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ORIGINAL PAPER

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# The early effects of partial outflow obstruction on contractile properties of diabetic and non-diabetic rat bladder

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**Abstract** The aim of this study is to determine the early effects of partial outflow obstruction (POO) on the detrusor contractility of diabetic (DM) and non-diabetic rats. A total of 67 adult female Wistar rats with average weight of 214 ± 3.1 g were randomized into five groups as control ($n = 6$), sham operated ($n = 6$), obstructed ($n = 18$), DM ($n = 19$), and DM with obstruction ($n = 18$). Intraperitoneal injection of 60 mg/kg streptozotocin was performed to achieve DM. Partial bladder neck obstruction was created surgically by ligating the urethra around a 3F feeding tube. Bladder strips were obtained and inspected on days 3, 7, and 14 of both the diabetic period and POO. Mean detrusor weights were measured and the maximal contractile responses to carbachol (Car), adenosine 5′-triphosphate (ATP), substance P (SP) and electrical field stimulation (EFS) of detrusor strips in all groups were studied in vitro. After 14 days of obstruction, no remarkable difference was observed between the maximal contractile responses to Car and SP of strips from obstructed-only (POO) and diabetic-obstructed (DM-POO) rats compared to the control group. The responses to EFS and ATP in the POO rats were significantly lower than the controls ($P < 0.01$, $P < 0.01$, respectively). In the DM-POO group however, the responses were significantly better than the POO group, reaching almost similar levels with the controls. The contractile responses of DM-POO rats were higher than the POO group but lower than the DM group. Better contractile responses of the rats with DM-POO than POO group can be explained by the early enhancing effects of DM on detrusor contractility. In early DM + POO period, the negative effects of POO on detrusor muscle contractility is masked by diabetes mellitus.

**Keywords** Partial outflow obstruction · Diabetes · Rat bladder · In vitro · Muscle contraction

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## Introduction

Partial outlet obstruction of the bladder secondary to benign prostatic hyperplasia (BPH) is known to be associated with increase in bladder mass, alteration in bladder capacity, decreased level of compliance and significantly decreased in vitro responses to field stimulation and pharmacological stimulation [7, 8, 19].

Urinary bladder function is also significantly altered in patients with diabetes mellitus. Many of these changes are also seen in the streptozotocin (STZ)-induced diabetic rats, the most commonly used animal model for diabetes mellitus [2, 9, 11, 13, 22]. Diabetes induced changes in micturition pattern occur within the first day after the onset of diabetes, reach the maximal values within 2 weeks, and remain relatively stable for as long as 2 months [3]. In addition to changes in the micturition pattern, STZ-induced diabetes also causes significant alterations in the contractile response of the bladder to field stimulation and contractile agonists [2, 9, 11, 13, 22].

Since diabetes and BPH increase in prevalence with age [5], it can be expected that a major fraction of patients with BPH concomitantly suffer from diabetes and vice versa. There are not a satisfactory number of studies which show how and to what degree the coexistence of these two pathologic processes affect bladder functions. The aim of this study was to investigate the effects of partial outlet obstruction on detrusor contractile responses in vitro, in STZ-induced diabetic rats.

## Materials and methods

### Animals

Studies on bladder outlet obstruction failed to demonstrate significant alterations in bladder weights and detrusor contractility in male rats [18]. This was thought to be due to urine reflux back to the seminal vesicles via the large ducts [18]. To our knowledge, there is no evidence on gender dependent changes of diabetes induced variations in contractile responses of obstructed detrusor. We therefore used female rats in the study.

Female albino rats ($n=67$) were obtained from the Animal Laboratories of Ankara University and housed under standard conditions for 1 week after delivery. The care and use of the animals were in accordance with the recommendations of the Guide for Care and use of Laboratory Animals (Ethics Committee of Ankara University, No:88–32, 1988).

### Induction of diabetes mellitus

Rats were fasted for 18–24 h. Diabetes was then induced with a single injection of streptozotocin (STZ, 60 mg/kg, intraperitoneally) in ice cold 0.02 m citrate saline. The STZ treated rats developed glycosuria and polyuria within 48 h of injection. Rats were considered diabetic and included in the study when random urine samples tested at least 3(+) on clinistix (corresponding to a glucose level of 1 g/100 ml or more) using Keto-Diastix test strips (Amos Division of Miles Laboratories, Elkhart, Ind., USA), and subsequent analysis of serum obtained at time of death showed glucose levels of at least 400 mg/100 ml.

