

Tadalafil, a phosphodiesterase type 5 inhibitor, improves bladder blood supply and restores the initial phase of lower urinary tract dysfunction in diabetic rats

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5 We appreciate the constructive and helpful comments.
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7 We have fully responded to the reviewers' comments as follows.
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10 **【Reviewer #1】**

11 1. Animal Models of Diabetic Uropathy. Firouz Daneshgari et al. (J Urol., Vol. 182,
12 S8-S13, December 2009) In the early phase (< 9 weeks) hyperglycemia induced
13 osmotic polyuria is the main mechanistic factor that causes compensatory bladder
14 hypertrophy, and associated myogenic and neurogenic alterations, which manifest
15 as storage problems (OAB). In the later phase (> 12 weeks) accumulation of
16 oxidative stress products during prolonged hyperglycemia causes decompensation of
17 bladder tissue and function, which manifest as bladder emptying problems (UAB).
18 However, the current article claimed STZ induced DM at 7 weeks is at the stage of
19 incomplete underactive bladder, which concept is obviated from the reference paper.
20 (Response)
21

22 The 7-week time point at which we performed the experiment corresponds to the
23 “early phase” when detrusor overactivity should be observed, according to a review
24 paper on animal models of diabetic uropathy (Ref #24). The hypothesis regarding
25 the time course of diabetic bladder dysfunction is partially derived from a paper
26 involving a mouse model (Ref #25). However in our present study, ICI was actually
27 prolonged and the void volume increased, which reflects the initial phase of
28 detrusor underactivity rather than detrusor overactivity. Our cystometry results
29 are consistent with the mouse-model paper, in which the bladder capacity was
30 greater and the ICI was longer at 9 weeks than at 3 weeks. The condition at 7 weeks
31 after diabetes induction is complicated. As the results show, the intravesical
32 pressure and urinary flow rate were higher in the D group, suggesting that the
33 detrusor was still sufficiently strong to compensate for the larger void volume and
34 urethral relaxation dysfunction. This may be one aspect of detrusor overactivity.
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46 2. The authors used the term urethra open pressure, but did not show the data of
47 contraction amplitude. From Fig 1, it seemed that the open pressure is similar to
48 contraction amplitude. Which data did not show UAB (Table 2).
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50 (Response)
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52 We stopped using the term “underactive” because, at 7 weeks after diabetes
53 induction, afferent nerve and urethral dysfunction may occur, but the detrusor may
54 increase in strength to compensate for larger void volume and urethral relaxation
55 dysfunction. In previous cystometry papers, the authors indicated that the
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5 “contraction amplitude” is the higher of the first or second peaks during voiding
6 reflex; they did not distinguish between them. The second peak may reflect the
7 power of detrusor contraction. At the second peak, urinary flow ends or almost ends;
8 thus, the amplitude does not correspond to detrusor pressure at the maximum flow
9 rate (P_{det} at Q_{max}) in clinical pressure flow studies.
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14 3. Abstract: The diabetes with tadalafil group was given oral tadalafil (2 mg/kg/day)
15 for 7 days before the experiments. Page 4 Six weeks after the induction of diabetes,
16 rats in the DT group were given tadalafil (2 mg/kg/day, oral) for 7 days. Please make
17 a clear description in the abstract.
18
19

20 (Response)

21 We revised the abstract to: “The rats were raised for an additional 7 weeks after
22 diabetes induction.”
23
24

25
26 4. The method of conscious CMG is not clear. Free moving or restricted??

27 (Response)

28 We performed conscious CMG on motion-restricted animals. We revised the
29 description to “Cystometry was performed in conscious, motion-restricted animals
30 in a Bollman cage”.
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34 5. “M” shape of pressure rising during CMG, are all voiding phase in rats showed
35 “M” shape, regardless of DM, normal, and DM with tadalafil?

36 (Response)

37 The “M”-shape of the pressure rise during CMG was observed in all voiding phases,
38 if the recording catheter was set at the appropriate bladder dome location,
39 regardless of whether the rats were DM, normal, or DM with tadalafil.
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44 6. As we known, diabetic bladder dysfunction would begin from the vesical
45 sensation loss and causing the bladder overdistention. A sign of this situation is a
46 large bladder capacity can be observed, as authors reported on table 2. At the early
47 stage of diabetic bladder dysfunction, the contractility of bladder did not be affected
48 profoundly, and this situation also can be observed on figure 2, which the voiding
49 pressure of STZ rat is similar to the control. In my opinion, this STZ model showed
50 the delayed sensation stage of diabetic bladder dysfunction and had no matter with
51 an underactive bladder.
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56 (Response)
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5 We agree with your opinion. We stopped using the term “underactive” because at 7
6 weeks after inducing diabetes, afferent nerve and urethral dysfunction may occur,
7 but the detrusor may strengthen to compensate for the larger void volume and
8 urethral relaxation dysfunction.
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13 7. The statement of bladder ischemia in the current STZ rat model is very important.
14 However, authors cannot indicate the etiology of the bladder ischemia in their STZ
15 model. Did this ischemia change result from vessel injury, failure of
16 microcirculation, or cyclic ischemia-reperfusion due to overdistention. It is probable
17 that this ischemia phenomenon be just from bladder overdistention, which would
18 cause the overexpression of HIF-1 α in the bladder of STZ rats as shown in Fig. 3.
19

20
21 (Response)

22 As the reviewer states, we cannot positively state the etiology of the bladder
23 ischemia. Overdistention can certainly cause an ischemia phenomenon and
24 hyperglycemia may influence vessel function. We measured bladder blood flow with
25 the bladder expanded to 50% of its functional capacity, based on cystometry results,
26 to maintain a constant bladder wall tension. The blood flow was significantly lower
27 in the diabetes group, suggesting that the ischemia involves both overdistention
28 and vessel dysfunction.
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35 8. Authors used the laser blood flow meter to determine the bladder hypoperfusion
36 of STZ rats. This laser measurement may have a wide range of variation. However,
37 as shown in Fig. 4, this study lacked of internal control, positive control, and
38 perfusion histogram. I suggest that authors could read the reference as follows: Sci
39 Rep 6:36110, 2016, in which they might find tips to improve the measurement.
40

41 (Response)

42
43 We used a different instrument and software (Omegazone OZ-2; Omegawave, Tokyo,
44 Japan) from those in the referenced article (Moor FLPI, Moor Instruments, Devon,
45 UK). We showed the mean blood flow values, based on the number of pixels in the
46 region of interest. The software calculates the blood flow as the absolute value; this
47 is the same method used in Ref #9.
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52 Reviewer: 2

53 1. “Incomplete” underactive bladder sounds odd (title, conclusion). Because UAB is
54 the symptom-based definition, “the incomplete phase of detrusor underactivity”
55 (page 11) seems better to describe the experimental condition of this study.
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6 (Response)

7 We agree with your suggestion. We stopped using the term “underactive” because, 7
8 weeks after inducing diabetes, afferent nerve and urethra dysfunction may occur,
9 but the detrusor may strengthen to compensate for the larger void volume and
10 urethral relaxation dysfunction. We altered the title accordingly.
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14 2. The major concern of this study is the lack of investigation regarding the site of
15 action of tadalafil. As discussed by the authors (page 13-14), the reduction in the
16 opening pressure may suggest that tadalafil, a PDE5 inhibitor, induces smooth
17 muscle relaxation in the urethra to facilitate the voiding; however, this aspect is not
18 studied. Thus, more detailed analyses of CNG parameters would be helpful, such
19 as threshold pressure for inducing micturition, bladder capacity, voiding efficiency,
20 bladder contraction pressure during voiding, voiding time and average flow rate
21 (voided volume/voiding time). Also, the data regarding PVR are shown in Table 2;
22 however, they are not described in the results or discussed in the Discussion section.
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26
27 (Response)

