Mechanisms of Action for Diabetic Bladder Dysfunction — State of the Art

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Abstract

Purpose of Review Diabetes (DM) is a common cause of lower urinary tract symptoms (LUTS), known as diabetic bladder dysfunction (DBD). The phenotype for DBD is described in the literature with considerable heterogeneity and includes poor sensation, increased compliance, detrusor underactivity, urinary retention, weak stream, hesitancy, but also urgency incontinence, and, for many, detrusor overactivity. Progress has been made in understanding DBD, yet a unified phenotype or classification system for DBD remains elusive. Much remains unknown about the underlying mechanisms.

Recent Findings No classification scheme to phenotype DBD has been proposed. Evidence from recent literature suggests four primary drivers: detrusor smooth muscle dysfunction, urothelial dysfunction, autonomic neurologic dysfunction, and circulating and systemic factors such as inflammation, oxidative stress, and microvascular damage. It is likely that these drivers have multi-factorial causes and inter-relate in complex ways. Recent findings in animal models lend new support to detrusor smooth muscle dysfunction as well as inflammation. Reports utilizing next-generation sequencing have begun to appear in the DBD literature and promise further insight.

Summary DBD currently lacks a unified classification scheme and a clear mechanism. The advent of new, more translatable large animal models and next-generation sequencing promises many exciting new tools and models that more closely translate to human disease.

Abbreviations

AUA	American Urological Association
DBD	Diabetic bladder dysfunction
DM	Diabetes mellitus
DM1	Type 1 diabetes mellitus
DM2	Type 2 diabetes mellitus
HgbA1C	Hemoglobin A1c
iNOS	Inducible nitric oxide synthase
IPSS	International prostate symptom score
JAK	Janus tyrosine kinase
LUTS	Lower urinary tract symptoms
MetS/D	Metabolic syndrome/diabetes
NLRP3	NACHT, LRR, and pyrin domains containing protein 3
OAB-q	Overactive bladder quantification questionnaire
$P2X_3$	Purine receptor involved in nociception and sensory hypersensitization
PVR	Post void residual
RT-PCR	Reverse transcription polymerase chain reaction
STAT-1	Signal transducers and activators of transcription-1
TLR-4	Toll-Like Receptor 4
TRPV1	Transient receptor potential cation channel subfamily V member 1

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Introduction

Diabetes (DM) is prevalent worldwide, particularly in affluent nations, having an estimated 382 million people affected in 2013 with 592 million projected by 2035 [1]. This leads to significant healthcare cost, patient disability, and even

premature death. Quality of life is severely impacted, and lower urinary tract symptoms (LUTS) that can result have been termed "diabetic cystopathy" or "diabetic bladder dysfunction" (DBD). Prevalence studies have estimated that DBD affects 80–93% of those with diabetes [2, 3••]. Currently, the clinical phenotype is heterogenous and the mechanism of DBD is poorly understood.

DBD was first described by Jordan et al. in 1935 and later popularized in 1976 by Frimodt-Moller to describe later-stage voiding dysfunction thought to be secondary to autonomic neuropathy in diabetic patients [4, 5]. The proposed natural history begins with impaired bladder sensation, ultimately progressing to impaired contractility and urinary retention [6]. Traditionally, DBD has been characterized as "a triad of decreased bladder sensation, increased bladder compliance and capacity, and impaired detrusor contractility" [7]. Currently, DBD can describe a wide range of LUTS, including detrusor overactivity (DO), as well as impaired bladder contractility, areflexic bladder, urinary retention, overflow incontinence, and urge incontinence. It is associated with decreased sensation to filling and increased bladder capacity, making the phenotype quite heterogenous [7, 8]. The purpose of this review is to summarize the clinical phenotypes of DBD as described in the literature and then outline the most recent evidence supporting 4 main hypotheses driving the clinically observed phenotypes.

Clinical Phenotype of Diabetic Bladder Dysfunction

Urodynamic studies have been a key method used to study DBD. Kaplan, Te, and Blaivas retrospectively examined 182 consecutive diabetic patients who underwent videourodynamic studies and found that detrusor *hyperreflexia* occurred in 55% which appears to contradict the dogma of poor sensation and detrusor underactivity (DU). In fact, they found that only 23% exhibited detrusor *underactivity* (DU), suggesting DBD is more likely to cause DO instead of DU [9]. Lee et al. prospectively enrolled 86 consecutive women from a diabetes care clinic and performed urodynamic studies. They found that, unlike Kaplan et al., more women exhibited DU than DO. Thirty-four percent of the DM2 women studied exhibited DU while 14% exhibited DO. Thirty-eight percent exhibited normal urodynamic findings [10•]. This heterogeneity of clinical phenotype is likely due to the progressive nature of DBD, which is thought to worsen with time and progression of DM, as noted by Lifford [11]. Moreover, it is *unlikely that duration and severity of DM at enrollment in future clinical studies will ever be controllable*. This makes the preclinical animal model crucial to moving forward in our understanding of DBD. The largest, prospective study of diabetic women with urodynamic outcomes examined 1640 consecutive women from Dujiangyan, China. All carried a diagnosis of DM. Urodynamic studies were prospectively performed after enrollment. Ninety-three percent self-reported LUTS and 88% had abnormal findings on urodynamic examination [3••]. Fifty-six percent exhibited DO and 48% exhibited DU by the authors' definition. Eight percent were found to have detrusor areflexia by the authors' urodynamic criteria, with 29% of the impaired contractility patients having diminished renal function with bilateral hydronephrosis [3••]. This underscores the potentially life-threatening consequences of DBD. All of the above clinical reports of urodynamic findings in DBD have lacked a comparison group of nondiabetic subjects. Malik et al. recently published a comparison of DM to non-DM women presenting to tertiary care centers for LUTS [12•] . The authors identified 384 subjects, of whom 88 (26%) self-reported DM, with no distinction between DM1 and DM2. Although there was no difference in the self-reported LUTS at presentation between groups, the DM group exhibited more DU, defined as not being able to achieve detrusor contraction >10 cm H2O from baseline and hold this for >10 s (30% vs. 18%, $p<0.05$). DM women also reported impaired sensation to filling and impaired desire to void more often than controls (17% vs. 5%, $p<0.005$). There was no difference in PVR (99 ± 46 for DM vs. 70 ± 147, $p= 0.224$). Hgb A1c >7.5% was associated with impaired sensation as well as impaired desire to void, underscoring the role of severity of DM. Duration of DM > 10 years was similarly associated with the impaired desire to void, elevated post void residual, lower detrusor pressures, and maximum urodynamic capacity.