### Surgical induction of partial outlet obstruction

To obtain a partial obstruction of the urethra, rats were anesthetized with ketamine, 100 mg/kg body weight, intraperitoneally. The abdomen was opened through a midline incision and the bladder and the proximal urethra were exposed. A ligature (nylon suture, 4–0) was placed around the urethra and tied in the presence of an intraluminally placed plastic rod with a diameter of 1 mm. The plastic rod was then removed. In sham-operated rats, the proximal urethra was circumferentially dissected but not ligated. The abdominal wall was closed and the animals were allowed to recover.

Rats were divided into five groups: 1. control $n=6$, 2. sham-operated $n=6$, 3. STZ-induced diabetic $n=9$, 4. obstructed $n=18$, 5. STZ-induced diabetic-obstructed $n=18$.

### Maintenance of animals

Each group of animals was caged separately and kept under precisely the same conditions as the other groups. All groups were kept in a temperature controlled room ($22\pm2$°C), artificially lit from 6.00 to 18.00 daily. The animals were fed with standard Purino chow (Ankara Purino, Turkey) and provided with water ad libitum. After the initial weights had been noted, each rat was routinely weighed once a week and the weights were recorded up to the end of the study.

### Preparation of tissues

Age and weight matched obstructed and/or STZ-induced diabetic rats were studied at 3, 7 and 14 days after obstruction and/or treatment with STZ. Rats were killed by decapitation. Blood was collected in ice-chilled tubes and serum separated and analyzed for serum glucose concentration using an Ames Glucometer 3 (Bayer Diagnostics, France).

After death, the entire bladder was removed and placed in Krebs solution of the following composition (mmol/l):NaCl, 113; KCl, 4.7; $NaH_2PO_4$, 1.4; $NaHCO_3$, 16.3; $MgSO_4$, 0.6; $CaCl_2$, 2.5 and glucose, 7.7. Bladder strips were prepared for pharmacological studies by the method described by Longhurst et al. [10]. An equally sized longitudinal strip measuring approximately $12\times3$ mm was cut from the center of the posterior and anterior surfaces of the bladder body. The tissues were placed into a 10 ml organ bath containing Krebs solution. One end of the strip was attached to a tissue holder and the other to a tension transducer (Ugo Basile, Milano, Italy) connected to pen recorder (Unirecord, Ugo Basile). The organ baths were constantly filtrated with 95% $O_2$/5% $CO_2$ and the organ baths and reservoirs of Krebs solution were maintained at 37°C. An initial load of between 0.5 g and 1.0 g tension was placed on urinary bladder preparations. Tissues were allowed to equilibrate for 60 min.

After a 60-min equilibration period, dose response curves were obtained using carbachol, ATP, SP. Dose response curves were obtained cumulatively by a step-wise increase in agonist concentration followed by at least two washes over a 15-min period.

Field stimulation used platinum electrodes set on both sides of the muscle strips in the organ bath. Transmural nerve stimulation was performed by using a Grass S-44 field stimulator delivering biphasic square wave pulses of 80 V, 1 ms duration and variable frequencies (0.5–64 Hz). Stimulation was maintained until the peak response was observed. The interval between stimulations was 2 min.

### Drugs used

Adenosine 5′-triphosphate, substance P, carbamylcholine chloride (carbachol), and streptozotocin were purchased from Sigma (St. Louis, Miss.). All drugs were prepared freshly before each experiment by dissolving them in control Krebs solution.

### Statistical analysis

All values are expressed as mean ± SEM. Statistical evaluation of the data was done using Student's *t*-test for unpaired observations. Differences were considered significant at $P<0.05$.

