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ORIGINAL ARTICLE

The Role of Dyslipidemia on Ocular Surface, Lacrimal and Meibomian Gland Structure and Function

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ABSTRACT

Purpose: Dyslipidemia is characterized by high lipid blood levels that are risk factors for cardiovascular diseases, which are leading causes of death. However, it is unclear whether dyslipidemia is a cause of the dry eye syndrome (DES). Therefore we determined in transgenic mice models of dyslipidemia, whether there is an association with DES development.

Methods: Dyslipidemic models included male and female adult mice overexpressing apolipoprotein CIII (Apo CIII), LDL receptor knockout (LDLR-KO) and ApoE knockout (ApoE-KO). They were compared with age- and gender-matched C57BL/6 mice. Ocular health was evaluated based on corneal slit lamp assessment, phenol red thread test (PRT) and impression cytology. Blood lipid profiles and histology of meibomian and lacrimal glands were also evaluated. Effects of high-fat diet and aging were observed in LDLR-KO and ApoCIII strains, respectively.

Results: Body weight and lacrimal gland weight were significantly higher in male mice compared to females of the same strain ($P < 0.05$). Body weight was significantly lower in LDLRKO mice receiving high lipid diet compared to their controls ($P = 0.0043$). ApoE-KO were hypercholesterolemic and ApoCIII hypertriglyceridemic while LDLR-KO showed increases in both parameters. The PRT test was lower in male LDLR-KO mice with high-fat diet than control mice with standard diet ($P = 0.0273$). Aging did not affect lacrimal structural or functional parameters of ApoCIII strain.

Conclusions: DES development is not solely dependent on dyslipidemia in relevant mice models promoting this condition. On the other hand, lacrimal gland structure and function are differentially impacted by lipid profile changes in male and female mice. This dissociation suggests that other factors beside dyslipidemia impact on tear film dysfunction and DES development.

Keywords: Apolipoprotein, Low density lipoprotein, Lacrimal gland, Meibomian gland, Dry eye, Ocular surface

Abbreviations: Apo, apolipoprotein; DES, dry eye syndrome; KO, knockout; KI, knockin, LDLR, low density lipoprotein receptor; LG, lacrimal gland; MG, meibomian gland; PRT, phenol red thread.

INTRODUCTION

Lipids are key tear film components and it has been suggested that disturbances in lipid metabolism resulting from systemic and environmental conditions, such as diet and medications, as well as hormonal status can elicit dry eye syndrome (DES) symptomolgy.[1–5] Despite the recognized importance of lipids on tear film composition and ocular surface maintenance,[6] it is unknown whether dyslipidemia resulting from systemic lipid disorders is associated with DES.

There are conflicting epidemiological studies about the association between the symptoms of dry eye and hyperlipidemia based on comparing data

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obtained from a group of individuals in Beaver Dam, Wisconsin, USA (risk ratio = 0.83) with those in Taiwan (odds ratio = 1,68).[7,8] Dyslipidemia in individuals with higher levels of lipoprotein A and in patients with Sjogren's syndrome frequently experience symptomology associated with DES. Up to 81% of the individuals in the former group present signs of dry eye and/or ocular surface changes.[9–11] To make a more definitive assessment of the role of dyslipidemia in DES symptomology development, transgenic mice can be used whose loss or gain of lipid mediator function cause dyslipidemia.

The models we used for this purpose include apolipoprotein-E knockout (APOE-KO), low density lipoprotein receptor knockout (LDLR-KO) and mouse model with over expression of human Apolipoprotein CIII (ApoCIIIKI). (knockin) They are useful because lipoproteins, such as the apolipoproteins (ApoA-ApoE), are responsible for lipid redistribution from the liver to peripheral tissues. Their other roles in lipid metabolism include detecting and interacting with cell receptors such as LDLR, supporting dietary linoleic acid absorption in the bowel, and enzyme lipogenic activity.[12,13] High-density lipoprotein (HDL) culture medium supplementation enhances *in vitro* acrimal gland (LG) acinar cell secretory activity.[14] Moreover, human and mouse LG express apolipoproteins, which suggests that lipoproteins have a role in supporting LG activity and maintaining its function needed for tear secretion.[15]

ApoCIII-KI mice present with high levels of blood triacyl glycerol and free fatty acids, which mimics human hyperliproteinemia type IV. This condition is very common in the general population, and has been associated with alterations in skin sebum content.[16,17]

LDLR-KO mice are healthy except for hypercholesterolemia (2–4 times higher than controls). They develop this condition because of delays in processing intermediate-density lipoproteins and low density lipoproteins (IDL and LDL) without changes in HDL.[18]

ApoE-KO mice develop spontaneous hypercholesterolemia and atherosclerosis. Moreover, this mouse model is susceptible to corneal viral infections, which may be related to imbalances in sex hormones.[19] Cross-breeding of LDLR-KO or ApoE-KO with ACAT1-KO mice and feeding them a hyperlipidemic diet generates mice with severe dry eye. However, the individual effects of ApoE-KO, LDLR-KO or ApoCIII-KI by themselves on tear film composition and on ocular surface health are unknown.[20]

The extensive evidence suggesting an association between hyperlipidemia and/or lipid metabolism disturbance and DES led us to further assess this linkage. This was done based on described increases in ocular surface alterations and corneal opacity seen under this condition. Accordingly, we determined here if in ApoCIII-KI, LDLR-KO and ApoE-KO mice there is a higher incidence of dry eye and/or ocular surface alterations involving modification of LG histology, tear secretion and ocular surface health that are related to their gender, diet or aging.

