

Longitudinal studies of time-dependent changes in both bladder and erectile function after streptozotocin-induced diabetes in Fischer 344 male rats

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OBJECTIVES

To provide sensitive physiological endpoints for the onset and long-term progression of deficits induced by diabetes mellitus (DM) in bladder and erectile function in male rats, and to evaluate parallel changes in urogenital and nerve function induced by hyperglycaemia over a protracted period as a model for chronic deficits in patients with diabetes.

MATERIALS AND METHODS

The study comprised in 877 male, 3-month-old, Fischer 344 rats; 666 were injected intraperitoneally with 35 mg/kg streptozotocin (STZ) and divided into insulin-treated and untreated diabetic groups. The rats were studied over 8 months and measurements made of both erectile and bladder function, as well as nerve conduction studies over the duration of the study.

RESULTS

There was an early (first month) abnormality of both erectile and bladder function that persisted through the 8 months of the study. The erectile dysfunction was manifest as reduced intracavernous pressure/blood pressure ratio, and the bladder dysfunction as a persistent increase in detrusor overactivity with no detrusor decompensation. Insulin treatment prevented or modified the abnormality in each organ. Hyperglycaemia caused a progressive decrease in caudal nerve conduction velocity. The mean digital sensory and tibial motor nerve conduction velocity did not deteriorate over time. Correlation measurements of nerve and organ function were not consistent.

CONCLUSIONS

The results of this extensive long-term study show early and profound effects of

hyperglycaemia on the smooth muscle of the penis and bladder, that were persistent and stable in surviving rats over the 8 months. The physiological changes did not correlate well with neurological measurements of those organs. Significantly, diverse smooth-muscle cellular and subcellular events antedated the measured neurological manifestations of the hyperglycaemia by several months. Although autonomic diabetic neuropathy is a primary life-threatening complication of long-term diabetes in humans, this rat model of STZ-induced diabetes showed that the rapid onset of physiological manifestations was based on many molecular changes in the smooth muscle cells in this model of type 1 DM.

KEYWORDS

diabetes mellitus, erectile dysfunction, detrusor overactivity, streptozotocin, diabetic autonomic neuropathy

INTRODUCTION

Autonomic peripheral neuropathies are common end-organ complications of hyperglycaemia that affect both the quality of life and the survival of patients with diabetes mellitus (DM) [1–3]. This condition progressively affects both the structure and function of components of the cardiovascular, gastrointestinal and urogenital systems. Until recently, the urological complications of DM, which include erectile (ED) and bladder dysfunction, have been under-appreciated

[4–7]. The incidence of bladder problems in DM is ≈33%, while almost half of men with DM have ED [8–10]. As defined by the ICS, DM-induced deficits in bladder function include diminished sensation and contractility, increase in residual volume, detrusor overactivity (DO) and urge incontinence [11]. Overall, the aetiology of the DO in patients with DM is not well understood, but this condition is believed to be associated with effects on the smooth muscle, nerves and urothelium of the bladder [7]. Similarly, the relationship between ED and

DM is also considered to be multifactorial, involving neural, hormonal, myogenic and vascular mechanisms. In most men with DM, the cause of ED might be related to heightened contractility and/or impaired relaxation of the corporal smooth muscle [12]. The clinical presentation is a diminished ability to obtain or maintain an erection after sexual arousal.

Although the healthcare burden of DM is appreciable, there are no longitudinal clinical studies focusing on the development,

progression and coincidence of the two principal genitourinary dysfunctions in this population. Furthermore, there have been few systematic studies that simultaneously explore the urogenital deficits in animal models of DM. The evaluation of the mechanism and extent of hyperglycaemia-induced deficits in bladder and erectile function in an animal model is a critical step to improved diagnosis, treatment and possible prevention of the urological complications of diabetes.

The present study was designed to: (i) provide sensitive physiological endpoints for the onset and progression of DM-induced deficits in bladder and erectile function; and (ii) to evaluate parallel changes in urogenital and nerve function induced by hyperglycaemia over a protracted period as a model for chronic deficits in patients with diabetes.

We have selected the streptozotocin (STZ) model of experimental DM, which is well established and recapitulates relevant aspects of DM-related genitourinary disease in humans. We previously explored the acute effects of STZ-induced hyperglycaemia on bladder and erectile function after 2 months [13]. In this report, we extend our observations to 8 months of DM and in a much larger sample of rats.

MATERIALS AND METHODS

Male Fischer-344 rats were used for the study (Table 1); 3-month-old male F-344 rats were made diabetic by an i.p. injection with 35 mg/kg of STZ dissolved in citrate buffer (60 mL of 0.1 M citric acid and 40 mL of 0.2 M Na₂HPO₄, pH 4.6). Once hyperglycaemia was confirmed (i.e. urinary glucose on days 3–5 of >500 mg/dL) the rats were subdivided into two groups. One group was given a daily injection of 2 units of long-acting insulin (Lantus 100 units/mL, Sanofi-Aventis, Bridgewater, NJ, USA) beginning at 1 week after the STZ injection; these rats were referred to as the insulin-treated (IT) group. The remaining STZ-treated rats received no treatment for their hyperglycaemia and constituted the diabetic group (DM). A series of age-matched rats received an injection of vehicle (citrate buffer) and were termed the age-matched control group (AMC). The rats were housed in individual cages and allowed free access to food and water. Urine glucose was measured with Diastix reagent strips (Bayer Corp, Elkhart, IN, USA) calibrated at levels of 100,

TABLE 1 The number of rats used in each segment of the study

Testing	Sample time, months						Total
	1	2	3	4	6	8	
Nerve conduction							
AMC	12	48	14	44	28	65	211
DM	31	107	82	112	72	58	462
IT	25	52	22	33	29	43	204
Nerve conduction + cystometry							
AMC	11	31	8	32	23	49	154
DM	27	97	66	92	57	57	396
IT	17	39	17	27	27	34	161
Nerve conduction + erectile function							
AMC	8	33	8	34	17	37	137
DM	19	82	54	74	46	40	315
IT	12	39	18	29	26	25	149

250, 500, 1000 and 2000 mg/dL for three consecutive days after STZ injection, when the somatic nerve conduction was measured, and at the time of urodynamic evaluation. Blood glucose was measured with the Ascensia Elite™ blood glucose meter (Bayer Health Care, LLC, Mishawaka, IN, USA) after completing the cavernous nerve stimulation assessment.

All experimental protocols were approved by the Animal Institute Committee of the Albert Einstein College of Medicine. The sequence of studies was as follows: on Wednesday, nerve-conduction studies; on Friday, surgery to place a the bladder catheter; on Monday, urodynamic studies; on Tuesday to Thursday, measurement of the intracavernous pressure (ICP).

