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Sex differences in lacrimal gland lesions in a streptozotocin-induced diabetic mouse model

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Abstract: Objective: To investigate gender-based differences in the progression of lacrimal gland lesions in diabetic mice.**Methods:** Sixty-four C57BL/6 mice of each sex (64 males, 64 females) were used. Mice of each sex were divided into a diabetic group and a control group (n = 32 per group). The diabetic group received intraperitoneal streptozotocin for five consecutive days; controls received citrate buffer. At 1, 2, 3, and 8 months post-injection, body weight, blood glucose, corneal sensitivity, and tear secretion were measured. Lacrimal glands were examined histologically and for inflammatory factor expression.**Results:** Diabetic mice showed significantly lower body weight and tear secretion, and higher blood glucose than controls at all time points. Male diabetic mice exhibited greater body weight loss than females throughout, and greater tear reduction at 1 month. Corneal sensitivity decreased significantly in diabetics from 2 months onward, with no sex difference. Lacrimal gland weight was significantly reduced in diabetics at 3 and 8 months, with greater reduction in males. Inflammatory cell infiltration appeared at 3 months and intensified by 8 months in both sexes. At 8 months, TNF-α and IL-1β expression was significantly elevated in diabetic males but not females.**Conclusion:** Both male and female diabetic mice developed dry eye-related pathologies, but males showed more severe lacrimal gland lesions, suggesting they may be a more suitable model for studying diabetic lacrimal gland complications.
Keywords: Diabetes; Mouse model; Lacrimal Gland Lesions; Sex Differences; Dry Eye-related Pathologies

Introduction

Diabetes is a metabolic disease characterized by chronic hyperglycemia, with a significant increase in incidence rate in recent years[1]. According to the latest national epidemiological survey, there are approximately 129.8 million individuals with diabetes in China, ranking first in the world[2]. Numerous studies have identified diabetes as a risk factor for lacrimal gland dysfunction, which contributes to the development of diabetes-related ocular surface diseases, including dry eye and corneal lesions[3,4]. Approximately 50% of diabetic patients experience dry eye complications, primarily presenting as reduced tear secretion and decreased corneal sensitivity[5]. Compared

to non-diabetic dry eye patients, those with diabetes exhibit a more pronounced decrease in tear secretion, which progressively worsens with prolonged diabetes duration[6]. As the lacrimal gland is the primary source of tear production, impairment of its physiological function can directly initiate and exacerbate dry eye[7,8]. The prevalence of dry eye is higher in women than in men, and research has confirmed that androgens exert predominantly positive effects on ocular surface tissues. Androgen deficiency can trigger pathological changes in ocular surface tissues, including elevated inflammatory cytokine levels and lacrimal gland secretory dysfunction[9,10]. However, the influence of sex on lacrimal gland pathological changes during the development of diabetes remains unclear. This study aims to evaluate sex-related differences in tear secretion, lacrimal gland weight, and inflammatory factor expression during the progression of lacrimal gland lesions in diabetic mice.

1. Materials and Methods

1.1 Laboratory animals

C57BL/6 mice (both male and female, 6-8 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animals were housed at the Animal Breeding Center of the Key Laboratory, adhering to the research animal usage guidelines established by the Association for Research in Vision and Ophthalmology (ARVO) and the Ethical Committee of the Shandong Eye Institute.

1.2 Establishment of the diabetic mouse model

A total of 128 C57BL/6 mice (64 males and 64 females; 6-8 weeks old) were used in this study. Body weight was measured prior to injection. For each sex, mice were randomly divided into a diabetic group and a control group (n = 32 per group) using a random number table. The diabetic group received an intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, USA; 50 mg/kg body weight) dissolved in citrate buffer once daily for five consecutive days. The control group received an equivalent volume of citrate buffer alone.

