Taurine Supplementation Improves Erectile Function in Rats with Streptozotocin-induced Type 1 Diabetes via Amelioration of Penile Fibrosis and Endothelial Dysfunction

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ABSTRACT

Introduction: For patients with diabetes, erectile dysfunction (ED) is common and greatly affects quality of life. However, these patients often exhibit a poor response to first-line oral phosphodiesterase type 5 inhibitors.

Aim: To investigate whether taurine, a sulfur-containing amino acid, affects diabetic ED (DED).

Methods: Type 1 diabetes mellitus was induced in male rats by using streptozotocin. After 12 weeks, an apomorphine test was conducted to confirm DED. Only rats with DED were administered taurine or vehicle for 4 weeks. Age-matched nondiabetic rats were administered saline intraperitoneally for 4 weeks.

Main Outcome Measures: Erectile function was evaluated by electrical stimulation of the cavernous nerve. Histologic and molecular alterations of the corpus cavernosum also were analyzed.

Results: Erectile function was significantly reduced in the diabetic rats compared with in the nondiabetic rats, and was improved in the diabetic rats treated with taurine. The corpus cavernosum of the rats with DED exhibited severe fibrosis and decreased smooth muscle content. Deposition of extracellular matrix proteins was increased in the diabetic rats, while expression of endothelial nitric oxide synthase/cyclic guanosine monophosphate/nitric oxide pathway–related proteins was reduced. Taurine supplementation ameliorated erectile response as well as histologic and molecular alterations.

Conclusion: Taurine supplementation improves erectile function in rats with DED probably by potential antiﬁbrotic activity. This finding provides evidence for a potential new therapy for DED.


Key Words: Taurine; Diabetes; Erectile Dysfunction; Fibrosis; Extracellular Matrix

INTRODUCTION

Erectile dysfunction (ED), defined as the inability to attain or maintain a penile erection sufficient for successful vaginal intercourse, is becoming more common worldwide.1 Approximately 15% to 20% of the general male population experiences ED.2,3 Patients with diabetes are nearly 3 times more likely to suffer from ED compared to patients without diabetes. In addition, ED appears to arise 10 years earlier in patients with diabetes and tends to be more severe, significantly decreasing health-related quality of life.4 Furthermore, the prevalence of ED is 95% in patients with diabetes above the age of 60.5

Introduction of phosphodiesterase type 5 (PDE5) inhibitors revolutionized the treatment of ED. PDE5 inhibitors enhance nitric oxide (NO)-mediated relaxation of the corpus cavernosum by preventing cyclic guanosine monophosphate (cGMP) degradation. Currently, PDE5 inhibitors are the first-line therapy for ED.3,6 However, because ED is caused by multiple factors, a certain proportion of patients, especially those with diabetes, exhibit a poor response to PDE5 inhibitors.7 Moreover, the failure rate for all PDE5 inhibitors is higher in men with diabetes than in men without.8 In addition, diabetes could induce a series of pathophysiologic changes that could contribute to the decreased response to PDE5 inhibitors. Therefore, it is urgent to develop novel treatment modalities for men with diabetic ED (DED).

Taurine (2-aminoethanesulfonic acid), a sulfur-containing amino acid, is one of the most abundant free amino acids in the
human body. It plays a role in several physiologic functions, including neuromodulation or neurotransmission, modulation of calcium flow, osmoregulation, stabilization of membranes, and bile formation in the liver. Data from existing studies have indicated that taurine might decrease cholesterol level, control high blood pressure, and have protective potential in the cardiovascular system. Previous studies also have shown a beneficial effect of taurine on diabetes-associated complications. Importantly, it has been demonstrated that taurine supplementation can enhance sexual response and mating ability in aged rats. However, whether taurine could mitigate DED has not been investigated.

In the present study, we evaluated the effect of taurine in rats with DED in vivo. Our results show that taurine improves erectile function in diabetic rats and could serve as an alternative therapy for ED.