MATERIALS AND METHODS

Five- to nine-month-old male and female mice were obtained from the Department of Physiology, Biology Institute, State University of Campinas, SP and the Department of Physiological Sciences, Health Sciences Center (ApoCIII-KI, LDLR-KO and C57BL/6) or Federal University of Espírito Santo, Vitória, ES, Brazil (ApoE-KO and C57BL/6). Animals were given free access to standard rodent chow and water, except where otherwise specified ($N = 5$/group).

All experimental procedures adhered to the "Principles of Laboratory Animal Care" (NIH publication no. 85-23) and ARVO statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the University Committee of Animal Experimentation.

Animals were compared at 5 months of age, for tear secretion and LG histology. LDLR-KO mice were also compared with or without a high-fat diet, and ApoCIII-KI were evaluated at 9 months of age with age-matched controls. The high-fat diet was given for 1 month to control and LDLR-KO strains, and consisted of 195.0 g/kg casein, 320.7 g/kg corn starch, 79.6 g/kg dextrin, 50.0 g/kg lard, 204.0 g/kg butter, 50.0 g/kg cellulose, 35.0 g/kg mineral mix AIN-93M, 10.0 g/kg vitamin mix AIN-93M, 1.8 g/kg L-cystine, 2.5 g/kg choline bitartrate, 51.4 g/kg sucrose and 0.008 g/kg butylated hydroxytoluene (BHT).

Plasma Biochemical Analysis

Blood samples were collected from the tail vein. Total cholesterol and triglycerides were measured in fresh plasma in the fasting state (12 h) using standard commercial kits, according to the manufacturer's instructions (Boehringer Mannhein®, Mannhein, Germany and Wako®, Neuss, Germany).

Eye Exam

The animals were weighed, a slit lamp exam was performed and the eyes were photographed (Carl Zeiss - Ltda, Germany and DSC-W5 Sony Ltda, Japan).

General anaesthesia consisting of a combination of ketamine (5 mg/100 g b.w.) (União Química Faramacêutica S.A, Embu-Guaçu, SP, Brazil) and xylazine (2 mg/100 g b.w.) (Laboratorio Callier S.A., Barcelona, Spain) was injected intraperitoneally. A

phenol red thread test was performed with a thread of 1-mm width and 20 mm length thread placed in the conjunctival fornix for 15 sec to measure tears secretion (Zone-Quick, Mericon America, Inc., San Mateo, CA). Additionally, epithelial cells were collected for impression cytology. After those sample collections, mice were euthanized with excess anaesthesia.

Impression Cytology

Corneal epithelial samples were obtained from the ocular surface of the three groups ($N = 5$/group), using 0.45 µm filter paper (Millipore Co, Billerica, MA), under anaesthesia. The samples were collected from the same area (temporal) and transferred to gelatin-coated slides, fixed with 70% ethanol glacial acetic acid and formalin, and stained with periodic acid-Schiff (PAS) and haematoxylin. Epithelial grading of squamous metaplasia was evaluated in a masked fashion, and epithelial cells were classified into four stages: stage 0 (for normal cell number, morphology and mucous staining), stage 1 (lower cell number and mucous staining), stage 2 (lower cell number, reduced size of nuclei, square shape of cells) and stage 3 (squamous metaplasia, including lower cell number, higher cytoplasmic volume and pycnotic or absent nuclei).[21] Photographic documentation was performed using an Olympus BX40 light microscope (Olympus Corporation, Tokyo, Japan) and a digital camera (Olympus Q-color 5) at 100 and 400× magnification.

Histology

LG and eye globes, including lids, and therefore meibomian glands, were collected, weighed, embedded in OCT compound (Sakura Fine Tek Inc., Torrance, CA) and frozen in dry ice. Blocks from the three groups ($N = 5$–10/group) were cut into 6 µm sections at –20°C and transferred to poly-L-lysine pre-coated glass slides (Perfecta, São Paulo, Brazil). The material was then stained with haematoxylin/eosin (HE) for histological examination. The images were recorded by light microscope, described above.

Statistical Analysis

Data are expressed as means ± SEM. Comparisons were made using Kruskal–Wallis test for continuous data comparison among several groups, the Mann–Whitney U test for continuous data comparison between models of disease and their respective controls, and the Fisher test for categorical data (Graphpad 5.0 software, Prism, San Diego, CA). The level of significance used was $P < 0.05$.

RESULTS

Comparative Analysis Between Dyslipidemic and Control Mice

Biometric Analysis

Body weight was significantly higher in male than in female mice in all strains studied ($P < 0.0001$, Kruskal–Wallis test). Comparing strains of age and sex-matched mice the body weight rank order was: control > ApoE-KO > LDLR-KO (Table 1).