The peripheral nerve conduction velocity (NCV) was measured in 877 male rats, with 204 in the IT group, 462 in the DM group and 211 in the AMC group (Table 1). In each rat, electrophysiology was assessed at one sample time, between 1 and 8 months after confirmed hyperglycaemia. During testing, rats were anaesthetized with isoflurane (2.5–3.5%/O₂) and placed on a water-circulating heating pad; the rectal temperature was maintained at 35.8–38.0 °C. Subcutaneous needle electrodes were used for both recording and stimulation procedures. NCV and the peak compound-action potential amplitude were assessed in both the caudal and digital nerves. These measures are sensitive to the integrity of myelin and axon, and to the transmembrane and nodal distributions of ions in large-

diameter fibres. Caudal and digital NCV were recorded orthodromically with recording sites at the proximal tail and at the lateral malleolus, respectively. Minimal F-wave latency was measured in the plantar muscles of the foot after stimulation of the tibial nerve at the ankle. This measure reflects motor-nerve conduction over both the distal and proximal segments of the nerve and within the ventral spinal root. Neural responses were induced by supramaximal stimulation at 1 Hz. Electrical stimuli were 100 µs square pulses, produced by the MP100 (BIOPAC Systems Inc., Santa Barbara, CA, USA). Caudal and digital nerve responses were averaged (20 samples). Neuroelectrical signals were impedance-matched and differentially amplified with a gain of 20 000 and a frequency band of 20 Hz to 3 kHz using a BIOPAC MP100 and AcqKnowledge software version 3.5.7 (BIOPAC Systems Inc.). Data were scanned for artefacts and digitized at a rate of >20 kHz.

In all rats the bladder catheter was placed under general anaesthesia (35 mg/kg of sodium pentobarbital, i.p.) using aseptic techniques. Anaesthesia was maintained during the course of the experimental protocol (1–2 h) and monitored by direct measurement of blood pressure (BP) or the toe-pinch reflex. A subsequent injection with pentobarbital (5–10 mg/kg) was given every 45–60 min, as required. The ventral abdominal wall and perineum were shaved and cleaned with 10% povidone-iodine. A lower abdominal midline incision was made, followed by a small incision in the bladder dome. A polyethylene catheter with a cuff (PE50) was inserted into the bladder and

secured in place using a purse-string suture. This indwelling catheter was tunneled through the s.c. space and exited through an orifice made on the back of the rat's neck, and fixed with a porous tape and suture. The abdominal incision was sutured and the free end of the catheter was sealed using fine-tip cautery.

Urodynamic studies were done in awake rats with the indwelling bladder catheter connected by a two-way valve to a pressure transducer and an infusion pump. The pressure transducer was connected via a transducer amplifier (ETH 400, CB Sciences Inc., Dover, NH, USA) to a data acquisition board (Mac Laboratory/8e, ADI Instruments, MA, USA). Pressure measurements were displayed in real-time and recorded on a Macintosh computer (Mac Laboratory software V3.4, ADI Instruments). The pressure transducers and analogue/digital board were calibrated in cmH₂O before each experiment. For the cystometric studies, the rate of infusion of room temperature saline was set at 10 mL/h on a programmable infusion pump (model PHD 2000, Harvard Instruments, MA, USA). Bladder activity was continuously recorded after the first micturition, and subsequently at least 2 h of data were recorded from each rat. Bladder function was evaluated using the following urodynamic criteria: bladder capacity (volume of infused saline at micturition); basal pressure (the lowest average bladder pressure recorded during cystometry); threshold pressure (bladder pressure immediately before micturition); micturition pressure (peak bladder pressure during micturition); micturition volume (the volume of urine discharged during micturition); residual volume (volume of infused saline minus the micturition volume); intermicturition pressure (IMP, the average pressure recorded between micturitions); spontaneous activity (SA, an approximate index of spontaneous bladder contractions between micturitions, i.e. bladder overactivity, calculated by subtracting the basal pressure from the IMP).

The surgery for *in vivo* erectile physiology studies was as follows: anaesthesia was induced and maintained as described above for cystometry. The rats were placed supine, and the bladder and prostate exposed through a midline abdominal incision. The inferior hypogastric plexus (i.e. the pelvic plexus or major pelvic ganglia), pelvic nerves and the cavernous nerve were identified

posterolateral to the prostate on both sides, and stainless-steel bipolar wire electrodes were placed around a cavernous nerve for electrical stimulation. The penis was then denuded of skin; both crura (corpus cavernosum) were exposed by removing part of the overlying ischiocavernosus muscle. To monitor the ICP, a 23 G PrecisionGlide® needle cannula was filled with 250 U/mL of heparin solution (1%), connected to polyethylene-60 tubing (Intramedic, Becton Dickinson, CA, USA) and inserted into the right corpus cavernosum. Systemic arterial BP was monitored via a 25 G cannula placed into the carotid artery. Both pressure lines were connected to a pressure transducer, and then via a transducer amplifier (ETH 400) to a data acquisition board, with real-time display and recording of ICP. The cavernous nerve was directly electrostimulated by a stainless-steel bipolar hook electrode attached to a multi-jointed clamp. Each probe was 0.2 mm in diameter; the two poles were separated by 1 mm. Monophasic rectangular pulses were delivered by a signal generator (custom-made and with integral constant-current amplifier). The stimulation parameters were 20 Hz, pulse width 0.22 ms, and duration 1 min; an increasing current of 1, 2, 4, 6 and 10 mA were used.

Continuous variables were summarized as the mean (SEM) or (SD). Between-group differences were evaluated by fitting ANOVA models, followed by Fisher's least-significant difference *post hoc* multiple comparison test for pair-wise comparisons. For electrophysiological measures, differences between groups were evaluated by ANOVA followed by Tukey's *post hoc* multiple-comparison test. Correlations between bladder and ED variables were estimated using Pearson's correlation coefficient. Correlations between measures of NCV and organ function (bladder or penis) were evaluated by estimates of slope of linear regression (SLR). In all tests, statistical significance was defined as $P < 0.05$.

RESULTS

Table 2 lists the measured changes in body weight, urine and blood glucose, 24-h urinary volume and final bladder weight in the three groups over time. The results indicate that the DM group lost weight during the 8-month period of the study. The IT rats maintained their weight, but were significantly lighter

than the AMC group at all sample times. Blood glucose levels were >400 mg/dL at every time in the DM group. In the IT group the blood glucose levels were significantly higher than in the AMC group at 2 and 3 months, but there were no significant differences at the other times. Urinary glucose concentrations were elevated in both the IT and DM groups at all sample times. The 24-h urine output for the AMC rats was 9–12 mL throughout the study. The 24-h urine output was always significantly higher than the AMC in the DM and IT groups. The bladder weight significantly increased in the first month for the DM group, reaching a maximum weight at 6 months, when the weight was greater for both the control and IT groups. The IT bladder weight was no different than the AMC in the first month, but increased to a significantly greater weight thereafter. There was no significant difference in the urine glucose concentration between the DM and IT groups at any of the sample times evaluated. However, those measurements were made in the morning the day after the long-acting insulin had been given to the rats and might not have been effective at that time. The urinary glucose concentration of the AMC rats was always zero.

