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ORIGINAL PAPER

Effect of moderate-intensity aerobic exercise on penile α-SMA and eNOS expression in diabetes mellitus rats model: A non-pharmacological approach to diabetic erectile dysfunction

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Summary

Background: *Diabetes mellitus (DM) is a chronic disease with globally increasing prevalence, significantly impacting quality of life, including erectile dysfunction (ED). Moderate-intensity aerobic exercise has been shown to improve metabolic and cardiovascular parameters, yet limited studies have examined its effect on erectile function. This study aimed to explore the effect of aerobic exercise on the expression of alpha-smooth Muscle Actin (α-SMA) and endothelial Nitric Oxide Synthase (eNOS) in the penile tissue of diabetic rat models.*

Methods: *This true-experimental study used 24 male Rattus norvegicus (Wistar), aged 12 weeks, randomly assigned to three groups: control (C), diabetic without intervention (NE), and diabetic with aerobic exercise (E). The aerobic exercise was performed for 60 minutes per session, 5 days a week, for 10 weeks. The expression of eNOS and α-SMA in penile tissues was analysed by immunohistochemistry and quantified by Image J Software Version 1.54p. Statistical analysis was performed using SPSS software version 29.0, including tests for homogeneity and normality, followed by one-way ANOVA with LSD or Tukey post hoc tests for normally distributed data, or the Kruskal Wallis test for non-normal distributions.*

Results: *There was no significant difference in eNOS expression among the three groups ($p > 0.05$). However, α-SMA expression showed a significant difference among the three groups ($p < 0.05$). Group E showed a significant increase in α-SMA expression compared to Group NE ($p = 0.034$).*

Conclusions: *Moderate-intensity aerobic exercise improves α-SMA expression in the penile tissue of diabetic rats, contributing to better erectile function. Although it did not affect eNOS expression, this finding supports the potential of exercise-based non-pharmacological therapy for managing ED in diabetic patients.*

KEY WORDS: *Diabetes mellitus; Erectile dysfunction; Aerobic exercise; alpha-Smooth Muscle Actin; eNOS.*

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INTRODUCTION

Diabetes mellitus (DM) has emerged as a global health crisis, with the number of affected individuals rising dramatically from 108 million in 1980 to 422 million in 2014, accompanied by an increase in global adult prevalence from 4.7% to 8.5% (1). This metabolic disorder is associated with severe complications, including cardiovascular, renal, ocular, and neural impairments, which significantly reduce functional capacity and quality of life (2, 3). Among these complications, *erectile dysfunction* (ED) is a prevalent yet often overlooked condition in diabetic males, with incidence rates two to three times higher than in the general population (4, 5).

The pathophysiology of ED in diabetes is multifactorial, involving central or autonomic neuropathy, endothelial and smooth muscle dysfunction in the corpus cavernosum, and hypogonadism (6). Hyperglycemia-driven mechanisms, such as the accumulation of *advanced glycation end-products* (AGEs), oxidative stress, and *reactive oxygen species* (ROS), play pivotal roles in vascular and neural damage (7). AGEs impair *endothelial nitric oxide synthase* (eNOS) activity, reduce *nitric oxide* (NO) bioavailability, and promote collagen cross-linking, leading to vascular stiffness and compromised erectile function (8, 9). Additionally, AGEs exacerbate oxidative stress by upregulating NADPH oxidase and downregulating antioxidant enzymes like Mn-SOD, further diminishing endothelial-dependent vasodilation (10).

While pharmacological interventions exist, non-pharmacological approaches, such as moderate-intensity aerobic exercise, have shown promise in mitigating diabetic complications. As demonstrated in hypercholesterolemic animal models, exercise enhances eNOS activity, NO synthesis, and endothelial function (9). However, the specific effects of aerobic exercise on molecular markers of erectile function, such as eNOS and α-smooth muscle actin (α-SMA) in diabetic corpus cavernosum, remain underexplored.

This study aims to investigate the impact of moderate-intensity aerobic exercise on eNOS and α-SMA expres-

sion in the penile tissue of diabetes mellitus rats model. By addressing this gap, we seek to elucidate exercise-induced mechanisms that may counteract ED progression in diabetes, offering a foundation for non-pharmacological therapeutic strategies.