To summarize, DBD lacks a standardized clinical definition and one can see the challenges of defining DBD based on urodynamic findings alone. The previously described series lack a consistent clinical phenotype. To help the reader better understand the landscape of medical literature with regard to DBD, Table 1 has been assembled. Table 1 lists key clinical studies having urodynamic outcomes, including those already described.

One can see that DBD lacks a predictable pattern when examining the published literature. Clinical research shows that the nature and severity of LUTS differ between DM1 and DM2, the duration of DM affects prevalence of LUTS, as does Hgb A1c, and so does the presence of end-organ damage such as neuropathy. Not surprisingly, the age of the patient also has a significant independent effect and must be controlled for in order to draw conclusions about the contribution of DM on DBD. No clinical report integrates all of these outcome measures (Table 1). Hypotheses can be generated based on clinical literature, and animal models can then be used to test them. Four key hypotheses will be explored focusing on the target tissue that is affected. It is thought that systemic factors such as inflammation, oxidative stress, and hyperglycemia contribute to damage in these

Table 1 Summary of key studies describing diabetic bladder dysfunction having urodynamic outcomes

Finding	Lead author	Participants
Detrusor overactivity (DO) in 55% Detrusor *underactivity* (DU) in 23% Detrusor areflexia in 10% Normal urodynamic findings (normal) in 1%	Kaplan 1995 [9]	n=182 retrospective urodynamic study, male and female, all diabetic, mixture of DM1 and DM2
Overall 11/29 had "voiding dysfunction" If motor nerve conduction velocity (MCV) of the peroneal nerve was Abnormal: "voiding dysfunction" in 11/16. If normal 0 had "voiding dysfunction"	Mitsui 1999 [51]	N=29 males and females with DM. voiding dysfunction was defined as elevated first sensation to void >300mL, PVR < 50mL, and maximum bladder capacity >500mL
PVR > 100cc in 13.9% of DM vs. 1.8% of controls Qmax 19.4cc/sec in DM vs. 25.9cc/sec in controls Emptying efficiency (voided volume /PVR) worse in DM with Peripheral neuropathy or duration of DM >10 and Age (34.6% vs. 21% of women with diabetes vs. controls)	Lee 2004 [17]	n=194 females with DM1 or DM2 vs. n=162 control females
DO in 25% of males DO in 31% of females BOO in 25% of males Urge incontinence in 12.5% of females Stress incontinence in 12.5% of females Males more likely to have DBD for age >64 years, DM duration >9 years, HgbA1c >7.9% Females more likely to have DBD for age >56 years, DM duration >8 years, HgbA1c >7	Kebapci 2007 [6]	n=54 males and females with DM2.
Neuropathy in 4% DO in 42% of those with DO, 76.5% were noted on MRI to have Multiple cerebral infarcts DU in 48% Impaired sensation (first sensation >300cc) in 32% PVR >30mL in 57%	Yamaguchi 2007 [50]	N=84 males and females with peripheral neuropathy retrospectively identified
DU in 34% Normal in 38%	Lee 2009 [10•]	n= 86 females with DM2
Impaired sensation (>250 mL): 23% Increased capacity (>600 mL): 25% DU in 78% DO in 38% PVR>30% capacity in 65% Bladder outlet obstruction (BOO, Abrams-Griffiths number >40) in 28% Neuropathy predicted urodynamic abnormality, increased further if abnormal sensory plus nerve conduction velocity	Bansal 2011 [15]	n=52 males, 51.9% demonstrated neuropathy based on sensory and nerve conduction velocity
DO in 56% DU in 48% mean PVR 323 mL Detrusor areflexia in 8%	Changxiao 2014 [3••]	n=1640 consecutive females with DM
Stress urinary incontinence in 48% DO in 23% DU in 11% Normal in 16%	Karoli 2014 [64]	N=46 women with DM2
3 categories of DM severity: Group 1 (no DR, no DN) n = 25 DO in 20% DHIC in 24% DU in 28% BCI 106 ± 31 PVR 7 ± 92 Group 2 (only DR, no DN) n = 18 DO in 0% DHIC in 44% DU in 38% BCI 74 ± 27 PVR 147 ± 151 Group 3 (both DR + DN) n=14 DO in 0% DHIC in 28% DU in 57% BCI 68 ± 31 PVR 150 ± 195	Majima 2019 [65]	n=57 men with DM2 categorized has having diabetic retinopathy (DR) and/or diabetic nephropathy (DN). Bladder contractility index (BCI) was also calculated

Table 1 (continued)

Finding	Lead author	Participants
DU in 30% vs 18% DO 23% vs 22% Impaired sensation 17% vs 5% Capacity 493 vs 409 $p=0.005$ PVR 99 vs 70, $p=0.224$ Duration of DM > 10 y impaired desire to void, elevated post void residual, lower detrusor pressures, and higher maximum urodynamic capacity A1C>7.5 conferred poor sensation, duration	Malik 2020 [12•]	Comparison of $n=88$ DM and $n=296$ non-DM women with urodynamic study results from multiple US institutions

target tissues and cause symptoms themselves independent of the target tissue. The evidence supporting these hypotheses will be explored.