1.3 Blood glucose measurement

At 1, 2, 3, and 8 months post-injection, 12 mice were randomly selected from each group (control and diabetic) for blood glucose measurement. Blood samples were collected from the tail vein. After calibrating the glucometer (Yuyue Blood Glucose Meter 580, Jiangsu Yuyue Medical Equipment Co., Ltd., China) and preparing the test strips, a 0.5 cm segment of the tail tip was clipped. Blood was gently expressed from the base to the tip of the tail, and a drop was applied to a test strip for reading. Only diabetic mice with blood glucose levels exceeding 16.7 mmol/L were included in subsequent experiments.

1.4 Body weight measurement

Body weight of mice from each group (control and diabetic) was measured at 1, 2, 3, and 8 months post-injection to compare weight changes among the groups. An electronic animal scale was placed on a flat, stable surface and leveled. After cleaning the weighing pan and ensuring it was properly installed, the scale was tared to zero. Each mouse was gently grasped (avoiding any wounds or bandages) and carefully placed on the pan. The body weight was recorded once the reading stabilized.

1.5 Measurement of corneal sensitivity

At 1, 2, 3, and 8 months post-injection, corneal sensitivity was measured in mice from the control and diabetic groups. While the mice were awake and quiet with eyes naturally open, the eyelids were gently pulled down. Corneal sensitivity was assessed using a Cochet-Bonnet Corneal Sensation Meter (Luneau Ophthalmologie, France). The length of the nylon filament was sequentially adjusted from 6.0 down to 1.0 cm, in 0.5 cm decrements. The filament tip was gently applied to the central cornea until a blink reflex occurred. The longest filament length that evoked a blink reflex was recorded as the corneal sensitivity threshold. For each length, three successive stimulations were performed; if the eyelids closed in two or more of these attempts, the response was considered valid. Each eye was measured three times to obtain valid readings, and the average value was recorded. All measurements were performed by the same operator to minimize variability.

1.6 Tear secretion measurement

At 1, 2, 3, and 8 months post-injection, tear secretion was measured in mice from the control and diabetic groups using phenol red cotton thread (Tianjin Jingming Co., Ltd., China) to compare changes among groups. Without general or local anesthesia, the lower eyelid was gently pulled down. Excess

tears in the conjunctival sac were first absorbed with a cotton swab, then the phenol red cotton thread was inserted into the middle-to-lower third of the eyelid conjunctiva using an ophthalmic forceps. An electronic stopwatch was immediately started for a continuous 15-second test. After 15 seconds, the lower eyelid was carefully opened, and the phenol red cotton thread was gently removed. The length of the thread wetted and discolored by tears was measured using the scale on the back of the packaging. Measurements were taken at the same time of day for three consecutive days, with each eye tested three times. The average value was recorded as the final length. After measurement, the mice were assisted in closing their eyelids to avoid excessive exposure. All procedures were performed by the same operator to minimize handling-related variability.

1.7 Lacrimal gland photography and weighing

After euthanasia by cervical dislocation, the skin and subcutaneous fascia were bluntly dissected at the intersection of the outer canthus and the extended line of the earlobe using ophthalmic scissors until a clearly demarcated yellow-white oval-shaped tissue was visible. The tissue was carefully separated with toothless micro-forceps to avoid damaging the ultrastructure of the lacrimal gland. Immediately after separation, the lacrimal gland wet weight was measured using an electronic balance (BT125D Analytical Balance, Sartorius, Germany). After weighing, the lacrimal glands from each group were gently placed flat on filter paper, and the length was measured along the largest flat surface using a ruler, followed by photography.

1.8 Lacrimal gland tissue collection and paraffin section preparation

At 3 and 8 months post-injection, mice from each group were euthanized by cervical dislocation. The bilateral lacrimal glands were carefully excised from control and diabetic mice, immediately placed in 10% neutral formalin solution, and fixed at room temperature for 24 hours. The tissues were then dehydrated through a graded ethanol series, cleared with xylene, embedded in paraffin, and sectioned at 4-5 μm thickness. The sections were then dried and stained with hematoxylin and eosin (HE).

