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Original Article

Effect of moderate-intensity aerobic exercise on bladder *TGF-β1* and type I collagen expressions in diabetic rat model

Haykal H. Fatahajjad[1,2], Mohammad A. Soebadi[1,3*], Rheza M. Putra[1,2], Nur Sulastri[4,5], Anny S. Rahaju[6,7]

[1]Department of Urology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [2]Department of Urology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia; [3]Department of Urology, Universitas Airlangga Teaching Hospital, Surabaya, Indonesia; [4]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [5]Department of Physical Medicine and Rehabilitation, Universitas Airlangga Teaching Hospital, Surabaya, Indonesia; [6]Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia; [7]Department of Anatomical Pathology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

*Corresponding author: yodisoebadi@gmail.com

Abstract

Transforming growth factor-beta 1 (*TGF-β1*) and type I collagen play crucial roles in the pathogenesis of diabetic bladder disease (DBD). Moderate-intensity aerobic exercise increases antioxidant activity to help manage DBD. The aim of this study was to evaluate the effect of moderate-intensity aerobic exercise on the expression of *TGF-β1* and type I collagen in the detrusor and lamina propria of the bladder in a type 2 diabetes mellitus (T2DM) rat model. A true experimental design with a post-test-only control group design was conducted with white rats (*Rattus norvegicus*), divided into three groups: a T2DM model group sacrificed after T2DM induction and diagnosed with T2D from a fasting blood glucose (FBC) test (Group C), a T2DM model group that did not receive exercise (Group NE), and a T2DM model group that received moderate-intensity aerobic exercise (Group E). Moderate-intensity aerobic exercise was conducted over six weeks, with a frequency of five days per week for 60 minutes per session. The findings revealed a significant reduction in *TGF-β1* expression in the lamina propria in Group E compared to Group C (p=0.004) Additionally, both Group E (p=0.002) and Group NE (p=0.028) showed a significant reduction in type I collagen expression in the lamina propria compared to Group C. These findings provide a basis for further investigation regarding the mechanism of non-pharmacologic DBD management by employing moderate-intensity exercise.

Keywords: Type 2 diabetes mellitus, aerobic exercise, *TGF-β1*, type I collagen, rat model

Introduction

Type 2 diabetes mellitus (T2DM) continues to increase in prevalence and incidence, as a leading cause of global morbidity and death [1]. Diabetes has a substantial negative impact on quality of life and functional ability [2]. There were 529 million individuals of all ages living with diabetes in 2021, with an age-standardized prevalence of 6.1%. It was also estimated that 485 million adults aged 20–79 years were living with diabetes in 2021 [3]. Global Burden Disease study report from 1990 to 2021 showed a 20% increase in each age group between 65–95 years [3].

In the urinary tract, diabetes-associated bladder dysfunction (referred to as diabetic bladder dysfunction, DBD) is a frequent urological problem. The prevalence of DBD among patients with diabetes could reach as high as 80% [4]. The prevalence of DBD found in a study conducted in

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2014 revealed that 93% of women with diabetes mellitus reported symptoms related to lower urinary tract symptoms, with 88% of them experiencing various abnormal urodynamic findings [5]. The condition causes difficulties with urine storage, bladder emptying, and maintaining proper bladder flexibility. Increased deposition of collagen fibers in the detrusor muscle is associated with reduced bladder compliance [6]. One of the fibrogenic cytokines, transforming growth factor-beta 1 (*TGF-β1*), is responsible for collagen formation in the bladder wall. *TGF-β1* is a regulator of collagen synthesis and degradation, including type I collagen [7]. *TGF-β1*-stimulated formation and stimulation of type I collagen plays an important role in the onset of bladder dysfunction in T2DM [8]. This condition eventually leads to decreased bladder function [8]. Moreover, elevated *TGF-β1* in the bladder contributes to increased fibrotic tissue production in the bladder wall, where the condition can be reversed by inhibiting *TGF-β1* [9].

In a previous study, exercise has been suggested to reduce oxidative stress by inhibiting the formation of free radicals, which are elevated in patients with diabetes [10]. A study reported that regular exercise reduces the incidence of various ROS-related diseases and helps the body adapt to the oxidative processes arising from physical activity [11]. Moderate-intensity exercise from a systematic review with human and animal subjects shows beneficial adaptive responses, including increased antioxidant activity, balanced redox reactions, and improved cellular function [12]. In a cross-sectional observational analytical study comparing male cyclists and sedentary male individuals, lower levels of *TGF-β1* in serum or plasma were found in male cyclists [13]. Unfortunately, evidence on the molecular mechanisms involved in the improvement of bladder functions by aerobic exercise has not been well established. Therefore, the aim of this study was to investigate the impact of moderate-intensity aerobic exercise on DBD using a rat model. The parameters evaluated include the expression of *TGF-β1* and type I collagen as markers of fibrotic changes, along with measurements of detrusor and lamina propria thickness to observe the anatomical changes.