All electrophysiological measures in control rats were within 'normal limits' and consistent with previously published maturational changes [14,15]. By contrast, hyperglycaemic rats showed marked evidence of peripheral neuropathy at each evaluation (Fig. 1). After only 1 month of hyperglycaemia, the caudal NCV was slowed in the DM group relative to that in the AMC group ($P = 0.037$) and the digital NCV was slowed in both the DM and IT groups relative to controls ($P = 0.001$). There was slowing of motor nerve conduction, evaluated by minimal F-wave latency after 2 months in the DM group ($P < 0.001$) and after 4 months in the IT group (Fig. 1D). There was progressive deterioration in measures of caudal nerve conduction after 4 months, with decreases in NCV of up to 35% (12 m/s) between the 4- and 8-month samples (Fig. 1A). The amplitude of the caudal nerve responses also progressively declined over 6–8 months (Fig. 1B). In some rats, responses were undetectable and often associated with necrotic processes at the end of the tail. The mean digital sensory and tibial motor nerve NCV did not deteriorate over the later times (4–8 months), most likely reflecting the selective death of the most severely affected rats. Except for the digital nerve, all measures

TABLE 2 The physical variables during the course of the study

Duration of DM, months; and group	Body weight, g		Glucose level, mg/dL		Urine volume, mL/24 h	Final bladder weight, mg
	Initial	Final	Blood	Urine		
1 DM	253.9 (4.4)	223.9 (6.3) ^f	426.8 (18.4) ^f	1034.5 (81.1)	51.1 (3.3) ^c	274.9 (11.5)
IT	244.5 (4.9)	263.7 (7.2) ^d	147.7 (36.9)	1039.5 (90.2) ^f	35.8 (7.1) ^f	238.5 (17.5)
AMC	247.0 (3.5)	312.5 (8.4) ^a	117.1 (8.4) ^a	0.0 (0.0) ^a	9.1 (1.2) ^a	204.7 (12.9) ^a
2 DM	251.9 (1.8)	200.7 (3.3) ^f	414.4 (8.1) ^f	1066.5 (48.3) ^e	49.2 (1.9) ^e	256.1 (7.6)
IT	253.4 (1.9)	250.6 (4.8) ^f	168.5 (21.2) ^f	1268.3 (76.3) ^f	58.9 (5.3) ^f	238.6 (9.3) ^f
AMC	250.9 (1.5)	323.3 (4.8) ^a	113.1 (6.1) ^a	0.0 (0.0) ^a	9.4 (0.8) ^a	176.9 (6.8) ^a
3 DM	249.3 (1.3)	200.9 (4.2) ^e	425.9 (10.3) ^f	1266.9 (57.4)	59.4 (2.9)	273.4 (8.6)
IT	250.4 (2.2)	247.2 (11.1) ^d	276.3 (36.6) ^d	1071.4 (71.4) ^d	50.6 (8.9) ^d	269.4 (10.7) ^d
AMC	240.1 (1.1)	343.2 (3.8) ^a	88.7 (8.9) ^a	0.0 (0.0) ^a	9.2 (1.5) ^a	173.5 (8.6) ^a
4 DM	249.9 (1.8)	194.7 (3.7) ^c	433.1 (9.1) ^f	1376.3 (58.9)	49.3 (1.4)	271.3 (7.2)
IT	249.1 (1.9)	267.0 (7.3) ^d	126.0 (19.3)	1181.0 (102.9) ^d	50.3 (4.6) ^d	251.4 (9.6) ^c
AMC	247.1 (0.9)	362.9 (3.6) ^a	104.4 (4.2) ^a	0.0 (0.0) ^a	10.3 (0.9) ^a	198.1 (11.0) ^a
6 DM	250.9 (1.5)	192.1 (4.5) ^d	430.7 (9.5) ^f	1336.4 (73.5) ^e	48.1 (2.4)	286.9 (12.8) ^c
IT	253.7 (2.8)	291.8 (9.2) ^d	119.4 (20.9)	924.0 (97.2) ^d	41.5 (4.4) ^d	249.3 (13.8) ^d
AMC	250.2 (1.5)	382.9 (3.9) ^a	110.2 (7.0) ^a	0.0 (0.0) ^a	11.9 (1.5) ^a	192.5 (7.9) ^a
8 DM	251.9 (2.0)	191.8 (4.5) ^f	447.5 (9.9) ^e	1491.2 (73.5) ^e	44.8 (2.3)	282.2 (12.7)
IT	244.1 (1.3)	305.6 (8.9) ^d	103.5 (13.9)	1140.6 (78.5) ^f	43.6 (2.9) ^d	269.2 (12.0) ^e
AMC	248.9 (1.3)	396.4 (3.6) ^a	117.0 (4.9) ^a	0.0 (0.0) ^a	9.6 (0.5) ^a	198.5 (4.5) ^a

^aP < 0.001 for DM vs AMC, ^bP < 0.05, ^cP < 0.01, ^dP < 0.001 for IT vs AMC, and ^eP < 0.01, ^fP < 0.001 for DM vs IT.

of nerve conduction in the IT group showed a milder degree of neuropathy, with values between the AMC and DM groups (Fig. 1).

The changes in the bladder dynamics with time showed an increase in bladder capacity in both the DM and IT groups, as reflected by the increase in urinary volume and bladder weight increase (Table 2). However, bladder capacity and micturition volume in the DM group was significantly greater than in the IT group, except at 2 months (Table 3). That difference might be reflected by the fact that at 3 months the 24-h urinary volume was greater in the IT group than the DM group (Table 2). At 3–8 months the 24-h urinary volumes were the same in both the IT and DM groups. Both were significantly greater than in the AMC group at all times. A representative urodynamic study from each of the three groups is shown in Fig. 2. Bladder compliance increased significantly at 2 months for both the DM and IT groups, and remained elevated at 4–8 months (Fig. 3). Similarly, voiding frequency was significantly lower for the IT and DM groups than the AMC group at every time point (Fig. 4). There was no residual volume at any time (data not shown) in any group. Thus, despite changes of detrusor dynamics over time in both the DM and IT groups there was no element of bladder

FIG. 1. The variables of nerve conduction in caudal (A,B), digital (C) and tibial (D) nerves compared among the three groups, i.e. AMC (open blue circles), DM (closed red triangles) and IT (open green triangles) rats after different periods of hyperglycaemia (1–8 months). All values are the mean (SD) except B, which is the mean (SEM); A, caudal NCV; B, maximum amplitude of caudal nerve response; C, digital NCV; D, tibial nerve F-wave minimal latency. *P < 0.05, **P < 0.01, ***P < 0.001 for DM vs AMC; +P < 0.05, ++P < 0.01, +++P < 0.001 for IT vs AMC; and \$P < 0.05, \$\$P < 0.01, \$\$\$P < 0.001 for DM vs IT.

