

Mitochondrial ATP synthase regulates corpus cavernosum smooth muscle cell function *in vivo* and *in vitro*

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Abstract. Adenosine triphosphate (ATP) levels are closely associated with diabetes-related erectile dysfunction (DMED). Mitochondrial ATP synthase serves a key role in ATP production. The present study aimed to investigate the relationship between F₁-ATP synthase and DMED *in vivo* and *in vitro*. The F₁-ATP synthase expression levels in corpus cavernosum tissues from rats with DMED were examined. F₁-ATP synthase expression was found to be lower in corpus cavernosum tissues from rats with DMED compared with healthy controls, suggesting a role for ATP synthase under high glucose conditions. In addition, the present study also demonstrated that hyperglycemia could downregulate F₁-ATP synthase expression in rat corpus cavernosum smooth muscle cells (CCSMCs) *in vitro*. The overexpression of F₁-ATP synthase in CCSMCs influenced the phenotypic CCSMC transformation, upregulated eNOS expression, increased cGMP levels and reduced CCSMC apoptosis under high glucose *in vitro*. In conclusion, the present study indicates that the upregulation of mitochondrial ATP synthase expression may improve CCSMC function,

suggesting that mitochondrial ATP synthase could serve as a potential therapeutic target for the treatment of DMED.

Introduction

Diabetes is one of the most common causes of erectile dysfunction (ED), such that the incidence of ED in patients with diabetes is four times higher compared with that in non-diabetic patients (1). During the early stages of diabetes, 50-75% of diabetic patients experience ED (2). Although effective pharmacological interventions for treating ED are available, there remains a lack of an effective treatment strategy for ED associated with diabetes (DMED).

Corpus cavernosum smooth muscle function involves a plethora of complex intracellular events and extracellular signals (3,4). Since smooth muscle cell (SMC) function depends heavily on mitochondrial activity a strong association exists between mitochondrial dysfunction and dysfunction in the corpus cavernosum smooth muscle (5). In particular, long-term hyperglycemia has been reported to reduce mitochondrial biogenesis, increase the ADP/ATP ratio, induce ultra-structural changes and markedly increase the production of reactive oxygen species (ROS) (6-8). These changes severely impair the physiological function of the corpus cavernosum smooth muscle.

Mitochondrial ATP synthase is the key enzyme of the mitochondrial energy machinery that is responsible for the synthesis of ATP in most eukaryotic cells. ATP synthase consists of the catalytic F₁ domain and the F₀ domain that is embedded in the inner mitochondrial membrane (9). It has been reported that low levels of ATP synthase in patient fibroblasts can significantly limit mitochondrial ATP production whilst increasing ROS production through the mitochondrial electron transport chain (10). Increases in ROS can cause damage to the diastolic function of penile cavernosum smooth muscle, resulting in penile vasculopathy (11).

The relationship between ATP synthase and DMED remains unclear. In the present study, F₁-ATP synthase expression in cavernosum smooth muscle under high glucose conditions was measured; subsequently, the effects of F₁-ATP synthase overexpression on cavernosum smooth muscle function under hyperglycemia were also investigated.

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Abbreviations: DMED, diabetes-related erectile dysfunction; SMCs, smooth muscle cells; ED, erectile dysfunction; ROS, reactive oxygen species; STZ, streptozotocin; APO, apomorphine; CCSMCs, corpus cavernosum smooth muscle cells; NOS, nitric oxide synthase

Key words: ATP synthase, erectile dysfunction, smooth muscle cells, apoptosis

Materials and methods

Animal models and groups. A total of 20 male Sprague-Dawley (SD) rats (weight range, 200–250 g), ~8 weeks old, were provided by Guangdong Medical Experimental Animal Centre (Guangdong, China). The animal procedures were approved by the Wenzhou Medical University Animal Policy and Welfare Committee (Zhejiang, China). All rats were housed with a 12 h light/dark cycle at 24±1°C, and provided with food and water *ad libitum* for a week before study. Apomorphine (APO; 100 µg/kg) was injected into the loose neck skin of the rats to verify normal erectile function previously (12). The rats were then randomly divided into the control (n=4) and diabetic groups (n=5). A diabetic rat model was generated by a single intraperitoneal injection of streptozotocin (STZ; Sigma Aldrich; Merck KGaA; 55 mg/kg) after an overnight fast (13). Plasma glucose levels in blood samples obtained from the tail vein were measured using a glucometer (Roche Diagnostics, Indianapolis, IN). A randomized blood glucose concentration ≥16.67 mmol/l was considered to indicate the successful establishment of the experimental diabetic rat model (14). Rats in the control group were injected with saline. During the experiment, all rats were fed with a normal diet.