Methods

Animal model

Male Wistar rats (*Rattus norvegicus*) aged six weeks old (n=30; 160–180 g), were obtained from Biochemistry Laboratories, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The rats were divided into three groups. The sample size was calculated using G*Power software [14] based on the following formula: $n_i = 2((Z_{1-\alpha/2} + Z_{1-ß})/d)^2$, where Z values correspond to a 5% research error (α), 5% statistical power (ß), and an effect size of 2 (strong effect). This calculation yielded a minimum of eight rats per group. To account for a 20% dropout risk, the final sample size was adjusted to 10 rats per group [15].

The rats were maintained in cages with a size of 12.5 × 20 × 30 cm, with two rats per cage, maintained at a temperature between 21°C and 25°C, under a 12-hour light-dark cycle. During the first seven days of the acclimation period, the rats were given a standard diet and subsequently given high-fat diets containing 18% kcal from protein, 61% kcal from fats, and 19% kcal from carbohydrates. The rats selected for this study were healthy, evidenced by smooth fur, bright and clear eyes, and being active, with no prior involvement in other studies. The final number of rats used in this study was 30, which were randomly distributed into three different groups.

Study design and group assignment

The aim of this study was to assess the effect of moderate-intensity aerobic exercise on *TGF-β1* and type I collagen in the detrusor and lamina propria. This study employed a true experimental design with a post-test-only control group. The rats were divided into three groups. The first group (n=10) was a T2DM model group terminated after diabetes induction with streptozotocin (STZ) as a control (Group C). The second group (n=10) was a T2DM model group that did not receive moderate-intensity aerobic exercise (Group NE) and was observed for six weeks. The third group (n=10) was a T2DM model group that received moderate-intensity aerobic exercise for six weeks (Group E). A diagram illustrating the study design framework of this research is presented in **Figure 1**.

Randomization and blinding

The study employed simple randomization, where all animals or samples were randomly assigned to the predetermined groups simultaneously without considering any other variables. Blinding in histological and immunohistochemical examination and measurement of lamina propria and detrusor thickness was conducted during outcome assessment.

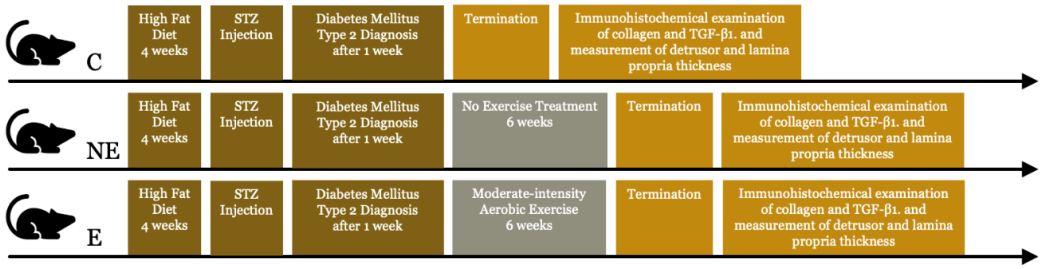

Figure 1. Illustration of the study design. C: control group; E: exercise group; NE: non-exercise group.

Type 2 diabetes mellitus rat model

The induction of T2DM in rats followed the recommendations from previous studies [16,17]. The rats were fed a custom purified rodent diet containing 60% of calories from fat (Diet DN 112252, Dyets, Inc., Bethlehem, PA, USA) for 28 consecutive days. Subsequently, a single injection of STZ in citrate buffer (pH 4.5) was administered intraperitoneally to the rats at a dose of 35 mg/kg BW to create the T2DM model. Seven days after the STZ injection, blood was collected from the tail tips of rats to determine the fasting blood glucose (FBG) levels. Rats were diagnosed with T2DM if FBG>150 mg/dL, as suggested by a published report [18].

Moderate-intensity aerobic exercise

Moderate-intensity aerobic exercise was performed by swimming in a container with a diameter of 75 cm and a height of 65 cm. Each exercise session lasted for 60 minutes, scheduled five days a week for six weeks during the light cycle [19]. FBG levels were examined at 2 and 4 weeks after the first exercise.