METHODS

Treatment of Animals

This study was approved by the Animal Care and Use Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China. Forty-five 8-week-old male Sprague-Dawley rats were used in the present study. After overnight fasting, the rats were injected with freshly prepared streptozotocin (60 mg/kg; Sigma-Aldrich, St Louis, MO, USA; n = 37) or vehicle (0.1 mol/L citrate-phosphate buffer; pH, 4.2; n = 8; nondiabetic control group) intraperitoneally. Blood glucose levels were measured 72 hours after streptozotocin injection using a blood glucose meter (ACCU-CHEK Performa; Roche Diagnostics, Shanghai, China). Only rats with a fasting glucose concentration ≥16.7 mmol/L were considered as diabetic rats (n = 35).

At 12 weeks, 30 diabetic rats survived. An apomorphine (APO) test was conducted to confirm DED. Eighteen APO-negative rats were used for further experiments. Rats with DED were administered taurine (400 mg/kg; Sigma-Aldrich; DED + taurine group) or vehicle (saline, DED group) intraperitoneally daily for another 4 weeks (n = 9 per group). This dose of taurine is within the range used in previous studies.

In Vivo Erection Studies

At 12 weeks after diabetes was induced, erectile function was evaluated by observing APO-induced erection in all surviving rats. APO was injected into the loose skin of the cervical vertebra subcutaneously (80 µg/kg). Then a camera was set in the bottom of the cage for 30 minutes, and the number of erections was recorded. The criteria for penile erection were based on previous studies as follows: stretching of the penis with a congested glans, repeated pelvic thrusts immediately followed by an upright position, and complete emergence of an engorged glans penis and distal penile shaft; otherwise, the result was negative.

At week 16, following a 2-day washout of taurine, intracavernosal pressure (ICP) was measured in all groups as described previously. Under proper anesthesia (pentobarbital sodium, 40 mg/kg, i.p.), the rats were fixed on the table, and the left carotid artery was exposed. A PE-50 tube filled with heparinized saline (100 IU/mL) was cannulated into the artery and connected to a pressure transducer to monitor mean arterial pressure (MAP) continuously. A 25-G needle was inserted into the left penile crura to monitor ICP. Pressure data were recorded by a data acquisition system (PowerLab/4SP; AD Instruments, Bella Vista, Australia). Erectile response was elicited by electrical stimulation using a bipolar electrode hooked to the cavernous nerve. Stimulation parameters were as follows: 7.5 v, 12 Hz, and 1.2 ms; this was judged to cause a maximal reaction. The ratio between maximum ICP and MAP (ICP max/MAP) was calculated to normalize for variations in systemic blood pressure. Total ICP was determined by the area under the curve (AUC, mmHg · s) during stimulation.

After finishing these in vivo studies, the rats were sacrificed by intraperitoneal overdose of pentobarbital. Penile midshafts were maintained in 4% paraformaldehyde overnight and embedded in paraffin for subsequent histologic studies. Remaining penile tissues were snap frozen in liquid nitrogen and stored at −80°C for subsequent molecular detection. Blood samples were obtained from rats via the vena cava. The total testosterone levels were measured in the central laboratory of our institution.

Western Blot Analysis

Equal amounts (40 µg/lane) of protein extracts were electrophoresed on 10% sodium dodecyl sulfate—polyacrylamide gels, transferred to polyvinylidene fluoride membranes (Immobilon-P Transfer Membrane; Millipore Corporation, Billerica, MA, USA), and probed with antibodies against endothelial nitric oxide synthase (eNOS; 1:500; BD Biosciences, San Jose, CA, USA), plasminogen activator inhibitor type 1 (PAI-1; 1:2500; BD Biosciences), collagen I (1:500; Boster, Wuhan, China), collagen IV (1:500; Proteintech, Wuhan, China), transforming growth factor β1 (TGF-β1; 1:500; Abcam, Cambridge, UK), α-smooth muscle actin (α-SMA; 1:1000; Abcam), or β-actin (1:500; Boster). Primary antibodies were incubated overnight at 4°C followed by incubation with horseradish peroxidase-labeled secondary antibody (1:10,000; Jackson ImmunoResearch, West Grove, PA, USA), then detected with Bio-Rad Clarity Western ECL Substrate (Bio-Rad Laboratories, CA, USA). The resulting image was analyzed using ImageJ software (http://rsb.info.nih.gov/ij) to determine the integrated density value of each protein band.

cGMP Measurement

cGMP was measured in extracts from penile samples using a commercial cGMP enzyme immunoassay kit (Cayman Chemicals Inc, Ann Arbor, MI, USA) following the manufacturer’s instructions. Protein content in each sample was measured using the BCA assay and expressed as range of pmol cGMP/mg protein. All samples were analyzed in triplicate.