Differences Between DM1 and DM2

There are differences between type 1 diabetes (DM1) and type 2 (DM2) with regard to DBD. DBD is seen in 43–87% of DM1 and 25% of those with DM2 [3••, 13]. The majority of reports in the literature are retrospective prevalence studies, with little insight as to mechanism, and most do not follow patients over time. Clinical retrospective studies have failed to consistently show the pattern expected by Frimodt-Moller [14, 15].

Incontinence has been primarily associated with DM2 rather than DM1. Analysis from the Nurse's Health Study and Nurse's Health Study II provides large-scale evidence of the differences in DBD. These are longitudinal prospective observational studies examining the health of 71,650 nurses ages 37–79 from 1976 to 2000. Nurses Health Study began in 1976 when 121,700 married female registered nurses aged 30–55 who lived in 11 US states completed a mailed questionnaire. Follow-up questionnaires are sent every 2 years, and the accuracy of the self-reported data has been validated by multiple independent studies. A wide range of medical conditions is queried, including DM1, DM2, and urinary incontinence. Danforth et al. performed pooled multiple logistic regression analysis and revealed a 20% increased prevalence of urinary incontinence affecting women with DM2. The investigators attempted to control for factors that also contribute to incontinence (OR 1.2, 95% CI 1–1.3) [16]. Incontinence was defined as 1+ leakage episodes in a 1-week period. Supporting the clinical results of Danforth et al., Lifford et al. also analyzed 81,845 women from the Nurses Health Study data set and appreciated a 50–200% increase in prevalence for urinary incontinence in women with DM2, compared to those reporting no DM2. DM1, gestational diabetes, and unconfirmed reports of DM were excluded from the analysis. Additionally, they noted that a longer duration of DM2 and the presence of microvascular disease increased the risk of incontinence [11, 17]. Not surprisingly, DM1 has also been associated with incontinence. The Epidemiology of Diabetes Interventions and Complications (EDIC) is another longitudinal prospective observational trial designed to provide insight into the complications of diabetes, specifically DM1. This project examined subjects from the diabetes control and complications trial (DCCT, 1983–1993) who were then enrolled in a follow-up longitudinal prospective observational study (EDIC, 1994–present). All subjects were women with DM1 who completed a urology-specific questionnaire after 10 years of involvement (2003) and after 17 years (2010). Investigators noted an increase in the odds ratio of 1.41 for every 1% Hgb A1c increase (95% CI 1.07–1.89) through the study period [18••]. Another prospective diabetes registry in Hong Kong noted a 19.7% incidence of chronic kidney disease over a median follow-up period of 7.2 years [19]. There is a link between DBD and other end-organ damage, such as peripheral neuropathy [20, 21]. Although a predictable pattern has not emerged, it is clear DM is associated with significant urologic complications [19–22].

Much more published data is available from animal models of DBD compared to human clinical data. The disease can be manipulated; animals can undergo invasive procedures not possible in humans and can be sacrificed for tissue analysis. Mouse models have a compressed lifespan and lower cost, allowing the efficient study of disease processes that can take years to develop in humans [23]. Animal models have been used to test hypotheses generated during clinical observation, with most investigators describing small animal bladder models for both DM1 and DM2, such as mice, rats, and rabbits. Large animal models of DBD are rare. One advantage to large animal models is that they may be better approximations to human urinary physiology in terms of the size of kidneys, ureter, and bladder which helps for device development, quantity of tissue available, and volume of urine voided. Urodynamic equipment and techniques developed for humans can be used on large animals instead of creating non-physiologic surgically implanted suprapubic

catheter-style urodynamic apparatuses. The Ossabaw miniature pig (*Sus scrofa domesticus, Ossabaw Island Hog*) was developed for this purpose, as a large animal model for diet-induced bladder complications of MetS and may more closely model the human bladder dysfunction caused by MetS not only in size but also because it can develop MetS through diet alone as humans do [24•].

Four Main Hypotheses Driving Diabetic Bladder Dysfunction

Smooth Muscle Dysfunction Hypothesis

Although the pathogenesis of DBD is likely multifactorial, it is critical to understand the key drivers and how they contribute. The earliest theories have hypothesized that neuropathy is the primary driver for DBD; however, recent evidence presents a strong case for smooth muscle dysfunction which carries important therapeutic implications.

The problem of heterogenous DBD phenotypes

Preclinical rodent models have revealed an early hypercontractile bladder phenotype followed by a late, hypo-contractile phenotype [25•]. This "temporal hypothesis" has been widely accepted but has not yet been demonstrated in humans [26]. It would explain why the clinical investigation of DBD has failed to produce a reproducible phenotype, as described above. Detrusor smooth muscle (DSM) Ca^{2+} transporters drive contractility and some have proposed alterations in Ca^{2+} transport underly the contractile phenotypes. There are significant parallels between detrusor smooth muscle (DSM) and coronary smooth muscle (CSM). With this in mind, Sturek and colleagues have demonstrated a similar biphasic phenotype in CSM using a preclinical Ossabaw pig metabolic syndrome/diabetes (MetS/D) model that is driven by alterations in Ca^{2+} influx through plasmalemmal Ca^{2+} channels and the size (Ca^{2+} content) of the sarcoplasmic reticulum (SR) Ca^{2+} store. Moreover, altered activity of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) drives these changes [27].

Just as seen in DM1 rats, there is an early hypercontractility that progresses to late hypocontractility as DM progresses in CSM. This mirrors the temporal alterations in contractility noted in DSM by Daneshgari and colleagues [25•, 27, 28]. McKenney-Drake, Sturek, and colleagues demonstrated this in CSM using Ossabaw metabolic pigs. First, a larger "compensated" sarcoplasmic reticulum Ca^{2+} store size drives the "early" phenotype, while after time, decompensation leads to a smaller sarcoplasmic reticulum Ca^{2+} store size which drives the "late" phenotype. The

second major driver is that differential activity in SERCA is a major regulator of the size of the sarcoplasmic reticulum Ca^{2+} store. The third major driver is that the decreased sarcoplasmic reticulum Ca^{2+} store drives DSM apoptosis in the "late" DM phenotype. Work in the diabetic Ossabaw pig model has shown detrusor underactivity associated with increased fibrosis in the DSM during the "late" DM phenotype. This has begun to establish the Ossabaw pig as a novel large animal model for DBD [24•].