Endpoint

After the three groups of rats completed a 1-week acclimatization phase, T2DM was induced with a high-fat diet for four weeks, followed by STZ injection. At this stage, rats diagnosed with T2DM in group C were terminated. Treatment with moderate-intensity aerobic exercise was administered for six weeks to group E, while group NE was monitored for six weeks without any treatment. Body weight was measured at the start of the study and before termination. The overdose of anesthetic drugs administered in this study followed the protocol approved by the American Veterinary Medical Association (AVMA) guidelines on euthanasia [20], using a combination of intraperitoneal injection of ketamine (300 mg/kg BW) and xylazine (30 mg/kg BW). The cervical dislocation was performed on sedated rats weighing less than 200 grams, and decapitation was carried out using a guillotine on the sedated animals.

Immunohistochemical examination

After being collected from the euthanized rats, bladder tissues were fixed in formalin and embedded in paraffin blocks. Sections of 5 µm thickness were mounted on slides and stained with the following antibodies: anti-collagen-1 monoclonal antibody (#BSM-33400M, Bioss) and anti-TGF-β1(6B9) monoclonal antibody (#BSM-33345M, Bioss). *TGF-β1* and type I collagen expression were observed under a light microscope at 400× magnification.

Scoring for *TGF-β1* and type I collagen expression was performed using the H-score method that combines staining intensity (i) and the percentage of cells stained at each intensity level (Pi), following the recommendation of a published report [21]. The intensity was graded as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). Pi values ranged from 0% to 100%. The H-score was calculated using the following formula: H-score = (0 × P0) + (1 × P1) + (2 × P2) + (3 × P3). The total score ranges from 0 to 300.

Measurement of detrusor and lamina propria thickness

The thickness of the detrusor and lamina propria was measured after the bladder tissues were fixed in 10% formaldehyde for 24 hours. The tissues were then stained with Hematoxylin and Eosin (HE). An anatomical pathologist examined the bladder tissue thickness using a light microscope with an eyepiece graticule and measured in micrometers (μm).

Statistical analysis

The normality of the data distribution was tested using the Shapiro-Wilk test. Data with a normal distribution were presented as mean ± standard deviation (SD), while those with abnormal distribution were presented as median (interquartile range, IQR). The one-way ANOVA test followed by least significant difference (LSD) post-hoc tests were performed for normally distributed data, whereas the Kruskal-Wallis test followed by Mann-Whitney post-hoc tests were used for non-normally distributed data. Software SPSS Version 27 (IBM, New York, USA) and a significance level of $p<0.05$ was used for all statistical tests.

Results

Body weight and fasting blood glucose level profiles

The body weight and FBG level profiles of the animals are presented in **Figure 2**. FBG levels in the three groups showed average values of >150 mg/dL. Body weights increased in Group NE ($p=0.001$) and Group E ($p=0.001$), which underwent six weeks of treatment and were examined at the time before termination. In this experiment, two rats in Group C and one rat in Group NE died less than one week after STZ injection, suspected to be caused by STZ acute toxicity. One rat in the NE group died in the second week of treatment caused by infection after STZ injection.

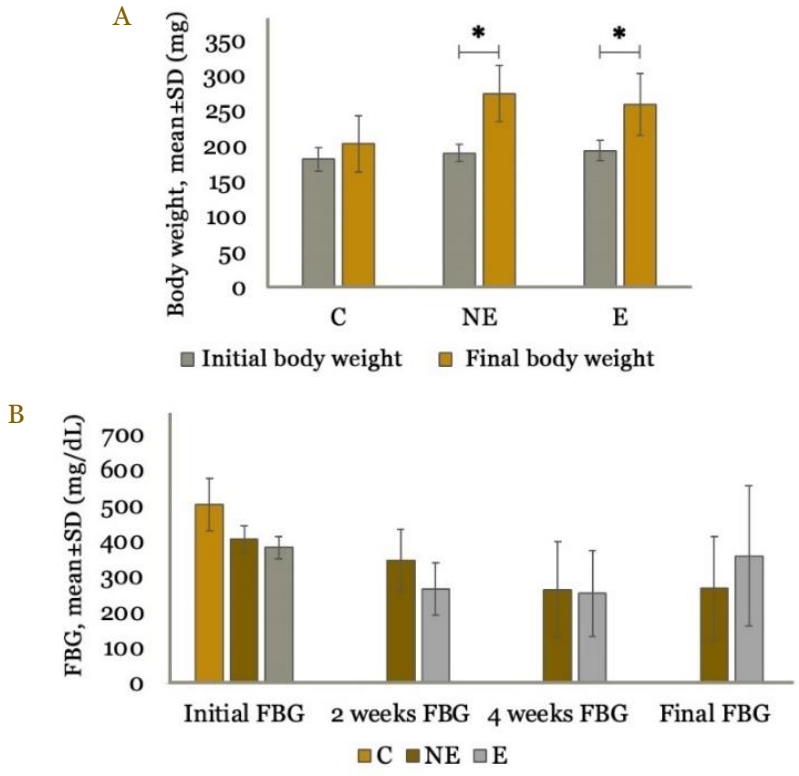

Figure 2. Initial body weight and final body weight of each group (A). Fasting blood glucose (FBG) in the timeline from the left to the right (B): initial, 2 weeks of treatment, 4 weeks of treatments, and final FBG before termination. *Significantly different at $p<0.05$. C: control group; E: exercise group; NE: non-exercise group.