1.9 Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from lacrimal gland tissues of control and diabetic groups using an animal tissue total RNA extraction kit. RNA purity and concentration were measured, and cDNA was synthesized by reverse transcription. β-actin was used as the internal reference gene. The primer sequences were as follows: TNF-α-forward, 5'-CAAGGTGCCCCGACTAC-3'; TNF-α-reverse, 5'-TGGGGCTCATACCAGGGTTTG-3'; IL-1β-forward, 5'-CTTCCCGTGGACCTTCA-3'; IL-1β-reverse, 5'-CTCGGCTCTGTGAGTGCAGTT-3'. SYBR Green I was used as the fluorescent dye for PCR amplification. The PCR cycling conditions were as follows: initial denaturation at 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Following the amplification, a melt curve analysis was performed to verify product specificity: 95 °C for 15 s, 60 °C for 1 min, followed by a continuous increase from 60 °C to 95 °C at a rate of 0.3 °C/s with continuous fluorescence acquisition. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression levels.

1.10 Statistical methods

Statistical analysis was performed using SPSS 26.0 software. The normality of all quantitative data was assessed using the Shapiro–Wilk test. Data conforming to a normal distribution were expressed as mean ± standard deviation (mean ± SD). Comparisons among groups were conducted using one-way analysis of variance (ANOVA). For indices involving both treatment and time factors, two-way ANOVA was performed within each sex to evaluate overall differences. When overall differences were statistically significant, the least significant difference (LSD) t-test was used for post hoc multiple comparisons. A P value < 0.05 was considered statistically significant.

2. Results

2.1 Comparison of body weight at different time points after STZ injection in male and female mice

As shown in **Table 1**, body weight was measured in both control and diabetic mice at multiple time points following STZ injection. The results showed that diabetic mice of both sexes exhibited significantly lower body weight than sex-matched controls at 1, 2, 3, and 8 months post-injection ($P < 0.001$). Notably, the extent of weight loss was more pronounced in male diabetic mice than in females at the same time points, and the differences were statistically significant ($P < 0.01$).

Table 1. Body weight changes in male and female mice at different time points after STZ injection

Group	*n*	Weight at different time points after injection (g)			
		1 month	2 months	3 months	8 months
Male					
NC	12	28.32 ± 1.53	29.21 ± 1.48	29.42 ± 1.43	34.13 ± 0.36
DM	12	19.65 ± 0.91	19.52 ± 1.60	20.35 ± 1.67	23.00 ± 2.90
P value		< 0.001	< 0.001	< 0.001	< 0.001
Female					
NC	12	19.68 ± 0.88	20.88 ± 0.69	20.93 ± 0.70	23.70 ± 0.70
DM	12	15.52 ± 1.17	15.73 ± 2.18	15.45 ± 2.36	20.60 ± 0.97
P value		< 0.001	< 0.001	< 0.001	< 0.001
Weight loss					
Male		8.59 ± 1.84	9.52 ± 1.93	9.07 ± 2.58	9.98 ± 1.40
Female		4.17 ± 1.16	4.90 ± 1.59	5.90 ± 1.84	2.00 ± 1.08
P value		< 0.0001	< 0.0001	0.0022	< 0.0001

Note: Data are presented as mean ± SD. NC: normal control; DM: diabetes mellitus.

2.2 Comparison of blood glucose levels at different time points after STZ injection in male and female mice

As shown in **Table 2**, blood glucose levels were compared between diabetic and control mice of both sexes at 1, 2, 3, and 8 months post-STZ injection. Diabetic mice (both male and female) exhibited significantly elevated blood glucose at all time points compared to their sex-matched controls, with all comparisons yielding $P < 0.001$. However, when comparing the magnitude of blood glucose elevation (diabetic value minus control value) between males and females, no significant differences were found at any time point ($P > 0.05$).

