

Full-length article

Effects of coffee and caffeine on bladder dysfunction in streptozotocin-induced diabetic rats¹Chao-ran YI^{2,5}, Zhong-qing WEI², Xiang-lei DENG⁴, Ze-yu SUN², Xing-rong LI⁴, Cheng-gong TIAN³²Department of Urology, ³Department of Endocrinology, the Affiliated Drum Tower Hospital, Nanjing University Medical School, Nanjing 210008, China; ⁴Department of Biochemistry and Molecular Biology, Medical College of Southeast University, Nanjing 210009, China**Key words**

caffeine; diabetic mellitus; bladder; urodynamics; muscle contraction; cyclic AMP

¹ Project supported by Nanjing Science and Medical Research Council Grant (No YKK0443).⁵ Correspondence to Dr Chao-ran YI.
Phn 86-25-8310-5518.
E-mail cryi7667@yahoo.com.cnReceived 2005-11-23
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Abstract

Aim: To explore the effects and mechanisms of caffeine and coffee on bladder dysfunction in streptozotocin-induced diabetic rats. **Methods:** Sprague-Dawley male rats were divided randomly into 4 groups: control, diabetes mellitus (DM), DM with coffee treatment, and DM with caffeine treatment. The diabetic rat was induced by intraperitoneal injection of streptozotocin (60 mg/kg). After 7 weeks of treatment with coffee and caffeine, cystometrograms, contractile responses to electrical field stimulation (EFS) and acetylcholine (ACh), and cyclic AMP (cAMP) concentration of the bladder body and base were measured. **Results:** The bladder weight, volume threshold for micturition and post-void residual volume (PVR) in the diabetic rats were significantly higher compared to those in the control animals. Coffee or caffeine treatment significantly reduced the bladder weight, bladder capacity and PVR in the diabetic rats. DM caused significant decreases in cAMP concentration of the bladder and coffee and caffeine caused upregulation of cAMP content in the diabetic bladder. In addition, coffee and caffeine tended to normalize the altered detrusor contractile responses to EFS and ACh in the diabetic rats. **Conclusion:** These results indicate that caffeine and coffee may have beneficial effects on bladder dysfunction in the early stage of diabetes by increasing cAMP content in the lower urinary tract, recovering the micturition reflex and improving the detrusor contractility.

Introduction

Urinary bladder dysfunction is a recognized complication of diabetes mellitus (DM) and has been attributed mostly to peripheral autonomic neuropathy. Functional alternations in autonomic neurotransmission, especially signal transduction pathways, have been reported in DM for adrenergic, cholinergic and non-adrenergic, non-cholinergic (NANC) nerves in the bladder of experimental animals^[1]. Based on urodynamic findings, the prevalence rate of DM-associated bladder dysfunction ranges from 40% to 100%^[1,2]. Diabetic cystopathy is characterized by impaired sensation of bladder fullness, increased bladder capacity, reduced bladder contractility and increased residual urine. However, the onset of diabetic cystopathy is usually insidious and develops silently in the early stage of DM, and these classic symptoms are not always observed in patients with DM. If it

continues, diabetic cystopathy eventually represents an atonic bladder with chronic urinary retention, urinary tract infection, vesicoureteral reflux and uremia in the advanced stage, which leads to a poor prognosis for the patient. Therefore, prevention and treatment in the early stage of DM is a key step to protect patients from renal failure and improve living standards.

Recently, several large epidemiologic investigations in different countries revealed that the consumption of coffee, especially caffeinated coffee, could lower the risk of type 2 DM^[3–5]. Although the exact mechanisms have not been recognized by researchers, they suggest that a preventive effect against diabetes may be related to the long-term effects of caffeine on glucose metabolism^[5]. There are less epidemiologic studies about the effect of caffeine or coffee on the risk of type 1 diabetes; however, several articles have recently reported that caffeine can be of benefit in prevent-

ing type 1 diabetic patients from getting severe nocturnal hypoglycemia^[6]. Caffeine may also promote insulin secretion in pancreatic islet β cells by activating Ca^{2+} release^[7]. Therefore, the effect of caffeine or coffee will most likely be of advantage to type 1 diabetes.