TGF-β1 expression

Photographed images of immunohistochemical staining for *TGF-β1* expression in the detrusor and lamina propria are presented in **Figure 2**. The H-scores for *TGF-β1* expression observed in

detrusor and lamina propria are presented in **Figures 3A** and **3B**. The expression of *TGF-β1* in detrusor among the three groups was not statistically different (p=0.095). Meanwhile, the *TGF-β1* expression in lamina propria was found to be significantly lower in Group E than in Group C (p=0.004).

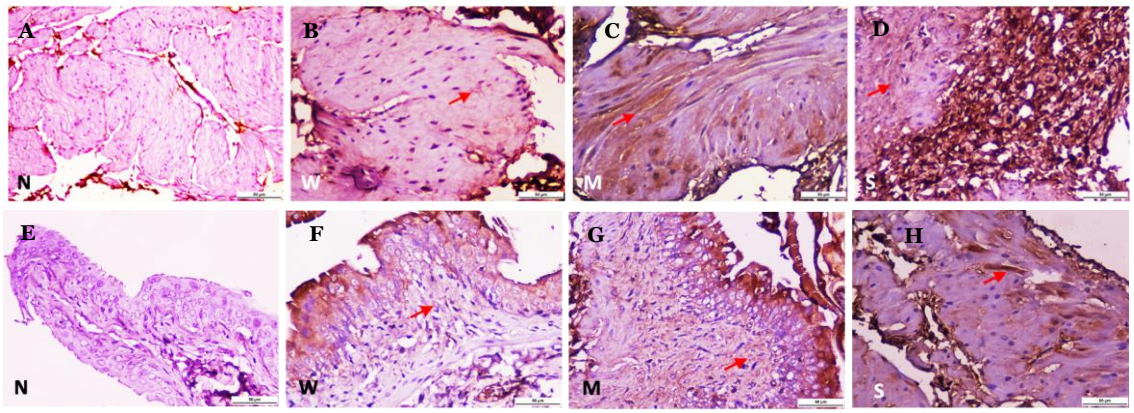

Figure 2. TGF-β1 staining in the detrusor (A-D) and lamina propria (E-H). From the left to the right panel, the stain intensity increases: negative (N), weak (W), moderate (M), and strong (S). Red arrow: *TGF-β1* staining.

Type I collagen expression

Photographed images of immunohistochemical staining for type I collagen expression in the detrusor and lamina propria are presented in **Figure 4**. The expression of type I collagen expression was calculated based on the H-score, where the results are presented in **Figures 3C** and **3D**. The expressions of type I collagen in the detrusor were not statistically significant among the tested group (p=0.685). However, significantly lower expressions of type I collagen were observed in lamina propria among Groups NE (p=0.028) and E (p=0.002), as compared to Group C. There was no significant difference in type I collagen expressions in the lamina propria between Groups E and NE (p=0.360).