28 We analyzed more detailed parameters associated with urethral function and added
29 data to Table 2. We paid attention to the complicated condition existing 7 weeks
30 after inducing diabetes. As the results show, the intravesical pressure and urinary
31 flow rate were higher in the D group, suggesting that the detrusor remained
32 sufficiently strong to compensate for the larger void volume and urethral relaxation
33 dysfunction.
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39 3. Is there any evidence showing that 6-7 weeks DM rats used in this study had
40 detrusor underactivity such as a reduction in bladder contraction pressure during
41 voiding or maximal contraction pressure during voiding or muscle strip contraction
42 pressures in response to agonists or electric field stimulation? If these parameters
43 are not reduced after DM or changed after tadalafil, the site of action to improve
44 micturition would be the urethra, rather than the bladder. The authors may wish
45 provide additional data and discuss these points.
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49 (Response)

50 We stopped using the term “underactive,” as stated above. We speculate that in this
51 DM model, afferent nerve function is disordered first, as evidenced by the longer ICI.
52 The urethral relaxation is disordered second because the opening pressure
53 increases. Finally, bladder contraction becomes disordered. In rats with DM for 6-7
54 weeks, the detrusor remained sufficiently stronger to compensate for the larger void
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5 volume and urethral relaxation dysfunction. We believe that this time point
6 corresponds to the initial phase of diabetic lower urinary tract dysfunction.
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10 4. It is described in Methods (page 6), the bladder blood flow was measured with the
11 bladder expanded to 50% of the functional bladder capacity based on the result of
12 cystometry. This means that the bladder was more extended in untreated DM rats
13 because of larger bladder capacity than in control rats or tadalafil-treated DM rats.
14 Because blood circulation is reduced as bladder is distended, should the
15 measurement of blood flow be done at the same bladder capacity? Please explain
16 why the authors used the relative bladder capacity (50%).
17

18
19 (Response)

20
21 In the untreated DM rats, the bladder volume is significantly larger, based on the
22 significantly increased bladder weight shown in Table 1. When the bladder empties,
23 the bladder wall becomes a little wrinkled. If we expand the bladder to 50% of the
24 functional capacity of control or tadalafil-treated DM rats, the bladder wall is much
25 less distended in untreated DM rats than in control or tadalafil-treated DM rats.
26 Therefore, we measured bladder blood flow with the bladder expanded to 50% of its
27 functional capacity, based on cystometry results, to maintain a constant tension. We
28 chose the 50% value because we could clearly observe the blood flow differences,
29 among the groups, at this tension.
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36 Reviewer: 3

37 Major concerns

38 1. Clinical dose of tadalafil for the treatment of BPH is normally 5 mg/day (≈ 0.1
39 mg/kg/day). In this study, 2 mg/kg/day was used, this was extremely higher dose
40 than that in the clinical dose. The author should address this point.
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43 (Response)

44 In a previous paper using tadalafil (Ref #10), the authors stated that a 0.5
45 mg/kg/day dose in rats corresponds to a clinical dose of 2.5 mg/day. Therefore, a dose
46 of 2 mg/kg/day may correspond to a clinical dose of 10 mg/day. Thus, the 2
47 mg/kg/day dose is double the clinical dose for treating BPH, but it is not extremely
48 high.
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53 2. Tadalafil was administered systemically. So, the author should address the site of
54 action and mechanisms of tadalafil on diabetic cystopathy in the present study.
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56 (Response)
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5 We speculate that systemic tadalafil administration allows the drug to act on the
6 urethra, vessels, and afferent nerves. The reduced ICI may reflect improved
7 afferent nerve activity. The reduced opening pressure reflects improved urethral
8 relaxation function. The reduction in ischemia reflects improved vessel function.
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12 3. The author used term of “incomplete underactive bladder”. In this study, which
13 parameter (result) was reflected as “incomplete underactive bladder”?

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15 (Response)

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17 We stopped using the term “underactive,” as stated above. We speculate that in this
18 DM model, the afferent nerve function becomes disordered first because ICI
19 lengthens. The urethral relaxation becomes disordered second because the opening
20 pressure increases. Finally, bladder contraction becomes disordered. In DM rats, at
21 6-7 weeks, the detrusor remained sufficiently strong to compensate for the larger
22 void volume and urethral relaxation dysfunction. We think that this time point
23 corresponds to the initial phase of diabetic lower urinary tract dysfunction.
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29 Minor concerns

30 4. There were few references in the Introduction section.

31
32 (Response)

33 We added a reference for the second sentence and moved sentences from the
34 Discussion section, as described in response to Comment #9.
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37 5. Non-diabetic rats treated with or without vehicle-injection?

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39 (Response)

40 Non-diabetic rats received intraperitoneal injections of saline, at volumes equal to
41 those for the tadalafil administrations.
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44 6. If residual volume expected, single cystometry was preferred.

45
46 (Response)

47 We expected the ICI would be longest in diabetic rats, even if we discharged the
48 post-void residual. The observed results were as expected, suggesting that the
49 existence of a post-void residual did not affect the understanding of the results. We
50 thought that showing repeated cystometry with less noise was important.
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54 7. Table 1 and 2: marks of asterisk and dagger was complicated in each table. Please
55 use same mark in the same group.
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5 (Response)
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7 We standardized the marks.
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10 8. Quantitative analysis of the immunohistochemical stainings was preferred.

11 (Response)

12 We agree with this comment. However, the immunohistochemical staining is
13 basically qualitative and the software for quantitative analyses is unreliable.
14 Therefore, we qualitatively analyzed the staining and added blood flow
15 measurements to supplement for the weakness.
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20 9. Discussion section should be described based on the present study. Some of
21 sentence should be moved to the Introduction section or removed. This section
22 needed to be more summarized.
23

24 (Response)

25 We removed 2 sentences from the first paragraph of the Discussion and moved the
26 paragraph to the Introduction. Thus, the Discussion section was focused on the
27 present results.
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ABSTRACT

Aims:

To investigate the effect of tadalafil on bladder blood flow and lower urinary tract function in a rat model of diabetes.

Materials and Methods:

We studied female Sprague-Dawley rats, and induced diabetes in some using a single intraperitoneal injection of streptozotocin. We divided the rats into non-diabetes (ND), diabetes (D), and diabetes with tadalafil (DT) groups. The rats were raised for an additional 7 weeks after diabetes induction. The DT group received oral tadalafil (2 mg/kg/day) for 7 days before the experiments. At 7 weeks after diabetes induction, we performed cystometry, resected the bladders for immunohistochemistry (hypoxia-inducible factor-1 α [HIF-1 α] and 8-oxo-2'-deoxyguanosine [8-OHdG] staining), and measured bladder blood supply using a laser blood flow meter.

Results:

The opening pressure, when the urethra opens and urine flow starts, was significantly lower in the DT group than in the D group (43.6 ± 12.3 vs. 24.9 ± 5.9 cmH₂O). The inter-contraction interval was significantly longer in the D group than in the ND and DT groups (1566.2 ± 168.7 vs. 702.9 ± 165.2 and 787.4 ± 148.8 s). Immunohistochemistry

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6 showed positive staining of the urothelial layer for both HIF-1 α and 8-OHdG in the D
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9 group, but not in the ND or DT groups. Bladder blood flow was significantly lower in the
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12 D group than in the ND or DT groups.

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15 Conclusions:

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18 Tadalafil improves bladder blood supply and lower urinary tract function in diabetic rats.