## Results

### Changes in serum glucose, body, bladder weight

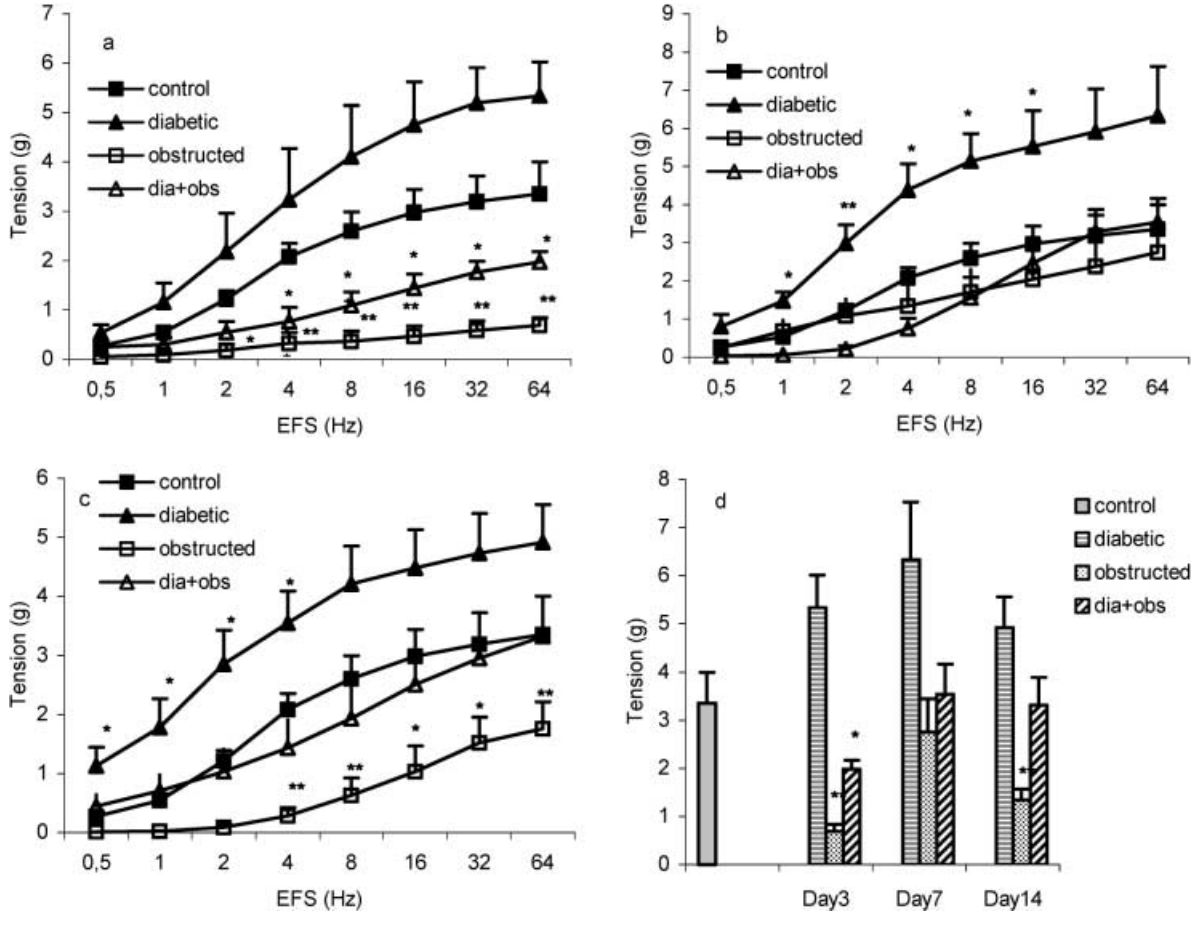
Characteristics of animals and tissues used in these experiments are shown in Table 1. The traditional symptoms of polyuria, polydipsia and polyphagia were observed in the diabetic animals. After death, the serum glucose of the diabetic animals was significantly higher than the other groups (Table 1). The body weights of all rats were similar before induction of diabetes. Diabetic rats weighted significantly lower after induction of diabetes than did the control or obstructed-only rats (Table 1). There were gradual increases in bladder weights in the DM, POO and DM-POO rats. Bladder weights increased faster in the POO and DM-POO rats than the DM group, but there were no significant differences in bladder weights between the POO and DM-POO groups.