Caffeine is probably the most widely used drug as a component of commonly consumed beverages such as coffee, tea and cola. It is well established that caffeine is the stimulant of the central nervous system and can influence the activities of sensory and motor neurons in various kinds of tissues. Moreover, by increasing intracellular cAMP concentration through phosphodiesterases (PDE) inhibition, caffeine can activate and promote many kinds of signal transduction pathways *in vivo*. In addition, previous clinical and experimental studies confirmed that, as an important antagonist of the adenosine receptor, caffeine can reduce the incidence of Parkinson's disease through neuroprotection^[8].

We infer from this evidence that coffee and caffeine might have potential effects against diabetic cystopathy. Therefore, the intention of the present study is to investigate the influences of coffee and caffeine on bladder dysfunction in streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Animal models Sprague-Dawley male rats weighing 200–240 g ($n=46$) were fasted for 18 h. Diabetes was randomly induced in 36 rats with a single intraperitoneal injection of STZ 60 mg/kg (Sigma, St Louis, USA) dissolved in ice-cold 0.1 mol/L citrate-phosphate buffer (pH 4.2). Another 10 rats as the control group received the same volume of the vehicle. After 48 h, 30 rats were accepted as diabetic by measuring the fasting serum glucose concentration which was more than 12 mmol/L and were divided randomly into 3 groups of 10 animals each: diabetes mellitus (DM), DM with coffee treatment (DM/coffee) and DM with caffeine treatment (DM/caffeine) group. Animal care was in accordance with the guidelines of Jiangsu Province Animal Research Advisory Committee.

Treatment of animals Caffeine (obtained from Sigma, USA) was dissolved in 2 mL distilled water and administered orally ($10 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, *po*) to diabetic rats of the DM/caffeine group for 7 weeks, beginning on the second day after the establishment of diabetes. Caffeine concentration of instant coffee (obtained from Nescafe Products, Dongwan, China) was around 35 mg/g. According to this concentration, diabetic rats of the DM/coffee group received corresponding coffee treatment ($286 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, *po*) for 7 weeks by the same method. The remaining two groups were given orally the

same volume of distilled water every day. All experimental rats received free access to food and water.

Cystometrogram After 7-weeks of treatment, every experimental rat was anesthetized with urethane (1.0 g/kg , *sc*)^[9]. The lower abdomen of the rat was opened and the bladder exposed. The cystometrogram was performed using two 24 G catheters inserted into the apex of the bladder dome for the purpose of recording bladder pressure and filling the bladder with physiological saline, respectively. One catheter was connected to an infusion pump (HL-2, Shanghai Xinbo Company, China) in order that external bladder filling was carried out at the constant rate of 0.6 mL/min until micturition was observed. The other catheter was connected to an external pressure transducer (YP100, Hebei Xinhang Device Company, China). Intravesical pressure was recorded by a portable computer (Toshiba A10, Shanghai, China) via a multiport controller (MedLab-U/4C, MedEase Company, Nanjing, China). During voiding, a 5-mL graduate was placed carefully at the meatus to measure the voided volume. To establish a reliable baseline, a period of at least 15 min was allowed to elapse after surgery before measurements were taken and cystometrograms were repeated three times in each rat. The following urodynamic parameters were compared among the groups: volume threshold for eliciting micturition, maximum intravesical pressure (P_v , max), voided volume, post-void residual volume (PVR) and V% ($100\times\text{PVR}/\text{bladder capacity}$).

Tissue preparation and contractile studies After the cystometrogram was completed, the bladder was removed from each rat, weighed and placed in modified Krebs-Henseleit solution of the following composition: NaCl 118 mmol/L, KCl 4.7 mmol/L, CaCl_2 2.5 mmol/L, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ 1.2 mmol/L, NaHCO_3 25 mmol/L, KH_2PO_4 1.2 mmol/L, glucose 11 mmol/L, pH 7.4. A full-thickness longitudinal strip (2 mm \times 8 mm) was cut from the posterior bladder body wall above the ureterovesical junction and used for contractile studies in an organ bath. The remaining part of the bladder body and base was prepared for biochemical analysis. The longitudinal strip was suspended on a 3-zero suture between a pair of platinum ring electrodes and placed in a 30 mL organ bath filled with modified Krebs-Henseleit solution. The suture was connected to a force displacement transducer (JZ100, Hebei Xinhang Device Company, China) and resting tension was adjusted to 0.8 g which was previously determined as the optimum resting tension for isometric contraction. Responses were also recorded on a portable computer via the MedLab-U/4C controller. The longitudinal strip was given a 40 min equilibration period, during which the strip was washed with modified Krebs-Henseleit solution twice