Materials and methods

Study design

This study was true experimental research with a post-test-only control group design. Animals were randomly assigned into three groups:
C: Non-diabetic control group (no treatment)
NE: Diabetic model group without exercise
E: Diabetic model group with moderate-intensity aerobic exercise intervention.
The intervention consisted of swimming for 60 minutes per session, 5 days a week, for 10 consecutive weeks. This study obtained ethical clearance for animal research under ethical number 66/EC/KEPK/FKUA/2025.

Animal model and sample size

The study utilized organ samples from a parallel project involving male Rattus norvegicus (Wistar strain), aged 12 weeks, weighing 140-180 grams, and meeting the following inclusion criteria: healthy, never used in prior experiments, and physically active. Animals were excluded if they became ill, died (not due to treatment), or failed three consecutive exercise sessions (11).
The sample size was calculated using the formula for comparing two means, with an effect size (d) of 1.8, α = 0.05, and β = 0.05 (power = 95%). A minimum of 8 rats per group was obtained, resulting in a total of 24 rats randomly assigned to 3 groups.

Experimental procedures

Acclimatization and Induction of Diabetes Mellitus
Rats underwent a 7-day acclimatization period under standard laboratory conditions (temperature 21-25°C, 12-hour light/dark cycle). Diabetes mellitus was induced using a single intraperitoneal injection of *streptozotocin* (STZ) at 40 mg/kg body weight dissolved in citrate buffer (pH 4.5). Fasting blood glucose was measured via the tail vein using a glucometer (Accu-Chek Instant), and rats with fasting glucose > 150 mg/dL were classified as diabetic (12). Table 1 presents the baseline and final characteristics of the rats, including body weight and fasting glucose levels, before and after the streptozotocin induction.

Table 1.
Animal characteristics before and after induction.

	C (Mean ± SD)	NE (Mean ± SD)	E (Mean ± SD)
Initial Body Weight	154.14 ± 10.88	150.29 ± 10.14	153.71 ± 9.84
Final Body Weight	235.71 ± 52.05	238.29 ± 24.79	187 ± 18.48
Initial Fasting Glucose		456.14 ± 137.001	382.57 ± 136. 15
Final Fasting Glucose	108.29 ± 9.84	490.43 ± 107.35	381.42 ± 138.12

Exercise protocol
Rats in the E group performed swimming exercises in a cylindrical tank (diameter 45 cm, water depth 55 cm) for 60 minutes/day, 5 days/week (Monday-Friday) for 10 weeks. Swimming was supervised, and sessions were terminated if rats could not keep their heads above water for more than 3 seconds without effort. Non-compliance in three consecutive sessions led to exclusion.

Euthanasia and tissue collection
At 24 hours following the completion of the 10-week intervention, all rats were anaesthetized with intraperitoneal injections of ketamine (300 mg/kg) and xylazine (30 mg/kg), followed by cervical dislocation and decapitation. Penile tissues were collected and preserved in formaldehyde for histological analysis.

Histological and immunohistochemical analysis

eNOS and α-Smooth Muscle Actin (α-SMA) expression
Penile tissue was processed into paraffin blocks, sectioned at 5 µm thickness, and mounted on slides.
Immunohistochemical staining was performed using monoclonal anti-eNOS (*#BSM-33176M, Bioss*) and polyclonal anti-α-SMA antibodies (*#BSM-33188M, Bioss*). Slides were processed via xylene and graded alcohol series, antigen retrieval, and incubation with antibodies, followed by chromogen development and counterstaining.
Slides were scanned using a high-resolution digital slide scanner, and representative images from the corpus cavernosum were selected for analysis. Image analysis was performed using ImageJ software version 1.54p to determine the area fraction of positive staining. Thresholding was used to identify and isolate the positively stained regions, and the area fraction of positive staining was calculated by dividing the stained area by the total area of the corpus cavernosum within the same field.
Measurement and all quantification were performed by a blinded observer to reduce bias. Data were reported as mean percentage area of positive staining per group.