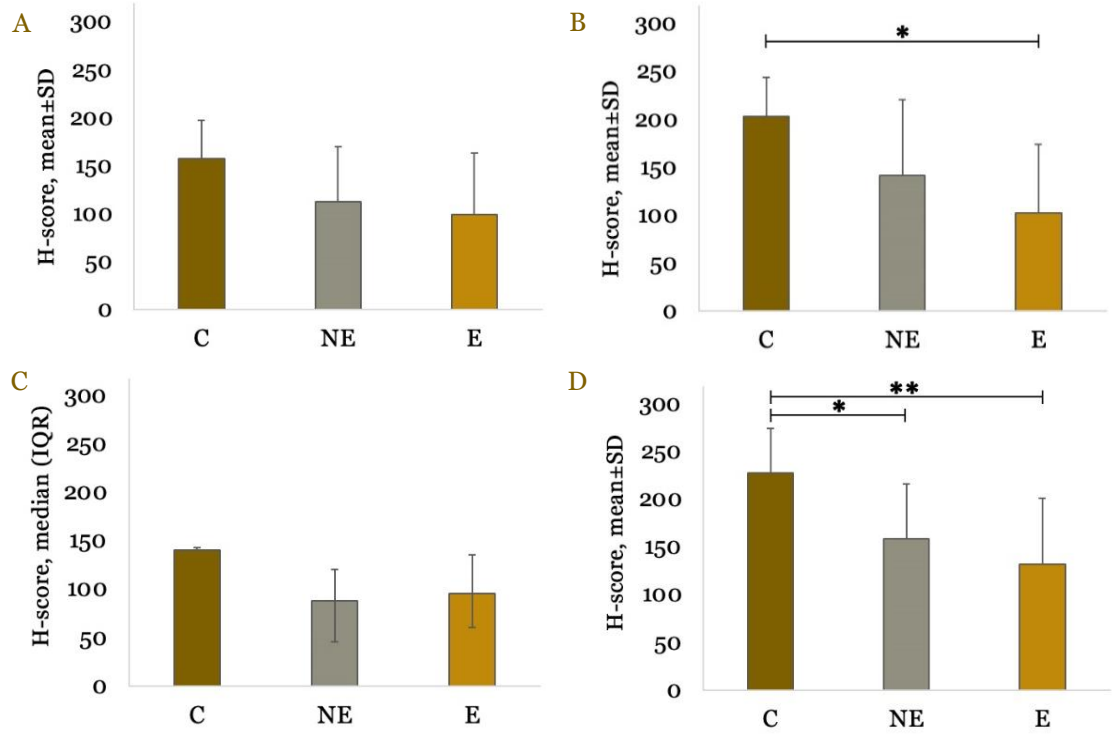

Figure 3. Expressions of *TGF-β1* in the detrusor (A) and lamina propria (B). Expressions of type I collagen in the detrusor (C) and lamina propria (D). Data are presented as mean±SD or median (IQR). *Significantly different at p<0.05. C: control group (n=8); E: exercise group (n=8); NE: non-exercise group (n=10).

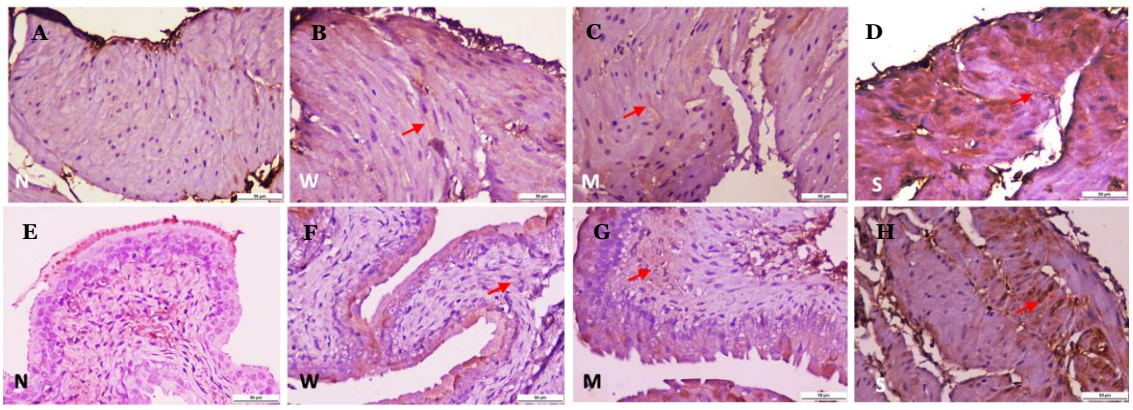

Figure 4. Type I collagen staining in the detrusor (A-D) and lamina propria (E-H). From the left to the right panel, the stain intensity increases: negative (N), weak (W), moderate (M), and strong (S). Red arrow: type I collagen staining.

Detrusor and lamina propria thickness

The photographed histological image of the thickness measurement is presented in **Figure 5**. Detrusor and lamina propria thickness observed in Groups E and NE are presented in **Figure 6**. There was no significant difference in the detrusor thickness among Groups C, NE, and E (p=0.176). Similarly, the lamina propria thickness across all groups was not significantly different (p=0.354).

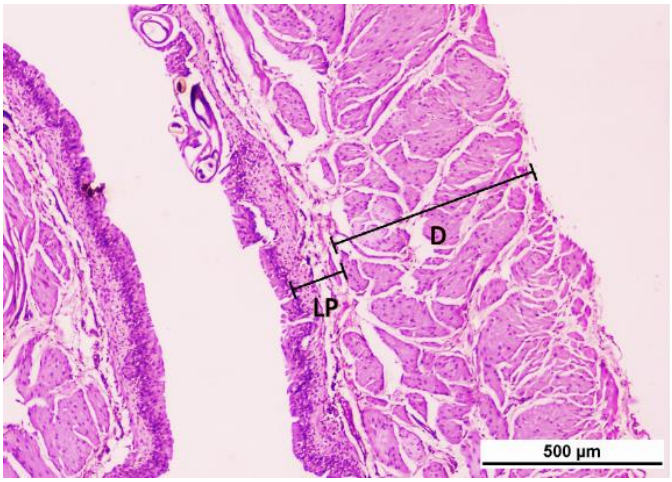

Figure 5. Thickness measurement of the detrusor (D) and lamina propria (LP).