and resting tension was maintained at 0.8 g. After this period, electrical field stimulation (EFS) was carried out. Frequency-response curves were elicited by EFS every 4 min for 10 s with 80 V and 1-ms duration using a stimulator (ST101, MedEase Company, Nanjing, China). Following field stimulation, the longitudinal strip was again given a 20-min equilibration period with fresh Krebs-Henseleit solution and resting tension remained 0.8 g. Subsequently, acetylcholine chloride (ACh; obtained from Sigma, St Louis, USA) stimulation was also carried out. Dose-response curves to ACh (1×10^{-7} – 1×10^{-3} mol/L) were obtained using noncumulative addition. Each concentration of ACh was left in touch with the strip for 1 min, followed by 2 washes with fresh Krebs-Henseleit solution, and resting tension was adjusted to 0.8 g over 5 min.

cAMP measurement The bladder body (50 mg) and the weighed bladder base were placed respectively in 2-mL ice-cold acetate buffer (50 mmol/L), pH 4.75, containing EDTA 4 mmol/L, NaAC 0.03 mol/L, and HAC 0.02 mol/L. The tissue was minced and homogenized with a homogenizer then the suspension was placed in a 10 mL centrifugal tube and centrifuged (3500×g, 15 min) followed by the supernatant, which was carefully poured into a 5 mL test tube without contamination. Subsequently, the homogenizer was washed with 2 mL alcohol (75%), which was poured into the same centrifugal tube, mixed with the precipitate and centrifuged (3500×g, 15 min) again. After that, the second supernatant was also poured into the same test tube for measuring cAMP concentration. cAMP concentration was quantified in the supernatant with a radioimmunoassay (γ radioimmunoassay meter, ZhongJia Company, Hefei, China) and expressed as picomoles of cAMP accumulated (increase over basal levels) using a standard curve run in parallel.

Statistical analysis

All results are expressed as the mean±SEM and compared by one way ANOVA, followed by SNK-*q* test between each two groups. *P*<0.05 was taken as statistically significant.

Results

Body weight, serum glucose, and bladder weight After 7-week of treatment all diabetic rats showed marked hyperglycemia and decreased body weight compared to the control rats. The bladder weight of diabetic rats was significantly greater than that of controls. Coffee and caffeine treatment did not have any effect on serum glucose levels and body weight during the whole procedure, but they significantly lowered the bladder weight of diabetic rats compared to that

of the untreated group. There was no significant difference between coffee-treated diabetic rats and caffeine-treated diabetic rats (Table 1).

Table 1. General characteristics of control, diabetic, coffee-treated and caffeine-treated diabetic rats. *n*=10. Mean±SEM. ^c*P*<0.01 vs control group. ^e*P*<0.05 vs DM group.

	Body weight (g)	Serum glucose (mmol/L) [before treatment]	Serum glucose (mmol/L) [after treatment]	Bladder weight (mg)
Control	351.7±16.4	3.9±0.4	4.25±0.27	191.5±15.8
DM	216.2±14.5 ^c	16.1±2.5 ^c	16.0±2.7 ^c	345.7±19.1 ^c
DM/coffee	218.2±11.6 ^c	16.8±2.2 ^c	16.8±2.9 ^c	277.9±15.1 ^{ce}
DM/caffeine	216.8±13.6 ^c	17.0±2.5 ^c	16.9±2.3 ^c	281.6±16.5 ^{ce}

Cystometrogram The volume threshold for micturition, PVR, and V% was significantly increased in the diabetic rats compared to those of control rats. Coffee and caffeine significantly decreased the volume threshold for micturition and reduced residual urine and V% in the diabetic rats. There was no significant difference in maximum intravesical pressure between the four groups (Figure 1, Table 2).

Contractile responses to EFS Peak force in response to EFS increased in the bladder strips of the four groups as the frequency of stimulation increased. Although the bladder strips from the diabetic rats produced more force in absolute terms than the same size strips from the control rats, the diabetic bladder strips (per 100 mg) actually produced less force at the different frequencies in terms of each strip weight than the control strips. The frequency-response curve was significantly shifted downward in the bladder strips of DM group. Coffee or caffeine treatment significantly potentiated the neurogenic contraction in the diabetic bladder (*P*<0.05, Figure 2).