Evaluation of erectile function. According to a method established by He *et al.* (13), rats in each group were placed in an observation cage after feeding. A period of 10 weeks after feeding, APO was then injected subcutaneously into the loose neck skin of the rat and each rat was videoed immediately with a camera for 30 min after injection, and the number of penile erections was observed and recorded. Each rat was examined at 8 am and 8 pm for three consecutive days.

Sample acquisition. After successful modelling, rats continued to be fed for 10 weeks. When APO experiment finished, rat corpus cavernosum tissues were cut into three sections and each section was ~2 mm. The middle section was fixed using 4% paraformaldehyde at 4°C overnight, whilst the remaining two sections were directly placed in cryovials and stored at -80°C.

Isolation of primary rat corpus cavernosum smooth muscle cells (CCSMCs). The corpus cavernosum tissues of normal SD rats were cut into pieces of ~0.5x1x1 mm and placed at 0.5 cm intervals into 25 ml sterile culture flasks containing 2 ml DMEM (Hyclone; GE Healthcare Life Sciences) with 20% FBS (Hyclone; GE Healthcare Life Sciences). Under light microscopy (magnification, x100), a small number of CCSMCs were observed to migrate out of the tissue following 3 days of incubation.

Cell culture. CCSMCs after passage number three were cultured in DMEM containing 10% heat-inactivated FBS (Hyclone; GE Healthcare Life Sciences) and 1% penicillin/streptomycin (Beijing Solarbio Science & Technology Co., Ltd.) and maintained in a humidified incubator at 37°C with 5% CO₂. Cells were seeded in six-well plates for immunocytochemical analysis, 12-well plates for western blotting and reverse transcription-PCR (RT-PCR), and 96-well plates for high-throughput flow cytometry and ELISA analysis.

Molecular cloning of *Atp5b* and cell transfection. Briefly, the *Atp5b* gene (Shanghai Shengggong Biology Engineering Technology Service, Ltd.) was cloned into the pcDNA3.1(+) carrier (Taihe Biotechnology Co., Ltd.) within BamH I (Thermo Fisher Scientific, Inc.) and Xho I (Thermo Fisher Scientific, Inc.) restriction enzyme to create a pcDNA3.1 plasmid encoding F₁-ATP synthase (pcDNA3.1-F₁-ATP). Following sequencing to verify integration, 2 µg pcDNA3.1-F₁-ATP or blank vector pcDNA3.1 was transfected into CCSMCs with Lipofectamine® 2000 (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's protocol. The medium was replaced with complete medium at 6 h following transfection. Transfected cells were collected for flow cytometry, western blotting or RT-PCR at 48 h after transfection. Finally, the cells were divided into the following groups: i) Control group, where CCSMC cultures were incubated in normal glucose medium; ii) high glucose group, where CCSMC cultures were incubated in medium containing high glucose (30 mmol/l); iii) high glucose + F₁-ATP synthase overexpression (OE), where CCSMCs transfected with the pcDNA3.1-F₁-ATP were cultured in medium containing high glucose; and iv) high glucose + blank vector groups, where the CCSMCs transfected with blank pcDNA3.1 vector were cultured in medium containing high glucose.

Analysis of apoptosis by TUNEL and flow cytometry. TUNEL staining was performed using a Tissue TUNEL apoptosis detection kit according to the manufacturer's protocol (Enjing Biotechnology). The corpus cavernosum tissues sections were fixed in 4% PFA overnight at 4°C. Slides were first immersed in 3% H₂O₂ for 10 min at room temperature. Equilibration buffer (100 µl) was then added and slides were incubated for 20 min at 37°C, followed by incubation with a TdT reaction mix (50 µl) for 60 min at 37°C. The cells were then stained with hematoxylin for 3 min at room temperature. Neutral balsam was used to mount the slides. Brown staining was considered as positive, and three fields of each slide were randomly selected under light microscopy (magnification, x400). The number of cells were counted and the percentage of positive cells was calculated under a light microscope (magnification, x400).

For flow cytometry experiment, cells were first digested with 0.25% trypsin without EDTA (15) for 2 min and then collected in centrifuges. Cells were centrifuged (1,000 x g) for 5 min at room temperature and resuspended in 500 µl binding buffer. Subsequently, cells were incubated with 5 µl Annexin V-FITC and 5 µl propidium iodide (Nanjing KeyGen Biotech Co., Ltd.) for 10 min at 37°C in darkness. Finally, cells were tested by flow cytometry within 2 h. Flow cytometry was performed using FACS Calibur (BD Biosciences), and FACS data were analyzed using CellQuest software (version 5.1; BD Biosciences)

Oxidative stress testing by ELISA. *In vivo* and *in vitro* samples were added to the wells, and biotin-labeled anti-endothelial nitric oxide synthase (eNOS; Bioswamp Wuhan Beiyinlai Biotechnology Co., Ltd; cat. no. RA20010) antibodies or biotin-labeled anti-cyclic guanosine monophosphate (cGMP; Bioswamp Wuhan Beiyinlai Biotechnology Co., Ltd; cat. no. RA20105) antibodies were added and cultured at room temperature, for 1 h, and then 50 µl enzyme-labeled reagent

was also added with the exception of the blank well. After incubation for 30 min at 37°C, Tetramethylbenzidine was performed in the darkness for 15 min at 37°C. Finally, stop solution (50 μ l) was added to each well to terminate the reaction. Absorbance (optical density at 450 nm) was subsequently measured using a microplate reader.