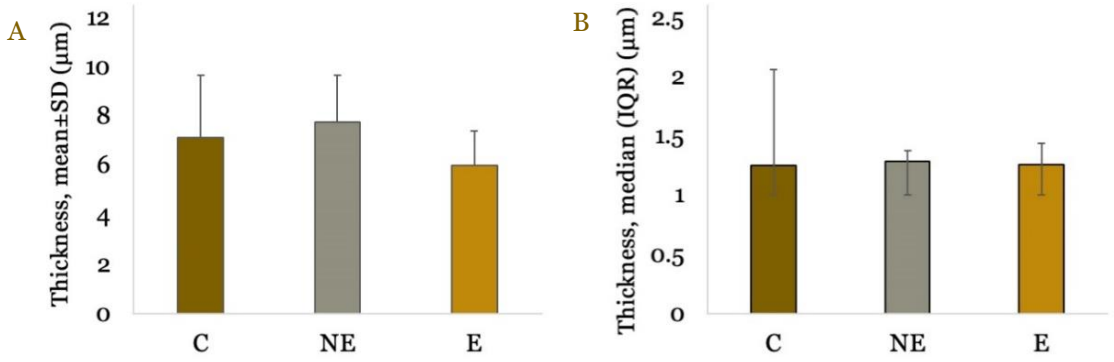

Figure 6. Thickness of the detrusor and lamina propria. Data are presented as mean±SD or median (IQR). C: control group (n=8); E: exercise group (n=8); NE: non-exercise group (n=10).

Discussion

Findings from the present study suggested that moderate-intensity aerobic exercise significantly reduced *TGF-β1* expression in the lamina propria compared to the control group. However, lower

expressions of the type I collagen, compared to the control group, were not associated with moderate-intensity aerobic exercise. Detrusor and lamina propria thickness were not affected by moderate-intensity aerobic exercise. This present study, utilizing a 6-week swimming intervention, is among the first to investigate these markers. Previously, a study conducted in South Korea in 2013 on women aged 69 to 72 showed that long-term physical exercise can be an effective method for significantly improving overactive bladder (OAB) [22]. In another study, physical exercise also improved bladder function in diabetic mice by enhancing urodynamic parameters, including reducing urination frequency and decreasing post-micturition residual urine volume [23].

The change in *TGF-β1* levels can be caused by various factors, including acids, bases, reactive oxygen species (ROS), proteases, and integrin-independent activation [24]. Regular physical exercise stimulates the activity of antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). This condition neutralizes free radicals and minimizes ROS production [11]. Routine exercise reduces the incidence of diseases associated with ROS and helps the body adapt to oxidative processes induced by physical activity [11]. Molecular adaptation results in improved physiological functions and increased resistance to oxidative stress [11]. Low ROS levels suppress the activation of *TGF-β1*, as evidenced by lower *TGF-β1* levels in serum/plasma [13]. In contrast, physically inactive individuals experience impaired physiological functions, reduced resistance to oxidative stress, and increased oxidative stress-related disease incidence [11].

TGF-β1 increases tissue inhibitor of metalloproteinases-1 (*TIMP-1*) mRNA expression while reducing mRNA expression of *MMP-1* and *MMP-3*. The disproportion between *TIMP-1* and *MMP-1* promotes the accumulation of extracellular matrix components, leading to bladder fibrosis in chronic diabetic rats. Matrix metalloproteinases (MMPs) can degrade extracellular matrix elements, including elastin, gelatin, collagen, proteoglycan, and matrix glycoproteins, and increased *TGF-β1* leads to a reduction in *MMP-1*, preventing the degradation of type I collagen, which subsequently accumulates [8]. A study on men aged 19–35 years showed that high resistance training led to an increase in *MMP-1*, *MMP-2*, *MMP-3*, and *MMP-4*, with this increase persisting even after eight weeks of training. This response appears to differ from that observed in calisthenic training, which showed a decrease in the response of the MMP [25].

In the present study, the reduction of type I collagen in both exercise and non-exercise groups is rather spontaneous due to the acute effect of STZ. Furthermore, we found that changes in type I collagen and *TGF-β1* in the lamina propria were more sensitive compared to those in the detrusor. The greater sensitivity of the lamina propria to changes is due to its structure, which contains a high amount of extracellular matrix (ECM), which is significantly affected by chronic inflammation, inflammatory cytokine and protease release [26,27]. Additionally, collagen in the bladder is primarily located in the lamina propria and between muscle bundles [28]. Type I collagen levels are associated with *TGF-β1* levels, which trigger fibrotic changes in the bladder [8]. Studies on the bladders of diabetic rats and those given diuretics showed that mRNA expression of type I collagen and *TGF-β1* was lower than in controls at weeks 2, 4, and 8 [29]. The decrease in collagen in the bladder of animals with diabetes mellitus is suspected to be caused by a reduction or cessation of collagen synthesis activity, rather than by maintaining or increasing collagen production in response to bladder hypertrophy [29].