Contractile responses to ACh Peak force in response to ACh increased in the bladder strips of four groups as the concentration of ACh increased. The bladder strips from the diabetic rats produced more force than the same size strips from the controls, even in terms of each strip weight [Control group: E_{max} 23.55 g, ED_{50} 3.092×10^{-5} mol/L (95% CI 2.751×10^{-7} to 3.477×10^{-3}); DM group: E_{max} 28.51 g, ED_{50} 1.693×10^{-5} mol/L (95% CI 1.320×10^{-5} to 2.172×10^{-5})]. The sigmoidal concentration-response curve was significantly shifted upward in the bladder strips of the DM group. Coffee and caffeine lowered contractile force mildly in the diabetic bladder strips [DM/

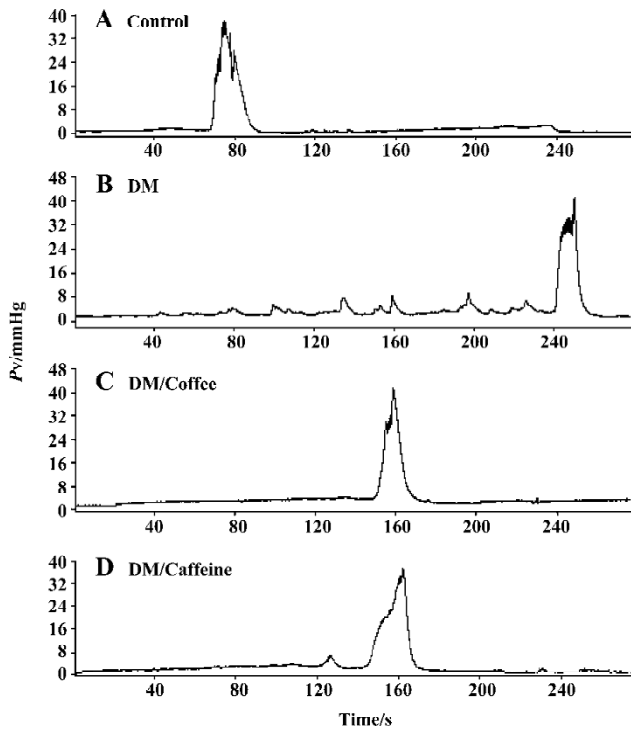


Figure 1. Typical recordings of cystometrogram of control (A), DM (B), DM/Coffee (C), and DM/Caffeine (D) groups. The diabetic rats showed a significant delay of the volume threshold for micturition and some unstable spontaneous contraction before micturition compared to the other rats.

coffee group: E_{max} 25.00 g, ED_{50} 1.830×10^{-5} mol/L (95% CI 4.294×10^{-6} to 7.795×10^{-5}); DM/cafeine group: E_{max} 25.92 g, ED_{50} 2.140×10^{-5} mol/L (95% CI 1.006×10^{-5} to 4.552×10^{-5}) ($P < 0.05$, Figure 3).

cAMP concentration of the bladder The concentration of cAMP in the diabetic bladder base and body was much lower than that of the controls ($P < 0.01$). Coffee or caffeine treatment significantly caused upregulation of the cAMP content in the bladder body and base compared with dia-

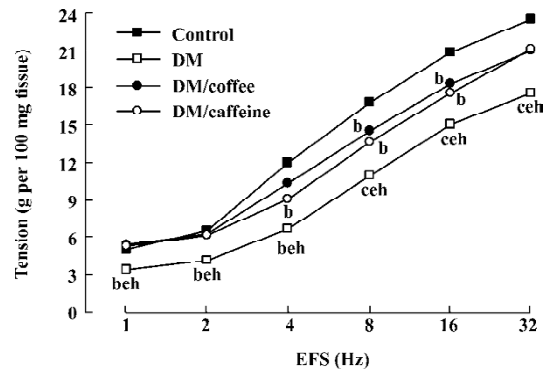


Figure 2. Frequency-response relationships of bladder body strips of control, diabetic, coffee-treated and caffeine-treated diabetic rats. ^b $P < 0.05$, ^c $P < 0.01$ vs control group. ^e $P < 0.05$ vs DM/coffee group. ^h $P < 0.05$ vs DM/cafeine group. EFS, electrical field stimulation.