Histologic Assessment

Penile tissue sections (5 µm thickness) were processed for immunohistochemistry as described previously. The primary
antibody was mouse anti-α-SMA (1:150; Boster). Masson’s trichrome staining was performed according to the standard protocol. Semiquantitative analysis was performed to evaluate intensity using Image-Pro plus software (Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

Results were analyzed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) and expressed as mean ± standard error of the mean. Statistical analyses were performed using 1-way analysis of variance followed by the Tukey-Kramer test for post hoc comparisons. Differences among groups were considered significant at \( P < .05 \).

RESULTS

Metabolic Variables and ICP/MAP Assessment

There were no significant differences in initial body weight or serum glucose concentration among the 3 groups. At 16 weeks after diabetes was induced, fasting glucose concentration was significantly higher, and body weight along with total testosterone levels were significantly lower in the diabetic rats than in the age-matched normal controls. No significant differences in body weight or glucose concentration were found between the DED and DED + taurine groups (Table 1).

Erectile function was evaluated by measuring maximum ICP, ICP max/MAP, and total ICP. All erectile function variables were significantly lower in the diabetic rats than in the control rats (Figure 1). Partial but significant recovery of erectile function was seen in the DED + taurine group. MAP did not differ significantly among the groups (Table 1, Figure 1).

Extracellular Matrix Protein Production

We performed Western blot analysis to evaluate production of extracellular matrix (ECM) proteins, including PAI-1, collagen I, and collagen IV. Cavernous ECM protein production was significantly higher in the DED group than in the normal controls (\( P < .05 \)); however, taurine reversed this change (Figure 2).

We also performed Western blot analysis to evaluate expression of α-SMA and TGF-β1 in each group. The diabetic rats showed lower α-SMA expression than the normal controls. Expression of α-SMA was higher in the DED + taurine group than in the DED group (all \( P < .05 \); Figures 4B and 4E). Meanwhile, expression of TGF-β1 was higher in the DED group than in the normal controls; however, taurine partially reversed its upregulation.

\( \text{Table 1. Metabolic and physiological variables} \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control(^a) (n = 8)</th>
<th>DED(^b) (n = 9)</th>
<th>DED + taurine(^a) (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>240.90 ± 11.47</td>
<td>233.10 ± 11.09</td>
<td>233.00 ± 12.06</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>522.90 ± 60.31</td>
<td>243.00 ± 42.66(^a)</td>
<td>257.50 ± 33.35(^a)</td>
</tr>
<tr>
<td>Initial fasting glucose (mmol/L)</td>
<td>6.25 ± 0.41</td>
<td>6.12 ± 0.44</td>
<td>6.11 ± 0.48</td>
</tr>
<tr>
<td>Final fasting glucose (mmol/L)</td>
<td>6.23 ± 0.47</td>
<td>30.54 ± 2.48(^a)</td>
<td>30.86 ± 2.11(^a)</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>2.55 ± 0.61</td>
<td>0.73 ± 0.05(^*)</td>
<td>1.60 ± 0.25(^+)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>112.50 ± 9.73</td>
<td>114.10 ± 10.30</td>
<td>115.80 ± 10.56</td>
</tr>
<tr>
<td>ICP (mm Hg)</td>
<td>97.58 ± 9.04</td>
<td>43.28 ± 12.10(^+)</td>
<td>71.99 ± 16.08(^b)</td>
</tr>
<tr>
<td>AUC (mm Hg · s)</td>
<td>5125 ± 723.6</td>
<td>1504 ± 505.2(^+)</td>
<td>3687 ± 1308(^*)</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; ICP = intracavernous pressure; AUC = area under the curve.

\(^a\) Data are presented as mean value ± standard deviation.