Instruments and materials
This study utilized a range of instruments and materials tailored to each phase of the experimental protocol. For the aerobic exercise intervention, a circular swimming tank with a diameter of 75 cm was employed to facilitate moderate-intensity swimming exercises in rats. Each session was monitored using a stopwatch to ensure consistent exercise duration, and a towel was used to dry the animals post-exercise before returning them to their cages.
Penile tissues were fixed in formalin, embedded in paraffin, and sectioned at 5 µm.
Immunohistochemistry was performed using Bioss antibodies against eNOS and α-SMA. Sections were processed with xylene, alcohol series, hydrogen peroxide, and chromogen substrates, then incubated for antibody binding. Stained tissues were scanned, and immunoreactive areas were quantified using ImageJ v1.54p to assess eNOS and α-SMA expression in erectile tissue after aerobic exercise in diabetic rats.

Study site and duration
The research was conducted over a period of six months in the *Biochemistry Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga*.

Data processing and statistical analysis
Data analysis was conducted using SPSS software version 29.0. Homogeneity and normality tests were performed initially. One-way ANOVA was used to compare normally distributed data between groups, followed by LSD or Tukey post hoc tests, while the Kruskal Wallis test was used for non-normally distributed data. A p-value < 0.05 was considered statistically significant.

Results

The basic data for this study were obtained from the rat penis preparations of male Wistar strain rats (Rattus norvegicus) aged 12 weeks from a previous study. All rats included in the previous study had their body weight measured at the beginning of STZ injection and at the end of the treatment before termination, along with fasting blood glucose measurements taken at both the beginning and end of the intervention. The C group received no treatment, NE group was the diabetic model group without treatment, E group received moderate-intensity aerobic exercise until the age of 22 weeks.
For the expression of eNOS in the corpus cavernosum smooth muscle, the immunohistochemical examination was performed using ImageJ software to assess the surface area of staining in the preparations. Homogeneity tests were conducted for all groups, and normality tests were performed on each group.
eNOS expression was found to be homogeneous and generally distributed in the smooth muscle of the cavernosum penis, allowing for analysis using a One-Way ANOVA. The results indicated no significant differences between groups ($p > 0.05$). Table 2 and Figure 1 show the mean eNOS expression, the results of the normality test, and the One-Way ANOVA test across three groups: C, NE, and E. In Group C, the mean eNOS expression was 0.115 ± 0.058, with a normality test p-value of

Group	Mean ± SD	Normality test	P value
C	0.115 ± 0.058	0.200	0.888
NE	0.099 ± 0.065	0.200	
E	0.108 ± 0.063	0.200	

Table 2.
Mean, normality test, and One-way ANOVA test on eNOS expression in the smooth muscle of the corpus cavernosum penis.