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21 Tadalafil may be a promising drug that restores lower urinary tract dysfunction in the
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24 early phase of diabetes.
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Introduction

The relationship between lower urinary tract ischemia and dysfunction has been studied since the late 1990's. Chronic bladder ischemia is caused by diabetes, arteriosclerosis, or bladder outlet obstruction due to benign prostatic hyperplasia (BPH).¹ Chronic bladder ischemia induces bladder overactivity in the early stage and bladder underactivity in the advanced stage. Several animal models of bladder ischemia have been described, including acute bladder ischemia due to ligation of the internal iliac arteries that decreases detrusor contractility in rats.^{2, 3} In rabbits, chronic bladder ischemia due to arteriosclerosis-like disease of the iliac arteries is associated with detrusor overactivity and increased detrusor contractility in response to carbachol and electrical field stimulation, if the ischemia is moderate, and impaired detrusor contraction, if the ischemia is severe.⁴

Chronic partial bladder outlet obstruction causes bladder ischemia leading to a progressive decrease in detrusor contractility.^{5, 6} According to previous reports, clinical causes, including insufficient blood supply (i.e., arteriosclerosis) and bladder outlet obstruction (i.e., BPH), induce ischemia and the ischemia/reperfusion cycle is accompanied by micturition and neurogenic and/or myogenic contractile dysfunction.⁷

Diabetes impairs vascular endothelial function due to nitric oxide (NO) hyposecretion

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6 and causes atherosclerosis, resulting in reduced blood supply. Tadalafil is a
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9 phosphodiesterase type 5 (PDE5) inhibitor approved for the treatment of BPH and
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12 erectile dysfunction. The drug relaxes smooth muscle by reducing PDE5 levels and
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15 increasing cyclic guanosine monophosphate (cGMP) levels and may improve pelvic
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18 organ blood supply and perfusion. PDE5 is expressed not only in the corpus
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21 cavernosum and vesicular-deferential artery, but also in the lower urinary tract, including
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24 the prostate, urethra, testis, and bladder.⁸ In this study, we investigated whether tadalafil
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27 improves bladder blood supply and lower urinary tract function using a model of lower
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30 urinary tract ischemia in diabetic rats.
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35 **Materials and Methods**

36 *Animals*

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39 We used female, 11–12-week-old Sprague-Dawley rats weighing 250–300 g. We
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42 maintained the rats under a 12-hour light/dark cycle (the lights turned on automatically
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45 at 8:00 a.m.) with free access to water and laboratory food. We induced diabetes using
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48 a single intraperitoneal injection of streptozotocin (65 mg/kg); control animals received
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51 an equivalent volume of saline. We performed the experiments 7 weeks after inducing
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60 diabetes. The animals were divided into non-diabetes (ND), diabetes (D), and diabetes

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6 with tadalafil (DT) groups.
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10 11 *Tadalafil administration* 12

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14 We suspended tadalafil in a 0.5% methylcellulose solution, using an agate mortar and
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16 pestle, to create a 2 mg/mL solution; the drug was administered (1 mL/kg body weight)
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18 according to a previous tadalafil report.⁹ Six weeks after inducing diabetes, rats in the
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20 DT group received oral tadalafil (2 mg/kg/day) daily for 7 days. The 2 mg/kg/day dose in
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22 rats may correspond to a clinical dose of 10 mg/day, which is double the clinical dose
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24 used for treating BPH; a 0.5 mg/kg/day dose in rats reportedly corresponds to a clinical
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26 dose of 2.5 mg/day.¹⁰
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38 *Cystometry* 39

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41 We anesthetized rats with isoflurane (Escain[®]; Mylan, Tokyo, Japan), and made a
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43 midline abdominal incision to insert a transvesical catheter with a fire-flared tip (PE-50)
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45 into the bladder dome; the catheter, secured with a silk thread, was used for bladder
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47 filling and pressure recording. We connected a three-way stopcock to the transvesical
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49 catheter to monitor bladder pressure. Cystometry was performed in conscious,
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51 motion-restricted animals in a Bollman cage. To restrict the use of invasive procedures
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6 on conscious animals, we did not remove the connective tissues and other accessory
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9 tissues surrounding the bladder during the in vivo measurements. We continuously
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12 infused (0.04 mL/min) room temperature saline through the transvesical catheter for at
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15 least 2 hours to record the cystometrogram. We measured the basal pressure (BP, the
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18 lowest pressure during the filling phase), threshold pressure for inducing micturition,
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21 opening pressure (OP, pressure at which the urethra opens and urine flow starts), nadir
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24 pressure (NP, pressure at which urine flow ends), closing pressure (CP, pressure at the
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27 second peak) (Fig. 1), inter-contraction interval (ICI), bladder capacity, void volume
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30 (VV), post-void residual (PVR), voiding efficiency, voiding time, and average flow rate
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33 (voided volume/voiding time) using PowerLab® and LabChart® software (ADInstrument
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36 Japan, Nagoya, Japan).

37 38 39 40 41 *Immunohistochemistry*

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44 We injected 0.4–1.0 mL of 10% formalin into the bladder with the outlet tied, kept the
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47 expanded bladder in 10% formalin, and embedded it in paraffin. We cut tissue sections
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50 (5- μ m thick) from the paraffin blocks and stained them with hematoxylin and eosin,
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53 hypoxia-inducible factor (HIF)-1 α as a hypoxia marker, and 8-oxo-2'-deoxyguanosine
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56 (8-OHdG) as an oxidation stress marker.
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Bladder blood flow measurements

We measured the bladder blood flow with the bladder expanded to 50% of its functional capacity, based on cystometry results, using a laser blood flow meter (Omegazone OZ-2®; Omegawave, Tokyo, Japan). We exposed the bladder through a midline abdominal incision, with the rat under isoflurane anesthesia. We analyzed image pixels to produce mean perfusion values, according to the bladder blood flow method described by Kawai et al.¹¹

Statistical analysis

All values are expressed as means \pm standard deviations. We used the Kruskal-Wallis one-way analysis of variance to analyze statistical differences and Dunn's post-hoc test.

A P-value < 0.05 was considered statistically significant.

Ethical information

All experiments were conducted in accordance with the institutional guidelines approved by the Nara Medical University Institutional Animal Care and Use Committee.

Results

Animal characteristics

The mean body weights were not significantly different among the groups. Bladder weights were significantly larger in the D group than in the ND or DT groups. Serum glucose levels were significantly higher in the D and DT groups than in the ND group (Table 1).

Cystometry

Typical cystometrograms showed longer ICIs and higher peak pressures in the D group than in the ND or DT groups (Fig. 2). The OP was significantly lower in the DT group than in the D group, suggesting that tadalafil administration restored the urethral opening function that was impaired due to diabetes. The ICI was significantly longer in the D group than in the ND or DT groups. Bladder capacity, voided volume per micturition, voiding efficiency, and post-void residual were significantly greater in the D group than in the ND group, but not compared with the DT group. The voiding time was significantly longer in the D group than in the DT group; the average flow rate was significantly higher in the D group than in the ND group (Table 2).

Immunohistochemistry

HIF-1 α and 8-OHdG stains were both positive in the urothelial layers in the D group, but not in the ND or DT groups (Fig. 3).

Bladder blood flow measurements

The arbitrary units of bladder blood flow were 40.0 ± 4.2 , 29.5 ± 4.3 , and 38.2 ± 2.1 in the ND, D, and DT groups, respectively ($n = 6$, each). The bladder blood flow was significantly lower ($P < 0.05$) in the D group than in the ND or DT groups (Fig. 4).

Discussion

Diabetes mellitus causes lower urinary tract dysfunction via a complex pathophysiological mechanism. Proposed mechanisms include hyperglycemia, free-radical influence, axonal transport impairment, and ischemia.¹² Additionally, diabetes is one of the major risk factors for peripheral artery disease,¹³ which may induce ischemia of the pelvic organs, including the lower urinary tract. In this study, we used diabetic rats as a model of lower urinary tract ischemia. In previous studies investigating diabetes-induced bladder dysfunction, a streptozotocin-induced model¹⁴⁻¹⁸ was used for type 1 diabetes, whereas Goto-Kakizaki rats¹⁹ and Torii rats²⁰ were used

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6 for type 2 diabetes. We used the streptozotocin-induced type 1 diabetes model because
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9 we hypothesized that tadalafil would improve the lower urinary tract dysfunction
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12 associated with NO secretory insufficiency, which we previously demonstrated in the
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15 same diabetes model^{14, 15}.