Table 2. Comparison of blood glucose levels in male and female mice at different time points after STZ injection (mean ± SD, mmol • L^{-1})

Group	*n*	1 month post injection	2 months post injection	3 months post injection	8 months post injection
Male					
NC	12	9.60 ± 1.70	10.63 ± 0.54	9.72 ± 1.68	11.10 ± 1.86
DM	12	33.23 ± 0.21	27.73 ± 6.32	32.78 ± 1.34	32.18 ± 2.25
P value		< 0.001	< 0.001	< 0.001	< 0.001
Female					
NC	12	10.69 ± 1.07	10.75 ± 1.01	10.58 ± 1.04	13.60 ± 2.12
DM	12	32.48 ± 1.78	28.19 ± 5.44	32.49 ± 1.74	32.70 ± 3.30
P value		< 0.001	< 0.001	< 0.001	< 0.001
Blood glucose elevation					
Male		23.63 ± 1.83	17.10 ± 6.79	23.06 ± 2.05	22.80 ± 1.26
Female		21.79 ± 2.38	17.44 ± 6.11	21.91 ± 2.05	21.18 ± 2.84
P value		0.0686	0.9076	0.2459	0.2317

Note: Data are presented as mean ± SD. NC: normal control; DM: diabetes mellitus.

2.3 Comparison of corneal sensitivity at different time points after STZ injection in male and female mice

As shown in **Table 3**, corneal sensitivity was measured in male and female mice at multiple time points following STZ injection. At 2, 3, and 8 months post-injection, both male and female diabetic mice exhibited significantly lower corneal sensitivity compared to their sex-matched controls, with all comparisons showing statistically significant differences ($P < 0.05$). At 1 month post-injection, no significant difference was observed

between diabetic and control mice in either sex ($P > 0.05$). When comparing the magnitude of decrease in corneal sensitivity between male and female diabetic mice, no statistically significant differences were found at any time point (all $P > 0.05$).

Table 3. Comparison of corneal sensitivity in male and female mice at different time points after STZ injection (mean ± SD, mm)

Group	*n*	1 month post injection	2 months post injection	3 months post injection	8 months post injection
Male					
NC	12	5.56 ± 0.42	5.83 ± 0.24	5.58 ± 0.40	5.17 ± 0.25
DM	12	5.77 ± 0.32	5.56 ± 0.42	4.70 ± 0.74	3.14 ± 0.92
P value		0.1108	0.0198	< 0.001	< 0.001
Female					
NC	12	5.43 ± 0.39	5.90 ± 0.20	5.47 ± 0.40	5.08 ± 0.20
DM	12	5.81 ± 0.24	5.48 ± 0.49	4.93 ± 0.65	3.91 ± 0.20
P value		0.4415	0.0022	< 0.001	< 0.001
Decrease in corneal sensitivity					
Male		−0.20 ± 0.67	0.25 ± 0.53	0.93 ± 0.88	1.75 ± 0.88
Female		−0.02 ± 0.33	0.38 ± 0.59	0.61 ± 0.83	1.67 ± 0.52
P value (male vs female)		0.1045	0.4268	0.2233	0.1918

Note: Data are presented as mean ± SD. NC: normal control; DM: diabetes mellitus.

2.4 Comparison of tear secretion at different time points after STZ injection in male and female mice

As presented in **Table 4**, tear secretion was measured in male and female mice at multiple time points following STZ injection. At 1, 2, 3, and 8 months post-injection, both male and female diabetic mice exhibited significantly reduced tear secretion compared to their respective same-sex control groups. Notably, the reduction in tear secretion was significantly greater in male mice than in female mice at the 1-month time point ($P = 0.0258$). However, this sex-related difference diminished over time, with no statistically significant differences observed at later time points ($P > 0.05$ for all).