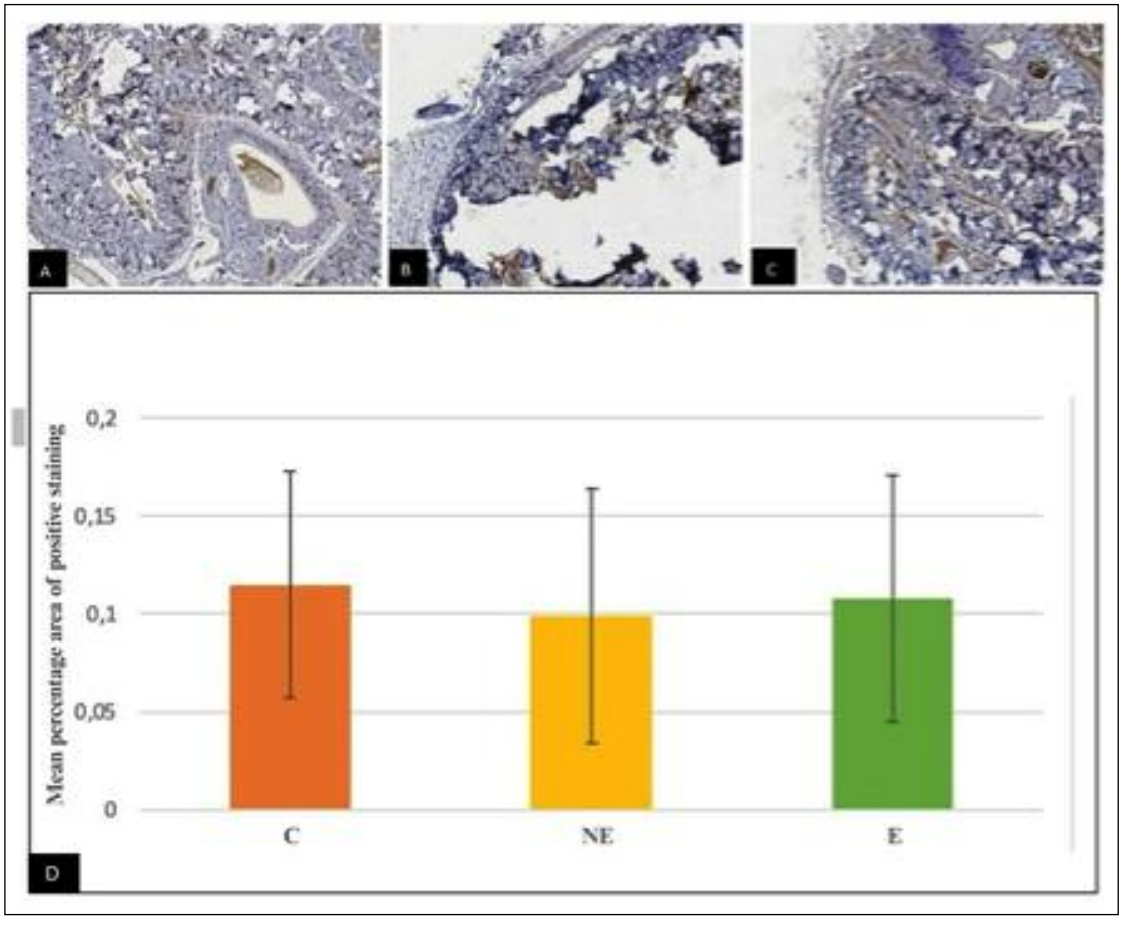

Figure 1.
Expression of eNOS in the smooth muscle of the corpus cavernosum penis,
A. Group C,
B. Group NE,
C. Group E,
D. Mean ± SD of eNOS expression in the smooth muscle of the penile cavernosum.

Group	Mean ± SD	Normality test	P value
C	0.118 ± 0.053	0.200	0.030
NE	0.048 ± 0.031	0.200	
E	0.106 ± 0.059	0.167	

Table 3.
Mean, normality test, and One-way ANOVA test on α-SMA expression in the smooth muscle of the corpus cavernosum penis.