\(^*\) \( P < .05 \) compared with the control group.

\(^+\) \( P < .05 \) compared with the DED + taurine group.

DISCUSSION

In this study, we demonstrated that administration of taurine, not as a food supplement, in rats with streptozotocin-induced diabetes significantly decreased penile fibrosis; significantly
decreased production of ECM proteins, including PAI-1, collagen I, and collagen IV; and upregulated eNOS expression; and that these changes were associated with physiologically relevant changes in erectile function.

Taurine is known to play a role in a variety of cellular processes, including calcium regulation, inflammation, osmoregulation, oxidative stress, and cysteine detoxification, all of which may contribute to cardiovascular homeostasis. Taurine has become a potential therapeutic target for cardiovascular disease, preventing stroke, atherosclerosis, and hypertension.22 Maia et al reported that taurine supplementation was beneficial to control high blood pressure and vascular remodeling induced via postweaning protein restriction.16 Besides, the protective effect of taurine on endothelial function in diabetic rats already has been proved.14 As a benchmark

**Figure 1.** Increase of erectile function in taurine-treated diabetic rats. (A) Representative tracing of intracavernosal pressure (ICP) and mean arterial pressure (MAP) for age-matched normal control, diabetic erectile dysfunction (DED) and taurine-treated DED groups. (B, C) Erectile function presented as maximum ICP/MAP and total ICP (AUC) in each group. *P < .05 compared with the control group. †P < .05 compared with the DED + Taurine group.

**Figure 2.** Taurine reduces cavernous extracellular matrix protein deposition. (A) Representative Western blot for plasminogen activator inhibitor type1 (PAI-1, 47 kDa), transforming growth factor beta 1 (TGF-β1, 37 kDa), collagen I (139 kDa), and collagen IV (161 kDa) in the control group, DED group and DED rats treated with taurine group. (B) Data are presented as the relative density of each protein compared with that of β-actin. *P < .05 compared with the control group. †P < .05 compared with the DED + Taurine group.
for cardiovascular disease, erectile function also has been inves-
tigated. A previous ex vivo study indicated that chronic treatment 
with taurine could prevent development of corpus cavernosum 
dysfunction after induction of diabetes. Dalaklioglu et al found 
that the protective effect of taurine might be associated with sup-
pression of nicotinamide adenine dinucleotide phosphate oxidase 
and Rho-kinase activity. However, the NO/cGMP pathway, 
which is the most classic pathway in the erectile process, has not 
been discussed. Our results suggest that taurine may partially 
restore endothelial function via upregulation of the eNOS/cGMP 
pathway. Our present work reproduced previous 
findings demonstrating improved NO production and/or bioavailability, 
and extended that knowledge by suggesting an anti-
hibrosis effect of 
this amino acid in the penis.