Table 4. Comparison of tear secretion at different time points after STZ injection in male and female mice (mean ± SD, mm)

Group	*n*	1 month post injection	2 months post injection	3 months post injection	8 months post injection
Male					
NC	12	6.27 ± 2.05	5.75 ± 0.92	5.69 ± 1.38	6.17 ± 0.82
DM	12	3.98 ± 1.25	3.31 ± 0.89	3.19 ± 0.85	2.91 ± 0.38
P value		< 0.001	< 0.001	< 0.001	< 0.001
Female					
NC	12	4.73 ± 1.25	4.47 ± 0.92	4.13 ± 1.45	4.25 ± 1.33
DM	12	3.52 ± 0.98	2.48 ± 1.15	2.35 ± 0.96	2.58 ± 0.49
P value		< 0.001	< 0.001	< 0.001	0.0055
Decrease in tear secretion					
Male	12	2.42 ± 2.08	2.44 ± 1.34	2.50 ± 1.66	2.46 ± 1.25
Female	12	1.21 ± 1.51	2.00 ± 1.44	1.77 ± 2.06	1.79 ± 1.50
P value		0.0258	0.2807	0.1836	0.3772

2.5 Comparison of tear gland weight in male and female mice after STZ injection

As shown in **Figure 1A, 1D**, the tear gland size at 3 and 8 months post-injection was significantly smaller in the diabetes group compared to the same-sex control group for both females and males. In both the control

and diabetes groups, the tear gland size of female mice was smaller than that of male mice. The comparison of tear gland weight at 3 and 8 months post-injection revealed that the diabetes group had significantly lower tear gland weight than the same-sex control group, regardless of sex (**Figure 1B, 1E**, $P < 0.01$). The decline in tear gland weight was significantly greater in males than in females at both 3 and 8 months post-injection, with statistically significant differences (**Figure 1C, 1F**, $P < 0.01$).