### Contractile responses to field stimulation

The contractile responses of strips from 3, 7 and 14 days DM rats to field stimulation were slightly higher than those of the controls, but no statistical significance was present. The contractile responses were significantly lower in strips from POO bladders on days 3 and 14 of obstruction when compared to controls (Fig. 1). The

**Table 1.** Changes in body weight, bladder weight and plasma glucose levels 14 days after STZ injection and/or obstruction. Significantly different from control, $*P < 0.05$, $**P < 0.01$

| | Day 3 | Day 7 | Day 14 |
|---|---|---|---|
| Rat weight (g) | | | |
| Control | | | 221 ± 7.8 |
| Sham | | | 226 ± 10.4 |
| Diabetic | 205 ± 4.2 | 194 ± 4.8* | 191 ± 5.7* |
| Obstructed | 214 ± 3.1 | 217 ± 5.4 | 235 ± 12.5 |
| DM + obstructed | 204 ± 5.2 | 197 ± 3.6 | 189 ± 8.3* |
| Bladder weight (mg) | | | |
| Control | | | 137 ± 12 |
| Sham | | | 142 ± 17 |
| Diabetic | 148 ± 1.2 | 176 ± 23 | 181 ± 16* |
| Obstructed | 334 ± 5.1** | 362 ± 4.9** | 577 ± 77** |
| DM + obstructed | 278 ± 46* | 369 ± 48** | 504 ± 11** |
| Glucose (mg/100 ml) | | | |
| Control | | | 121 ± 8 |
| Sham | | | 132 ± 10 |
| Diabetic | 338 ± 19** | 407 ± 11** | 511 ± 13** |
| Obstructed | 118 ± 18 | 124 ± 9 | 128 ± 14 |
| DM + obstructed | 324 ± 12** | 398 ± 17** | 482 ± 15** |




obstruction of the diabetic rats caused increased contractility in response to field stimulation. The responses to field stimulation were significantly lower than controls on day 3, whereas the responses to field stimulation were similar by the day 7 following obstruction, in diabetic rats (Fig. 1).

**Fig. 1a–d.** Frequency-response curves of bladder body strips from control (*filled squares*), diabetic (*filled triangles*), obstructed (*empty squares*) and diabetic + obstructed (*empty triangles*) to field stimulation. Each represents mean ± SEM of 6–7 individual bladders. Day 3 (**a**), day 7 (**b**), day 14 (**c**) after STZ injection and/or obstruction. Effects of obstruction and/or diabetes on maximal response of bladder body to field stimulation (**d**). Significantly different from control, $*P < 0.05$, $**P < 0.01$

## Contractile responses to carbachol

The maximal responses of strips from 3, 7 and 14 days DM rats to carbachol were significantly higher than the controls (Fig 2).

Contractile responses of strips from 3 and 14 days POO and DM-POO rats were not significantly different from those of the controls. However, after 7 days, the responses of strips from POO and DM-POO rats to carbachol were significantly greater than those of controls (Fig 2).