Loss of collagen deposits leads to bladder hypertrophy, causing an imbalance in the muscle-to-collagen ratio, which alters bladder compliance in diabetic rats [29]. Rats that were recently induced with diabetes, had increased elastin synthesis, as well as decreased collagen synthesis, contributing to increased bladder compliance [29]. In the later phase, a significant increase in the protein expression of collagen I was found compared to control rats after 44 weeks of treatment [8]. Noncompliant bladders contain more collagen compared to normal control rats [30]. Functional decompensation of the bladder is associated with detrusor hypertrophy and an increase in bladder weight [31]. The increase in bladder weight also indicates the thickening of the detrusor muscle [32]. Glucosuria and osmotic diuresis lead to increased bladder stretch and intravesical pressure, resulting in bladder hypertrophy, which at the decompensation stage can lead to increased residual urine volume [33]. The bladder is changing from a compensated to a decompensated condition, characterized by a decrease in peak detrusor leak pressure and an

increase in resting pressure from the nine weeks after diabetes induction [34]. A study on histological examination after 16 weeks of intervention has found that T2DM rat bladders resulted in significant thickening of the detrusor muscle layer [35]. Another study with 16 weeks of treatment shows that diabetic rat bladders are larger and heavier compared to controls [36]. The non-significant results in detrusor and lamina propria thickness might be due to the shorter treatment duration compared to previous studies that lasted for 16 weeks [35,36], while the intervention in this study only lasted for six weeks.

The limitation of this study is the use of an animal model to replicate T2DM conditions in humans. Using only one strain or species could limit the ability to observe different physiological effects. The DBD model employed in the study may lack robustness. Future studies could use more quantitative methods to measure *TGF-β1* and type I collagen. Additionally, the exercise regimen was limited to swimming, and other types of exercise should be explored. In future research, a longer intervention period or a different exercise frequency is needed to observe *TGF-β1* and type I collagen change in the bladder of rats.

Conclusion

Moderate-intensity aerobic exercise significantly decreases the expression of *TGF-β1* in the lamina propria. Future research is needed for specific mechanisms and effects of aerobic exercise on the structural and molecular expression in T2DM model rats and their implications for developing exercise therapies for patients with type 2 diabetes.

Ethics approval

The Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, granted ethical approval for this study following the Council for International Organizations of Medical Sciences (CIOMS) 2016 guidelines. Registration number: 70/EC/KEPK/FKUA/2024.

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Competing interests

All the authors declare that there is no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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References

1. Khan MAB, Hashim MJ, King JK, *et al*. Epidemiology of type 2 diabetes - global burden of disease and forecasted trends. J Epidemiol Glob Health 2019;10(1):107-111.
2. Ramtahal R, Khan C, Maharaj-Khan K, *et al*. Prevalence of self-reported sleep duration and sleep habits in type 2 diabetes patients in South Trinidad. J Epidemiol Glob Health 2015;5(4 Suppl 1):S35-S43.
3. Ong KL, Stafford LK, McLaughlin SA, *et al*. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: A systematic analysis for the Global Burden of Disease Study 2021. Lancet 2023;402(10397):203-234.