Fibrosis is involved in most diabetic complications, such as 
cardiomyopathy and nephropathy. Corpopal fibrosis also has 
emerged as the predominant underlying cause of DED, especially 
in poor responders to PDE5 inhibitors. Although it differs in or-
gans and cells, fibrosis shares some common characteristics. In 
patients with diabetes, hyperglycemic conditions may serve as the 
initial insult throughout the corpora and penile arteries, which is 
typically followed by an inflammatory response. Profibrotic 
Factors are subsequently released. It has been demonstrated in 
animal models that diabetes leads to increased expression of TGF-
β1 and penile fibrosis. These studies suggest that upregula-
tion of TGF-β1 and its downstream effectors might play a pivotal 
role in diabetes-induced structural changes. In line with these 
previous studies, our work suggests that in diabetic rats, TGF-β1 
and PAI-1 are both upregulated. It is well documented that TGF-
β1 has multiple biologic functions, including cell proliferation, 
differentiation, apoptosis, autophagy, and ECM protein produc-
tion. The role of TGF-β1 in penile fibrosis has been confirmed 
by measuring mRNA expression in penile tissues of young and 
aging rats. TGF-β1 expression was higher in older rats than in 
young rats. In diabetes models, accumulation of myofibroblasts 
and the switch to a synthetic phenotype producing ECM proteins 
due to TGF-β1 activation have been shown to exacerbate penile 
fibrosis. As such, collagen and ECM proteins accumulate in the 
penis, accompanied by loss of functional cells. PAI-1, the most 
potent cellular inhibitor of the urokinase-type plasminogen 
activator/tissue-type plasminogen activator/plasmin fibrinolytic 
system, plays a prominent role in wound healing. However, under 
certain circumstances (eg, diabetes), excessive PAI-1 expression 
contributes to induced fibrogenesis by suppressing cellular proteo-
lytic activity and increasing ECM protein stability. Taken 
together, elevated TGF-β1 and PAI-1 expression promotes excess-
egive deposition of collagen and ECM proteins in cavernous tissue. 
However, both in vivo and in vitro studies have demonstrated that 
taurine can inhibit expression of TGF-β1. In addition, Lee et al reported that taurine significantly reduced PAI-1 expression in rats with streptozotocin-induced diabetes at the mRNA and protein 
level. Here, taurine administration reversed the elevated expres-
sion of TGF-β1 and PAI-1. As a result, collagens and ECM pro-
teins, including collagen I and collagen IV, were reduced.
Cavernous smooth muscle cells compose the predominant structure of erectile tissue and are responsible for blood flow control into the corpus cavernosum. In patients with DED, loss of smooth muscle cells further aggravates penile fibrosis. Increased apoptosis and reduced α-SMA expression qualifies this view. Moreover, insulted smooth muscle cells may serve as fibrogenic effector cells, which in return, produce a variety of mediators, cytokines, and other factors involved in inflammation. Preservation of taurine for smooth muscle cells can break this vicious circle. In summary, taurine, as an anti-fibrosis agent, not only reduced ECM protein deposition but also protected functional cells from apoptosis.

As DED is closely related to hypogonadism, we determined plasma testosterone levels. In line with the results of previous studies, DED rats had a marked decrease in plasma testosterone levels. Moreover, taurine affects hormonal parameters in aged rats. In the present study, the levels of testosterone were increased in DED rats treated with taurine but were still lower compared to those of normal controls. Accordingly, the increase in plasma testosterone levels might have an indirect effect on erectile function.

Our previous work (unpublished, doctorate dissertation of Dr Jun Yang) noted that APO-positive rats had only mild to moderate ED, whereas APO-negative rats’ sexual function was impaired severely and often had a poorer response to PDE5 inhibitors. In this study, we used an APO-negative model to mimic severe ED. Our results indicate that taurine could at least partially ameliorate ED in APO-negative rats. This finding provides evidence for a potential novel therapy for severe ED. Future studies are needed to validate the combination of PDE5 inhibitors and taurine, which could improve the response rate in nonresponders to PDE5 inhibitors.

A major limitation of the current research is that our streptozotocin-induced diabetes model reflects type 1 diabetes but may not necessarily represent the pathologic processes that occur in the more common type of diabetes. Furthermore, we did not thoroughly investigate the mechanisms involved in the benefits of taurine for DED. Therefore, future studies should examine the effect of taurine in vitro and determine the correlation with erectile function and penile fibrosis during the course of diabetes.

CONCLUSION

Data from the present study suggest that taurine supplementation may improve erectile function in rats with DED and ameliorate penile fibrosis as well as endothelial dysfunction. This

Figure 4. Semiquantitative analyses of smooth muscle and collagen ratio and α-smooth muscle actin (α-SMA) expression in all groups. (A) Masson trichrome and immunohistochemical staining with antibody to α-SMA (magnification ×100). (B) Representative Western blot analysis of α-SMA expression. (C) Data are expressed as a ratio of smooth muscle to collagen. (D) Quantitative analysis of α-SMA-positive area in cavernous tissue was performed with an image analyzer. (E) Data are presented as the relative density values of α-SMA to β-actin loading control. The relative ratio measures in the control group is arbitrarily presented as 1. *P < .05 compared with the control group. †P < .05 compared with the DED + Taurine group. Figure 4 is available in color at www.jsm.jsexmed.org.
finding provides evidence that taurine supplementation may be an alternative therapy for nonresponders to PDE5 inhibitors.

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REFERENCES


SUPPLEMENTARY DATA

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