study are available from the corresponding author D.A.D. on request.

References
1. Schaumberg, D. A., Dana, R., Buring, J. E. & Sullivan, D. A. Prevalence of dry eye disease among US men: Estimates from the Physicians' Health Studies. *Arch. Ophthalmol.* **127**, 763–768 (2009).
2. Tellefsen Nøland, S. *et al.* Sex and age differences in symptoms and signs of dry eye disease in a Norwegian cohort of patients. *Ocul. Surf.* **19**, 68–73 (2021).
3. Sullivan, D. A. *et al.* TFOS DEWS II sex, gender, and hormones report. *Ocul. Surf.* **15**, 284–333 (2017).

4. Meester, I. *et al.* SeXY chromosomes and the immune system: Reflections after a comparative study. *Biol. Sex Differ.* **11**, 3 (2020).
5. Yang, J.-H. *et al.* Hormone replacement therapy reverses the decrease in natural killer cytotoxicity but does not reverse the decreases in the T-cell subpopulation or interferon-gamma production in postmenopausal women. *Fertil. Steril.* **74**, 261–267 (2000).
6. Liesegang, T. J. Hormone replacement therapy and dry eye syndrome. Schaumberg DA. *Am. J. Ophthalmol.* **133**, 435–436 (2002).
7. Orucoglu, F., Akman, M. & Onal, S. Analysis of age, refractive error and gender related changes of the cornea and the anterior segment of the eye with Scheimpflug imaging. *Cont. Lens Anterior Eye* **38**, 345–350 (2015).
8. Strobbe, E., Cellini, M., Barbaresi, U. & Campos, E. C. Influence of age and gender on corneal biomechanical properties in a healthy Italian population. *Cornea* **33**, 968–972 (2014).
9. Sullivan, D. A., Jensen, R. V., Suzuki, T. & Richards, S. M. Do sex steroids exert sex-specific and/or opposite effects on gene expression in lacrimal and meibomian glands?. *Mol. Vis.* **15**, 1553–1572 (2009).
10. Bukhari, A. A., Basheer, N. A. & Joharjy, H. I. Age, gender, and interracial variability of normal lacrimal gland volume using MRI. *Ophthal. Plast. Reconstr. Surg.* **30**, 388–391 (2014).
11. Sullivan, B. D., Evans, J. E., Dana, M. R. & Sullivan, D. A. Influence of aging on the polar and neutral lipid profiles in human meibomian gland secretions. *Arch. Ophthalmol.* **124**, 1286–1292 (2006).
12. Wang, S. B. *et al.* Estrogen negatively regulates epithelial wound healing and protective lipid mediator circuits in the cornea. *FASEB J.* **26**, 1506–1516 (2012).
13. Gao, Y. *et al.* Female-specific downregulation of tissue polymorphonuclear neutrophils drives impaired regulatory T cell and amplified effector T cell responses in autoimmune dry eye disease. *J. Immunol.* **195**, 3086–3099 (2015).
14. Sullivan, D. A., Block, L. & Pena, J. D. Influence of androgens and pituitary hormones on the structural profile and secretory activity of the lacrimal gland. *Acta Ophthalmol. Scand.* **74**, 421–435 (1996).
15. Ebeigbe, J. A. & Ebeigbe, P. N. The influence of sex hormone levels on tear production in postmenopausal Nigerian women. *Afr. J. Med. Med. Sci.* **43**, 205–211 (2014).
16. Suzuki, T. *et al.* Estrogen's and progesterone's impact on gene expression in the mouse lacrimal gland. *Invest. Ophthalmol. Vis. Sci.* **47**, 158–168 (2006).
17. English, J. T., Norris, P. C., Hodges, R. R., Dartt, D. A. & Serhan, C. N. Identification and profiling of specialized pro-resolving mediators in human tears by lipid mediator metabolomics. *Prostaglandins Leukot. Essent. Fatty Acids* **117**, 17–27 (2017).
18. Li, D., Jiao, J., Shatos, M. A., Hodges, R. R. & Dartt, D. A. Effect of VIP on intracellular [Ca2+], extracellular regulated kinase 1/2, and secretion in cultured rat conjunctival goblet cells. *Invest. Ophthalmol. Vis. Sci.* **54**, 2872–2884 (2013).
19. Hodges, R. R. & Dartt, D. A. Tear film mucins: Front line defenders of the ocular surface; comparison with airway and gastrointestinal tract mucins. *Exp. Eye Res.* **117**, 62–78 (2013).
20. Saban, D. R. *et al.* Resolvin D1 treatment on goblet cell mucin and immune responses in the chronic allergic eye disease (AED) model. *Mucosal Immunol.* **12**, 145–153 (2019).
21. Li, D. *et al.* Resolvin D1 and aspirin-triggered resolvin D1 regulate histamine-stimulated conjunctival goblet cell secretion. *Mucosal Immunol.* **6**, 1119–1130 (2013).
22. Ríos, J. D. *et al.* Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion. *Invest. Ophthalmol. Vis. Sci.* **40**, 1102–1111 (1999).
23. Serhan, C. N. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* **510**, 92–101 (2014).