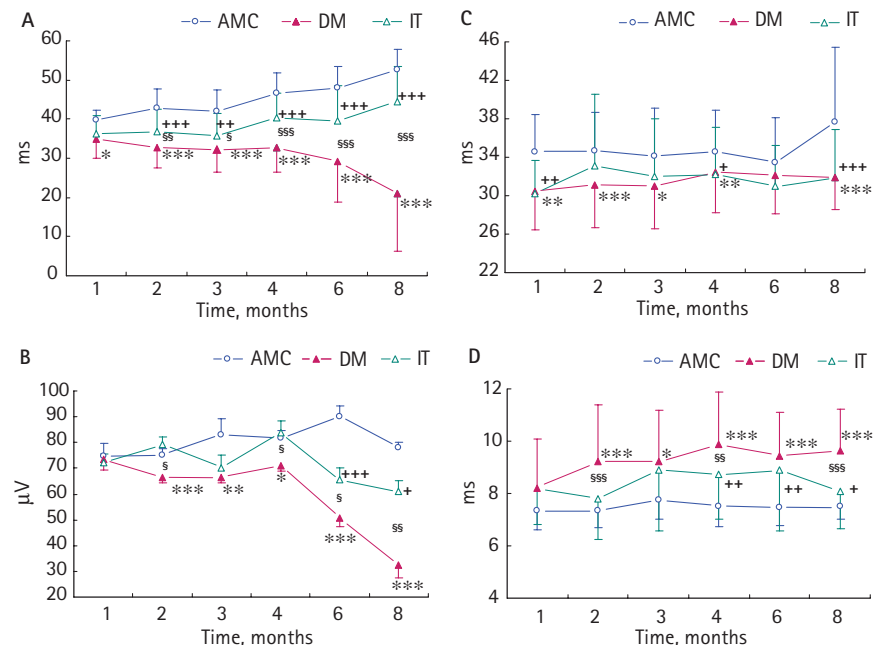
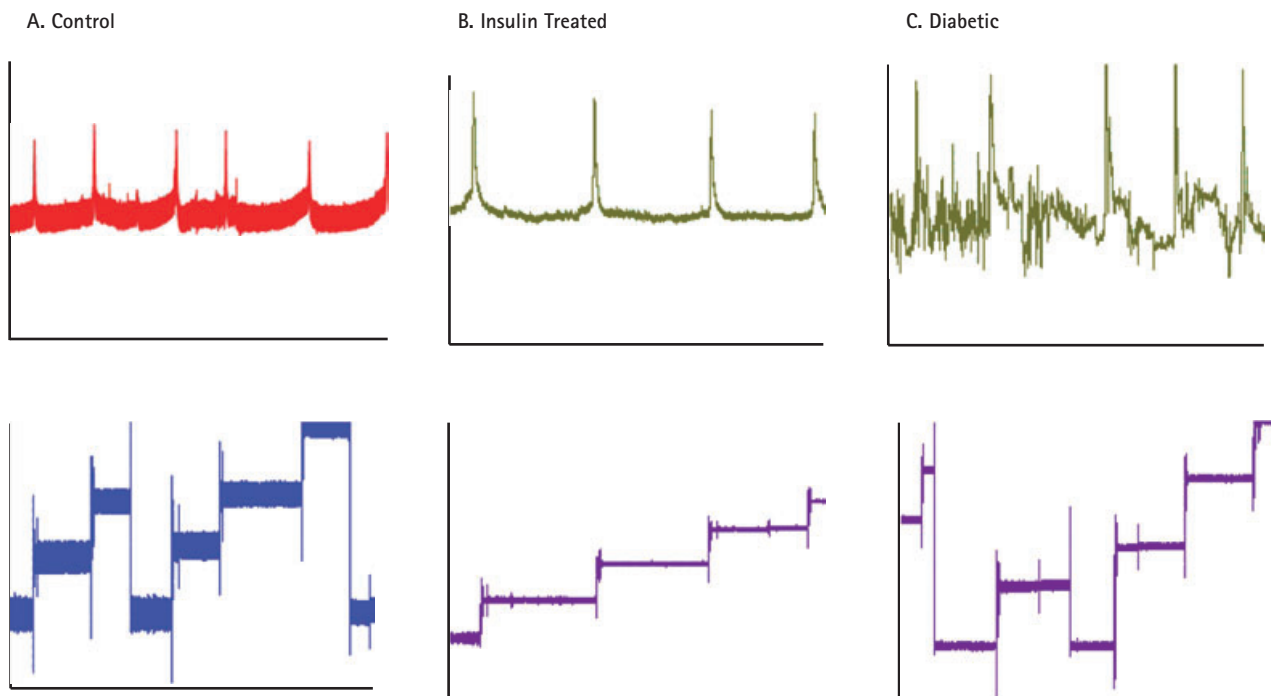


TABLE 3 The urodynamic variables during the course of the study

Duration of DM, months, and group	Bladder capacity, mL	Micturition volume, mL	Pressure, cmH ₂ O			
			Basal	Threshold	Micturition	IMP
1 DM	2.5 (0.1) ^j	2.5 (0.1) ^j	11.4 (1.3)	31.2 (2.6)	73.7 (5.4)	22.1 (2.2)
IT	1.8 (0.2) ^f	1.7 (0.2) ^f	6.6 (1.1)	20.1 (1.8)	56.2 (8.9)	13.0 (1.6)
AMC	1.1 (0.1) ^c	1.0 (0.1) ^c	8.7 (1.2) ^a	22.7 (1.7) ^c	50.4 (5.5) ^a	14.6 (1.0) ^b
2 DM	3.1 (0.1) ^j	3.0 (0.1) ^j	7.8 (0.7) ^h	24.8 (1.1)	68.4 (4.0)	17.6 (1.2)
IT	2.3 (0.1) ^j	2.2 (0.1) ^f	6.7 (1.0)	23.1 (2.0)	54.2 (4.4)	14.1 (1.8)
AMC	1.2 (0.1) ^c	1.2 (0.1) ^c	11.5 (1.2) ^a	26.2 (1.8)	49.3 (2.6) ^b	18.8 (1.7)
3 DM	3.2 (0.1) ^f	3.1 (0.1)	7.5 (0.7)	24.3 (1.3)	71.9 (5.0)	16.6 (1.2)
IT	3.4 (0.2) ^j	3.3 (0.2) ^f	5.6 (1.1)	22.4 (2.3)	65.4 (8.7)	13.5 (1.8)
AMC	1.1 (0.1)	1.0 (0.1) ^c	11.1 (2.5)	28.6 (2.9)	57.6 (6.7)	17.5 (3.8)
4 DM	3.1 (0.1) ^j	3.0 (0.1) ^j	8.1 (0.6)	24.4 (1.2)	75.7 (3.5) ⁱ	20.1 (1.3)
IT	2.7 (0.2) ^j	2.6 (0.2) ^f	6.2 (1.2)	20.0 (2.0)	58.9 (4.7)	13.7 (2.2)
AMC	1.4 (0.1) ^c	1.4 (0.1) ^c	10.1 (1.0) ^a	25.8 (1.3)	49.3 (2.6) ^c	16.7 (1.2) ^a
6 DM	3.2 (0.1) ^j	3.1 (0.1) ^j	8.3 (0.9)	25.5 (1.6)	71.5 (4.0)	19.5 (1.8)
IT	2.4 (0.2) ^f	2.4 (0.2) ^f	5.9 (1.0)	22.5 (2.8)	66.5 (10.4)	13.6 (2.4)
AMC	1.4 (0.1) ^c	1.4 (0.1) ^c	7.5 (1.0)	21.3 (1.3)	45.8 (2.6) ^b	12.4 (1.2) ^b
8 DM	3.3 (0.1) ^j	3.2 (0.1) ^j	8.8 (0.8)	27.7 (1.9)	76.7 (4.6) ⁱ	22.1 (1.9)
IT	2.8 (0.2) ^f	2.7 (0.2) ^f	5.4 (0.9) ^d	21.2 (1.4)	51.5 (3.2)	13.1 (1.4)
AMC	1.5 (0.1) ^c	1.4 (0.1) ^c	9.9 (1.0) ^a	25.8 (1.2) ^a	54.2 (2.9) ^c	18.2 (1.6) ^c