Western blotting. Cells were collected in lysis buffer (Beyotime Institute of Biotechnology) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) (Beyotime Institute of Biotechnology) for lysis at 4°C for 20 min. Next, the samples were centrifuged (12,000 \times g) for 10 min at 4°C and the supernatant was used to detect the protein concentration using a Bicinchoninic Acid protein assay kit (Beyotime Institute of Biotechnology). Equal amounts (40 μ g) of protein samples were separated by 10% SDS-PAGE and transferred to a PVDF membrane (EMD Millipore). After blocking with 5% non-fat milk in TBST at room temperature for 1 h, the membranes were incubated with primary anti-ATP synthase (1:1,000; cat. no. NBP1-91573; Bio-Techne) or anti-GAPDH (1:10,000; cat. no. KC5G5; Aksomics) primary antibodies at 4°C overnight. The next day, the membranes were incubated with horseradish peroxidase (HRP)-conjugated Goat anti-Rabbit (1:10,000; cat. no. AP307P; Merck KGaA) antibody at room temperature for 2 h. The bands were visualized using enhanced chemiluminescence (Pierce; Thermo Scientific, Inc.) and quantified by measuring the intensity of the signals using ImageJ software (1.8.0; National Institutes of Health).

Fibrotic detection in vivo. To measure rat cavernosum collagen/smooth muscle ratio, tissue sections were stained with Masson's trichrome stain (16), followed by light microscopy (magnification, \times 100) examination. The area ratio of the collagen fibres to muscle was analyzed using Image-Pro Plus software (6.0, Media Cybernetics, Inc.).

Immunocytochemistry. In aseptic conditions, dry glass slides were placed in a six-well plate, onto which the 2×10^6 cells were subsequently seeded and cultured overnight. The cells were then fixed using 4% paraformaldehyde for 30 min at room temperature followed by permeabilization with 0.2% Triton X-100 for another 5 min at room temperature. After blocking with 10% goat serum (Beijing Solarbio Science & Technology Co., Ltd.) in PBS for 1 h at room temperature, the cells were incubated with primary α -SMA antibody (1:200; cat. no. 55135-1-AP; Proteintech Group, Inc.) overnight at 4°C. This was followed by incubating with HRP-conjugated secondary antibody (1:400; cat. no. AP307P; Merck KGaA) for 1 h at room temperature, and then 3,3'-diaminobenzidine (Beijing Solarbio Science & Technology Co., Ltd.) was incubated for another 5 min at room temperature subsequently. Finally, hematoxylin (Beijing Solarbio Science & Technology Co., Ltd.) was used to stain the nuclei and neutral for 2 min at room temperature and gum was used to seal the samples. Images of the cells were captured under light microscopy (magnification, \times 100) and the extent of immunostaining was analyzed using Image-Pro Plus software (6.0, Media Cybernetics, Inc.).

RT-PCR. Total RNA was isolated from CCSMCs using TRIzol[®] (Invitrogen; Thermo Fisher Scientific, Inc.) and

reversely transcribed to cDNA using a GenestarScriptRT cDNA Synthesis kit (Shanghai Jixing Biotechnology Co., Ltd.), the thermocycling conditions for reverse transcription were as follows: 37°C for 15 min and 85°C for 5 sec. RT-PCR analysis was performed to measure the mRNA levels using SYBR Green Clon Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) in an ABI 7500 Rapid Thermal Cycler. The sequences of the primers used were as follows: α -smooth muscle actin (α -SMA) forward, 5'-ATCTGGCAC CACTCCTTCTA-3' and reverse, 5'-GTCCAGCACAATACC AGTTG-3'; smooth muscle myosin heavy chain (SMMHC) forward, 5'-CCGCTGCCTATGACAAACT-3' and reverse, 5'-CGCATACTTGGAGGAGATGT-3'; calponin forward, 5'-GGAACATCATTGGCCTACAG-3' and reverse, GCGTGT CACAGTGTTCAT-3'; osteopontin (OPN) forward, 5'-GCT ATCAAGGTCATCCCAGTT-3' and reverse, 5'-GTTTCC ACGCTTGGTTCAT-3'; β -actin forward, 5'-AGGGAAATC GTGCGTGACAT-3' and reverse, 5'-GAACCGCTCATTGCC GATAG-3'. The thermocycling conditions for RT-PCR were as follows: 1 cycle of 95 °C for 5 min, 40 cycles of 50°C for 2 min, 95°C for 2 min, and 60°C for 32 sec. The thermocycling conditions for melting curves were: 95 °C for 15 sec, 60°C for 1 min and 95°C for 15 sec. Final values were computed using the $2^{-\Delta\Delta Cq}$ method (17).