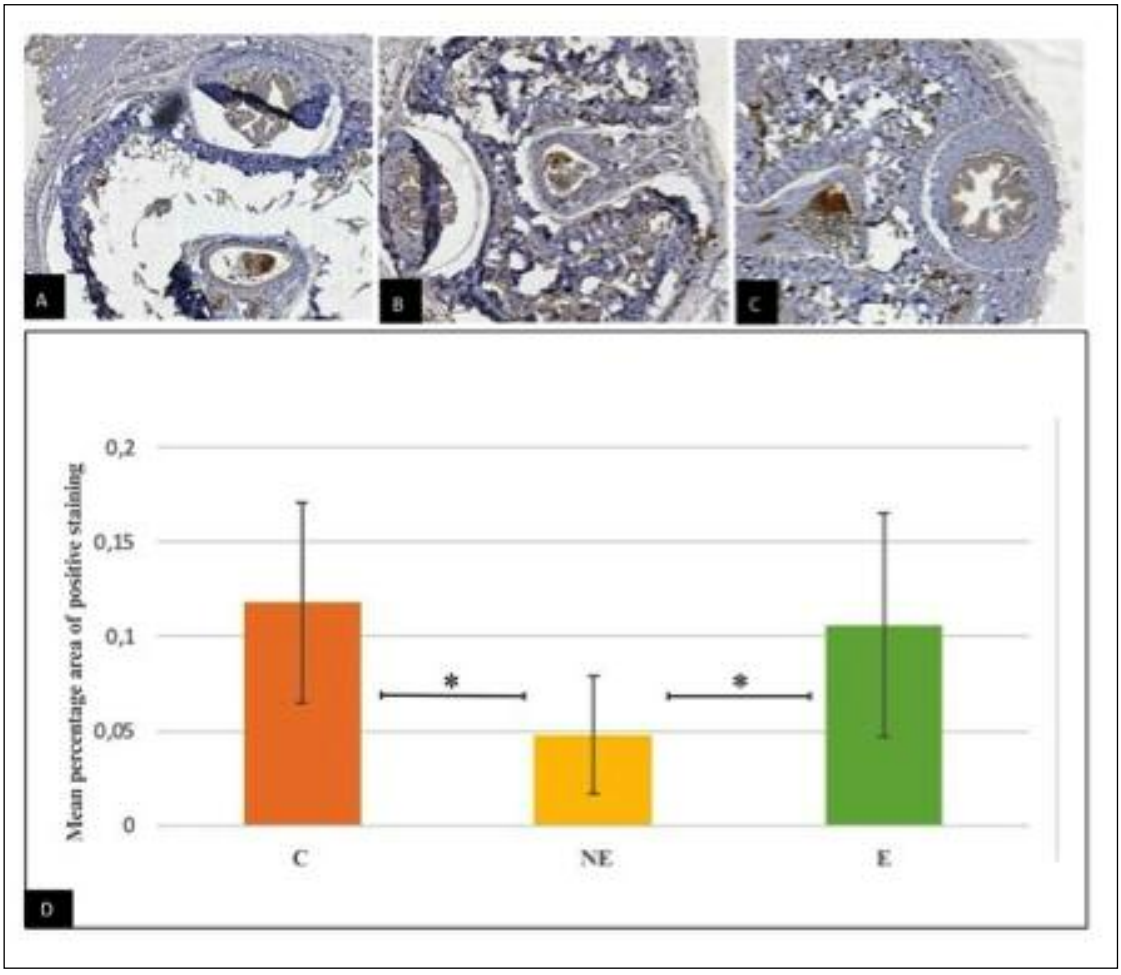

Figure 2.
Expression of α-SMA in the smooth muscle of the corpus cavernosum penis in Group C, Group NE, and Group E. as mean ± SD of α-SMA expression in the smooth muscle of the corpus cavernosum penis.
** Significant difference in post hoc LSD analysis of α-SMA expression at p < 0,05.*

0.200, indicating that the data follows a normal distribution. In group NE, mean eNOS expression was 0.099 ± 0.065, with a normality test p-value of 0.200, indicating a normal distribution.
Similarly, Group E showed a mean eNOS expression of 0.108 ± 0.063, with a normality test p-value of 0.200, again indicating normal distribution. The One-Way ANOVA test resulted in a p-value of 0.888, suggesting no statistically significant difference in eNOS expression between the groups (since the p-value is greater than 0.05).
Table 3 and Figure 2 shows mean α-SMA expression, normality test, and One-Way ANOVA test across three groups: C, NE, and E. For Group C, the mean α-SMA expression was 0.118 ± 0.053. The normality test p-value for this group was 0.200, indicating that the data follow a normal distribution. In Group NE, the mean α-SMA expression was 0.048 ± 0.031, with a normality test p-value of 0.200, also indicating normal distribution. Group E showed a mean α-SMA expression of 0.106 ± 0.059 with a normality test p-value of 0.167, indicating the data also follows a normal distribution. The ANOVA test yielded a p-value of 0.030, suggesting a statistically significant difference among the groups regarding α-SMA expression. Post hoc LSD analysis of α-SMA expression between groups revealed significant differences between C and NE (p = 0.013) as well as between NE and E (p = 0.034). In contrast, no significant difference was found between C and E (p = 0.641). The results of post hoc LSD analysis are shown in Table 4.

Table 4.
Post hoc LSD test analysis of α-SMA expression between groups in the smooth muscle of the penile cavernosum.

Group	C	NE	E
C		0.013	0.641
NE	0.013		0.034
E	0.641	0.034	

Discussion

Diabetes mellitus is a chronic metabolic disease with rising global prevalence, increasing from 108 million in 1980 to 422 million in 2014.1 One notable complication is *erectile dysfunction* (ED), which is 2-3 times more common in diabetic men (13). The multifactorial pathophysiology involves vascular damage, neuropathy, and hormonal imbalances. Chronic hyperglycemia leads to oxidative stress and accumulation of *advanced glycation end-products* (AGEs), contributing to endothelial dysfunction by decreasing eNOS and α-SMA (14-16).