4. Daneshgari F, Liu G, Birder L, *et al*. Diabetic bladder dysfunction: Current translational knowledge. J Urol 2009;182(6 Suppl):S18-S26.
5. Changxiao H, Zhengyong Y, Shibing Y, *et al*. Clinical and urodynamic evaluation of women referred with diabetes mellitus. Int Urogynecol J 2014;25(7):979-983.
6. Zhang HY, Ji HX, Li W, *et al*. Correlation between bladder compliance and the content of detrusor collagen fibers. Chin Nurs Res 2016;3(2):83-85.
7. Pohlers D, Brenmoehl J, Löffler I, *et al*. TGF-β and fibrosis in different organs - molecular pathway imprints. Biochim Biophys Acta 2009;1792(8):746-756.
8. Elrashidy RA, Liu G. Long-term diabetes causes molecular alterations related to fibrosis and apoptosis in rat urinary bladder. Exp Mol Pathol 2019;111:104304.
9. Györfi AH, Matei AE, Distler JHW. Targeting TGF-β signaling for the treatment of fibrosis. Matrix Biol 2018;68-69:8-27.
10. Stranahan AM, Lee K, Martin B, *et al*. Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. Hippocampus 2009;19(10):951-961.
11. Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med 2008;44(2):153-159.
12. Meng Q, Su CH. The impact of physical exercise on oxidative and nitrosative stress: Balancing the benefits and risks. Antioxidants (Basel) 2024;13(5):573.
13. Eka Widiastuti IA, Arsyad A, Idris I, *et al*. Exercise adaptations and TGF-β1 levels in recreational cyclists. Ann Med Surg (Lond) 2021;70:102872.
14. Kang H. Sample size determination and power analysis using the G*Power software. J Educ Eval Health Prof 2021;18:17.
15. Althubaiti A. Sample size determination: A practical guide for health researchers. J Gen Fam Med 2023;24(2):72-78.
16. Quranayati Q, Iqhrammullah M, Saidi N, *et al*. Extracts from *Phyllanthus emblica* L stem barks ameliorate blood glucose level and pancreatic and hepatic injuries in streptozotocin-induced diabetic rats. Arab J Chem 2023;16(9):105082.
17. Syukri M, Zaini LM, Kartasasmita AS, *et al*. Effects of SGLT2 inhibitor administration on blood glucose level and body weight in type 1 diabetes rat model. Narra J 2023;3(2):e194.
18. Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed Pharmacother 2017;95:605-613.
19. Wang R, Tian H, Guo D, *et al*. Impacts of exercise intervention on various diseases in rats. J Sport Health Sci 2020;9(3):211-227.
20. American Veterinary Medical Association. AVMA guidelines for the euthanasia of animals: 2020 edition. 2020. Available from: https://www.avma.org/resources-tools/avma-policies/avma-guidelines-euthanasia-animals. Accessed: 14 May 2024.
21. Nicholson RI, Bouzubar N, Walker KJ, *et al*. Hormone sensitivity in breast cancer: Influence of heterogeneity of oestrogen receptor expression and cell proliferation. Eur J Cancer Clin Oncol 1991;27(7):908-913.
22. Ko IG, Lim MH, Choi PB, *et al*. Effect of long-term exercise on voiding functions in obese elderly women. Int Neurourol J 2013;17(3):130-138.
23. Vadhavkar M, Golbidi S, Sea J, *et al*. Exercise improves bladder function in diabetic mice. Neurourol Urodyn 2011;30(1):174-182.
24. Deng Z, Fan T, Xiao C, *et al*. TGF-β signaling in health, disease, and therapeutics. Signal Transduct Target Ther 2024;9(1):61.
25. Urso ML, Pierce JR, Alemany JA, *et al*. Effects of exercise training on the matrix metalloprotease response to acute exercise. Eur J Appl Physiol 2009;106(5):655-663.
26. Sorokin L. The impact of the extracellular matrix on inflammation. Nat Rev Immunol 2010;10(10):712-723.
27. Pompili S, Latella G, Gaudio E, *et al*. The charming world of the extracellular matrix: A dynamic and protective network of the intestinal wall. Front Med (Lausanne) 2021;8:610189.
28. Fathollahi A, Daneshgari F, Hanna-Mitchell AT. Effect of polyuria on bladder function in diabetics versus non-diabetics: An article review. Curr Urol 2015;8(3):119-125.
29. Gray MA, Wang CC, Sacks MS, *et al*. Time-dependent alterations of select genes in streptozotocin-induced diabetic rat bladder. Urology 2008;71(6):1214-1219.

30. Deveaud CM, Macarak EJ, Kucich U, *et al*. Molecular analysis of collagens in bladder fibrosis. J Urol 1998;160(4):1518-1527.
31. Zhang EY, Stein R, Chang S, *et al*. Smooth muscle hypertrophy following partial bladder outlet obstruction is associated with overexpression of non-muscle caldesmon. Am J Pathol 2004;164(2):601-612.
32. Qiao LY, Xia C, Shen S, *et al*. Urinary bladder organ hypertrophy is partially regulated by Akt1-mediated protein synthesis pathway. Life Sci 2018;201:63-71.
33. Golbidi S, Laher I. Bladder dysfunction in diabetes mellitus. Front Pharmacol 2010;1:136.
34. Daneshgari F, Liu G, Imrey PB. Time dependent changes in diabetic cystopathy in rats include compensated and decompensated bladder function. J Urol 2006;176(1):380-386.
35. Masuda K, Aizawa N, Watanabe D, *et al*. Pathophysiological changes of the lower urinary tract behind voiding dysfunction in streptozotocin-induced long-term diabetic rats. Sci Rep 2020;10(1):4182.
36. Hindi EA, Williams CJ, Zeef LAH, *et al*. Experimental long-term diabetes mellitus alters the transcriptome and biomechanical properties of the rat urinary bladder. Sci Rep 2021;11(1):15529.