Rabbit models have also demonstrated diminished smooth muscle strength. Response to KCl bath as well as bethanechol (both expected to elicit DSM contraction) was diminished in the rabbit model of DM1 in the work of Changolkar et al. supporting the hypothesis of smooth muscle dysfunction driving DBD [29]. Using KCl and bethanechol stimulation eliminated the contribution of potentially defective autonomic innervation. This was also associated with significant oxidative stress. Klee examined the temporal hypothesis further in high fat diet, streptozocin-treated DM1 rats using urothelium-denuded smooth muscle strips and stimulating them at 3 months (compensated, early DM1) and 6 months (decompensated, late DM1). Electrical field stimulation (EFS) was applied, effectively bypassing any contribution from neuropathy, and then EFS in the presence of atropine was used to determine the muscarinic component of detrusor contractility, which was not noted to be different in compensated nor decompensated state in DM1. Detrusor smooth muscle was also stimulated with ATP and carbachol with increased force at the early, compensated stage but lost the increased force to carbachol at the late stage compared to non-DM1 controls. This relative decrease in cholinergic-mediated contraction in the late, decompensated state supports the temporal hypothesis for increasing, then decreasing bladder contractility. Unlike urothelium, which will be discussed in the next section, muscarinic M3 receptors were not more prevalent in the early DM1 smooth muscle but were increased in the late (decompensated) smooth muscle [30].

Hypocontractility is not the only driver for smooth muscle DBD. It has been demonstrated that loss of functioning detrusor smooth muscle mass by apoptosis plays a contributory role. Wang et. al demonstrated that endoplasmic reticulum (ER) stress has been directly linked to detrusor smooth muscle apoptosis in a DM1 rat model employing streptozocin (STZ)-treated rats [31]. The Ossabaw pig model supported this finding by Wang et. al *but in metabolic syndrome, not DM1*, by noting an increased percentage of collagen found in the muscularis layer and no difference in thickness of the layers between lean and metabolic syndrome groups [24•]. Apoptosis causing loss of smooth muscle mass is a well-documented driver for smooth muscle dysfunction in cardiovascular disease related to diabetes, *but its role in DBD is less well studied* [31, 32]. DSM contractility can also be modulated directly by hyperglycemia as noted by Xue and

colleagues. They noted increased apoptosis as well as oxidative stress (superoxide dismutase) and decreased α - smooth muscle actin (contractility marker) when rat detrusor smooth muscle cells were cultured in high glucose solution [33].

In summary, decreased contractility due to Ca^{2+} signaling, apoptosis due to ER stress, and high glucose environment all contribute to DSM driving DBD.

Role of Interstitial Cells in Humans

The abovementioned differential expression of matrix metalloproteases relates to an evolving understanding of the role interstitial cells play in the regulation of smooth muscle cell contraction. There is new evidence that interstitial cells situated among detrusor smooth muscle may become dysfunctional in DM. Canda and colleagues have noted a decrease in the quantity of C-kit positive interstitial cells in comparing 10 bladders of diabetic men vs. 11 non-diabetic male bladders removed for bladder cancer [35]. This is important in that C-kit positive interstitial cells have been shown to affect muscarinic receptor-induced phasic bladder contractions in the diabetic rat [36].

In summary, significant evidence in the basic sciences points to smooth muscle dysfunction/damage as a key driver in DBD. It is important to note that the role of urothelium as an active sensory organ has become widely recognized, leading to its recognition as a second hypothesis driving DBD.

Urothelial Dysfunction Hypothesis

Urothelial dysfunction has recently been suggested as a contributing factor in DBD. The urothelium functions as both a passive barrier that regulates permeability, transport, and endocytosis across the bladder wall as well as a sensor that modulates bladder function [8]. It is widely accepted that M3 receptors play the most significant role in urothelial sensory function. With this in mind, Tong noted an increase in M3 receptor protein and mRNA expression for both urothelium and detrusor smooth muscle (DSM) in fructose-fed rats. M2 and M3 receptors were also noted to be upregulated in DM1 rats induced with streptozosin [37]. This supports a role for urothelial dysfunction in DBD. Lee et. al provided evidence of altered urothelial function and not just protein expression in rats fed a high fructose diet, noting increased contractility on cystometry as well as decreased protein expression of TRPV1, $P2X_3$, and iNOS in the urothelium compared to normal diet rats [38]. The work of Santoso demonstrates that ATP as well as urothelium both have an inhibitory effect on the strength of detrusor muscle contraction. Their elegant work was done using rats, where strips of detrusor smooth muscle were stimulated to contract with carbachol in the presence of ATP, which decreased the strength of contraction, with and without urothelium intact. The urothelium

further decreased the strength of contraction, demonstrating an important modulatory function of urothelium [39]. Urothelium in DM1 rats was less responsive to bradykinin than controls, and although thicker in the diabetic rats, contained less prostaglandin by weight than controls, suggesting that, similar to Lee above, P2X receptor subtypes in urothelium suffer diminished activity in DM1 [40].

Just as urothelium appears to have an inhibitory influence on detrusor function in the mouse, human urothelium in DM has also demonstrated increased M3 receptor expression on Western blot compared to controls and, in this regard, is similar to OAB [41].

Infection is also a chief concern for diabetics. The urothelium is also the chief adhesion and entry point for uropathogenic *Escherichia coli*. It is known that urinary tract infections are more prevalent in diabetic patients, and this has been often attributed to glucosuria as well as presumed urinary retention. Recent evidence by Ho and colleagues has demonstrated a plausible mechanism by which glucose might promote *E. coli* bladder infection. Increased glucose in the environment was noted to enhance the toll-like receptor (TLR) response, but only with bacteria present. Increased intracellular pathogenic *E. coli* bacterial loads were noted in glucose-treated urothelial cell cultures, and the intracellular load increased with glucose concentration. TLR-4 was increasingly transcribed as well, as was JAK/ STAT-1, further supporting the hypothesis that glucose enhances the inflammatory cascade via the TLR-4 pathway and facilitates intracellular infection. STAT-1 inhibitor fludarabine reduced intracellular *E. coli* load, regardless of glucose treatment [42].