Moderate-intensity aerobic exercise has been shown to improve endothelial function, enhance eNOS expression, and reduce AGEs (17, 18). This study examined the effect of moderate-intensity swimming exercise on the expression of eNOS and α-SMA in the corpus cavernosum of diabetic Wistar rats. Tissues were obtained from a previous study and divided into three groups: healthy control (C), diabetic without intervention (NE), and diabetic with exercise intervention (E).

Immunohistochemical analysis was used to assess biomarker expression.

The study found no significant difference in eNOS expression between groups, although the highest expression was in the control group (C), suggesting diabetes impairs eNOS levels and potentially contributes to ED. This aligns with previous studies showing exercise alone may not restore eNOS levels in diabetic models (16, 19) though conflicting evidence exists in other tissues like the heart (20, 21).

Conversely, α-SMA expression showed significant differences between groups. Diabetic rats (NE) had lower α-SMA expression than controls (C), indicating structural deterioration of smooth muscle. Moderate-intensity exercise (E) increased α-SMA levels in diabetic rats, suggesting partial restoration, although not to control levels. This supports the potential of exercise to reverse structural changes in penile tissue (22). While the observed increase in α-SMA expression in the exercise group may suggest a beneficial effect of moderate-intensity aerobic activity on smooth muscle preservation in diabetic penile tissue, it is important to note that α-SMA is also a marker of myofibroblast activation involved in early fibrotic processes. Therefore, the elevation in α-SMA could reflect either tissue restoration or the onset of fibrosis. Without the inclusion of additional fibrosis-specific markers – such as TGF-β1 or collagen subtypes – it is difficult to fully interpret the nature of these changes. Future studies are recommended to incorporate a broader panel of fibrosis biomarkers and histological analyses to distinguish between adaptive remodelling and pathological fibrosis, thereby providing a more comprehensive understanding of exercise-induced tissue changes in diabetic erectile dysfunction (23-25).

Limitations of this study include the use of archived tissue samples, which may have undergone some degree of degradation, and the assessment of only two biomarkers (eNOS and α-SMA), limiting the scope of molecular insights. Additionally, the exercise protocol employed was limited to a single intensity and duration (moderate-intensity swimming for 10 weeks), without comparisons to other intensities or frequencies. As a result, it remains unclear whether lower or higher-intensity activity, or different training frequencies, could elicit more pronounced or distinct effects on penile tissue markers. Although the 10-week intervention yielded some molecular changes, it may still represent a relatively short duration for modelling chronic conditions such as diabetes. Longer-term interventions may be necessary to observe more sustained or significant endothelial and smooth muscle improvements. Future research should incorporate a range of exercise intensities and durations and assess a broader spectrum of molecular markers such as *reactive oxygen species* (ROS), *advanced glycation end-products* (AGEs), and inflammatory cytokines to more comprehensively elucidate the therapeutic potential of exercise in diabetic erectile dysfunction.

Conclusions

This study demonstrated that moderate-intensity aerobic exercise did not significantly affect eNOS expression in the corpus cavernosum of diabetic rats. However, moderate-intensity aerobic exercise was associated with a significant increase in α-SMA expression compared to the untreated diabetic group, suggesting restoration of smooth muscle integrity. These findings support the potential of aerobic exercise in mitigating structural deterioration in diabetic erectile dysfunction, although further research with additional biomarkers and longer intervention durations is warranted to clarify the underlying mechanisms.

Declarations

Ethical approval: *This study has been ethically approved and registered under 66/EC/KEPK/FKUA/2025.*

Availability of data and material: *All data generated or analyzed during this study are available upon request.*

Competing interests: *The authors declare that they have no competing interests.*

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Authors' contributions: *AAA: Conceptualization, methodology, and supervision of the study. JR: Data collection, analysis, and manuscript drafting. MAS: Data collection, analysis, and manuscript drafting. ASR: Data analysis, manuscript drafting, and review.*

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