Statistical analysis. All statistical data are expressed as the mean \pm standard deviation. The differences among groups were analysed using one-way ANOVA followed by Bonferroni correction. $P < 0.05$ was considered to indicate a statistically significant difference. GraphPad Prism software (version 5.0; GraphPad Software, Inc.) was used to perform the statistical analyses.

Results

General characteristics of the diabetic rats. The characteristics of diabetic and healthy control rats are compared in Fig. 1. Diabetic rats exhibited significantly elevated blood glucose levels and lower body weights compared with normal control rats ($P < 0.05$; Fig. 1E). Following injection with APO, the erectile response in diabetic rats was significantly lower compared with that in the control group ($P < 0.01$; Fig. 1A).

High glucose increases the collagen/smooth muscle ratio and induces SMC apoptosis in diabetic rats. The collagen/smooth muscle ratio was observed to be significantly increased in diabetic rats compared with the control group ($P < 0.05$; Fig. 1B), whilst the number of TUNEL-positive cells in the diabetic group was significantly higher compared with that in the control group ($P < 0.01$; Fig. 1C).

F_1 -ATP synthase expression is reduced in diabetic rats. To evaluate the activity of F_1 -ATP synthase in cavernosum SMCs, western blot analysis was performed to determine the expression of F_1 -ATP synthase protein in the two groups. The expression of F_1 -ATP synthase was found to be significantly lower in the diabetic group compared with the control group ($P < 0.01$; Fig. 1D).

F_1 -ATP synthase overexpression promotes the expression of ATP synthase under high glucose conditions in vitro. CCSMCs