^aP < 0.05, ^bP < 0.01, ^cP < 0.001 for DM vs AMC, ^dP < 0.05, ^eP < 0.01, ^fP < 0.001 for IT vs AMC, and ^gP < 0.05, ^hP < 0.01, ⁱP < 0.001 for DM vs IT.

FIG. 2. A composite of three representative urodynamic evaluations of one AMC, IT and DM rat. The upper panels are the pressure measurements in the bladder and the lower panels show the cumulative voided urine volumes. Note the recurrent spontaneous contractions indicative of DO in the DM bladder, not present in either of the other rats.



decompensation at any time. The micturition pressure was higher in DM group than in the AMC at every sample time, and was higher than in the IT group at 4 and 8 months. Despite the similarity in the micturition volume in the IT and DM groups at 3–8 months there was a significant increase in DO (as SA) only in the DM group at 1, 4, 6 and 8 months, compared with the AMC group and at 6 and 8 months compared with the IT group (Fig. 5).

The results indicate an early (1 month) and persistent decrease in erectile function in the DM group. The IT group was not protected at 1 month (Fig. 6B), which might relate to the fact that the insulin therapy was begun 1 week after the STZ injection. The diminished erectile function imposed by hyperglycaemia was corrected from 2 months by the IT. Thus, two measures of myogenic impairment, erection and spontaneous detrusor activity, showed an early and persistent sustained effect in the diabetic rats.

We examined the correlation with the effects of DM in the penis and bladder. In the DM group, specific bladder measurements were correlated with ED measurements, but the strengths of the associations tended to diminish over time. For example, at 1 month, ICP/BP (at 4 mA) was positively correlated with bladder capacity ($r = 0.57$; $P = 0.02$) and micturition volume ($r = 0.63$; $P = 0.01$), but at 3 months both correlations were reduced to 0.35 ($P = 0.01$). The ICP/BP was also positively correlated with SA at 6 months ($r = 0.29$; $P = 0.06$), but the direction of the association reversed at 8 months ($r = -0.18$; $P = 0.26$). Bladder and ED measurements (4 mA) were less correlated in the IT group; the only significant correlation was at 2 months with SA ($r = -0.40$; $P = 0.04$). Thus, although there were significant mean differences between the AMC, IT and DM groups in both the ED and bladder studies throughout the study, variation in the individual rat organ response to hyperglycaemia limits a statistically significant correlation between the organs, and as a consequence was not useful for physiological predictions.

Correlations between measures of caudal nerve conduction and variables of bladder (capacity, IMP, SA and threshold pressure) or erectile function (ICP/BP) were evaluated by estimates of the SLR for each group of rats. All estimates of SLR were of a low value, and only a few were statistically significant. In the DM

FIG. 3. The bladder compliance in the AMC, IN and DM groups over time. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for DM vs AMC, + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$ for IT vs AMC, and § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ for DM vs IT.

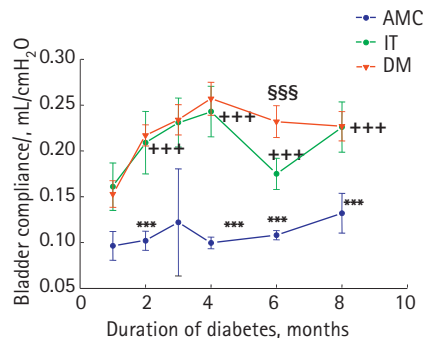
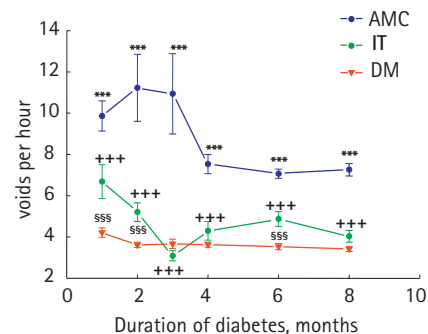


FIG. 4. The micturition frequency of the three groups during urodynamic testing over 8 months. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for DM vs AMC, + $P < 0.05$, ++ $P < 0.01$ and § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ for DM vs IT.



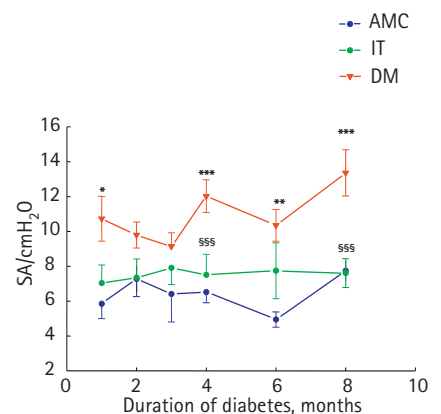
group, of 10 comparisons marked as statistically significant, nine were associated with ED. Caudal NCV correlated with values of ICP/BP at 2 (SLR 0.016, $P < 0.005$), 4 and 8 months of hyperglycaemia (SLR 0.012, $P < 0.01$). Caudal nerve amplitude correlated with measures of ICP/BP at values of SLR 10 times lower than for NCV comparisons. The IT group had characteristics of SLR similar to those in the DM group, but fewer were statistically significant. Therefore, similar to correlations across organ measures, both DM and IT groups had a weak association across neurophysiological and organ measures at the time points evaluated.