In summary, urothelium has not only sensory and inhibitory roles but also plays an important role in preventing infection in DBD. This leads to the third hypothesis that drives DBD, neuropathy.

Diabetic Neuropathy Hypothesis

Neuropathy remains a primary driver for DBD, and there is significant evidence to support this. Animal models for DBD demonstrate metabolic derangement of Schwann cells leading to segmental demyelination and decreased nerve conduction velocity, which was also confirmed in human biopsy specimens by assessing acetylcholinesterase activity (decreased) and S100 stain positivity (increased Schwann cell myelin synthesis) suggesting that Schwann cells growth is compensating for impaired axonal transmission in DBD [43–45].

Nerve growth factor (NGF) has been noted as a urinary marker for an overactive bladder associated with urinary incontinence [46]. NGF levels are increased in rats with early-stage DM, while late-stage DM sees a decrease in bladder NGF levels [47]. Furthermore, supplementing rats

deficient in NGF using gene therapy has improved bladder emptying in a streptozocin-induced DM1 model of DBD [47].

In humans, NGF levels decrease after onabotulinumtoxin A treatment as well as sacral neuromodulation [48, 49]. Decreasing levels of NGF in diabetics may explain detrusor underactivity and impaired sensation but does not address the recently appreciated phenomenon of DO in DBD. This might be explained by biphasic smooth muscle dysfunction (see above "smooth muscle dysfunction hypothesis") and could also be explained by supra-pontine neurologic injury, such as from cerebral infarction. Some have noted a remarkable 76% rate of multiple cerebral infarctions in diabetics exhibiting DO, based on MRI, as an alternative cause for the hypercontractile phenotype, yet this does not explain why animal models progress to underactivity after a period of hyperactivity [50].

Elevated PVR (surrogate for emptying efficiency) is related to peripheral neuropathy in women with diabetes when compared to age-matched, parity-matched, and BMI-matched women without diabetes. Lee et al. examined 194 diabetic women compared to 162 women without diabetes. PVR and uroflow were the primary outcome measures. PVR > 100cc was noted in 13.9% of diabetic patients and only 1.8% of controls. The mean uroflow maximum flow rate was 19.4cc/s for diabetic women compared to 25.9cc/s in controls ($p<0.001$). Age also affected PVR [17]. These findings support the traditional triad of decreased bladder sensation, increased bladder compliance and capacity, and impaired detrusor contractility described in the introduction.

Although PVR and flow are easy to measure, it is difficult to measure sensation. This is often done by filling and asking the patient for a self-report, which is not standardized and suffers from subjectivity. A novel electrical stimulation technique was used by Lee et al. to assess poor bladder sensation. Eighty-six women with DM2 at a diabetes treatment clinic in Taiwan were stimulated during urodynamics to assess current perception. A frequency of 250 Hz was intended to represent a sensory response in A delta fibers, and a frequency of 5 Hz was intended to elicit a sensory response in C fibers. While 38.4% demonstrated normal urodynamic studies, 34.9% demonstrated detrusor underactivity. Impaired current perception thresholds were noted in both A delta and C fibers, and those with impaired sensation also exhibited average lower voided volume, voided velocity, and higher PVR. This is important because it is the first study to link impaired A delta and C fiber sensory function in the bladder with urodynamically proven detrusor underactivity [10•].

Motor nerve conduction velocity (sural nerve, peroneal nerve), similarly, was associated with urodynamic-proven voiding dysfunction in DBD, but patients did not report increased LUTS [51]. The authors concluded that impaired nerve conduction velocity is highly correlated with the delayed first desire to void and elevated bladder capacity in DBD, but LUTS were not [51]. There might be a role for motor nerve conduction velocity to screen for DBD before clinical symptoms are apparent. Impaired temperature sensation, as a surrogate for DBD, does not appear to be linked to urodynamic findings and LUTS in DBD [52].

Peripheral neuropathy can also predict DBD. Kebapci et al. investigated 54 Turkish men and women with DM2 and noted peripheral neuropathy increased odds of DBD. They also found that age, duration of diabetes since diagnosis, and Hgb A1c were correlated with diabetic cystopathy. Men and women had different thresholds for exposure [6]. Yamaguchi et al. reported only 4% of subjects in a large urodynamic database had evidence of diabetic neuropathy. Initially identified by neurologic exam (84/2300), 71% of the neuropathic patients reported poor flow, 59% reported hesitancy, and 38% with the sensation of residual urine. Urodynamics revealed DU in 48%, impaired sensation in 32% (the first sensation occurred at 300cc), and PVR > 100 in 32%. Unfortunately, this was confounded by 21/84 (25%) patients who were determined to have suffered cerebral infarction. Diabetic neuropathy was also described in 26% of 182 consecutive diabetics undergoing urodynamic studies by Kaplan, Blaivas, and colleagues [9]. As described previously, this series noted that 42% had DO while 48% reported urinary urgency [50]. Although the Kaplan and Yamaguchi investigations both involve diabetics, the rate of diabetic neuropathy was very different (4% vs 26%) and neuropathy was defined differently. Differing definitions of key independent and dependent variables as well as varying stages of diabetes severity at the time of analysis make finding common elements in DBD challenging. To summarize, evidence for neuropathy in DBD includes decreased Ach activity, increased nerve synthesis, increased NGF levels in those complaining of overactive bladder, impaired sensation including impaired current threshold for A delta and C fibers, and peripheral neuropathy. Thus far, smooth muscle dysfunction, urothelial dysfunction, and neuropathy have considerable evidence of contributing to DBD. The final contributor, circulating and systemic factors, is likely a causative factor in the three abovementioned hypotheses in addition to being a primary driver itself.