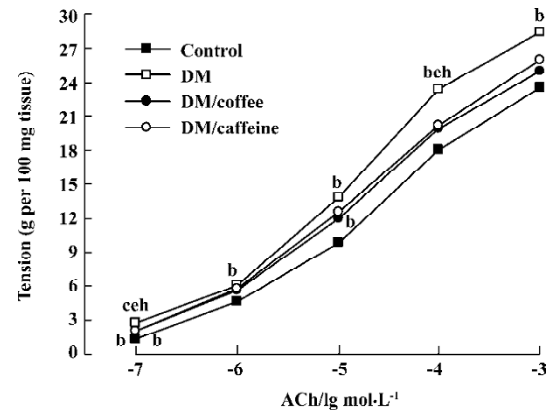


Figure 3. Concentration-response relationships for bladder body strips from control, diabetic, coffee-treated and caffeine-treated diabetic rats. ^b $P < 0.05$, ^c $P < 0.01$ vs control group. ^e $P < 0.05$ vs DM/coffee group. ^h $P < 0.05$ vs DM/cafeine group.

betic rats ($P < 0.05$). However, there was still a significant difference between them and that of the controls ($P < 0.05$, Table 3).

Table 2. Comparison of the urodynamic parameters among the groups. $n=10$. Mean \pm SEM. ^b $P < 0.05$, ^c $P < 0.01$ vs control group. ^e $P < 0.05$ vs DM group.

	Volume threshold for micturition (mL)	Voided volume (mL)	PVR (mL)	V%	P_v , max (mmHg)
Control	0.77 \pm 0.17	0.65 \pm 0.09	0.12 \pm 0.04	15.58 \pm 1.64	39.96 \pm 5.21
DM	2.09 \pm 0.37 ^c	0.84 \pm 0.11	1.25 \pm 0.16 ^c	59.81 \pm 11.42 ^c	39.88 \pm 6.62
DM/coffee	1.68 \pm 0.26 ^{be}	1.25 \pm 0.24 ^b	0.43 \pm 0.07 ^e	25.60 \pm 6.91 ^e	38.46 \pm 6.07
DM/cafeine	1.64 \pm 0.22 ^{be}	1.18 \pm 0.22 ^b	0.46 \pm 0.09 ^e	28.04 \pm 5.51 ^e	40.46 \pm 5.77

PVR, post-void residual volume; V%, 100 \times PVR/bladder capacity; P_v , max, maximum intravesical pressure.

Table 3. cAMP concentration in the bladder body and base in different groups. *n*=10. Mean±SEM. ^b*P*<0.05, ^c*P*<0.01 vs control group. ^e*P*<0.05 vs DM group.

	cAMP concentration of the bladder body (pmol/mg)	cAMP concentration of the bladder base (pmol/mg)
Control	0.67±0.05	0.44±0.02
DM	0.31±0.05 ^c	0.18±0.03 ^c
DM/coffee	0.48±0.04 ^{bc}	0.36±0.06 ^{bc}
DM/caffeine	0.51±0.06 ^{bc}	0.34±0.03 ^{bc}

Discussion

Caffeine is a potent pharmacological and psychotropic agent that is metabolized by demethylation and oxidation *in vivo*. Potential effects of caffeine at the cellular level are mediated by three main mechanisms of action which include: (i) intracellular mobilization of calcium from sarcoplasmic reticulum (SR) and increased sensitivity of myofibrils to calcium; (ii) inhibition of PDE leading to increases in cAMP and cGMP in various tissues including muscle; and (iii) antagonism at the level of adenosine receptors, mainly in the central nervous system^[10]. Due to such pharmacological actions, caffeine can influence activities of cells as well as functions of various tissues and organs, which in turn lead to physiological and pathologic changes in either humans or animals, in terms of different doses. Data from Australia suggest that the average intake in adults is approximately 232 mg/person per day from all sources in Australia, which is similar to the USA^[11,12]. This average dose equates to approximately 3.5 mg/kg for a 70 kg human (approximately 10 mg/kg in a rat due to a high metabolic rate)^[11]. Australian experts defined that low, moderate and high doses of caffeine referred to daily intakes in the range of 80–250 mg/day (1.1–3.5 mg/kg per day), 300–400 mg/day (4–6 mg/kg per day) and over 500 mg/day (7 mg/kg per day) in 70 kg adults^[11]. However, most clinical and animal experiments showed that consumption of caffeine at low to moderate doses on the long run was much safer and more healthy than consumption of caffeine at high doses because of its toxicological effects. So we applied the upper limit of low dose caffeine, which was equal to the average intake in adults in most western countries to the diabetic rats (approximately 10 mg/kg in rat) in the present study in order to provide a safe and reasonable investigation. In our study, we focused on the diabetic complication regardless of diabetic rats types. The fact is that most researchers in the field adopt the STZ-

induced diabetic rat model to study diabetic complications since it has been proven to be best model available.