LG weight was similar in all groups compared to sex-matched controls, but was greater in males than females in all strains (Table 1). The ratio between LG weight and body weight revealed that male mice have a relatively larger LG compared to females. There were no differences in the ratio between LG weight and body weight when we compared age and sex-matched controls of the different genotypes (Table 1).

Biochemical Analysis

LDLR-KO mice had significantly higher blood cholesterol and triacylglyceride levels and both measurements were higher in females compared to male LDLR-KO mice (Table 2).

ApoE-KO mice presented with higher cholesterol, but similar triacylglyceride levels compared to control mice (Mann–Whitney U) (Table 2). Male ApoE-KO presented with higher cholesterol levels than male LDLK-RO ($P = 0.019$), Mann–Whitney U test) (Table 2).

TABLE 1 Body weight and lacrimal gland (LG) weight of males and females of Control, ApoE-KO and LDLR-KO, of 5 months of age.

Groups	Sex	Control	LDLR-KO	ApoE-KO	*P*
Body weight (g)	Male	31.0 ± 1.1*	25.2 ± 1.0*	28.3 ± 1.0*	*0.003
	Female	21.1 ± 0.2	20.8 ± 0.5	24.9 ± 1.2	
LG weight (mg)	Male	16.7 ± 1.8#	13.1 ± 0.7#	19.4 ± 0.7#	#Control 0.0038, LDLRKO 0.0003, ApoE-KO 0.0022
	Female	7.8 ± 1.1#	7.7 ± 0.9#	10.8 ± 1.5#	
LGW/BW	Male	0.55 ± 0.08	0.51 ± 0.05	0.71 ± 0.03	
	Female	0.29 ± 0.02	0.34 ± 0.05	0.43 ± 0.08	

LGW/BW, ratio between lacrimal gland weight and body weight.
Data are reported as mean ± SEM.
*$P < 0.05$ (Kruskal–Wallis test, Dunn's *post hoc* test), #$P < 0.05$ for comparison between genders.

TABLE 2 Blood level of cholesterol and triacylglycerol in Control, LDLR-KO and ApoE-KO mice of 5 months of age.

Groups	Sex	Control	LDLR-KO	ApoEKO	*P*
Cholesterol (mg/dl)	Male	99.7 ± 2.6*&	317.9 ± 15.1*#	432.3 ± 18.1&	*0.0003 &0.0167
	Female	57.3 ± 8.0*&	376.1 ± 14.3*#	317.5 ± 25.1&	#0.0335 *0.0043 &0.0004
Triacylglycerol (mg/dl)	Male	60.5 ± 3.9*	146.0 ± 14.5*#	62.3 ± 1.8	*0.0002 #0.0295
	Female	37.1 ± 8.6*	195.9 ± 4.7*#	49.9 ± 3.6	*0.00043

Data are reported as mean ± SEM.
*&$P < 0.05$ for comparison between control and LDLR-KO, #$P < 0.05$ for comparison between genders (Mann–Whitney U test).

Body and Ocular Examination

Regarding other tissues of ectodermal origin, it was of interest that LDLR-KO mice had limited areas of alopecia, without skin lesions, which was more prevalent in mice fed a high-fat diet (Figure 1).

Slit lamp examination did not show any ocular surface alterations in either gender of any of the following groups: control, LDLR-KO and ApoE-KO (Figure 2).

Tears secretion was unchanged between the controls and any of the transgenic KO groups. Their values are: A) male control: 7.0 ± 1.5 mm, female control: B) 5.0 ± 1.5 mm, male LDLR-KO: 4.0 ± 0.6 mm, female LDLR-KO: 6.0 ± 1.6 mm, male; C) ApoE-KO: 5.0 ± 0.4 and female ApoE-KO: 6.0 ± 2.1 mm ($P > 0.05$, Kruskal–Wallis test).

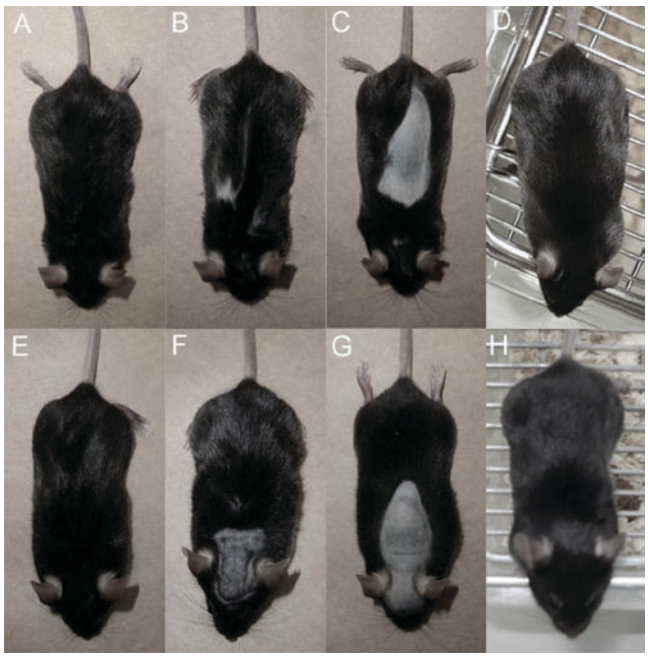

FIGURE 1 Representative photos of pelage in mice showing dorsal alopecia in LDLR-KO, more evident in females and under high fat diet, A) male control, B) male LDLR-KO, C) male LDLR-KO with high-fat diet, D) male ApoE, E) female control, F) female LDLR-KO, G) female LDLR-KO with high-fat diet and H) female ApoE.