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18 The topic of “underactive bladder” is becoming increasingly popular in the literature, but
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21 the absence of a formal International Continence Society (ICS) definition hinders clinical
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24 research advancements relating to this topic. The Congress on Underactive Bladder
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27 (CURE-UAB) proposed that underactive bladder is a complex symptom suggestive of
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30 detrusor underactivity, and is usually characterized by prolonged urination times, with or
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33 without a sensation of incomplete bladder emptying, and usually involves hesitancy,
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36 reduced sensation upon filling, a slow stream, a palpable bladder, always straining to
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39 void, enuresis, and/or stress incontinence.²¹ CURE-UAB defines detrusor underactivity
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42 as a voiding efficiency of <90%, and which is characterized by impaired detrusor
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45 contraction with weak or absent flow in urodynamic studies.²¹ The diabetic rat is a useful
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48 animal model for investigating detrusor underactivity. In the streptozotocin-induced type
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51 1 diabetes model, the post-void residual volume started to increase (0.11 ± 0.06 mL)
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54 and the voiding efficiency started to decrease ($93.2 \pm 2.9\%$) 6 weeks after diabetes
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57 induction.¹⁸ Using the CURE-UAB definition of detrusor underactivity, the 6-week
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6 diabetic bladder corresponds to the initial phase of detrusor underactivity. We speculate
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9 that, in the streptozotocin-induced type 1 diabetes model, the afferent nerve function
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12 becomes disordered first as evidenced by the longer ICI. The urethral relaxation
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15 becomes disordered next because of the increased OP. At this time, however, detrusor
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18 activity remains sufficiently strong to compensate for the urethral relaxation dysfunction.
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21 Finally, the detrusor contraction becomes disordered. We focused on the initial phase of
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24 diabetic lower urinary tract dysfunction rather than complete diabetic cystopathy
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27 because, in our clinical experience, the latter does not resolve. In fact, in our preliminary
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30 experiment, the response to tadalafil decreased with time in the later phases of diabetes
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33 (data not shown). Hence, we administered tadalafil 6 weeks after inducing diabetes, and
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36 observed its effect on the bladder.

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38 Improvements in bladder blood flow are associated with improved bladder function. In
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41 the spontaneously hypertensive rat model, bladder blood flow and void volume per
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44 micturition significantly decrease compared with control Wistar rats. Silodosin, an
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47 α 1-blocker, improves bladder blood flow, increases void volume per micturition, and
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50 decreases the number of non-voiding contractions. These results suggest that silodosin
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53 is effective for treating overactive bladder associated with impaired blood supply.²²
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56 Diabetes impairs the vascular endothelium by causing NO hyposecretion while also
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6 causing atherosclerosis,²³ which results in reduced blood flow. Recent studies have
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8 provided increasing evidence that chronic bladder ischemia induces bladder overactivity,
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10 during the early stage, and bladder underactivity during the advanced stage.²⁴ In
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12 patients with diabetic cystopathy, the afferent nerve function is impaired.¹⁸ The voided
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14 volume per micturition is larger in the severe phase of diabetes, suggesting impairment
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16 of the voiding reflex. Bladder overdistension during the storage phase also leads to
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18 reduced blood supply. Thus, the 7-week time point used in the present study
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20 corresponds to the “early phase” when detrusor overactivity should be observed,
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22 according to a review paper describing animal models of diabetic uropathy.²⁵ The
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24 hypothesis regarding the time course of diabetic bladder dysfunction is partially derived
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26 from a paper investigating mice.²⁶ However, in our present study, the ICI was actually
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28 prolonged and the void volume increased, reflecting the initial phase of detrusor
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30 underactivity rather than detrusor overactivity. Our cystometry results are consistent
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32 with the mouse paper, which indicated that bladder capacity was greater and the ICI
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34 was longer at 9 weeks than at 3 weeks.
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49 In tadalafil-treated rats, the void volume per micturition decreased (partially normalized),
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51 suggesting improvement in the voiding reflex. Furthermore, our cystometry studies
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53 revealed an “M”-shaped pressure rise during voiding. The first peak of the rise
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6 corresponded to the start of urine discharge, which reflects the opening of the urethra.
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9 The OP was high in diabetic rats but lower in tadalafil-treated diabetic rats. This
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11 suggests that the diabetic urethra is more resistant to opening, resulting in higher
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13 intravesical pressures and, therefore, bladder blood flow insufficiency during the voiding
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15 phase. We previously demonstrated that urethral relaxation during micturition is
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17 impaired in diabetic rats and that the administration of L-arginine, as an NO donor,
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19 improves urethral function.¹⁴ Tadalafil inhibits PDE5, increases smooth muscle cGMP
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21 levels, and induces smooth muscle relaxation; therefore, tadalafil also acts as an NO
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23 donor. Hence, the administration of tadalafil may induce an appropriate start of
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25 micturition (urethral opening) and improved urine flow. A complicated condition exists 7
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27 weeks after diabetes induction. As the results show, the intravesical pressure and
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29 urinary flow rate were higher in the D group, suggesting that the detrusor was
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31 sufficiently strong to compensate for the larger void volume and urethral relaxation
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33 dysfunction. We speculate that systemic tadalafil administration acts on the urethra,
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35 vessels, and afferent nerves, with the reduced ICI possibly reflecting improved afferent
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37 nerve activity. Further, the reduced OP reflects improved urethral relaxation function,
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39 and the reduced ischemia reflects improved vessel functioning.
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55 This study has some limitations. First, in our practice, chronic type 2 diabetes patients
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6 are more common than acute type 1 diabetes. Therefore, to improve the clinical
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9 relevance of this study, Goto-Kakizaki or Torii rats should have been used, and we
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12 should have investigated tadalafil's effects during different phases of diabetes. However,
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15 we chose the acute type 1 diabetes model for this initial experiment to confirm that
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18 tadalafil improves diabetic cystopathy. Second, we performed cystometry and did not
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21 directly assess urethral function. Although we believe that tadalafil also improved
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24 urethral functioning, direct urethral pressure measurements, during the voiding reflex,
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27 are needed in future experiments.
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32 **Conclusions**

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35 Tadalafil improves bladder blood supply and lower urinary tract function in diabetic rats.
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38 Thus, tadalafil may be a promising drug that restores lower urinary tract dysfunction in
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41 the early phase of diabetes.
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Figure Legends

Fig. 1 Representative cystometry. The first peak occurs when the urethra opens (opening pressure). The nadir pressure is the pressure at which urine flow ends. The second peak of pressure is the closing pressure at which the detrusor contracts while the urethra is closing.

Fig. 2 Typical cystometry charts in non-diabetes (ND, A), diabetes (D, B), and diabetes with tadalafil (DT, C) groups. The inter-contraction interval is longer in the D group than in the ND or DT groups.

Fig. 3 Hypoxia-inducible factor-1 α (HIF-1 α) immunohistochemistry in the non-diabetes (ND, A1), diabetes (D, A2), and diabetes with tadalafil (DT, A3) groups. 8-oxo-2'-deoxyguanosine (8-OHdG) immunohistochemistry in the ND (B1), D (B2), and DT (B3) groups. Photographs are at $\times 100$ magnification. Positive staining is only observed in the urothelial layer of the D-group animals.