## Contractile responses to ATP

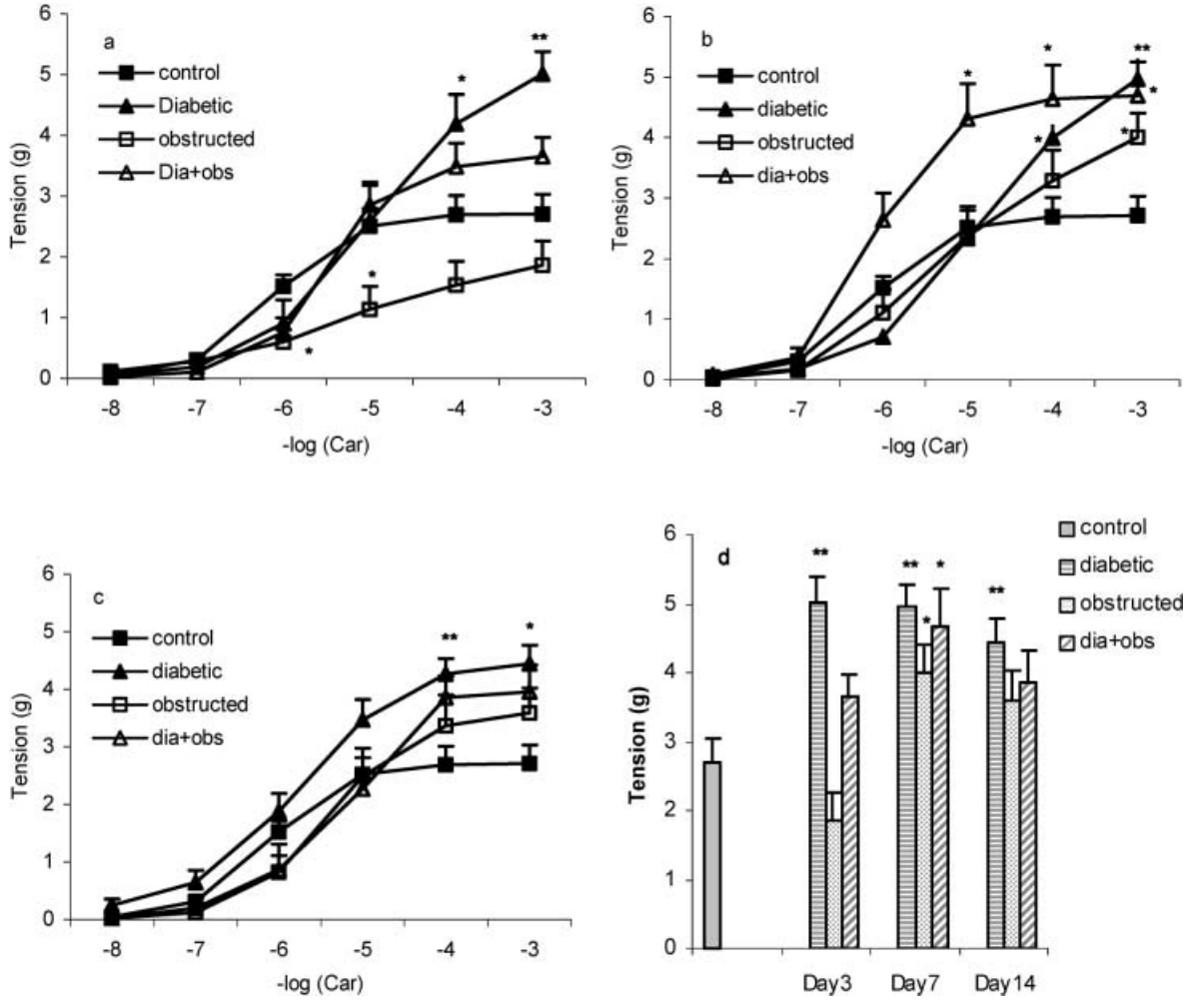
The contractile responses of the DM rats to ATP were similar to the responses of the control rats (Fig. 3).

The maximal contractile responses to ATP significantly decreased 3 days after obstruction, but they increased and almost reached the control levels by day 14 (Fig. 3). Contractile responses of strips from 3 and 14 days DM-POO rats were not significantly different from those of the controls. However, at day 7, the responses of strips from DM-POO rats to ATP were significantly lower than those of the controls (Fig. 3).

## Contractile responses to substance P

Although the maximal response to substance P increased until 14 days following the induction of diabetes, responses were significantly different from those of the controls only on day 7 (Fig. 4). Responses of the 7 days obstructed strips were significantly different from controls (Fig. 4). Responses were diminished in strips from 14 days obstructed rats. Although the obstruction of the rats resulted in decreased contractile responses of bladder strips to substance P, obstruction of the diabetic rats caused increased contractility in response to substance P.

**Fig. 2a–d.** Concentration-response curves of bladder body strips from control (*filled squares*), diabetic (*filled triangles*), obstructed (*empty squares*) and diabetic + obstructed (*empty triangles*) to carbachol. Each represents mean ± SEM of 6–7 individual bladders. Day 3 (**a**), day 7 (**b**), day 14 (**c**) after STZ injection and/or obstruction. Effects of obstruction and/or diabetes on maximal response of bladder body to carbachol (**d**). Significantly different from control, $*P < 0.05$, $**P < 0.01$