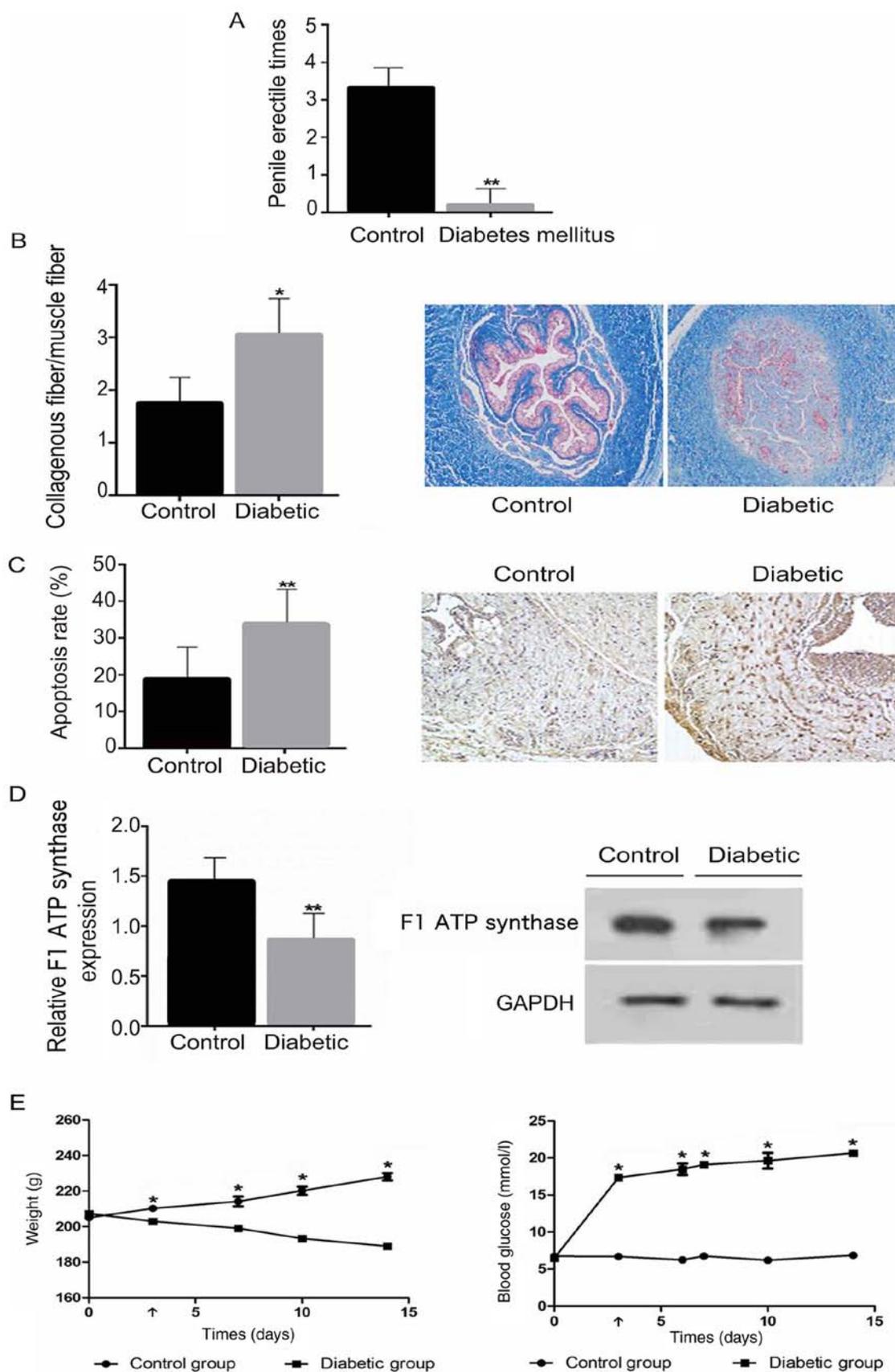


Figure 1. Comparison of diabetic rats with healthy controls. (A) Evaluation of erectile function. Apomorphine experiments were performed to evaluate the erectile function of rats in each group. ** $P < 0.01$ vs. Control. (B) Red represents collagen and blue represent muscle. Collagenous fiber/muscle fiber in each group according to Masson's trichrome staining in each group. Magnification, $\times 100$. * $P < 0.05$ vs. Control. (C) The apoptotic rate in each group according to TUNEL staining. Representative images of TUNEL staining in tissue sections from rats in each group are shown. Magnification, $\times 100$. ** $P < 0.01$ vs. Control. (D) Western blot analysis showing the protein expression levels of F₁-ATP synthase in rat corpus cavernosum tissues. GAPDH is the loading control and data are presented as the band density of F₁-ATP synthase relative to GAPDH. ** $P < 0.01$ vs. Control. (E) Body weight and blood glucose levels measured in rats from each group during the study. Arrow on the x-axis indicates the day in which respective testing (day 3) commenced. * $P < 0.05$ vs. Control.