24. Yang, M., Bair, J. A., Hodges, R. R., Serhan, C. N. & Dartt, D. A. Resolvin E1 reduces leukotriene B4-induced intracellular calcium increase and mucin secretion in rat conjunctival goblet cells. *Am. J. Pathol.* **190**, 1823–1832 (2020).
25. Hodges, R. R. *et al.* Lipoxin A4 activates ALX/FPR2 receptor to regulate conjunctival goblet cell secretion. *Mucosal Immunol.* **10**, 46–57 (2016).
26. Dartt, D. A. *et al.* Conjunctival goblet cell secretion stimulated by leukotrienes is reduced by resolvins D1 and E1 to promote resolution of inflammation. *J. Immunol.* **186**, 4455–4466 (2011).
27. Yang, M. *et al.* RvE1 uses the LTB4 receptor BLT1 to increase [Ca2]i and stimulate mucin secretion in cultured rat and human conjunctival goblet cells. *Ocul. Surf.* **18**, 470–482 (2020).
28. Wickham, L. A. *et al.* Identification of androgen, estrogen and progesterone receptor mRNAs in the eye. *Acta Ophthalmol. Scand.* **78**, 146–153 (2000).
29. Pache, M., Glatz-Krieger, K., Sauter, G. & Meyer, P. Expression of sex hormone receptors and cell cycle proteins in melanocytic lesions of the ocular conjunctiva. *Graefes Arch. Clin. Exp. Ophthalmol.* **244**, 113–117 (2006).
30. Spelsberg, H. *et al.* Detection of oestrogen receptors (ER) alpha and beta in conjunctiva, lacrimal gland, and tarsal plates. *Eye* **18**, 729–733 (2004).
31. Sullivan, D. A. *et al.* Androgen deficiency, meibomian gland dysfunction, and evaporative dry eye. *Ann. N. Y. Acad. Sci.* **966**, 211–222 (2002).
32. Azcarate, P. M. *et al.* Androgen deficiency and dry eye syndrome in the aging male. *Invest. Ophthalmol. Vis. Sci.* **55**, 5046–5053 (2014).
33. Jin, X., Lin, Z., Liu, Y., Lin, L. & Zhu, B. Hormone replacement therapy benefits meibomian gland dysfunction in perimenopausal women. *Medicine* **95**, e4268 (2016).
34. Azzarolo, A. M., Eihausen, H. & Schechter, J. Estrogen prevention of lacrimal gland cell death and lymphocytic infiltration. *Exp. Eye Res.* **77**, 347–354 (2003).
35. Ahmad, N., Chen, S., Wang, W. & Kapila, S. 17β-estradiol induces MMP-9 and MMP-13 in TMJ fibrochondrocytes via estrogen receptor α. *J. Dent. Res.* **97**, 1023–1030 (2018).
36. Nalvarte, I. *et al.* Estrogen receptor β controls MMP-19 expression in mouse ovaries during ovulation. *Reproduction* **151**, 253–259 (2016).
37. Rahimi Darabad, R. *et al.* Does estrogen deficiency cause lacrimal gland inflammation and aqueous-deficient dry eye in mice?. *Exp. Eye Res.* **127**, 153–160 (2014).
38. Suzuki, T., Schirra, F., Richards, S. M., Jensen, R. V. & Sullivan, D. A. Estrogen and progesterone control of gene expression in the mouse meibomian gland. *Invest. Ophthalmol. Vis. Sci.* **49**, 1797–1808 (2008).
39. Chen, Z. *et al.* Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J. Clin. Invest.* **103**, 401–406 (1999).
40. Liclican, E. L. & Gronert, K. Molecular circuits of resolution in the eye. *Sci. World J.* **10**, 1029–1047 (2010).
41. Gronert, K. *et al.* A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *J. Biol. Chem.* **280**, 15267–15278 (2005).
42. Biteman, B. *et al.* Interdependence of lipoxin A4 and heme-oxygenase in counter-regulating inflammation during corneal wound healing. *FASEB J.* **21**, 2257–2266 (2007).
43. Erdinest, N., Ovadia, H., Kormas, R. & Solomon, A. Anti-inflammatory effects of resolvin-D1 on human corneal epithelial cells: In vitro study. *J. Inflamm.* **11**, 6 (2014).
44. Hirakata, T. *et al.* Dietary ω-3 fatty acids alter the lipid mediator profile and alleviate allergic conjunctivitis without modulating T2 immune responses. *FASEB J.* **33**, 3392–3403 (2019).
45. Troisi, F. *et al.* Sex hormone-dependent lipid mediator formation in male and female mice during peritonitis. *Front. Pharmacol.* **12**, 818544 (2021).

Author contributions

Conceptualization, M.Y. and D.A.D.; Investigation, M.Y.; student intern, H.F, K.F., N.W.; writing-original draft, M.Y.; writing-review and editing, D.A.D., C.N.S. and T.P.U.; funding acquisition, D.A.D.; supervision, D.A.D.; H.F and K.F contributed equally.

Funding

Competing interests

Dr. T. P. Utheim is a TFOS Global Ambassador for Norway, a board member of the International Ocular Surface Society and a Faculty Member of the European School for advanced studies in ophthalmology. The rest of the authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-20177-9.

Correspondence and requests for materials should be addressed to D.A.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.