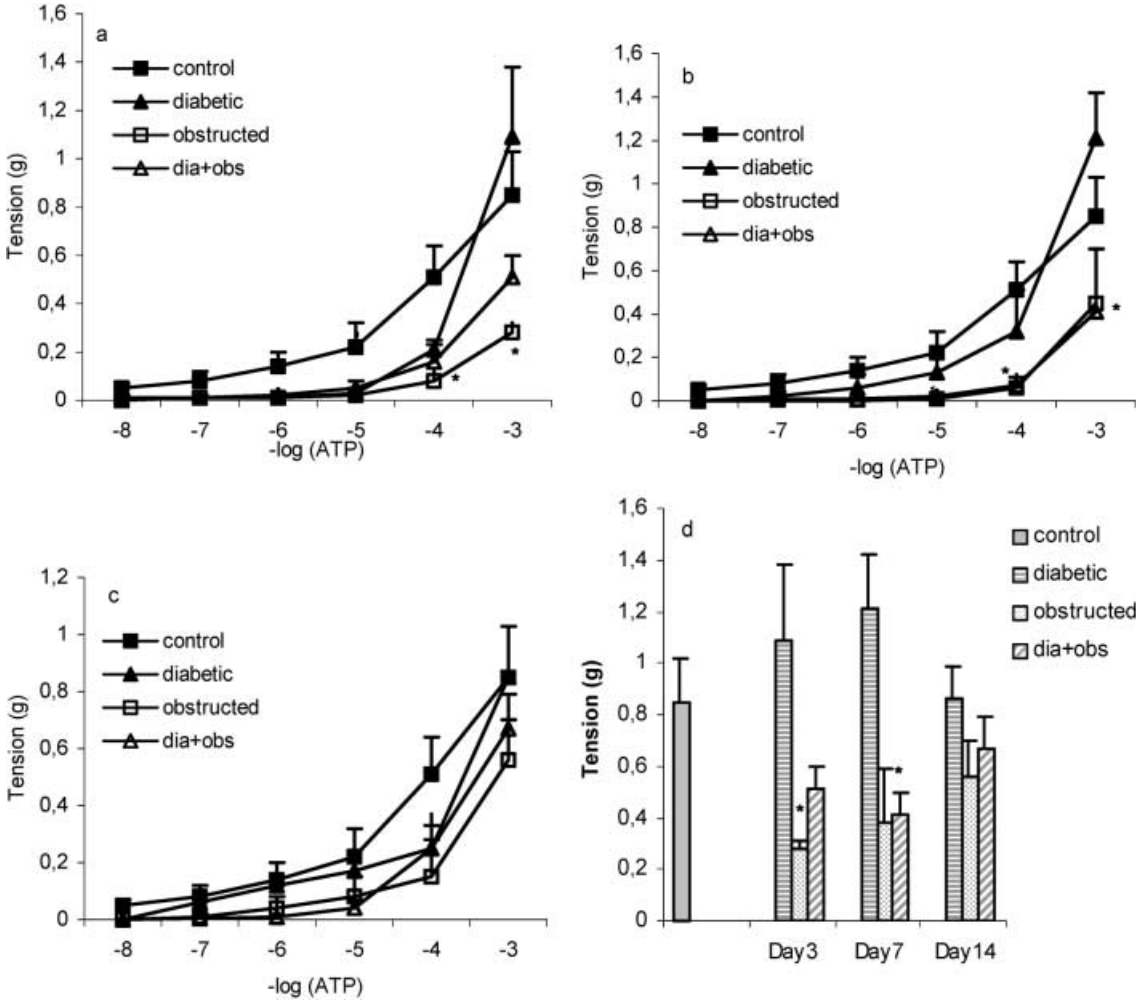



**Fig. 3a–d.** Concentration-response curves of bladder body strips from control (*filled squares*), diabetic (*filled triangles*), obstructed (*empty squares*) and diabetic + obstructed (*empty triangles*) to ATP. Each represents mean ± SEM of 6–7 individual bladders. Day 3 (**a**), day 7 (**b**), day 14 (**c**) after STZ injection and/or obstruction. Effects of obstruction and/or diabetes on maximal response of bladder body to ATP (**d**). Significantly different from control, $*P < 0.05$, $**P < 0.01$

Sham operated controls

The bladder weights and contractile responses of all sham groups were similar, with no difference from non-operated controls.

## Discussion

Outflow obstruction is one of the most common urological problems in elderly male patients [14, 19, 21]. Outflow obstruction secondary to BPH induces various bladder dysfunctions. Since diabetes and BPH increase in prevalence with age [5], it can be expected that a major proportion of the patients with BPH concomitantly suffer from diabetes and vice versa. There are not enough data on how and to what extent the coexistence of these two pathologic conditions affects bladder functions. We studied the effect of partial outlet obstruction on detrusor contractile responses of STZ-induced diabetic rats.