Circulating and Systemic Factors hypothesis: Oxidative stress, Acute Glycation End Products, Inflammation, and Microvascular Damage

The prior three sections have outlined significant evidence supporting end-organ damage, such as smooth muscle, urothelial, and neurologic changes in DBD. Systemic and circulating factors likely cause the damage in these tissues and also contribute directly to symptoms. Polyuria from increased osmotic load [23, 53], oxidative stress to nerve

and muscle tissue [29, 54], and microvascular damage [55] all contribute to DBD. Urinary indicators of oxidative stress, such as thiobarbituric acid reactive substances (TBARS), are elevated in pigs exhibiting metabolic syndrome vs. controls [24•]. Mitochondria also play a role and are a prime source of reactive oxygen species, implicated in tissue damage due to hyperglycemia [56]. Advanced glycation end products (AGEs) have been widely studied in animal models for DBD but recently were noted in human subjects with various LUTS, with an increase in serum AGEs being associated with higher IPSS (international prostatic symptoms score) as well as OAB-q (overactive bladder questionnaire) scores [57]. Obesity is associated with oxidative stress, is well correlated with DM2 but not DM1, and has been associated with urinary incontinence in multiple studies [58–60].

In addition to the abovementioned systemic drivers contributing to DBD, systemic inflammation has recently been shown to play a key role in the development of overactive bladder in DBD. It has also been implicated in progression to underactive bladder. In fact, DBD may not be possible without inflammation. Hughes, Purves, and colleagues demonstrated that knocking out NLRP3, a gene associated with inflammation isolated to the urothelium, prevented the progression to the underactive bladder in "late" DM1 [61]. The investigators previously demonstrated bladder hypertrophy and overactive bladder in the Akita mouse genetically modified to exhibit DM1 [62]. The Akita DM1 mouse was cross-bred with a knockout mouse model lacking NLRP3. Investigators noted that while the Akita DM1 mouse progressed to OAB at 15 weeks and then underactive bladder at 30 weeks, this progression failed to occur in the mouse lacking NLRP3.

On a genetic level, DM1 can cause inhibition of gene expression, promoting inflammation and preventing nerve growth and repair. Hindi and colleagues used RNA microarray analysis of DM1 rat detrusor vs. controls as well as vs. sucrose-fed rats and appreciated 1461 gene transcripts that were differentially expressed when compared to controls (including sucrose-fed controls). Further analysis focused on the most pronounced differences and identified 7 pathways in which DM1 rats differed from both controls and sucrose-fed rats. These pathways included inhibition of matrix metalloproteases and axonal guidance signaling pathways supporting the case for extracellular matrix dysregulation as well as neuropathy in DBD [34].

Next-generation sequencing promises to advance this further than what is possible with microarray analysis. Identification and sampling of single cells for whole-genome sequencing allows for analysis of extremely small samples of cells with precision. To demonstrate the utility of next-generation single-cell sequencing, Abedeni

and colleagues have successfully identified and sequenced small numbers of sloughed cells from the kidney as well as bladder urothelial cells from voided urine as a part of the TRIDENT diabetic nephropathy research project using single-cell RNA sequencing [63]. Although not directly related to the bladder, one can see future applications of this technology in investigating sloughed urothelial cells. The authors characterized gene expression in 5 patients with diabetic kidney disease and created a reference gene expression library using pooled urine from healthy controls. This new application of an emerging technique promises new and less invasive ways to study the differential expression of genes to further elucidate the mechanism of DBD. Investigators in that work appreciated differential expression of genes associated with nephrotic syndrome in podocytes as well as genes found in collecting duct cells associated with kidney stones.

Clearly, systemic factors contribute to abnormalities in the end organ, such as detrusor smooth muscle, urothelium, and nerves, but also appear to independently contribute to the DBD phenotype. The DBD phenotype appears dependent on the duration of DM and severity of DM, with a still-evolving understanding.

Conclusion

The etiology of DBD is clearly multifactorial, but recent evidence suggests smooth muscle dysfunction is a strong driver. Animal models are helpful in testing clinically observed hypotheses, as well as isolating complex inter-relationships between contributing factors. These include neuropathy, polyuria, smooth muscle dysfunction, urothelial-nerve-muscle interactions, inflammation, oxidative stress, AGEs, and microvascular compromise. These factors change with the duration and severity of DM. Well-designed, prospective human observational studies are needed.

Declarations

Conflict of Interest Dr. Powell is an investigator for Micron Medical LLC, Medtronic, and Neuspera LLC.

Human and Animal Rights and Informed Consent This article does discuss studies performed by the authors that were done in accordance with human and animal rights rules and approved by the respective Institutional Review Boards with human or animal subjects.

References

Papers of particular interest, published recently, have been highlighted as:
● Of importance
●● Of major importance