Fig. 4 Laser measurement of blood flow in non-diabetes (ND, A), diabetes (D, B), and diabetes with tadalafil (DT, C) groups. The bladder blood flow is lower in the D group than in the ND or DT groups.

ABSTRACT

Aims:

To investigate the effect of tadalafil on bladder blood flow and lower urinary tract function in a rat model of diabetes.

Materials and Methods:

We studied female Sprague-Dawley rats, and induced diabetes in some using a single intraperitoneal injection of streptozotocin. We divided the rats into non-diabetes (ND), diabetes (D), and diabetes with tadalafil (DT) groups. The rats were raised for an additional 7 weeks after diabetes induction. The DT group received oral tadalafil (2 mg/kg/day) for 7 days before the experiments. At 7 weeks after diabetes induction, we performed cystometry, resected the bladders for immunohistochemistry (hypoxia-inducible factor-1 α [HIF-1 α] and 8-oxo-2'-deoxyguanosine [8-OHdG] staining), and measured bladder blood supply using a laser blood flow meter.

Results:

The opening pressure, when the urethra opens and urine flow starts, was significantly lower in the DT group than in the D group (43.6 ± 12.3 vs. 24.9 ± 5.9 cmH₂O). The inter-contraction interval was significantly longer in the D group than in the ND and DT groups (1566.2 ± 168.7 vs. 702.9 ± 165.2 and 787.4 ± 148.8 s). Immunohistochemistry

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6 showed positive staining of the urothelial layer for both HIF-1 α and 8-OHdG in the D
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9 group, but not in the ND or DT groups. Bladder blood flow was significantly lower in the
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12 D group than in the ND or DT groups.

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15 Conclusions:

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18 Tadalafil improves bladder blood supply and lower urinary tract function in diabetic rats.

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21 Tadalafil **may be** a promising drug that restores **lower urinary tract dysfunction** in the
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24 early phase of diabetes.
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Introduction

The relationship between lower urinary tract ischemia and dysfunction has been studied since the late 1990's. Chronic bladder ischemia is caused by diabetes, arteriosclerosis, or bladder outlet obstruction due to benign prostatic hyperplasia (BPH).¹ Chronic bladder ischemia induces bladder overactivity in the early stage and bladder underactivity in the advanced stage. **Several animal models of bladder ischemia have been described, including acute bladder ischemia due to ligation of the internal iliac arteries that decreases detrusor contractility in rats.^{2, 3} In rabbits, chronic bladder ischemia due to arteriosclerosis-like disease of the iliac arteries is associated with detrusor overactivity and increased detrusor contractility in response to carbachol and electrical field stimulation, if the ischemia is moderate, and impaired detrusor contraction, if the ischemia is severe.⁴ Chronic partial bladder outlet obstruction causes bladder ischemia leading to a progressive decrease in detrusor contractility.^{5, 6} According to previous reports, clinical causes, including insufficient blood supply (i.e., arteriosclerosis) and bladder outlet obstruction (i.e., BPH), induce ischemia and the ischemia/reperfusion cycle is accompanied by micturition and neurogenic and/or myogenic contractile dysfunction.⁷ Diabetes impairs vascular endothelial function**

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6 due to nitric oxide (NO) hyposecretion and causes atherosclerosis, resulting in reduced
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9 blood supply. Tadalafil is a phosphodiesterase type 5 (PDE5) inhibitor approved for the
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11 treatment of BPH and erectile dysfunction. The drug relaxes smooth muscle by reducing
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13 PDE5 levels and increasing cyclic guanosine monophosphate (cGMP) levels and may
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15 improve pelvic organ blood supply and perfusion. PDE5 is expressed not only in the
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17 corpus cavernosum and vesicular-deferential artery, but also in the lower urinary tract,
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19 including the prostate, urethra, testis, and bladder.⁸ In this study, we investigated
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21 whether tadalafil improves bladder blood supply and lower urinary tract function using a
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23 model of lower urinary tract ischemia in diabetic rats.
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35 **Materials and Methods**

36 *Animals*

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38 We used female, 11–12-week-old Sprague-Dawley rats weighing 250–300 g. We
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40 maintained the rats under a 12-hour light/dark cycle (the lights turned on automatically
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42 at 8:00 a.m.) with free access to water and laboratory food. We induced diabetes using
43
44 a single intraperitoneal injection of streptozotocin (65 mg/kg); control animals received
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46 an equivalent volume of saline. We performed the experiments 7 weeks after inducing
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48 diabetes. The animals were divided into non-diabetes (ND), diabetes (D), and diabetes
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6 with tadalafil (DT) groups.
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10 11 *Tadalafil administration* 12

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14 We suspended tadalafil in a 0.5% methylcellulose solution, using an agate mortar and
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16 pestle, to create a 2 mg/mL solution; the drug was administered (1 mL/kg body weight)
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18 according to a previous tadalafil report.⁹ Six weeks after inducing diabetes, rats in the
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20 DT group received oral tadalafil (2 mg/kg/day) daily for 7 days. **The 2 mg/kg/day dose**
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22 **in rats may correspond to a clinical dose of 10 mg/day, which is double the**
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24 **clinical dose used for treating BPH; a 0.5 mg/kg/day dose in rats reportedly**
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26 **corresponds to a clinical dose of 2.5 mg/day.**¹⁰
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38 *Cystometry* 39

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41 We anesthetized rats with isoflurane (Escain[®]; Mylan, Tokyo, Japan), and made a
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43 midline abdominal incision to insert a transvesical catheter with a fire-flared tip (PE-50)
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45 into the bladder dome; the catheter, secured with a silk thread, was used for bladder
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47 filling and pressure recording. We connected a three-way stopcock to the transvesical
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49 catheter to monitor bladder pressure. Cystometry was performed in conscious,
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51 **motion-restricted animals** in a Bollman cage. To restrict the use of invasive
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6 procedures on conscious animals, we did not remove the connective tissues and other
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9 accessory tissues surrounding the bladder during the in vivo measurements. We
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11 continuously infused (0.04 mL/min) room temperature saline through the transvesical
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13 catheter for at least 2 hours to record the cystometrogram. We measured the basal
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15 pressure (BP, the lowest pressure during the filling phase), **threshold pressure for**
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17 **inducing micturition**, opening pressure (OP, pressure at which the urethra opens and
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19 urine flow starts), **nadir pressure (NP, pressure at which urine flow ends), closing**
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21 **pressure (CP, pressure at the second peak)** (Fig. 1), inter-contraction interval (ICI),
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23 **bladder capacity**, void volume (VV), post-void residual (PVR), **voiding efficiency,**
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25 **voiding time, and average flow rate (voided volume/voiding time)** using PowerLab[®]
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27 and LabChart[®] software (ADInstrument Japan, Nagoya, Japan).
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41 *Immunohistochemistry*

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43 We injected 0.4–1.0 mL of 10% formalin into the bladder with the outlet tied, kept the
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45 expanded bladder in 10% formalin, and embedded it in paraffin. We cut tissue sections
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47 (5- μ m thick) from the paraffin blocks and stained them with hematoxylin and eosin,
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49 hypoxia-inducible factor (HIF)-1 α as a hypoxia marker, and 8-oxo-2'-deoxyguanosine
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51 (8-OHdG) as an oxidation stress marker.
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Bladder blood flow measurements

We measured the bladder blood flow with the bladder expanded to 50% of its functional capacity, based on cystometry results, using a laser blood flow meter (Omegazone OZ-2®; **Omegawave**, Tokyo, Japan). We exposed the bladder through a midline abdominal incision, with the rat under isoflurane anesthesia. We analyzed image pixels to produce mean perfusion values, according to the bladder blood flow method described by Kawai et al.¹¹

Statistical analysis

All values are expressed as means \pm standard deviations. We used the Kruskal-Wallis one-way analysis of variance to analyze statistical differences and Dunn's post-hoc test.