DISCUSSION

The recent reports of the unexpected negative effect of intensive targeted therapy to control

FIG. 5. The changes in SA (calculated by subtracting the basal pressure from the IMP) of the bladder over the 8 months of the trial in the three groups.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for DM vs AMC, + $P < 0.05$, ++ $P < 0.01$ and § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ for DM vs IT. The effect of DM on erectile function as determined by cavernous nerve stimulation at two of the levels of stimulation on the same three groups of rats is shown in Fig. 6.



hyperglycaemia on cardiovascular events in an epidemiological study of type 2 diabetes underscores the fact that many aspects of the effect of diabetes and its therapy remain to be elucidated [16]. The goal of the present study was to compare the simultaneous physiological manifestation of experimental DM on two urogenital organs known to be affected by DM, and to correlate those changes with objective measures of nerve function (i.e. NCV) over a period extending up to 8 months. A key finding was that various measures of urogenital function showed deficits by 1 month, which was the earliest sample examined after inducing DM. These changes were concomitant with the initial significant deficits in somatic NCV. Further, the bladder, ED and NCV effects induced by hyperglycaemia were persistent throughout the study, but for each measure, the progression over the 8 months was less severe than anticipated. For some measures (i.e. bladder capacity), the magnitude of the deficit at 8 months was similar to that detected at 2 months. One possibility for this relative lack of progression is that genetic variation among the rats resulted in only the 'strongest' or 'most adaptable' rats surviving hyperglycaemia over the extended period of this investigation. Thus in the later sample times, the mean values reflected a type of 'survivor' bias, despite the documented presence of persistent hyperglycaemia.

FIG. 6. The effect of cavernous nerve electrostimulation at 4 (A) and 10 mA (B) on the ICP/BP in the three groups of rats over the duration of the study. Note that the ICP/BP ratio of the DM rats was below the threshold of 0.6 considered to represent ED, at all sample time except 6 months. The ICP/BP in each of the other groups was always higher than 0.6, the ratio generally needed to achieve a minimal erection sufficient for coitus. *P < 0.05, **P < 0.01, ***P < 0.001 for DM vs AMC, +P < 0.05, ++P < 0.01 and SP < 0.05, \$\$\$P < 0.01 and \$\$\$SP < 0.001 for DM vs IT.

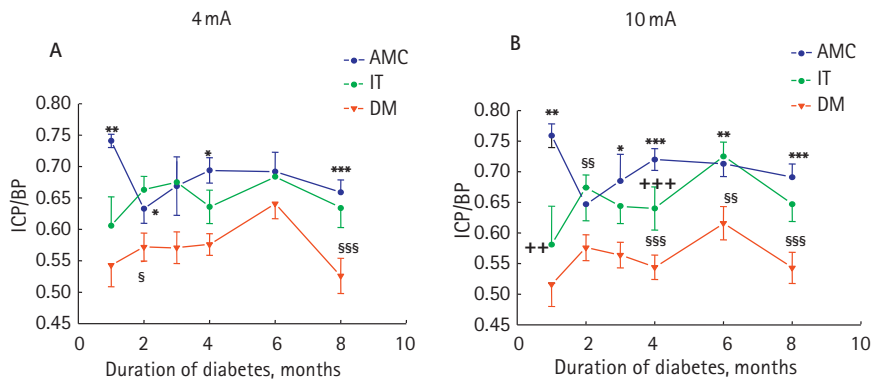


TABLE 4 Cellular and subcellular findings from previous reports

Effect	Corpora	Bladder	Reference
C _α 43	decreased	up-regulated	[22]
P ₂ Y ₂ R	decreased	up-regulated	[22]
P ₂ X ₁ R	increased	unchanged	[22]
P ₂ Y ₄ R	unchanged	up-regulated	[22]
P ₂ X ₄ R	decreased	unchanged	[22]
P ₂ X ₇ R	unchanged	unchanged	[22]
Maxi-K	decreased activity	not measured	[27]
K _{ATP}	decreased activity	not measured	[27]
VCSA1 Expression	down-regulated	down-regulated	[21]
RhoA	increased	not measured	[19]
ROK α & ROK β	increased	not measured	[19]
Endothelin-1	increased	not measured	[19]
ECE-1	increased	not measured	[19]

The effect of DM on the penis was to reduce the maximum ICP (i.e. the rats had the functional equivalent of ED). The effect of DM on the bladder was a volume effect of increased bladder weight and micturition volume, but heightened detrusor smooth muscle activity, as shown by an increase in threshold pressure and SA. This finding is equivalent to DO in clinical patients, a symptom that is becoming increasingly recognized as being prevalent in people with DM [17]. Of great importance is that there was no deterioration or decompensation of bladder function, as shown by the lack of change in residual volume in the DM rats during the 8 months of the study. This finding differs from the results reported by Daneshgari *et al.* [18], where after the ninth

week of a 20-week study of STZ-induced DM there was a decreased 'peak detrusor leak pressure' and increased resting pressure. The discrepancy across studies might be related to the use of Sprague-Dawley rats and anaesthesia for urodynamic testing in the earlier study [18]. In the present study, the dual urogenital organ dysfunction, ED and DO, as a consequence of hyperglycaemia, was prevented or modified by IT begun 1 week after the onset of the hyperglycaemia. We reported similar changes in organ function after 2 months of DM [13]. The novelty of the present report is the long-term (8 months) study in a very large number of rats. Our findings indicate that, in the STZ-induced DM rat model, the early changes in organ function that occur within a month do not continue to

diminish, despite deterioration in both peripheral and autonomic nerve function and progressive weight loss in the DM rats. A panorama of changes at the cellular and subcellular level, that were a component of the overall examination of those same rats by other members of our research group, produced findings at the tissue, molecular and gene expression level (published elsewhere) that were consistent with the physiological manifestation of heightened smooth muscle tone and the subsequent impaired organ function that would be expected from those changes [19–26]. Examination of the corpus cavernosum from the same male diabetic rats used in the current study revealed an increase in the expression of the endothelin/Rho-kinase (ROK) contractile pathway [19]. In particular, the expression of the two known isoforms of ROK (ROK α and β), as well as the ROK activator RhoA, were significantly increased at both mRNA and protein levels after 2 and 6 months of STZ-induced DM when normalized to smooth muscle α -actin expression. In addition, the expression of the potent vasoconstricting peptide endothelin-1, as well as endothelin-converting enzyme-1 (ECE-1, that converts Big endothelin-1 to mature endothelin-1), were also significantly increased after both 2 and 6 months of DM. Endothelin-1 stimulates Rho kinase, which is involved in calcium sensitization of corpus cavernosal smooth muscle, tipping the balance in favour of a contracted, flaccid, penile state. Thus, the early and long-term up-regulation of ECE-1, endothelin-1 and both ROK isoforms might partly explain the pathogenesis of DM-related ED. A summary of those findings is shown in Table 4 [19,22,27].