1. Federation, I.D., *IDF Diabetes atlas, 6th edn*. 2013, International Diabetes Federation: Brussels, Belgium.
2. Daneshgari F, Moore C. Diabetic uropathy. Semin Nephrol. 2006;26(2):182–5.
3.●● Changxiao H, et al. Clinical and urodynamic evaluation of women referred with diabetes mellitus. Int Urogynecol J. 2014;25(7):979–83. **The largest prospective population-based study exploring the urodynamic characteristics and prevalence of DBD. Some of the outcome variables occurred at a very high rate compared to other studies.**
4. Frimodt-Moller C. Diabetic cystopathy A review of the urodynamic and clinical features of neurogenic bladder dysfunction in diabetes mellitus. Dan Med Bull. 1978;25(2):49–60.
5. Jordan, W.R. and H.H. Crabtree, *Paralysis of the bladder in diabetic patients.* Archives of Internal Medicine, 1935. **55**(1): p. 17-25 %@ 0730-188X.
6. Kebapci N, et al. Bladder dysfunction in type 2 diabetic patients. Neurourol Urodyn. 2007;26(6):814–9.
7. Wittig L, et al. Diabetic bladder dysfunction: a review. Urology. 2019;123:1–6.
8. Liu G, Daneshgari F. Diabetic bladder dysfunction. Chin Med J (Engl). 2014;127(7):1357–64.
9. Kaplan SA, Te AE, Blaivas JG. Urodynamic findings in patients with diabetic cystopathy. J Urol. 1995;153(2):342–4.
10.● Lee WC, et al. Investigation of urodynamic characteristics and bladder sensory function in the early stages of diabetic bladder dysfunction in women with type 2 diabetes. J Urol. 2009;181(1):198–203. **This work demonstrates impaired sensory thresholds with a novel device and correlates with detrusor.**
11. Lifford KL, et al. Type 2 diabetes mellitus and risk of developing urinary incontinence. J Am Geriatr Soc. 2005;53(11):1851–7.
12● Malik RD, et al. A modern comparison of urodynamic findings in diabetic versus nondiabetic women. Female Pelvic Med Reconstr Surg. 2020;26(1):44–50. **The first comparison of urodynamic findings in a prospectively collected series of urodynamic studies on diabetic women vs. non-diabetic women. This revealed more prevalent DO in DM, impaired sensation, and higher PVR. Duration of DM as well as severity were associated with impaired sensation.**
13. Frimodt-Moller C. Diabetic cystopathy: epidemiology and related disorders. Ann Intern Med. 1980;92(2 Pt 2):318–21.
14. Hong GS, et al. Correlation between metabolic syndrome and lower urinary tract symptoms of males and females in the aspect of gender-specific medicine: a single institutional study. Korean J Urol. 2010;51(9):631–5.
15. Tai HC, et al. Metabolic syndrome components worsen lower urinary tract symptoms in women with type 2 diabetes. J Clin Endocrinol Metab. 2010;95(3):1143–50.
16. Danforth KN, et al. Type 2 diabetes mellitus and risk of stress, urge and mixed urinary incontinence. J Urol. 2009;181(1):193–7.
17. Lee WC, et al. Effects of diabetes on female voiding behavior. J Urol. 2004;172(3):989–92.
18.●● Lenherr SM, et al. Glycaemic control and risk of incident urinary incontinence in women with type 1 diabetes: results from the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study. Diabet Med. 2016;33(11):1528–35. **Diabetes Control and Complications Trial (DCCT) is the first and largest prospective, interventional population-based study on the complications of diabetes, while the Epidemiology of Diabetes Interventions and Complications (EDIC) project is an observational follow-up study of these patients specifically examining urologic complications of diabetes, noting urinary incontinence was related to worsening Hgb A1c.**
19. Luk, A.O., et al., *Risk association of HbA1c variability with chronic kidney disease and cardiovascular disease in type 2 diabetes: prospective analysis of the Hong Kong Diabetes Registry.* Diabetes Metab Res Rev, 2013.
20. Bansal R, et al. Urodynamic profile of diabetic patients with lower urinary tract symptoms: association of diabetic cystopathy with autonomic and peripheral neuropathy. Urology. 2011;77(3):699–705.
21. Beylot M, Marion D, Noel G. Ultrasonographic determination of residual urine in diabetic subjects: relationship to neuropathy and urinary tract infection. Diabetes Care. 1982;5(5):501–5.
22. Wessells H, et al. Burden of urological complications in men and women with long-standing type 1 diabetes in the diabetes control and complications trial/epidemiology of diabetes interventions and complications cohort. Diabetes Care. 2018;41(10):2170–7.
23. Daneshgari F, et al. Animal models of diabetic uropathy. J Urol. 2009;182(6 Suppl):S8-13.
24.● Powell CR, et al. Ossabaw pig demonstrates detrusor fibrosis and detrusor underactivity associated with oxidative stress in metabolic syndrome. Comp Med. 2020;70(5):329–34. **This is the first large animal model (Ossabaw pig) that spontaneously develops metabolic syndrome by dietary modification alone. Histologic change in detrusor smooth muscle was demonstrated as well as in-vivo urodynamic dysfunction. This fills a need since most investigations on DBD historically have focused on mice and rats.**
25.● Daneshgari F, et al. Temporal differences in bladder dysfunction caused by diabetes, diuresis, and treated diabetes in mice. Am J Physiol Regul Integr Comp Physiol. 2006;290(6):R1728-35. **DBD likely changes over time. The authors provide the first evidence that the LUTS seen in DBD might evolve and change in a predictable time-dependent manner.**
26. Kirschner-Hermanns R, et al. Does diabetes mellitus-induced bladder remodeling affect lower urinary tract function? ICI-RS 2011. Neurourol Urodyn. 2012;31(3):359–64.
27. McKenney-Drake ML, et al. Biphasic alterations in coronary smooth muscle Ca^{2+} regulation in a repeat cross-sectional study of coronary artery disease severity in metabolic syndrome. Atherosclerosis. 2016;249:1–9.
28. Samuel J Francis, D.Y., Richa Sharma, Mouhamad Alloosh, Michael S Sturek and C Subah Packer, *Effects of metabolic syndrome (MetS) on male and female ossabaw miniature pig detrusor muscle contractility and compliance.* The Journal for

the Federation of American Societies for Experimental Biology 2009(23): p. 781.12.

29. Changolkar AK, et al. Diabetes induced decrease in detrusor smooth muscle force is associated with oxidative stress and over-activity of aldose reductase. J Urol. 2005;173(1):309–13.

30. Klee NS, Moreland RS, Kendig DM. Detrusor contractility to parasympathetic mediators is differentially altered in the compensated and decompensated states of diabetic bladder dysfunction. Am J Physiol Renal Physiol. 2019;317(2):F388–98.

31. Wang D, et al. Endoplasmic reticulum stress is involved in apoptosis of detrusor muscle in streptozocin-induced diabetic rats. Neurourol Urodyn. 2017;36(1):65–72.

32. Maamoun, H., et al., *Endoplasmic reticulum stress: a critical molecular driver of endothelial dysfunction and cardiovascular disturbances associated with diabetes.* Int J Mol Sci, 2019. **20**(7).