The two functions of the urinary bladder are to store and subsequently expel urine. During the storage phase, the bladder detrusors distend appropriately with the increasing urine at lower intravesical pressure, meanwhile urethral sphincters close continuously. In contrast, in micturition the detrusors rapidly contract to empty urine at high intravesical pressure together with the relaxation of urethrae. Owing to neurogenic and myogenic lesions, DM influenced the functions of the bladder and urethra, which made the patients suffer from manifold vesicoureteral dysfunctions, such as urinary urgency, frequent urination, urinary incontinence, dysuria and urinary retention. Many similar changes were observed in the STZ-induced diabetic rat^[1,9]. More evidence confirmed that functional alternations in autonomic neurotransmission, especially signal transduction pathways, contributed mostly to urinary bladder dysfunction in humans, rats and rabbits^[1,13,14].

cAMP is an important intracellular second messenger that is synthesized from its corresponding nucleoside triphosphate by adenylyl cyclase and is degraded by PDE, which plays a critical role in modulating the levels of cyclic nucleotides and their duration of action inside the cell via their ability to hydrolyze these compounds^[1,13,14]. The action of many hormones and neurotransmitters involved in the regulation of smooth muscle tone is mediated through specific receptors coupled to these enzymes. Currently, cAMP and cGMP are recognized as secondary messengers mediating detrusor and urethral smooth muscle relaxation respectively^[1]. Previous studies showed that the production of cAMP was reduced in the bladder of the diabetic rabbit and the formation of cGMP also occurred in the urethra^[1]. The results of the present study demonstrated that cAMP concentration in the bladder body and base of diabetic rats was significantly lowered compared to that of age-matched controls. Impaired cAMP formation in the bladder body and base may alter bladder compliance and detrusor contractility as well as impair bladder outlet relaxation leading to inadequate bladder emptying and residual urine^[1], which is recognized as an important measurement of bladder function. This was supported by our data in which the volume threshold for micturition, PVR and V% significantly increased in diabetic rats compared to those of control rats. Caffeine is an important inhibitor of PDE and may result in upregulation of cAMP and cGMP content in various tissues. The present study demonstrated that coffee or caffeine treatment significantly increased cAMP concentration of the bladder body and base in diabetic rats. In addition, these two compounds signifi-

cantly improved the delay of the volume threshold for micturition and decreased PVR and V% in diabetic rats during cystometrogram, which may represent the recovery of the micturition reflex^[9]. Mumtaz *et al*^[11] demonstrated that cGMP formation was reduced dramatically in the bladder neck and urethra of the diabetic rabbit compared to age-matched controls, and the cGMP-dependent NO-mediated relaxation of the bladder neck and urethral smooth muscle was significantly impaired in the diabetic rabbit. Although we did not measure cGMP concentration in the urethra of the diabetic rat in the present study, we predicted that caffeine might increase cGMP content in the urethra of the diabetic rat by the inhibition of PDE, which in turn activate the NO-cGMP pathway to relax the urethra and reduce residual urine.

Watanabe and Miyagawa^[15] suggested that residual urine in diabetics was related to a decrease in the detrusor contraction strength and an enlarged bladder, so prevention of and treatment for overdistention to maintain the normal size of the bladder are important in addition to good control of hyperglycemia. Our study showed that hyperglycemia caused a significant increase in the bladder weight and a dramatic decrease in the bladder strip contraction (per 100 mg) caused by EFS, and coffee or caffeine treatment was able to significantly decrease the bladder weight and potentiate the strip contraction (per 100 mg) in the diabetic rat. These results indicate that such potentiated neurogenic bladder contraction may be attributable to the activation of Ca²⁺ mobilization from intracellular Ca²⁺ stores. Elevation of the intracellular Ca²⁺ concentration ([Ca²⁺]_i) is an essential process for many different cellular functions in a variety of cell types^[16]. Two types of Ca²⁺ release operate in the SR of smooth muscle cells: ryanodine-receptor mediated Ca²⁺ release and inositol 1,4,5-trisphosphate-induced Ca²⁺ release^[16,17]. It is well established that caffeine as a specific agonist of ryanodine receptors triggered Ca²⁺ release through intracellular Ca²⁺ store in SR and increased sensitivity of myofibrils to Ca²⁺ to promote smooth muscle contraction. Therefore, we inferred that caffeine might have a positive effect on the detrusor contraction strength in the diabetic rat by elevating [Ca²⁺]_i.