Sullivan *et al.* [28] recently reported that in the cavernosum of STZ-induced DM F-344 rats there was changed expression of at least 529 transcripts compared to controls. Gene ontology classification indicated that there was a decrease in numerous extracellular matrix genes and an increase in oxidative stress-associated genes in the DM rat cavernosum. Recent studies by our group also showed that in the cavernosum and bladder the onset of STZ-induced DM is associated with changes in many genes, and that gene ontology analysis identifies the expression of oxidative stress-associated genes as a significantly altered ontological theme (Hipp *et al.* unpublished, and data not shown). Overall, the combined gene expression and

physiological studies highlight the potential complexity of these DM-induced urogenital disorders [28]. Because both sensory distal symmetric polyneuropathy and distal autonomic neuropathy are such an important component of the human manifestations of the disease, nerve function was carefully measured in the experimental rats in the present study.

Electrophysiological evaluation of nerve conduction in hyperglycaemic rats confirmed the presence of neuropathy in all three somatic nerves evaluated, with the initial effects evident at 1 month. These observations were expected and consistent with previous publications [15,26,29–33]. The unique feature of the electrophysiology in the present study was the scope of the assessment, which included >800 rats, three distinct nerves, velocity, amplitude and F-wave endpoints, and evaluation over a period of 8 months. NCV in the distal segments of the digital and caudal nerves, dominated by sensory axons, showed the earliest onset of deficits, followed by changes in motor nerve conduction in the tibial nerve. This sequence is consistent with a predominate sensory, length-dependent distal axonopathy [29,32,34]. Also as expected, there was a clear progression of the slowing in the caudal nerve, which continued for the period between 4 and 8 months, accompanied by continued reduction in the response amplitude. The caudal nerve in the distal tail is substantially longer than any other nerve in the rat. However, contrary to expectations, the measures of digital NCV and F-wave did not significantly worsen over the later sample times. As indicated above, this relative lack of progression might be due to a 'survivor effect', with loss of measures in the most severely affected rats at the later times.

Previously published reports that compared somatic nerve electrophysiology and measures of autonomic dysfunction in DM have reported inconsistent results. Some studies reported a close relationship between DM-induced pathology in somatic NCV and autonomic/visceral functions [33,35,36], while others failed to identify a clear correlation in these measures [37–39]. In our previous studies, we found a strong correlation between measures of cystometry and caudal NCV in a population which included both DM and non-DM rats, and a clear association between abnormal

electrophysiological responses in both somatic and autonomic nerves (i.e. distal cavernous nerve) and ICP reflecting ED [26,33].

In the present study, the initial deficits in NCV were concomitant with abnormalities in cystometry and with ED. However, the progressive deterioration of caudal NCV and amplitude after 4 months of hyperglycaemia was in contrast to findings in bladder and for ED that appeared to plateau after the initial deficits detected over the first few months. This apparent discrepancy might have three overlapping explanations. (i) The effects of prolonged hyperglycaemia might be relatively greater on the vulnerable segments of peripheral nerves than on whole organ systems. (ii) Neuropathy is clearly a contributing element in urogenital dysfunction associated with DM, but it is only one of many factors controlling bladder function. The system might compensate for deficits in one or more of the controlling factors [40]. (iii) The progressive effects on the very long somatic nerves measured in the present study are probably greater than the induced changes in the shorter autonomic axons innervating the bladder and cavernosa. This possibility is supported by the recent report of histopathological changes in the distal shaft of the penis that were limited to abnormal accumulations of glycogen granules in isolated unmyelinated and pre-terminal axons, and necrosis of scattered smooth muscle fibres in rats even after prolonged periods of hyperglycaemia [26].

In summary, the results of this extensive, long-term study show early and profound effects of hyperglycaemia on the smooth muscle of the penis and bladder that were persistent and stable in the surviving rats over the 8 months of study. Those physiological changes did not correlate well with neurological measurements of those organs. Of significance is that diverse smooth muscle cellular and subcellular events antedated the measured neurological manifestations of the hyperglycaemia by several months. Although autonomic diabetic neuropathy is a primary life-threatening complication of long-term DM in humans, the rat model of STZ-induced DM shows that the rapid onset physiological manifestations are based on many molecular changes in the smooth muscle cells themselves in this model of type 1 DM.

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CONFLICT OF INTEREST

None declared.

REFERENCES

- 1 **Boulton AJ, Vinik AI, Arezzo JC et al.** Diabetic neuropathies: a statement by the American Diabetes Association. *Diabetes Care* 2005; **28**: 956–62
- 2 **Fox CS, Coady S, Sorlie PD et al.** Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. *Circulation* 2007; **115**: 1544–50
- 3 **Vinik AI, Maser RE, Mitchell R, Freeman R.** Diabetic autonomic neuropathy. *Diabetes Care* 2003; **26**: 1553–79
- 4 **Brown JS, Wessells H, Chancellor MB et al.** Urologic complications of diabetes. *Diabetes Care* 2005; **28**: 177–85
- 5 **Kaplan SA, Te AE, Blaivas JG.** Urodynamic findings in patients with diabetic cystopathy. *J Urol* 1995; **153**: 342–4
- 6 **Sasaki K, Yoshimura N, Chancellor MB.** Implications of diabetes mellitus in urology. *Urol Clin North Am* 2003; **30**: 1–12
- 7 **Yoshimura N, Chancellor MB, Andersson KE, Christ GJ.** Recent advances in understanding the biology of diabetes-associated bladder complications and novel therapy. *BJU Int* 2005; **95**: 733–8
- 8 **Bacon CG, Hu FB, Giovannucci E, Glasser DB, Mittleman MA, Rimm EB.** Association of type and duration of diabetes with erectile dysfunction in a large cohort of men. *Diabetes Care* 2002; **25**: 1458–63
- 9 **Ellenberg M.** Sexual function in diabetic patients. *Ann Intern Med* 1980; **92**: 331–3
- 10 **Ellenberg M.** Diabetes and sexual function. *N Y State J Med* 1982; **82**: 927–30
- 11 **Abrams P, Cardozo L, Fall M et al.** The standardisation of terminology of lower

- urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *NeuroUrol Urodyn* 2002; **21**: 167–78
- 12 **Chang S, Hypolite JA, Changolkar A, Wein AJ, Chacko S, DiSanto ME.** Increased contractility of diabetic rabbit corpora smooth muscle in response to endothelin is mediated via Rho-kinase beta. *Int J Impot Res* 2003; **15**: 53–62
 - 13 **Christ GJ, Hsieh Y, Zhao W et al.** Effects of streptozotocin-induced diabetes on bladder and erectile (dys)function in the same rat in vivo. *BJU Int* 2006; **97**: 1076–82
 - 14 **Schmelzer JD, Low PA.** Electrophysiological studies on the effect of age on caudal nerve of the rat. *Exp Neurol* 1987; **96**: 612–20
 - 15 **Stevens MJ, Li F, Drel VR et al.** Nicotinamide reverses neurological and neurovascular deficits in streptozotocin diabetic rats. *J Pharmacol Exp Ther* 2007; **320**: 458–64
 - 16 **Gerstein HC, Miller ME, Byington RP et al.** Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008; **358**: 2545–59
 - 17 **Yu HJ, Lee WC, Liu SP, Tai TY, Wu HP, Chen J.** Unrecognized voiding difficulty in female type 2 diabetic patients in the diabetes clinic: a prospective case-control study. *Diabetes Care* 2004; **27**: 988–9
 - 18 **Daneshgari F, Liu G, Imrey PB.** Time dependent changes in diabetic cystopathy in rats include compensated and decompensated bladder function. *J Urol* 2006; **176**: 380–6
 - 19 **Chua R, Tar M, Melman A, DiSanto ME.** Streptozotocin-induced diabetes results in time-dependent upregulation of the endothelin/Rho-kinase pathway in rat corpus cavernosum smooth muscle. *J Sex Med* 2006; **4** (Suppl. 1): 25
 - 20 **Davies KP, Zhao W, Tar M et al.** Diabetes-induced changes in the alternative splicing of the slo gene in corporal tissue. *Eur Urol* 2007; **52**: 1229–37
 - 21 **Hipp JD, Davies KP, Tar M et al.** Using gene chips to identify organ-specific, smooth muscle responses to experimental diabetes: potential applications to urological diseases. *BJU Int* 2007; **99**: 418–30
 - 22 **Suadicani SO, Urban-Maldonado M, Tar MT, Melman A, Spray DC.** Effects of ageing and streptozotocin-induced diabetes on connexin 43 and P2 purinoceptor expression in the rat corpora cavernosa and urinary bladder. *BJU Int* 2009 Jan 14. [Epub ahead of print]
 - 23 **Tong Y, Tiplitsky SI, Tar M, Melman A, Davies KP.** Transcription of G-protein coupled receptors in corporeal smooth muscle is regulated by the endogenous neutral endopeptidase inhibitor sialorphin. *J Urol* 2008; **180**: 760–6
 - 24 **Tong Y, Tar M, Melman A, Davies K.** The opiorphin gene (ProL1) and its homologues function in erectile physiology. *BJU Int* 2008; **102**: 736–40
 - 25 **Yohannes E, Chang J, Christ GJ, Davies KP, Chance MR.** Proteomics analysis identifies molecular targets related to diabetes mellitus-associated bladder dysfunction. *Mol Cell Proteomics* 2008; **7**: 1270–85
 - 26 **Zotova EG, Schaumburg HH, Raine CS et al.** Effects of hyperglycemia on rat cavernous nerve axons: a functional and ultrastructural study. *Exp Neurol* 2008; **213**: 439–47
 - 27 **Zhao W, Brink P, Christ G.** Alterations in second messenger regulation of potassium channel activity in corporal smooth muscle cells following w months of streptozotocin (STZ)-induced diabetes. *J Urol* 2008; **179**: 223
 - 28 **Sullivan CJ, Teal TH, Luttrell IP, Tran KB, Peters MA, Wessells H.** Microarray analysis reveals novel gene expression changes associated with erectile dysfunction in diabetic rats. *Physiol Genomics* 2005; **23**: 192–205
 - 29 **Arezzo JC, Zotova E.** Electrophysiologic measures of diabetic neuropathy: mechanism and meaning. *Int Rev Neurobiol* 2002; **50**: 229–55
 - 30 **Kato N, Makino M, Mizuno K, Suzuki T, Shindo M.** Serial changes of sensory nerve conduction velocity and minimal F-wave latency in streptozotocin-induced diabetic rats. *Neurosci Lett* 1998; **244**: 169–72
 - 31 **Kato N, Mizuno K, Makino M, Suzuki T, Yagihashi S.** Effects of 15-month aldose reductase inhibition with fidarestat on the experimental diabetic neuropathy in rats. *Diabetes Res Clin Pract* 2000; **50**: 77–85
 - 32 **Li F, Abatan OI, Kim H et al.** Taurine reverses neurological and neurovascular deficits in Zucker diabetic fatty rats. *Neurobiol Dis* 2009; **22**: 669–76
 - 33 **Zotova EG, Christ GJ, Zhao W, Tar M, Kuppam SD, Arezzo JC.** Effects of fidarestat, an aldose reductase inhibitor, on nerve conduction velocity and bladder function in streptozotocin-treated female rats. *J Diabetes Complications* 2007; **21**: 187–95
 - 34 **Schaumburg HH, Berger AR.** Anatomic classification of peripheral nervous system disorders. In Schaumburg HH, Berger AR eds, *Disorders of Peripheral Nerves*. Philadelphia: FA Davis Co, 1992: 10–24
 - 35 **Mitsui T, Kakizaki H, Kobayashi S, Morita H, Matsumura K, Koyanafi T.** Vesicourethral function in diabetic patients: association of abnormal nerve conduction velocity with vesicourethral dysfunction. *NeuroUrol Urodyn* 1999; **18**: 639–45
 - 36 **Watanabe T, Miyagawa I.** Effects of long-chain fatty alcohol on peripheral nerve conduction and bladder function in diabetic rats. *Life Sci* 2002; **70**: 2215–24
 - 37 **Tentolouris N, Pagoni STA, Katsilambros N.** Peripheral neuropathy does not invariably coexist with autonomic neuropathy in diabetes mellitus. *Eur J Int Med* 2001; **12**: 20–7
 - 38 **Töyry JP, Partanen JV, Niskanen LK, Lansimies EA, Uusitupa MI.** Divergent development of autonomic and peripheral somatic neuropathies in NIDDM. *Diabetologia* 1997; **40**: 953–8
 - 39 **Winkler AS, Ejskjaer N, Edmonds M, Watkins PJ.** Dissociated sensory loss in diabetic autonomic neuropathy. *Diabetes Med* 2000; **17**: 457–62
 - 40 **Ellenberg M.** Development of urinary bladder dysfunction in diabetes mellitus. *Ann Intern Med* 1980; **92**: 321–3
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- Abbreviations:** AMC, age-matched control; BP, blood pressure; DM, diabetes mellitus; IT, insulin-treated; ED, erectile dysfunction; DO, detrusor overactivity; STZ, streptozotocin; ICP, intracavernous pressure; NCV, nerve conduction velocity; IMP, intermicturition pressure; SA, spontaneous activity; SLR, slope of linear regression; ROK, Rho-kinase; ECE-1, endothelin-converting enzyme-1.