Differential Effects of Hyperlipidemic Diet in Mouse Models

Considering that no ocular or tear secretion alterations were observed among the groups, and alopecia was only noticeable in LDLR-KO mice on a high-fat diet, further functional and histological analyses were performed to determine if feeding LDLR-KO and LDLR-KO mice a high-fat diet had any impact on anterior ocular surface phenomena

No changes were observed in the ocular surface of LDLR-KO mice on a high-fat diet (data not shown). On the other hand, tear secretion fell in LDLR-KO male mice receiving a high-fat diet relative to their controls (4.0 ± 0.9 versus 7.0 ± 1.5 mm, respectively, $P = 0.0273$, Mann–Whitney U test), whereas no such change was detected amongst females of the same strain, based on a comparison of the effect of high-fat diet with that of a control on tear flow, respectively 6.0 ± 2.1 mm versus 5.0 ± 1.5 mm, $P > 0.05$, Mann–Whitney U test).

Corneal impression cytology did not reveal any signs of metaplasia or other epithelial cell alterations in either gender of controls or LDLR-KO either on or off the high-fat diet ($P > 0.05$, Fisher test) (Figure 3).

Histological analysis to identify gross histological differences in tissues from dyslipidemic and control groups on the cornea, MG and LG stained with HE did not show any structural differences among the controls, LDLR-KO or LDLR-KO receiving the high-fat diet based on either corneal thickness, lipid deposits, inflammatory

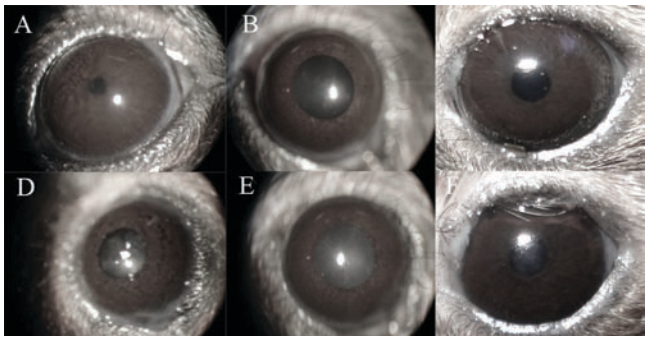

FIGURE 2 Slit lamp exam of mice eyes A) male control, B) male LDLR-KO, C) male ApoE, D) female control, E) female LDLR-KO and F) female ApoE.

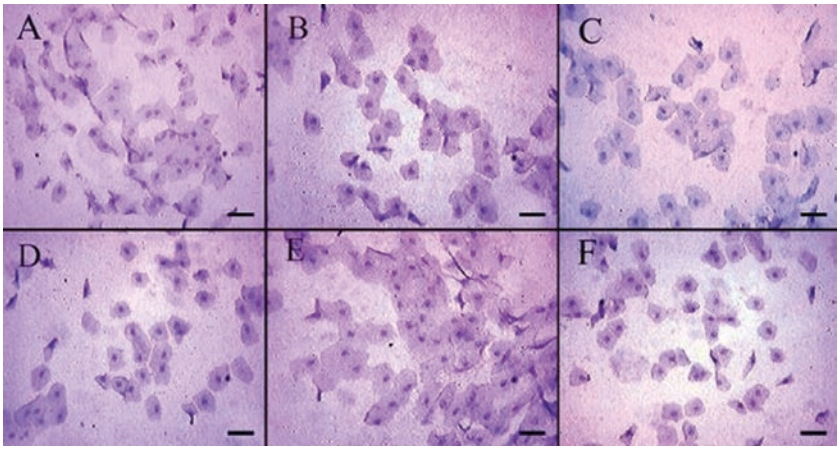

FIGURE 3 Representative microphotographs of impression cytology of corneal epithelial cells from control A&D male and female control, B&E male and female LDLR-KO, C&F male and female LDLR-KO with high-fat diet mice (C) ($N=5$/group) (scale bar = 50 μm). Grades 0 to 3 were assigned to each sample in a masked manner, considering cell, shape, nucleus size and presence of mucus. Data did not differ significantly between the three groups ($P>0.05$; Fisher test).