A P-value < 0.05 was considered statistically significant.

Ethical information

All experiments were conducted in accordance with the institutional guidelines approved by the Nara Medical University Institutional Animal Care and Use Committee.

Results

Animal characteristics

The mean body weights were not significantly different among the groups. Bladder weights were significantly larger in the D group than in the ND or DT groups. Serum glucose levels were significantly higher in the D and DT groups than in the ND group (Table 1).

Cystometry

Typical cystometrograms showed longer ICIs and higher peak pressures in the D group than in the ND or DT groups (Fig. 2). The OP was significantly lower in the DT group than in the D group, suggesting that tadalafil administration restored the urethral opening function that was impaired due to diabetes. The ICI was significantly longer in the D group than in the ND or DT groups. **Bladder capacity, voided volume per micturition, voiding efficiency, and post-void residual were significantly greater in the D group than in the ND group, but not compared with the DT group. The voiding time was significantly longer in the D group than in the DT group; the average flow rate was significantly higher in the D group than in the ND group** (Table 2).

Immunohistochemistry

HIF-1 α and 8-OHdG stains were both positive in the urothelial layers in the D group, but not in the ND or DT groups (Fig. 3).

Bladder blood flow measurements

The arbitrary units of bladder blood flow were 40.0 ± 4.2 , 29.5 ± 4.3 , and 38.2 ± 2.1 in the ND, D, and DT groups, respectively (n = 6, each). The bladder blood flow was significantly lower ($P < 0.05$) in the D group than in the ND or DT groups (Fig. 4).

Discussion

Diabetes mellitus causes lower urinary tract dysfunction via a complex pathophysiological mechanism. Proposed mechanisms include hyperglycemia, free-radical influence, axonal transport impairment, and ischemia.¹² Additionally, diabetes is one of the major risk factors for peripheral artery disease,¹³ which may induce ischemia of the pelvic organs, including the lower urinary tract. In this study, we used diabetic rats as a model of lower urinary tract ischemia. In previous studies investigating diabetes-induced bladder dysfunction, a streptozotocin-induced model¹⁴⁻¹⁸

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6 was used for type 1 diabetes, whereas Goto-Kakizaki rats¹⁹ and Torii rats²⁰ were used
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9 for type 2 diabetes. We used the streptozotocin-induced type 1 diabetes model because
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12 we hypothesized that tadalafil would improve the lower urinary tract dysfunction
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15 associated with NO secretory insufficiency, which we previously demonstrated in the
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18 same diabetes model^{14, 15}.

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21 The topic of “underactive bladder” is becoming increasingly popular in the literature, but
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24 the absence of a formal International Continence Society (ICS) definition hinders clinical
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27 research advancements relating to this topic. The Congress on Underactive Bladder
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30 (CURE-UAB) proposed that underactive bladder is a complex symptom suggestive of
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33 detrusor underactivity, and is usually characterized by prolonged urination times, with or
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36 without a sensation of incomplete bladder emptying, and usually involves hesitancy,
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39 reduced sensation upon filling, a slow stream, a palpable bladder, always straining to
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42 void, enuresis, and/or stress incontinence.²¹ CURE-UAB defines detrusor underactivity
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45 as a voiding efficiency of <90%, and which is characterized by impaired detrusor
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48 contraction with weak or absent flow in urodynamic studies.²¹ The diabetic rat is a useful
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51 animal model for investigating detrusor underactivity. In the streptozotocin-induced type
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54 1 diabetes model, the post-void residual volume started to increase (0.11 ± 0.06 mL)
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57 and the voiding efficiency started to decrease ($93.2 \pm 2.9\%$) 6 weeks after diabetes
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6 induction.¹⁸ Using the CURE-UAB definition of detrusor underactivity, the 6-week
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9 diabetic bladder corresponds to the initial phase of detrusor underactivity. **We**
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11 **speculate that, in the streptozotocin-induced type 1 diabetes model, the afferent**
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13 **nerve function becomes disordered first as evidenced by the longer ICI. The**
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15 **urethral relaxation becomes disordered next because of the increased OP. At this**
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17 **time, however, detrusor activity remains sufficiently strong to compensate for the**
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19 **urethral relaxation dysfunction. Finally, the detrusor contraction becomes**
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21 **disordered.** We focused on **the initial phase of diabetic lower urinary tract**
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23 **dysfunction** rather than complete diabetic cystopathy because, in our clinical
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25 experience, the latter does not resolve. In fact, in our preliminary experiment, the
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27 response to tadalafil decreased with time in the later phases of diabetes (data not
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29 shown). Hence, we administered tadalafil 6 weeks after inducing diabetes, and
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31 observed its effect on the bladder.
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44 Improvements in bladder blood flow are associated with improved bladder function. In
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46 the spontaneously hypertensive rat model, bladder blood flow and void volume per
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48 micturition significantly decrease compared with control Wistar rats. Silodosin, an
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50 α 1-blocker, improves bladder blood flow, increases void volume per micturition, and
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52 decreases the number of non-voiding contractions. These results suggest that silodosin
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6 is effective for treating overactive bladder associated with impaired blood supply.²²
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13 provided increasing evidence that chronic bladder ischemia induces bladder overactivity,
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15 during the early stage, and bladder underactivity during the advanced stage.²⁴ In
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17 patients with diabetic cystopathy, the afferent nerve function is impaired.¹⁸ The voided
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19 volume per micturition is larger in the severe phase of diabetes, suggesting impairment
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21 of the voiding reflex. Bladder overdistension during the storage phase also leads to
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23 reduced blood supply. **Thus, the 7-week time point used in the present study**
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25 **corresponds to the “early phase” when detrusor overactivity should be observed,**
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27 **according to a review paper describing animal models of diabetic uropathy.²⁵ The**
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29 **hypothesis regarding the time course of diabetic bladder dysfunction is partially**
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31 **derived from a paper investigating mice.²⁶ However, in our present study, the ICI**
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33 **was actually prolonged and the void volume increased, reflecting the initial phase**
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35 **of detrusor underactivity rather than detrusor overactivity. Our cystometry results**
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37 **are consistent with the mouse paper, which indicated that bladder capacity was**
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39 **greater and the ICI was longer at 9 weeks than at 3 weeks.**
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55 In tadalafil-treated rats, the void volume per micturition decreased (partially normalized),
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6 suggesting improvement in the voiding reflex. Furthermore, our cystometry studies
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9 revealed an “M”-shaped pressure rise during voiding. The first peak of the rise
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12 corresponded to the start of urine discharge, which reflects the opening of the urethra.
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15 The OP was high in diabetic rats but lower in tadalafil-treated diabetic rats. This
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18 suggests that the diabetic urethra is more resistant to opening, resulting in higher
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21 intravesical pressures and, therefore, bladder blood flow insufficiency during the voiding
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24 phase. We previously demonstrated that urethral relaxation during micturition is
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27 impaired in diabetic rats and that the administration of L-arginine, as an NO donor,
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30 improves urethral function.¹⁴ Tadalafil inhibits PDE5, increases smooth muscle cGMP
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33 levels, and induces smooth muscle relaxation; therefore, tadalafil also acts as an NO
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36 donor. Hence, the administration of tadalafil may induce an appropriate start of
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39 micturition (urethral opening) and improved urine flow. **A complicated condition exists**
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42 **7 weeks after diabetes induction. As the results show, the intravesical pressure**
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45 **and urinary flow rate were higher in the D group, suggesting that the detrusor**
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48 **was sufficiently strong to compensate for the larger void volume and urethral**
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51 **relaxation dysfunction. We speculate that systemic tadalafil administration acts**
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54 **on the urethra, vessels, and afferent nerves, with the reduced ICI possibly**
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57 **reflecting improved afferent nerve activity. Further, the reduced OP reflects**
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6 improved urethral relaxation function, and the reduced ischemia reflects
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9 improved vessel functioning.
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11 This study has some limitations. First, in our practice, chronic type 2 diabetes patients
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13 are more common than acute type 1 diabetes. Therefore, to improve the clinical
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15 relevance of this study, Goto-Kakizaki or Torii rats should have been used, and we
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17 should have investigated tadalafil's effects during different phases of diabetes. However,
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19 we chose the acute type 1 diabetes model for this initial experiment to confirm that
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21 tadalafil improves diabetic cystopathy. Second, we performed cystometry and did not
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23 directly assess urethral function. Although we believe that tadalafil also improved
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25 urethral functioning, direct urethral pressure measurements, during the voiding reflex,
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27 are needed in future experiments.
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41 Conclusions