Many of the signs and symptoms of partial outflow obstruction can be reproduced in animal models [4, 7, 23]. An increase in bladder mass is observed in all experimental studies on partial outflow obstruction [4, 7, 23]. A similar significant increase in bladder mass was observed in diabetic rats beginning by day 7 of diabetes [3, 20]. In our study, while the significant increase in the bladder mass of the diabetic rats was on day 14, it could be observed on the day 3 of the obstructed and obstructed-diabetic group. This increase in the bladder mass of the obstructed and obstructed-diabetic groups on day 14 reached almost four times the bladder mass of the control group. There were no significant differences among the bladder masses of the obstructed and obstructed-diabetic groups. From these and the other studies, one can conclude that the amount of increase in bladder mass is affected by the severity of the obstruction and directly relates to the contractile dysfunctions observed in the presence of severe partial outlet obstruction.

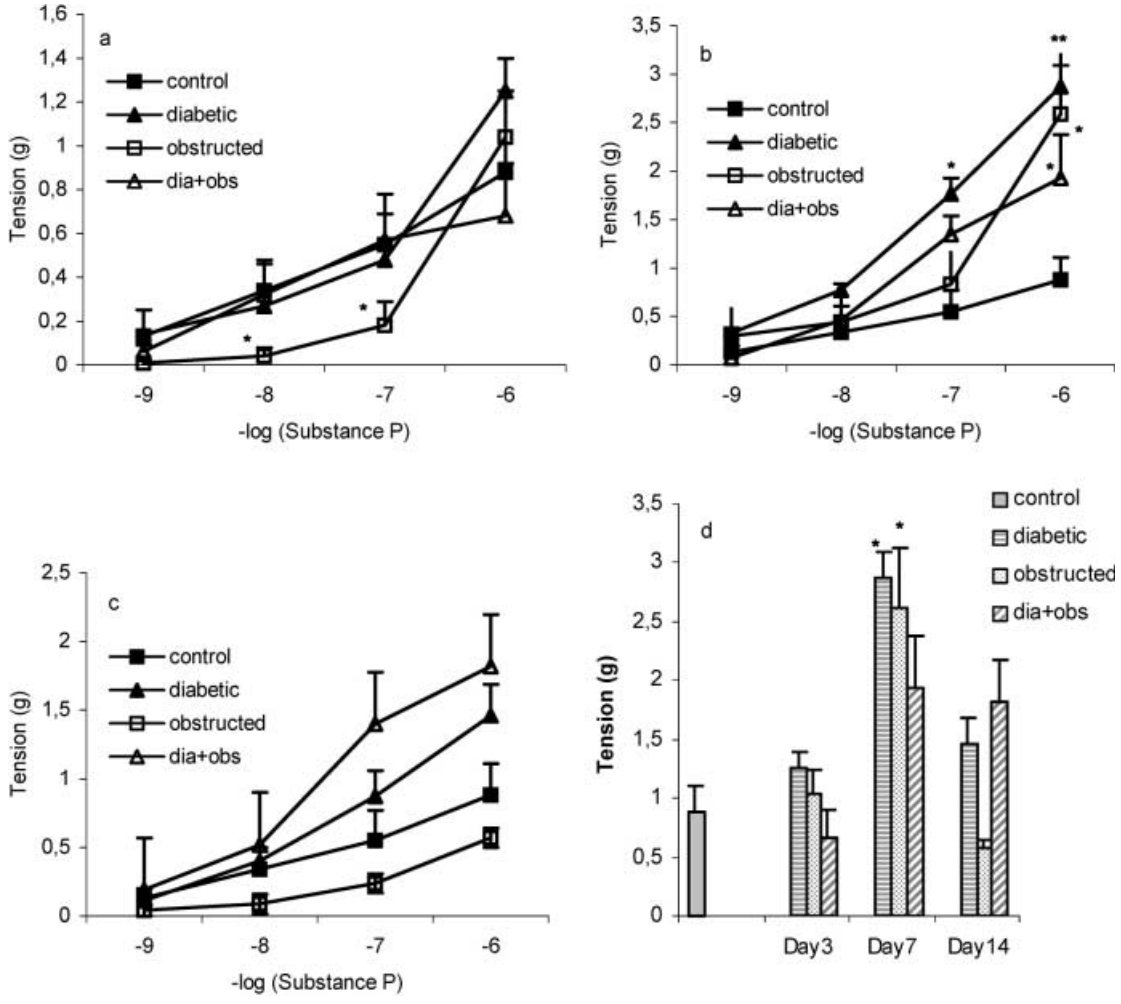



**Fig. 4.** Concentration-response curves of bladder body strips from control (*filled squares*), diabetic (*filled triangles*), obstructed (*empty squares*) and diabetic + obstructed (*empty triangles*) to substance P. Each represents mean ± SEM of 6–7 individual bladders. Day 3 (**a**), day 7 (**b**), day 14 (**c**) after STZ injection and/or obstruction. Effects of obstruction and/or diabetes on maximal response of bladder body to substance P (**d**). Significantly different from control, $*P < 0.05$, $**P < 0.01$

Previous studies reported significant increases in the contractile responses of bladder strips from diabetic rats to field stimulation, carbachol and ATP (by 7 days after induction of diabetes) [20]. However, Saito et al. reported significantly reduced responses to field stimulation and ATP on days 7 and 14 in severely obstructed bladder compared to controls, although the response to carbachol was similar for control and the severely obstructed groups [16].

In our study, after 14 days of diabetes we observed increased contractile responses to field stimulation, carbachol and ATP, but the changes were significant only with carbachol. By contrast, in the obstructed group after the same period, significant decreases in the contractile responses to field stimulation and ATP were observed. It was seen that contractile responses to field stimulation and ATP in the diabetic-obstructed group improved markedly and almost reached the control levels when compared to the decreased responses of the obstructed group. The detrusor contractile responses to pharmacologic agents and EFS is usually reported to start decreasing on day 7 of obstruction and reach the lowest measured levels at about day 14 [16]. An early fall in contractility was shown in the sham operated rats however, which was usually observed before day 7 and thought to arise due to the trauma caused by the surgical intervention. This was also shown to be a reversible phenomenon. So, in our study, early decrease of contractile responses to EFS on