Previous studies showed that upregulation of bladder muscarinic receptors led to hyper-contractility of the bladder smooth muscle in the early stage of the diabetic rat^[18-20]. In our study, a few unstable spontaneous contractions before micturition were observed in some diabetic rats during cystometrogram and the bladder strip contraction stimulated by ACh was significantly increased in the diabetic rat compared to control animals. ED₅₀ value (1.693×10⁻⁵ mol/L) in the DM group was less than that in the control group (3.092×10⁻⁵

mol/L). These data suggested that the affinity between bladder muscarinic receptors and ACh or the amount of muscarinic receptors is upregulated in the DM group. This was partly in accordance with Latifpour *et al*'s observations^[20]. However, coffee and caffeine treatment can partly reverse this kind of increased strip contraction and reduce such spontaneous contraction overactivity as well as increase ED₅₀ value in diabetic rats. The latest study showed that caffeine may reduce spontaneous contraction overactivity in a detrusor instability model. So caffeine can actually benefit patients with lower urinary tract syndrome or overactive bladder^[21]. According to clinical and experimental investigations, alterations of bladder dysfunction are manifold and related with duration of DM^[22,23]. Diabetes induces time-dependent pathologic changes in the urinary bladder of rats^[24,25] and the change of detrusor pressure (P_{det}) is also time-dependent. In an early stage of compensation, P_{det} indeed increases even beyond a normal range, and then reduces to a normal level. When it comes to a decompensatory stage, P_{det} usually descends, leading to an atonic bladder. In our study there was no significant difference in the maximum intravesical pressure among the four groups. We suggested that urinary bladder dysfunction in 7-week diabetic rats was still recognized as the compensatory change. Although the decreased bladder strip contraction (per 100 mg) by EFS was seen in the diabetic rat, in terms of the whole bladder weight, the diabetic bladder produced absolute contractile force more than the control bladder. Coffee or caffeine treatment may recover the micturition reflex and potentiate the detrusor contractility in the diabetic rat to elongate the duration and reverse the degree of the compensation. This may result from over-expression of the muscarinic receptors by the pharmacological effect that we discovered, but it needs further investigation.

As was stated above, we suggest that the mechanism for the effect on the bladder is as follows: (i) by increasing cAMP content in the bladder recovery of the micturition reflex and improvement of the detrusor contractility was shown; (ii) during the storage phase, caffeine may reduce spontaneous contraction overactivity in a detrusor instability model^[21], and thus benefits bladder compliance; (iii) histopathological studies showed that caffeine could protect the ultrastructures of intracellular sarcoplasmic reticulum, mitochondria, and so on, in the bladder muscle of diabetic rats (unpublished our data). In addition, previous studies showed that caffeine may have protective and nutritive effects on neurons to prevent and treat Parkinson's disease and migraine; stimulate release of calcitonin gene-related peptide^[26], which is recognized as one of the most important sensory neurotrans-

ducers and neuromodulators in the bladder to improve the impaired sensation of bladder fullness in diabetic rats^[27], and influence neuromuscular functions as the antagonist of adenosine receptors *in vivo*.

Although consumption of large amounts of coffee has been shown to decrease the incidence of type 2 diabetes^[3-5], coffee and caffeine did not have any effect on the blood glucose levels of the STZ-treated rat in our study. On the one hand, the effect of coffee on type 2 diabetes may mainly prevent or decrease risks, but not cure type 2 diabetes. On the other hand, it might be related to the long-term effects of caffeine on glucose metabolism^[5]. Further research is needed in order to evaluate possible therapeutic effects of caffeine on diabetic animals over a period of time.

Conclusion

In conclusion, these results indicate that the consumption of coffee or caffeine may have a beneficial effect on urinary bladder dysfunction in 7-week STZ-induced diabetic rats by increasing cAMP content in the bladder, recovery of the micturition reflex and improvement of the detrusor contractility.

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