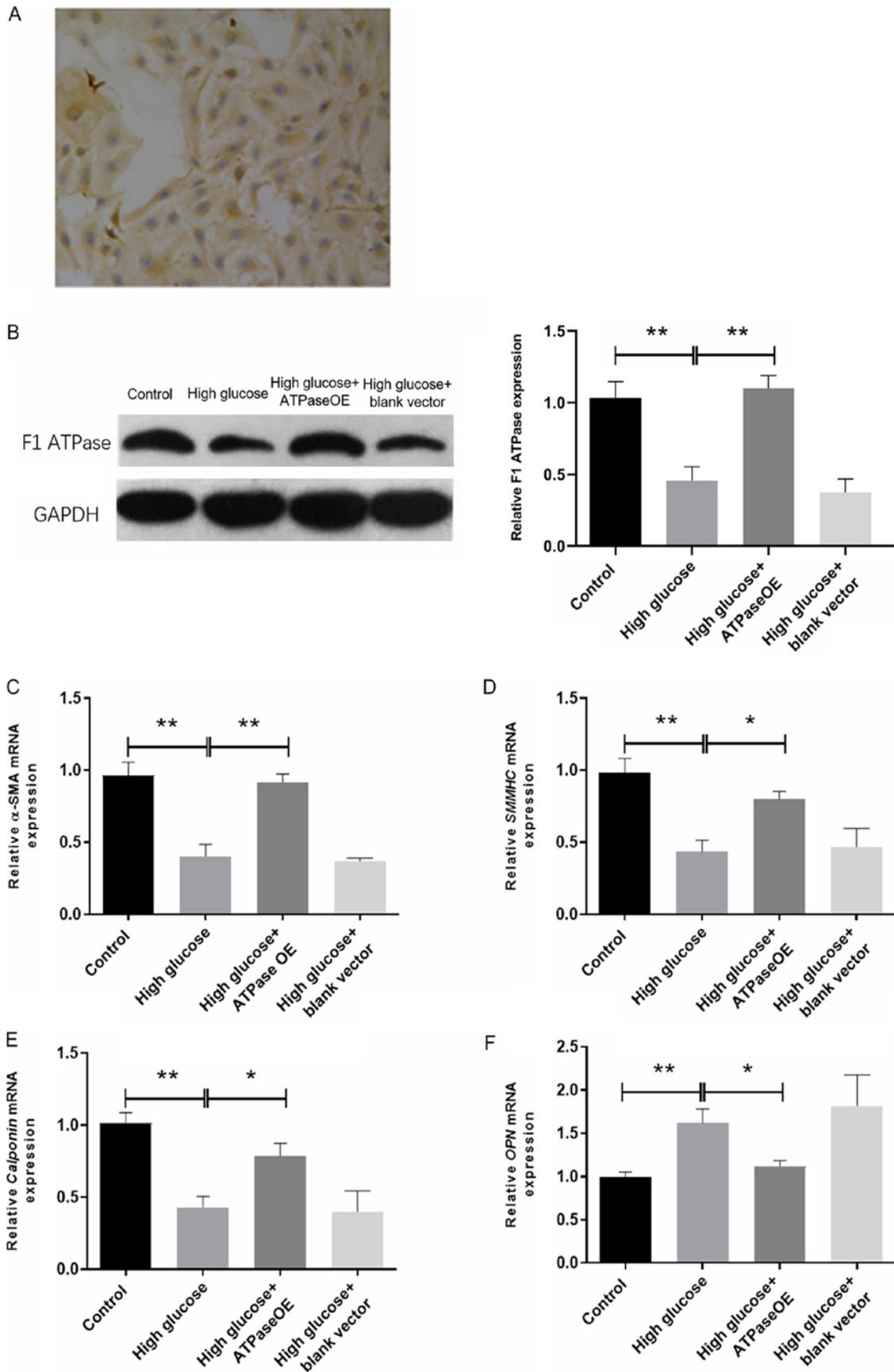


Figure 2. Effects of high glucose and ATP synthase on smooth muscle markers in cultured rat CCSMCs. (A) The α -SMA immunocytochemical staining of cultured rat CCSMCs. Magnification, $\times 100$. (B) Western blot analysis of F₁-ATP synthase expression in cultured rat CCSMCs from the Control, high glucose, high glucose + ATP synthase OE and high glucose + blank vector groups. (C-F) mRNA expression of smooth muscle markers in CCSMCs from control, high glucose, high glucose + ATP synthase and high glucose + blank vector groups as measured using reverse transcription-quantitative PCR. (C) α -SMA expression, (D) SMMHC expression, (E) calponin expression and (F) OPN expression. * $P < 0.05$ and ** $P < 0.01$ as indicated. CCSMCs, corpus cavernosum smooth muscle cells; OE, overexpression; OPN, osteopontin; α -SMA, α -smooth muscle actin; SMMHC, smooth muscle myosin heavy chain.