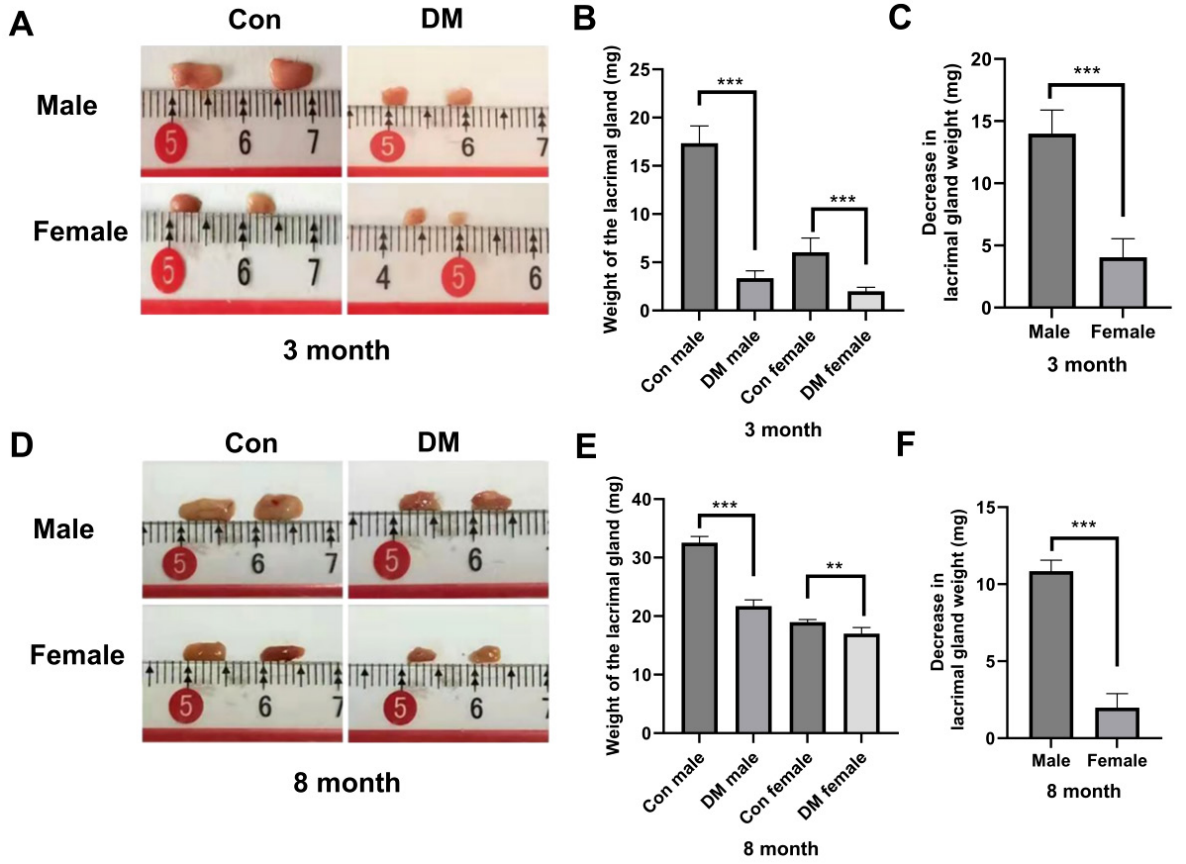

Figure 1. Sex differences in the effect of diabetes on tear gland weight in mice.

(A) Tear gland size in male and female mice at 3 months after STZ injection; (B) Tear gland weight changes in male and female mice at 3 months after STZ injection; (C) Reduction in tear gland weight in male and female mice compared to same-sex controls at 3 months after STZ injection; (D) Tear gland size in male and female mice at 8 months after STZ injection; (E) Tear gland weight changes in male and female mice at 8 months after STZ injection; (F) Reduction in tear gland weight in male and female mice compared to same-sex controls at 8 months after STZ injection. $^{**}P < 0.01$, $^{***}P < 0.001$.

2.6 Infiltration of inflammatory cells and expression changes of inflammatory factors in the lacrimal gland of male and female mice after STZ injection

As shown in **Figure 2A-B**, the lacrimal gland tissue structure of both male and female mice in the control group remained normal at 3 and 8 months after injection, with no obvious inflammatory cell infiltration. In contrast, inflammatory cell infiltration was observed in both male and female diabetic mice at 3 months post-injection (**Figure 2A**), and this infiltration became more pronounced at 8 months post-injection (**Figure 2B**). The expression levels of the inflammatory factors TNF-α and IL-1β in lacrimal gland tissues were analyzed by qRT-PCR at 8 months after STZ injection. The results showed that the expression of TNF-α and IL-1β in the lacrimal gland of male diabetic mice was significantly higher than that in male control groups (**Figure 2C-D**, $P < 0.05$). In female diabetic mice, the expression of TNF-α and IL-1β was also elevated compared to same-sex controls, but the differences did not reach statistical significance (**Figure 2C-D**, $P > 0.05$).