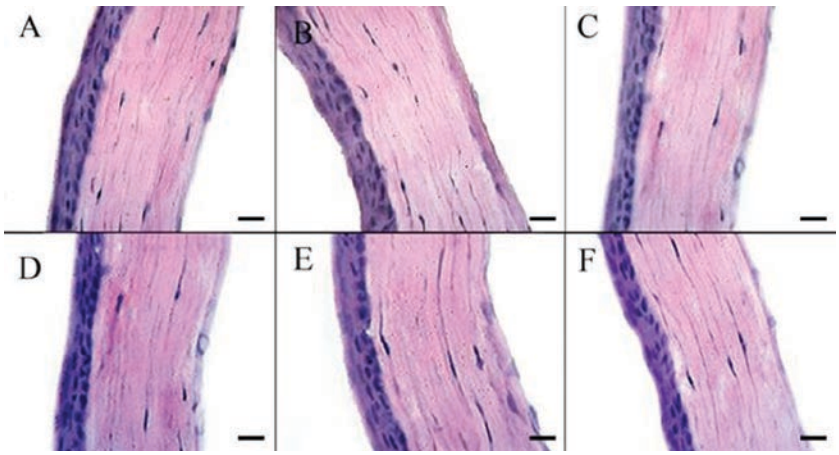

FIGURE 4 Histological comparison of male and female corneas of 5 months old mice from control (A, D), LDLR-KO (B, E) and LDLR-KO with high-fat diet (C, F) groups. The material was stained with HE, scale bar = 12.5 μm.

cell infiltration, MG or LG acinar size. Light microscopy does not have the needed resolution to detect individual cellular differences (Figures 4, 5 and 6).

Effect of Aging on ApoCIII-KI Mice

Chronic dyslipidemia in 9-month-old ApoCIII-KI mice resulted in higher cholesterol and blood triacylglyceride levels in comparison to young (5 months old) and age-matched controls. Moreover, the body weight of male ApoCIII-KI mice was significantly higher than age-matched controls and APOCIII-KI females. LG weight was higher in both male and female ApoCIII-KI groups compared to their respective controls (Table 3). However, the LG/Body weight ratio was similar between gender- and age-matched ApoCIII-KI and controls ($P=0.65$ for females and $P=0.18$ for males, Mann–Whitney U test).

A body exam did not show any pelage or skin alteration. The ocular exam did not show corneal alteration, but cataracts were present in female ApoCIII-KI (Figure 7). Neither aging nor elevated triacylglyceride blood levels affected tear secretion in 5-month-old or 9-month-old controls of both sexes (Table 3). Similarly, histology of LG, MG and cornea of aging ApoCIII-KI of both sexes did not show any structural differences in comparison with 5- or 9-month-old control mice (Figure 7).

DISCUSSION

Even though hyperlipidemia and/or a change in lipoproteins distribution was induced by the use of different mouse knockout and knockin models fed high lipid diets, this study indicates that hyperlipidemia does not elicit DES *per se.* This assessment is based on the finding that LDLR, ApoCIII and ApoE knockouts developed hyperlipidemia, but this condition was not a contributing factor for dry eye or organ related inflammation. This negative correlation is in contrast with some other diseases such as atherosclerosis whose severity correlates with levels of blood lipoprotein imbalance.[22]

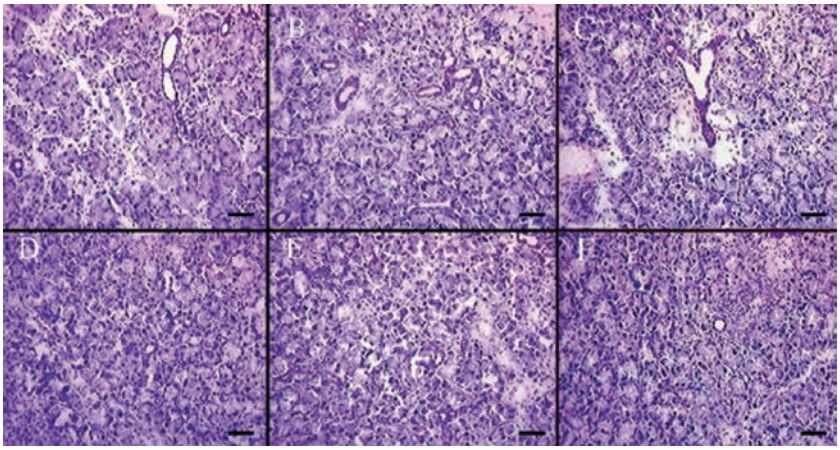

FIGURE 5 Histological comparison of male and female lacrimal gland of 5 months old mice from control (A, D), LDLR-KO (B, E) and LDLR-KO with high-fat diet (C, F) groups. The material was stained with HE, scale bar = 50 μm.

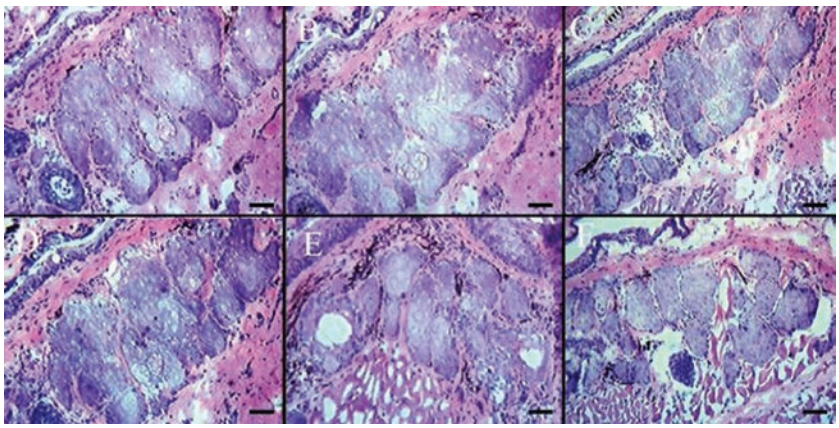

FIGURE 6 Histological comparison of male and female meibomian gland of 5 months old mice from control (A, D), LDLR-KO (B, E) and LDLR-KO with high-fat diet (C, F) groups. The material was stained with HE, scale bar = 12.5 μm.