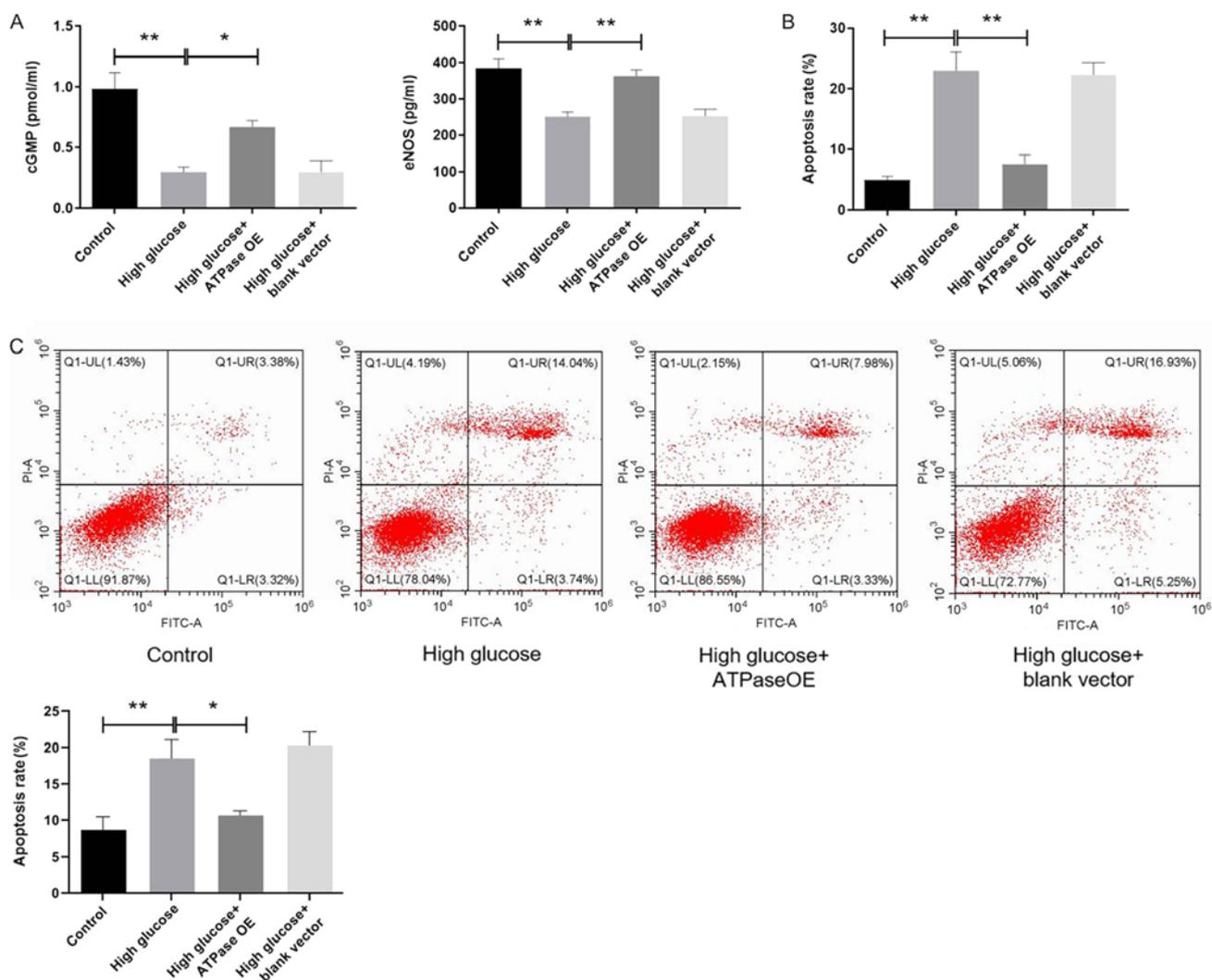


Figure 3. Effects of high glucose and ATP synthase on cGMP, eNOS and apoptosis in cultured rat CCSMCs. (A) ELISA analysis of cGMP and eNOS levels in cultured rat CCSMCs from Control, high glucose, high glucose + ATP synthase OE and high glucose + blank vector groups. * $P < 0.05$ and ** $P < 0.01$ as indicated. (B) Apoptosis rates in the four groups. (C) Images from the Annexin V-FITC/PI staining flow cytometry assay. * $P < 0.05$ and ** $P < 0.01$ as indicated. cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; PI, propidium iodide; OE, overexpression.

were isolated and cultured prior to α -SMA immunocytochemical staining. Positive α -SMA cellular staining was observed, suggesting that the isolation of CCSMCs was successful (Fig. 2A). Consistent with the findings of the *in vivo* experiment, F_1 -ATP synthase expression in the CCSMCs incubated with high glucose was markedly reduced compared with that in the control CCSMCs (Fig. 2B), and the high glucose-induced effect was reversed by transfection with the vector encoding F_1 -ATP synthase (Fig. 2B). No notable difference was observed in the expression of F_1 -ATP synthase between the high glucose and high glucose + blank vector groups.

Overexpression of F_1 -ATP synthase promotes CCSMC phenotypic transformation under high glucose. The mRNA expression levels of α -SMA, SMMHC and calponin were significantly lower in the high glucose group compared with the control ($P < 0.01$; Fig. 2C-E). By contrast, OPN mRNA expression in the high glucose group was significantly higher compared with that in the control group ($P < 0.01$; Fig. 2F). All of the aforementioned changes were significantly reversed following transfection

with the vector encoding F_1 -ATP synthase ($P < 0.05$; Fig. 2C-F), and no significant differences in α -SMA, SMMHC, calponin and OPN mRNA expression were observed between the high glucose and high glucose + blank vector groups.

Overexpression of F_1 -ATP synthase increases cGMP levels and eNOS expression in CCSMCs under high glucose. In cultured rat CCSMCs, the levels of eNOS and cGMP were measured using ELISA. Compared with the control group, the eNOS and cGMP levels were significantly lower in the high glucose group ($P < 0.01$; Fig. 3A). Although no significant differences were observed in the levels of eNOS expression and cGMP between the high glucose and high glucose + blank vector groups, eNOS expression and cGMP levels in the high glucose group transfected with the F_1 -ATP synthase vector were found to be significantly higher compared with those in the high glucose group ($P < 0.05$; Fig. 3A).

Overexpression of F_1 -ATP synthase reduces the apoptosis of rat CCSMCs under high glucose. A significant increase in the

number of TUNEL-positive cells was observed in the high glucose group compared with the control group ($P < 0.01$; Fig. 3B), and this increase was significantly reversed by F_1 -ATP synthase overexpression ($P < 0.01$; Fig. 3B). No significant differences were observed in the number of TUNEL-positive cells between the high glucose + F_1 -ATP synthase overexpression and control groups (Fig. 3B). According to the annexin V/PI assay, the apoptotic rate was significantly higher in the high glucose group compared with the control group, and the increase was markedly reversed by F_1 -ATP synthase overexpression (Fig. 3B and C). No notable changes were observed in the number of TUNEL-positive or apoptotic cells between the high glucose and high glucose + blank vector groups (Fig. 3B and C).