42 Tadalafil improves bladder blood supply and lower urinary tract function in diabetic rats.

43 Thus, tadalafil **may be** a promising drug that restores **lower urinary tract dysfunction**
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48 in the early phase of diabetes.
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51 References

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10 11 **Figure Legends**

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14 Fig. 1 Representative cystometry. The first peak occurs when the urethra opens
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16 (opening pressure). **The nadir pressure is the pressure at which urine flow ends.**
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21 **The second peak of pressure is the closing pressure at which the detrusor**
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23 **contracts while the urethra is closing.**

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26 Fig. 2 Typical cystometry charts in non-diabetes (ND, A), diabetes (D, B), and diabetes
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28 with tadalafil (DT, C) groups. The inter-contraction interval is longer in the D group than
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32 in the ND or DT groups.

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35 Fig. 3 Hypoxia-inducible factor-1 α (HIF-1 α) immunohistochemistry in the non-diabetes
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37 (ND, A1), diabetes (D, A2), and diabetes with tadalafil (DT, A3) groups.
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8-oxo-2'-deoxyguanosine (8-OHdG) immunohistochemistry in the ND (B1), D (B2), and
DT (B3) groups. Photographs are at $\times 100$ magnification. Positive staining is only
observed in the urothelial layer of the D-group animals.

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Fig. 4 Laser measurement of blood flow in non-diabetes (ND, A), diabetes (D, B), and
diabetes with tadalafil (DT, C) groups. The bladder blood flow is lower in the D group
than in the ND or DT groups.

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For Peer Review

Table 1 Animal characteristics at 7 weeks after diabetes induction

	Non-diabetes	Diabetes	Diabetes with Tadalafil
	n=6	n=5	n=6
Body weight (g)	275.0 ± 20.7	299.0 ± 22.5	261.7 ± 19.4
Bladder weight (mg)	115.2 ± 10.0	231.2 ± 50.0†	122.0 ± 28.5*
Serum glucose (mg/mL)	95.6 ± 9.3	486.4 ± 30.4†	442.8 ± 55.0†

* p <0.05: compared with Diabetes group

† p <0.05: compared with Non-diabetes group

Table 2 The comparison of cystometry parameters

	Non-diabetes n=6	Diabetes n=5	Diabetes with Tadalafil n=6
Basal pressure (cmH ₂ O)	7.9 ± 7.8	4.8 ± 2.6	3.4 ± 2.4
Threshold pressure (cmH ₂ O)	11.9 ± 2.1	11.0 ± 3.7	8.7 ± 2.9
Opening pressure (cmH ₂ O)	29.8 ± 5.7	43.6 ± 12.3	24.9 ± 5.9*
Nadir pressure (cmH ₂ O)	23.2 ± 5.4	33.7 ± 12.8	22.5 ± 7.1
Closing pressure (cmH ₂ O)	30.6 ± 2.7	37.5 ± 12.0	25.8 ± 7.0
Inter-contraction interval (sec)	702.9 ± 165.2	1566.2 ± 168.7 [†]	787.4 ± 148.8*
Bladder capacity (mL)	0.6 ± 0.1	2.7 ± 0.4 [†]	1.0 ± 0.3
Voided volume (mL)	0.6 ± 0.1	2.4 ± 0.4 [†]	1.0 ± 0.3

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Post-void residual (mL)	0.0 ± 0.0	0.3 ± 0.1 [†]	0.0 ± 0.1
Voiding efficiency (%)	100.0 ± 0.0	87.7 ± 5.4 [†]	96.2 ± 5.8
Voiding time (sec)	2.4 ± 1.2	3.1 ± 0.9	1.6 ± 0.5 [*]
Average flow rate (mL/sec)	0.3 ± 0.2	0.8 ± 0.3 [†]	0.7 ± 0.1

* p <0.05: compared with Diabetes group

† p <0.05: compared with Non-diabetes group

For Peer Review

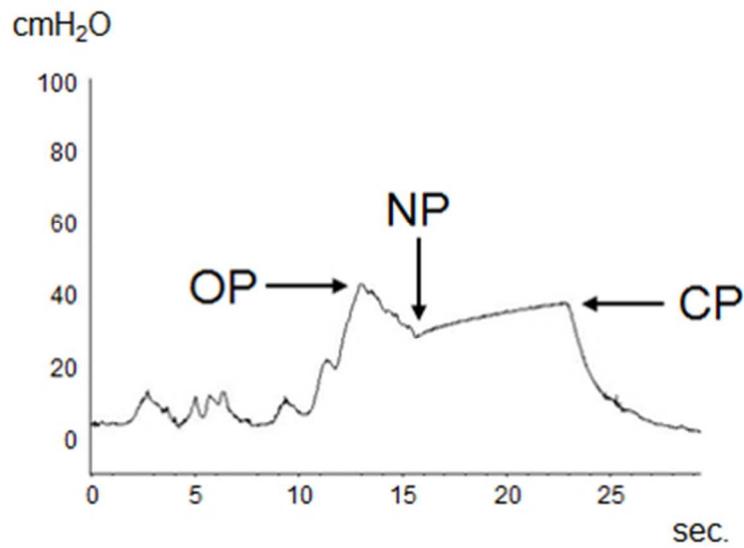


Fig. 1 Representative cystometry. The first peak occurs when the urethra opens (opening pressure). The nadir pressure is the pressure at which urine flow ends. The second peak of pressure is the closing pressure at which the detrusor contracts while the urethra is closing.

127x94mm (78 x 78 DPI)

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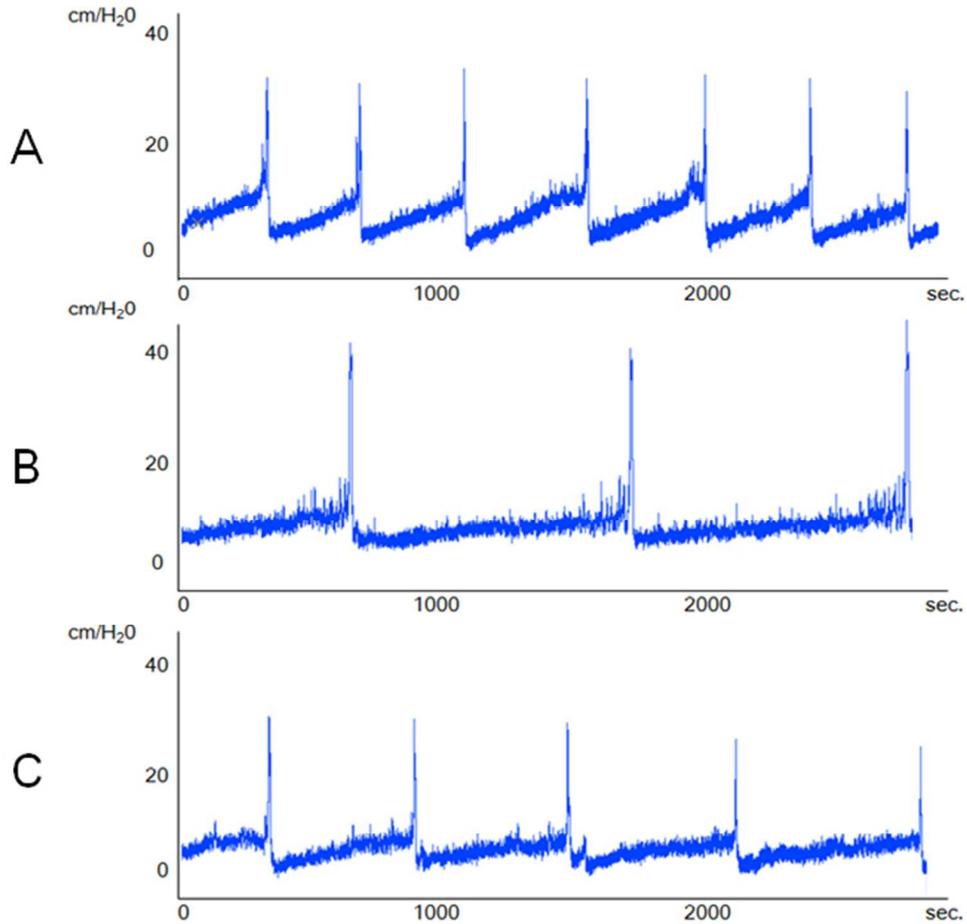