This disconnect between DES and hyperlipidemia and lipoprotein imbalance agrees with recent reports on genetic induced MGD pathophysiology In this case, MG metabolism was perturbed disrupting lipid biosynthesis, but without eliciting DES.[23] Similarly, the role of inflammation in inducing MGD remains unclear.[23]

It was described before that in mouse liver and skin that over expressing human apolipoprotein C1 (ApoCIKI) resulted in elevated cholesterol and free fatty acid serum levels. This lipid profile change is similar to what we observed in ApoE-KO and LDLR-KO mice. However, ApoCIKI mice have thicker, inflamed skin and atrophic meibomian glands. These findings suggest that ApoCIKI mice are more susceptible to developing DES than ApoE-KO and LDLR-KO, despite rises in their lipid profile. These increases may be due to impaired lipid (or lipid fractions) uptake, or possibly local action of APOC1 on ocular surface tissues.[24]

Among various lipids present in the tear film are cholesterol esters.[25–27] Higher meibomian cholesterol ester levels and fatty acids are associated with meibomian gland disease in humans.[28] However, blocking cholesterol esterification in ACAT-1 KO mice led to atrophy of the skin, MG and ocular surface disturbance, a clear model of DES. These changes were even more pronounced by breeding the ACAT-1 KO mice with ApoE-KO or LDLR-KO mice, and feeding them a high-fat diet.[24,29]

Interestingly, MG disease patients and type IV hyperlipoproteinemic subjects have similar lipid profiles. This correspondence is seen when the lipids obtained from MG disease patients are compared with those obtained from skin sebaceous glands in the latter condition. In particular, both groups have higher levels of cholesterol ester and wax ester. Despite this similarity, no linkage has been reported between the two different conditions.[16]

Disruptions of cholesterol biosynthesis are associated with sebaceous/MG dysfunction. Statin therapy (used to reduce blood cholesterol levels) is associated with hordeolum (MG obstruction and inflammation) and skin sebaceous gland hypotrophy. This suggests that the actions of statins are not limited to reducing liver cholesterol biosynthesis. Lowering local cholesterol

TABLE 3 Comparison of males and females mice of Control and ApoCIII-KI groups of 9 months of age.

Groups	Sex	Control	ApoCIII-KI	*P*
Body Weight (g)	Male	25.6 ± 0.5*	31.7 ± 0.9*§	*0.0012, §0.0238
	Female	24.9 ± 1.0	25.1 ± 1.4§	
LG weight (mg)	Male	16.1 ± 1.5§	17.9 ± 1.0#	§0.0025, # 0.012
	Female	6.0 ± 0.4§	6.7 ± 0.6#	
LGW/BW	Male	0.63 ± 0.06	0.51 ± 0.03	
	Female	0.24 ± 0.01	0.27 ± 0.03	
Phenol red thread test (mm)	Male	4.0 ± 0.9	5.0 ± 1.1	
	Female	5.0 ± 1.1	6.0 ± 3.4	
Cholesterol mg/dl	Male	62.6 ± 5.6*	88.8 ± 2.7**	*0.0043
	Female	Not measured	Not measured	
Triacylglycerolmg/dl	Male	72.8 ± 5.7*	257.3 ± 47.5*#	*0.0080
				#0.0476
	Female	65.9 ± 7.4*	552.3 ± 86.3*#	*0.0091

Data are reported as mean ± SEM.
*$P < 0.05$ for comparison of parameters between strains, §#$P < 0.05$ for comparison between genders (Mann–Whitney U test).
LGW/BW, ratio between lacrimal gland weight and body weight.