day 3 was probably due to the effects of surgery on the detrusor, while day 7 was the time when the statistically insignificant early impact of obstruction was observed, which in turn, reached the significant level on day 14. The seventh day of obstruction probably stands within a ''window period'' with a mild insignificant decrease of EFS response observed in the period between the impact of surgery and the significant effects of obstruction on the detrusor. Why the changes in responses to ATP, carbachol and SP are observed on day 7 is a point of question in which related mechanisms can not be understood and explained solely by the findings of this study. On the other hand, it should be kept in mind that surgery has proven early

impacts on detrusor contractility which would have certain contributions to the bias mentioned above.

The decrease of contractions with EFS in the obstructed-only group was more significant than the decrease in the contractions induced by the pharmacologic agents. This is consistent with the morphological demonstration that outlet obstruction induced a specific degeneration of neural elements within the bladder smooth muscle. This change might be induced by the bladder wall ischemia [15]. The mid-contractile responses in the early phase diabetic rats may be due to increase in the density of the muscarinic and purinergic receptors. This up-regulation of the receptors may be because of diuresis and/or hypertrophy regulated changes in these receptor systems. In diabetic-obstructed rats, increase in the contractile responses induced by diabetes may mask the decrease caused by obstruction.

Substance P has been reported to be one of the sensory neurotransmitters in the urinary bladder [12, 17]. It has been demonstrated that the contractile responses to SP increase significantly in the 8–11 weeks diabetic rats. This increase was claimed to be because of the functional changes in the bladder smooth muscle [6]. On the other hand, it has also been shown that in the 6 week obstructed rats, contractile responses to SP decrease markedly [1]. In our study, while the responses to SP of the 14 days diabetic and diabetic-obstructed rats increased, a decrease was observed in the obstructed-only group. But these changes lacked statistical significance with respect to the controls. These results suggest that the induction of DM causes functional changes in the bladder smooth muscle and improves the deceased contractile responses to SP in obstructed rats.

## Conclusions

Although the obstruction of the rats resulted in decreased contractile responses of the bladder strips, the coexistance of diabetes with the obstructed state minimized these effects and markedly improved the responses almost to control levels. Diabetes may delay the transition of the bladder to the decompensated state in obstructed rats. It could therefore be proposed that the symptoms of diabetic BPH patients would be milder than in the patients without diabetes. Nevertheless, further long-term and urodynamic studies are needed to confirm this hypothesis.

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