Fig. 2 Typical charts of cystometry in non-diabetes (ND) (A), diabetes (D) (B), and diabetes with tadalafil (DT) (C) groups. Inter-contraction interval (ICI) is longer in the D than in the ND and DT groups.

177x168mm (96 x 96 DPI)

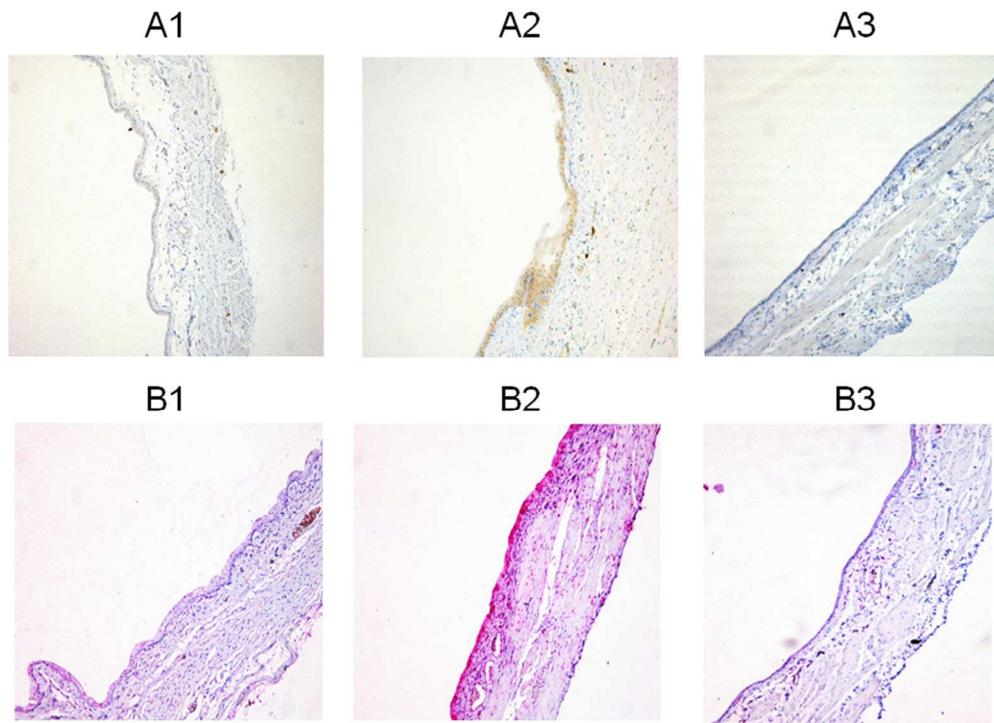


Fig. 3 Immunohistochemistry for HIF-1 α in non-diabetes (ND) (A1), diabetes (D) (A2), and diabetes with tadalafil (DT) (A3) groups and, for 8-OHdG in ND (B1), D (B2), and DT (B3) groups. Photographs are at $\times 100$ magnification. Positive staining is observed at the urothelial layer only in the D group.

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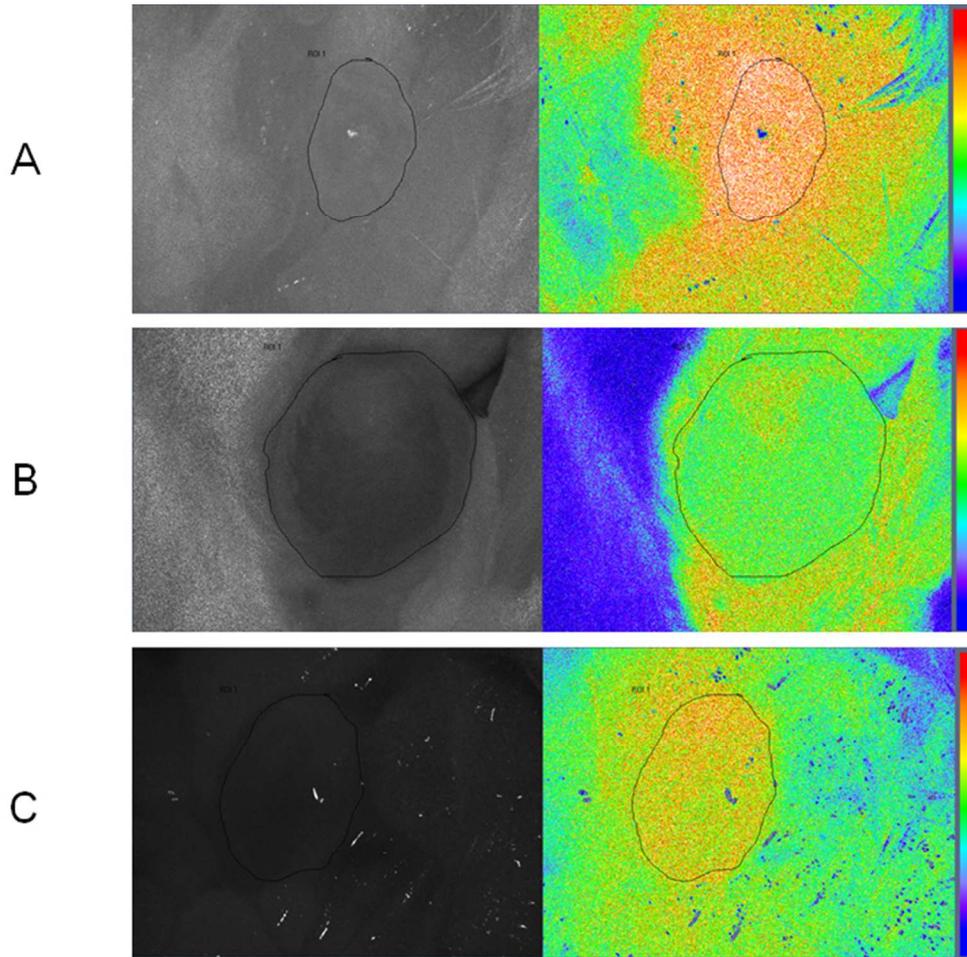


Fig. 4 Measurement of the laser blood flow meter in non-diabetes (ND) (A), diabetes (D) (B), and diabetes with tadalafil (DT) (C) groups. The left-side photograph of each pair shows the measurement field and the right-side photograph shows the bladder blood flow. The bladder blood flow is lower in the D than in the ND and DT groups.

193x185mm (96 x 96 DPI)