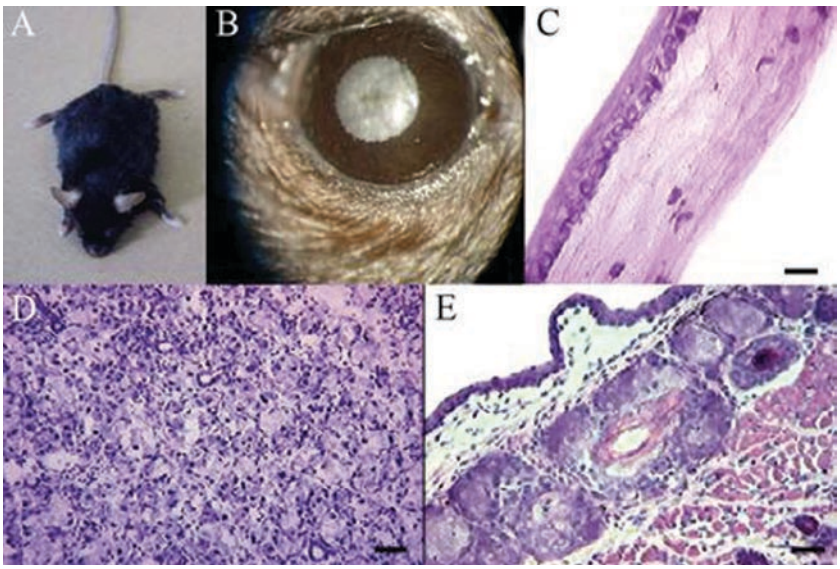

FIGURE 7 Representative images of APOCIII mice at 9 months of age: A) dorsal area of body showing normal pelage, B) female eye at the slit lamp showing cataracts, C) histology of cornea, stained with HE, scale bar = 12.5 μm, D) histology of LG stained with HE, scale bar = 50 μm, E) histology of meibomian gland, stained with HE, scale bar = 12.5 μm.

production in sebaceous/meibomian glands through inhibition of the 3-hydroxy-3-methyl-glutaryl-CoA mevalonate pathway, does indeed impact on ocular surface and skin health.[30,31]

We did not employ any drugs to reduce cholesterol levels. It is remarkable that only in LDLR-KO males fed the hyperlipidemic diet tear secretion declined. However, no changes were seen.in LDLR-KO females or ApoCIII-KO as a function of aging in both sexes.

Previous studies found that lipogenic enzymes expressed in mouse lacrimal and meibomian glands are regulated by androgens. Such a hormonal effect could induce gender-related differences in hyperlipidemic models.[32] However, we did not observe any relevant gender differences in meibomian gland structure or ocular surface characteristics even though male LDLR-KO model fed a high-fat diet had different blood lipid levels and lower tear flow than their female counterpart.

Taken together, these findings suggest that blood lipid profile and tissue-specific responses to environmental conditions (e.g. fat content in the diet) are not the only contributing factors affecting ocular surface health. In other words, in the mouse models we evaluated hyperlipidemic background or high levels of cholesterol in the blood are not the only determinants controlling tear film stability and meboimian gland functions and/or corneal attributes.

Our identification of normal corneas, and cataracts only in the aging group, also suggest that the several reports of corneal opacities related to hyperlipidemia in humans are instead local manifestations of genetic errors, rather than a simple consequence of peripheral deposition of excessive lipids.[13,33–35]

Clinical ocular surface and tear film exams and their use in experimental studies may lack sufficient sensitivity to detect the subtle changes identified in the current study that are associated with DES.[36] Moreover, this study did not evaluate lipids secretion in the tear film or tissue samples. As recently described, state of the art laboratories and sensitive methods are necessary to adequately evaluate tear lipid samples.[27]

The finding that feeding a high lipid diet to male LDLR-KO mice led to reduced tears secretion offers indirect support for maintaining a diet containing certain lipid profiles to ameliorate DES. In recent years, vitamin E and essential fatty acids, such as ω-3, have been advocated, based on their potential anti-inflammatory and/or anti-oxidant activity in the MG and improvement of oil secretion quality in the tear film.[37,38]

In summary, although different genetic backgrounds in mice induced differences in hyperlipidemic status, they did not in any case independently affect structural, histological or functional characteristics of meibomain, lacrimal glands and corneas. Therefore, the present work confirms the hypothesis that local lipid biosynthesis is not strictly dependent on the systemic lipid transport system and that DES is caused by a combination of genetic and environmental conditions rather than merely a hyperlipidemic condition associated with increases in tear film cholesterol esters and an imbalance in lipoprotein profiles.