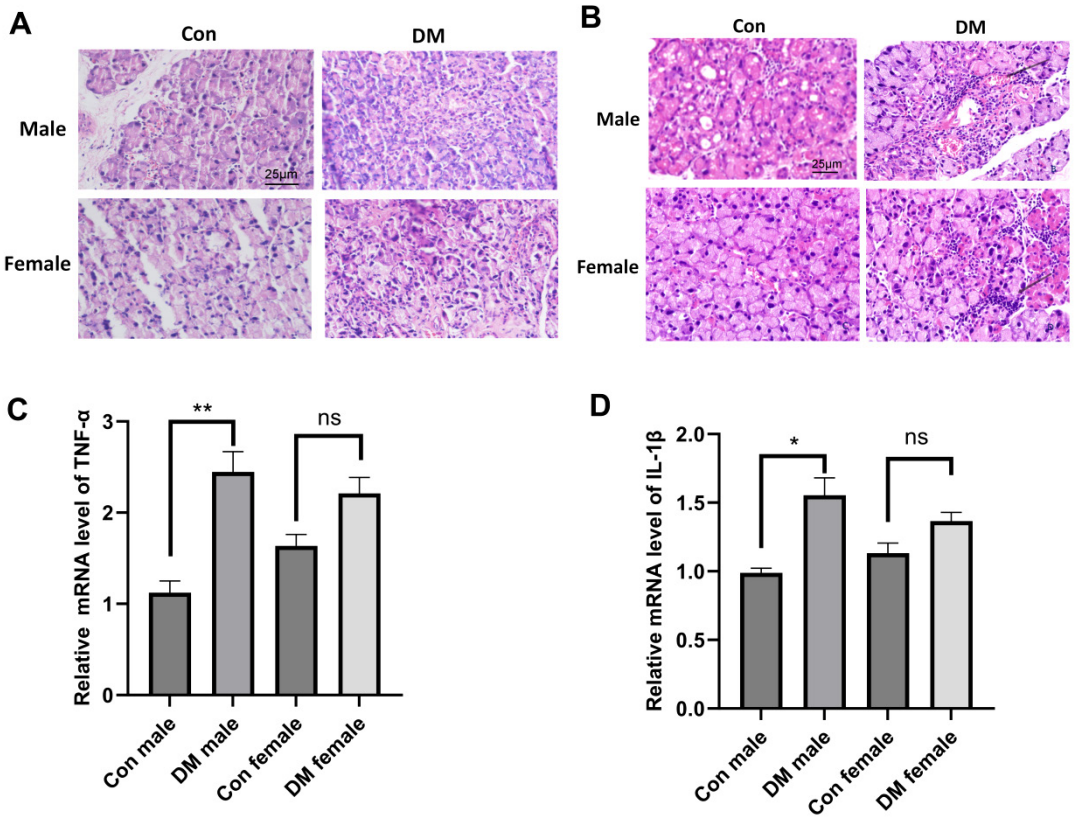

Figure 2. Histological analysis of lacrimal glands and expression changes of inflammatory factors in each experimental group. (A) H&E-stained sections of lacrimal glands from male and female mice in control and diabetic groups at 3 months after STZ injection (×400). (B) H&E-stained sections of lacrimal glands from male and female mice in control and diabetic groups at 8 months after STZ injection (×400). (C) Expression levels of TNF-α in the lacrimal glands of male and female diabetic mice at 8 months after STZ injection. (D) Expression levels of IL-1β in the lacrimal glands of male and female diabetic mice at 8 months after STZ injection. $^{**}P < 0.01$, $^{*}P < 0.05$. ns: not significant.

3. Discussion

The global prevalence of diabetes continues to rise annually, accompanied by numerous and complex complications[5]. Among these, dry eye is a common ocular complication in diabetic patients, who typically exhibit more severe dry eye symptoms than non-diabetic individuals. Moreover, the severity of dry eye in diabetic patients is closely associated with the progression of the disease[11]. Accumulating evidence indicates that the lacrimal gland plays a critical role in both the physiological and pathological processes of the ocular surface, and that lacrimal gland dysfunction contributes to the onset and progression of dry eye[12]. In the context of diabetes, abnormal lacrimal gland function has been identified as a key factor in the development of diabetes-associated dry eye[7]. Previous studies have implicated chronic hyperglycemia, oxidative stress, neural alterations, and disruptions in insulin action as important contributors to lacrimal gland damage in diabetes[13]. However, the influence of sex on diabetes-related lacrimal gland lesions remains unclear. To address this gap, this study established STZ-induced diabetes models in both female and male mice to investigate the effects of sex on lacrimal gland lesions associated with diabetes.

Reduced tear secretion from the lacrimal gland is a key clinical feature of dry eye. In the present study, we observed that under normal physiological conditions, basal tear secretion was lower in female mice than in male mice. Following diabetes induction, both male and female mice exhibited significantly reduced tear secretion compared to their sex-matched controls as early as one month after STZ injection, with decreases of approximately 36% in males and 25% in females. The reduction in tear secretion was significantly more pronounced in male mice during the early stage of

diabetes, suggesting that male mice may be more susceptible to diabetes-induced impairment of tear secretion, possibly related to their higher baseline secretion levels. As diabetes progressed to the middle and late stages, although the absolute decrease in tear secretion remained lower in females than in males, the difference between the two sexes was no longer statistically significant. These findings indicate that male mice are more sensitive to hyperglycemia-induced reductions in tear secretion during the early phase of diabetes, whereas in later stages, the magnitude of reduction stabilizes and the sex difference becomes less pronounced.