Discussion

To the best of our knowledge, the present study is the first to observe significantly reduced ATP synthase expression in CCSMCs from the penile tissues of rats with DMED, and to demonstrate that the upregulation of F_1 -ATP synthase expression can increase eNOS expression and cGMP levels whilst suppressing the apoptosis of rat CCSMCs under high glucose conditions.

ATP synthase is the terminal enzyme in the oxidative phosphorylation pathway and ATP formation in the mitochondria. Previous studies have demonstrated that ATP synthase exists on the outer surface of the plasma membrane of endothelial cells and tumor cells (18,19). ATP synthase dysfunction can reduce mitochondrial ATP production and lead to severe consequences for all energy-dependent cellular activities in the human body. However, the role of ATP synthase in human diseases remains elusive. Previous studies have shown that the myocardial ATP synthase levels in patients with ischemic cardiomyopathy are significantly decreased along with the exhaustion of myocardial contractile function, and the artificial restoration of ATP synthase levels can restore the contractile function of cardiomyocytes (20,21). It has also been reported that downregulation of ATP synthase activity was detected in the skeletal muscle of diabetic patients following the cessation of insulin treatment (22). These previous observations suggest that ATP synthase may be a promising target for the regulation of muscle contractility.

In the penis, SMCs account for 40-52% of cells in the cavernosum and serve to maintain penile contractility; in particular, maintenance of the contractile phenotype in SMCs is essential for cavernous space relaxation and penile erection (12). Chronic hyperglycaemia results in dysfunction of the vascular and nervous systems in the corpus cavernosum, in addition to inducing CCSMC apoptosis and changes in fiber and muscle content (23,24). Under harmful external stimulation, including hypoxia and hyperglycemia, CCSMCs display a tendency towards transforming from a contractile to proliferative phenotype (25,26). SMMHC, α -SMA and calponin are considered as molecular markers for contractile CCSMCs, whereas OPN is considered as a molecular marker for synthetic or proliferative CCSMCs (13,27). In the present study, the expression of α -SMA, SMMHC and calponin in CCSMCs incubated with high glucose group was significantly reduced, whilst the expression of the OPN was significantly increased compared with that in control CCSMCs. However, under high glucose conditions, the changes

in the expression levels of α -SMA, SMMHC, calponin and OPN were reversed following F_1 -ATP synthase transfection. These results suggest that under high glucose treatment, rat CMSCs underwent phenotypic transformation from the contractile to the proliferative phenotype, in a manner that could be reversed by F_1 -ATP synthase overexpression. ATP serves an important role in maintaining smooth muscle tone in the corpus cavernosum. In a previous study, hyperglycemia has been demonstrated to impair mitochondrial function and inhibit ATP synthase activity, leading to reduced levels of ATP production (28). Liu *et al* (5) showed that high glucose resulted in abnormal lipid metabolism, in turn leading to a decrease in ATP synthase expression through the Akt phosphorylation pathway in CCSMCs, suggesting that ATP synthase is essential for CCSMC function in hyperglycemia. In the present study, continuous exposure to high glucose reduced the expression of the F_1 -ATP synthase protein in both rat CCSMCs and smooth muscle tissues from the corpus cavernosum in diabetic rats. Additionally, the present study also confirmed that the overexpression of F_1 -ATP synthase could switch the cavernosum smooth muscle from a proliferative to a contractile phenotype under high glucose conditions.

Impaired ATP synthase function as a result of hyperglycemia can induce the production of excessive mitochondrial ROS (29). Increased ROS levels may inhibit the use of L-arginine, which is a substrate for NOS (30). The present study showed that the overexpression of F_1 -ATP synthase elevated eNOS levels under hyperglycemia. NOS activates the NO-cGMP pathway, which induces penile smooth muscle relaxation and the subsequent initiation and maintenance of penile erection (31). This observation suggests that ATP synthase can serve as a novel target for DMED treatment.

However, some limitations remain attached to the present study. Additional studies are required to understand the regulatory mechanism of ATP synthase activity in this DMED animal model. Since chronic hyperglycemia not only impairs CCSMC function but also leads to vascular sclerosis and neurological dysfunction (32,33), further studies are required to elucidate the relationship between ATP synthase activity and the pathophysiological vascular and neurological features of DMED.

In conclusion, ATP synthase activity is closely associated with CCSMC function under hyperglycemia. Further understanding of ATP synthase may provide new therapeutic options for treating DMED.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZGW and LZ conceived and supervised the study; JC and ZGW designed experiments; ZQX, JHC and SWC performed the experiments; YBX and QQW developed new software and performed the simulations; JHC and ZQX analyzed the data and wrote the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

The animal procedures were approved by the Wenzhou Medical University Animal Policy and Welfare Committee (Zhejiang, China). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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