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REFERENCES

[1] Krenzer KL, Dana MR, Ullman MD et al. Effect of androgen deficiency on the human meibomian gland and ocular surface. *J Clin Endocrinol Metab* 2000;85:4874–4882.
[2] Cella W, Urbano AP, Vinhadelli WS, Donatti M, Rocha EM. Xerophthalmia secondary to short bowel syndrome. *J Pediatr Ophthalmol Strabismus* 2002;39:125–127.
[3] Sullivan BD, Evans JE, Dana MR, Sullivan DA. Impact of androgen deficiency on the lipid profiles in human meibomian gland secretions. *Adv Exp Med Biol* 2002;506:449–458.
[4] Duerden JM, Tiffany JM. Lipid synthesis *in vitro* by rabbit Meibomian gland tissue and its inhibition by tetracycline. *Biochim Biophys Acta* 1990;1042:13–18.
[5] Caffery BE. Influence of diet on tear function. *Optom Vis Sci* 1991;68:58–72.
[6] Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res* 2004;78:347–360.
[7] Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol* 2000;118:1264–1268.
[8] Wang TJ, Wang IJ, Hu CC, Lin HC. Comorbidities of dry eye disease: A nationwide population-based study. *Acta Ophthalmol* 2010. [Epub a head of print]
[9] Lodde BM, Sankar V, Kok MR, Leakan RA, Tak PP, Pillemer SR. Serum lipid levels in Sjögren's syndrome. *Rheumatology (Oxford)* 2006;45:481–484.
[10] Wiedemann R, Reuter W, Meier T, Wiedemann P. Keratoconjunctivitis sicca in patients with lipoprotein (a) elevation. *Klin Monbl Augenheilkd* 1997;210:33–37.
[11] Ramos-Casals M, Brito-Zerón P, Sisó A et al. High prevalence of serum metabolic alterations in primary Sjögren's syndrome: Influence on clinical and immunological expression. *J Rheumatol* 2007;34:754–761.
[12] Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. *Circulation* 2004;109:III2–III7.
[13] Schaefer EJ, Ordovas JM, Law SW et al. Familial apolipoprotein A-I and C-III deficiency, variant II. *J Lipid Res* 1985;26:1089–1101.
[14] Hann LE, Kelleher RS, Sullivan DA. Influence of culture conditions on the androgen control of secretory component production by acinar cells from the rat lacrimal gland. *Invest Ophthalmol Vis Sci* 1991;32:2610–2621.
[15] Wu K, Joffre C, Li X et al. Altered expression of genes functioning in lipid homeostasis is associated with lipid deposition in NOD mouse lacrimal gland. *Exp Eye Res* 2009;89:319–332.
[16] Güldür T, Bayraktar N, Kaynar O, Beker G, Koçer M, Ozcan H. Excretion rate and composition of skin surface lipids on the foreheads of adult males with type IV hyperlipoproteinemia. *J Basic Clin Physiol Pharmacol* 2007;18:21–35.
[17] Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science* 1990;249:790–793.
[18] Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest* 1993;92:883–893.
[19] Hill JM, Bhattacharjee PS, Neumann DM. Apolipoprotein E alleles can contribute to the pathogenesis of numerous clinical conditions including HSV-1 corneal disease. *Exp Eye Res* 2007;84:801–811.
[20] Plump AS, Smith JD, Hayek T et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 1992;71:343–353.
[21] Módulo CM, Jorge AG, Dias AC et al. Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats. *Endocrine* 2009;36:161–168.
[22] Steinberg D. Thematic review series: The pathogenesis of atherosclerosis: An interpretive history of the cholesterol controversy, part III: Mechanistically defining the role of hyperlipidemia. *J Lipid Res* 2005;46:2037–2051.
[23] Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: Report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci* 2011;52:1938–1978.
[24] Jong MC, Gijbels MJ, Dahlmans VE et al. Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J Clin Invest* 1998;101:145–152.

[25] van Haeringen NJ, Glasius E. Cholesterol in human tear fluid. *Exp Eye Res* 1975;20:271–274.
[26] Cenedella RJ, Fleschner CR. Cholesterol biosynthesis by the cornea. Comparison of rates of sterol synthesis with accumulation during early development. *J Lipid Res* 1989;30:1079–1084.
[27] Butovich IA, Millar TJ, Ham BM. Understanding and analyzing meibomian lipids—a review. *Curr Eye Res* 2008;33:405–420.
[28] Shine WE, McCulley JP. The role of cholesterol in chronic blepharitis. *Invest Ophthalmol Vis Sci* 1991;32: 2272–2280.
[29] Yagyu H, Kitamine T, Osuga J et al. Absence of ACAT-1 attenuates atherosclerosis but causes dry eye and cutaneous xanthomatosis in mice with congenital hyperlipidemia. *J Biol Chem* 2000;275:21324–21330.
[30] Jang SB, Lee YJ, Lim LA et al. Pharmacokinetic comparison of controlled-release and immediate-release oral formulations of simvastatin in healthy Korean subjects: A randomized, open-label, parallel-group, single- and multiple-dose study. *Clin Ther* 2010;32:206–216.
[31] Cakmak S, Gül U, Gönül M, Demiriz M, Cakmak A. Statin therapy and diabetic skin. *Adv Ther* 2008;25:17–22.
[32] Schirra F, Richards SM, Sullivan DA. Androgen influence on cholesterogenic enzyme mRNA levels in the mouse meibomian gland. *Curr Eye Res* 2007;32:393–398.
[33] Winder AF, Garner A, Sheraidah GA, Barry P. Familial lecithin:cholesterol acyltransferase deficiency. Biochemistry of the cornea. *J Lipid Res* 1985;26:283–287.
[34] Chu FC, Kuwabara T, Cogan DG, Schaefer EJ, Brewer HB Jr. Ocular manifestations of familial high-density lipoprotein deficiency (Tangier disease). *Arch Ophthalmol* 1979;97:1926–1928.
[35] Carlson LA. Fish eye disease: A new familial condition with massive corneal opacities and dyslipoproteinaemia. *Eur J Clin Invest* 1982;12:41–53.
[36] Barabino S, Chen W, Dana MR. Tear film and ocular surface tests in animal models of dry eye: Uses and limitations. *Exp Eye Res* 2004;79:613–621.
[37] Kawashima M, Kawakita T, Okada N et al. Calorie restriction: A new therapeutic intervention for age-related dry eye disease in rats. *Biochem Biophys Res Commun* 2010;397: 724–728.
[38] Fonseca EC, Arruda GV, Rocha EM. Dry eye: Etiopathogenesis and treatment. *Arq Bras Oftalmol* 2010;73:197–203.