33. Xue J, et al. CGRP protects bladder smooth muscle cells stimulated by high glucose through inhibiting p38 MAPK pathway in vitro. Sci Rep. 2021;11(1):7643.

34. Hindi EA, et al. Experimental long-term diabetes mellitus alters the transcriptome and biomechanical properties of the rat urinary bladder. Sci Rep. 2021;11(1):15529.

35. Canda AE, et al. Does diabetes affect the distribution and number of interstitial cells and neuronal tissue in the ureter, bladder, prostate, and urethra of humans? Cent European J Urol. 2014;67(4):366–74.

36. Vahabi B, et al. The role of c-kit-positive interstitial cells in mediating phasic contractions of bladder strips from streptozotocin-induced diabetic rats. BJU Int. 2011;107(9):1480–7.

37. Tong YC, Cheng JT. Alterations of M2,3-muscarinic receptor protein and mRNA expression in the bladder of the fructose fed obese rat. J Urol. 2007;178(4 Pt 1):1537–42.

38. Lee WC, et al. Sensory dysfunction of bladder mucosa and bladder oversensitivity in a rat model of metabolic syndrome. PLoS One. 2012;7(9):e45578.

39 Santoso AG, et al. The role of the urothelium and ATP in mediating detrusor smooth muscle contractility. Urology. 2010;76(5):1267 e7-12.

40. Pinna C, Zanardo R, Puglisi L. Prostaglandin-release impairment in the bladder epithelium of streptozotocin-induced diabetic rats. Eur J Pharmacol. 2000;388(3):267–73.

41. Wang CC, Kuo HC. Urothelial dysfunction and chronic inflammation in diabetic patients with overactive bladder. Low Urin Tract Symptoms. 2017;9(3):151–6.

42. Ho CH, et al. Enhanced uropathogenic Escherichia coli-induced infection in uroepithelial cells by sugar through TLR-4 and JAK/STAT1 signaling pathways. J Microbiol Immunol Infect. 2021;54(2):193–205.

43. Mastri AR. Neuropathology of diabetic neurogenic bladder. Ann Intern Med. 1980;92(2 Pt 2):316–8.

44. Van Poppel H, et al. Diabetic cystopathy: neuropathological examination of urinary bladder biopsies. Eur Urol. 1988;15(1–2):128–31.

45. Nadelhaft I, Vera PL. Reduced urinary bladder afferent conduction velocities in streptozocin diabetic rats. Neurosci Lett. 1992;135(2):276–8.

46. Liu HT, Chen CY, Kuo HC. Urinary nerve growth factor in women with overactive bladder syndrome. BJU Int. 2011;107(5):799–803.

47. Sasaki K, et al. Diabetic cystopathy correlates with a long-term decrease in nerve growth factor levels in the bladder and lumbosacral dorsal root Ganglia. J Urol. 2002;168(3):1259–64.

48. Liu HT, Chancellor MB, Kuo HC. Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol. 2009;56(4):700–6.

49. Shalom DF, et al. Sacral nerve stimulation reduces elevated urinary nerve growth factor levels in women with symptomatic detrusor overactivity. Am J Obstet Gynecol. 2014;211(5):5611–5.

50. Yamaguchi C, et al. Overactive bladder in diabetes: a peripheral or central mechanism? Neurourol Urodyn. 2007;26(6):807–13.

51. Mitsui T, et al. Vesicourethral function in diabetic patients: association of abnormal nerve conduction velocity with vesicourethral dysfunction. Neurourol Urodyn. 1999;18(6):639–45.

52. Ishigooka M, et al. Thermoreceptor mediated bladder sensation in patients with diabetic cystopathy. Int Urol Nephrol. 1997;29(5):551–5.

53. Liu G, Daneshgari F. Alterations in neurogenically mediated contractile responses of urinary bladder in rats with diabetes. Am J Physiol Renal Physiol. 2005;288(6):F1220-6.

54. Beshay E, Carrier S. Oxidative stress plays a role in diabetes-induced bladder dysfunction in a rat model. Urology. 2004;64(5):1062–7.

55. Brown JS. Diabetic cystopathy–what does it mean? J Urol. 2009;181(1):13–4.

56. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol. 2006;212(2):167–78.

57. Gali A, et al. Correlation between advanced glycation end-products, lower urinary tract symptoms and bladder dysfunctions in patients with type 2 diabetes mellitus. Low Urin Tract Symptoms. 2017;9(1):15–20.

58. Goldberg RP, et al. Delivery mode is a major environmental determinant of stress urinary incontinence: results of the Evanston-Northwestern Twin Sisters Study. Am J Obstet Gynecol. 2005;193(6):2149–53.

59. Danforth KN, et al. Risk factors for urinary incontinence among middle-aged women. Am J Obstet Gynecol. 2006;194(2):339–45.

60. Han MO, Lee NY, Park HS. Abdominal obesity is associated with stress urinary incontinence in Korean women. Int Urogynecol J Pelvic Floor Dysfunct. 2006;17(1):35–9.

61. Hughes FM Jr, et al. Diabetic bladder dysfunction progresses from an overactive to an underactive phenotype in a type-1 diabetic mouse model (Akita female mouse) and is dependent on NLRP3. Life Sci. 2022;299:120528.

62. Inouye BM, et al. Diabetic bladder dysfunction is associated with bladder inflammation triggered through hyperglycemia, not polyuria. Res Rep Urol. 2018;10:219–25.

63. Abedini A, et al. Urinary single-cell profiling captures the cellular diversity of the kidney. J Am Soc Nephrol. 2021;32(3):614–27.

64. Karoli R, et al. A study of bladder dysfunction in women with type 2 diabetes mellitus. Indian J Endocrinol Metab. 2014;18(4):552–7.

65. Majima T, et al. Urodynamic analysis of the impact of diabetes mellitus on bladder function. Int J Urol. 2019;26(6):618–22.