Tears are secreted by the lacrimal gland, and previous studies have shown that lacrimal gland growth is impaired during the early stages of diabetes[7], suggesting that the reduction in tear secretion in diabetic individuals may be directly attributable to diabetes-induced lacrimal gland damage. In the present study, we analyzed lacrimal gland weight in male and female diabetic mice at 3 and 8 months after STZ injection. Under normal physiological conditions, the lacrimal gland weight of female mice was significantly lower than that of male mice, which may explain the lower basal tear secretion observed in females. At both time points examined, diabetic mice of both sexes exhibited a significant reduction in lacrimal gland weight compared to their same-sex controls, consistent with the marked decline in tear secretion observed in these animals. Notably, the reduction in lacrimal gland weight was significantly greater in male diabetic mice than in female diabetic mice, indicating that hyperglycemia exerts a more pronounced effect on the lacrimal glands of males, potentially related to their larger baseline gland size. This finding suggests that the growth restriction of the lacrimal gland in diabetic male mice is more severe, which may contribute to the more substantial decline in tear secretion observed in males during the early phase of diabetes. Furthermore, although both male and female mice showed a significant decrease in corneal sensitivity beginning 2 months after STZ injection, no significant sex differences in corneal sensitivity were detected under either normal or diabetic conditions. This suggests that diabetes-induced changes in lacrimal gland weight may occur independently of alterations in corneal sensitivity.

Dry eye is recognized as an inflammatory disease, and the infiltration of inflammatory cells as well as the expression of inflammatory factors in the lacrimal gland are closely associated with its onset and progression[14]. In the present study, H&E staining revealed inflammatory cell infiltration in the lacrimal glands of both male and female diabetic mice. The inflammatory cytokines TNF-α and IL-1β are commonly used indicators for evaluating ocular surface inflammation. We examined the expression of TNF-α and IL-1β in the lacrimal glands of diabetic mice at 8 months after STZ injection. The results showed that the expression levels of both TNF-α and IL-1β were significantly elevated in the lacrimal glands of male diabetic mice compared to their male controls. In female diabetic mice, although the expression of these inflammatory factors was also increased relative to female controls, the differences did not reach statistical significance. These findings suggest that diabetes-induced upregulation of inflammatory factors in the lacrimal gland may be more pronounced in males, potentially contributing to the sex-related differences observed in lacrimal gland pathology.

Epidemiological studies have consistently shown that dry eye is more prevalent in women, particularly among perimenopausal and postmenopausal populations. This sex-related difference is generally attributed to alterations in sex hormone balance, including declining hormone levels, changes in feedback mechanisms, and modifications in receptor sensitivity, which collectively disrupt ocular surface homeostasis and predispose women to dry eye[15–17]. Based on the findings of the present study, we propose that the inherently smaller lacrimal gland size and lower basal tear secretion observed in females may represent an additional contributing factor to the higher clinical prevalence of dry eye in women. However, for basic research focusing on diabetes-associated dry eye, our results demonstrate that diabetes-induced pathological changes in the lacrimal gland are more pronounced in male mice. Therefore, we suggest that the male mouse model of diabetes may be more suitable for investigating lacrimal gland lesions resulting from diabetes, as it provides a more pronounced phenotype for further study and therapeutic interventions.

In summary, this study demonstrates that the STZ-induced diabetic male mouse model exhibits a more pronounced phenotype, making it a more suitable model for future research into diabetes-associated

lacrimal gland diseases.

Conflict of Interest

The authors declares no conflict of interest.

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