Diabetic Bladder Dysfunction: Current Translational Knowledge

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Abbreviations and Acronyms

DBD = diabetic bladder dysfunction
DM = diabetes mellitus
DSM = detrusor smooth muscle
LUT = lower urinary tract
MLC = myosin light chain
MLCP = MLC phosphatase
PCR = polymerase chain reaction
ROS = reactive oxygen species
STZ = streptozotocin

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Purpose: Diabetes mellitus, a metabolic disorder caused by an absolute or relative deficiency of insulin, is a debilitating and costly disease with multiple serious complications. Lower urinary tract complications are among the most common complications of diabetes mellitus. The most common, bothersome lower urinary tract complication of diabetes mellitus is diabetic cystopathy or diabetic bladder dysfunction. We reviewed the current translational knowledge of diabetic bladder dysfunction.

Materials and Methods: We performed a search of the English literature through PubMed®. The key words used were diabetes and bladder dysfunction or cystopathy. Our data and perspective are provided for consideration of the future direction of research.

Results: Despite traditional recognition of diabetic bladder dysfunction as a voiding problem characterized by poor emptying and overflow incontinence, recent clinical and experimental evidence indicate storage problems such as urgency and urge incontinence in diabetes mellitus cases. Recent experimental evidence from studies of diabetic bladder dysfunction in small animal models of diabetes mellitus show a temporal effect on diabetic bladder dysfunction. Early phase diabetes mellitus causes compensated bladder function and the late phase causes decompensated bladder function. The temporal theory could plausibly provide the scientific road map to correlate clinical and experimental findings, and identify the role of mechanisms such as polyuria, hyperglycemia, oxidative stress, autonomic neuropathy and decompensation of the bladder contractile apparatus in the creation of clinical and experimental manifestations of diabetic bladder dysfunction.

Conclusions: Diabetic bladder dysfunction includes time dependent manifestations of storage and emptying problems. Identifying mechanistic pathways would lead to the identification of therapeutic intervention.

Key Words: urinary bladder, diabetes mellitus, urination disorders, complications, epidemiology

About 1/14 Americans, including almost 1/7 black Americans and 1/5 Americans 65 years old or older, has DM, of whom about 30% are undiagnosed. Type 1 DM accounts for 5% to 10% of all diagnosed cases. About 1/500 children and adolescents has type 1 DM.[1] The incidence of type 2 DM, which accounts for 90% to 95% of cases, increased by 33% between 1990 and 1998, and by 75% in patients 30 to 39 years old.[2] The United States Centers for Disease Control and Prevention estimated in 2005 that 20.8

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million individuals in the United States (7% of the population) had DM. The total medical and indirect costs of DM and its complications were estimated to be $132 billion in the United States in 2005, accounting for about 10% of total health care costs.[1] This trend is expected to continue due to the continuing increase in obesity, a major risk factor for type 2 DM. Diabetics live decades with the disease and are susceptible to numerous burdensome, costly complications. The complications of DM render it a debilitating, devastating disease. LUT complications are among the most common complications of DM. The most common, bothersome LUT complication of DM is diabetic cystopathy or DBD.[3] We reviewed the current translational knowledge of DBD.

DIABETIC BLADDER DYSFUNCTION

LUT complications are found in more than 80% of individuals diagnosed with DM, a higher rate than that of widely recognized complications such as neuropathy and nephropathy, which affect less than 60% and 50%, respectively.[4] The most common, bothersome LUT complication of DM is DBD. Although DBD is not life threatening, it substantially affects quality of life. However, little is known about the natural history and pathophysiology of DBD. The paucity of knowledge is a barrier to developing the best prevention and treatment methods.

Is DBD Storage or Voiding Problem?

The bladder has 2 major, distinct functions, including urine storage and disposal. A simplified categorization of bladder dysfunction into storage or voiding problems is widely accepted.[5] Urodynamic studies are often done to provide more information on the storage or voiding nature of bladder dysfunction (see Appendix). DBD is traditionally described as a triad of decreased sensation, increased capacity and poor emptying but many inconsistencies have been found in those classic findings. In most of the asymptomatic patients with diabetes whom they studied Ueda et al found increased bladder volume at first voiding sensation and decreased detrusor contractility with resultant increased post-void residual urine volume as well as a 25% incidence of detrusor overactivity.[6] A review by Kaplan et al of urodynamic findings in 182 diabetes cases revealed that 55% had detrusor overactivity with 10% areflexic and 11% indeterminate.[7] The mixed clinical picture of DBD was also revealed in recent large-scale studies, in which DM was associated with a 40% to 80% increased risk of urge incontinence and a 30% to 80% increased risk of overflow incontinence on controlled multivariate analyses.[8] Thus, it is now clear that DBD manifestations are a combination of storage and voiding bladder problems.

DBD Temporal Theory as Potential Unifying Mechanism of Pathogenesis

When examining DBD natural history, we observed that morphological and functional manifestations of DBD in studies of STZ induced DM are time dependent. Bladder hypertrophy and remodeling, increased contractility and associated neurogenic changes occur soon after the onset of DM,[9–11] while decreased peak voiding pressure in the cystometric measure develops only at a later stage of DM.[12,13] Time dependent alterations in DBD served as the basis of our temporal hypothesis of DBD with mixed clinical manifestations, in which we propose that DM causes the bladder to undergo 2 phases of alterations via 2 main mechanisms (fig. 1). In the early phase hyperglycemia induced osmotic polyuria is the main mechanistic factor that causes compensatory bladder hypertrophy, and associated myogenic and neurogenic alterations. In the later phase accumulation of oxidative stress products during prolonged hyperglycemia causes decompensation of bladder tissue and function. This temporal hypothesis of DBD pathophysiology provides a potentially unifying theory by which the complex interaction among seemingly confusing bladder dysfunctions may be explained. Furthermore, it provides a scientific road map with which the timing and specific roles of various components, such as detrusor, urothelium, autonomic nerves and urethra, may be explored.

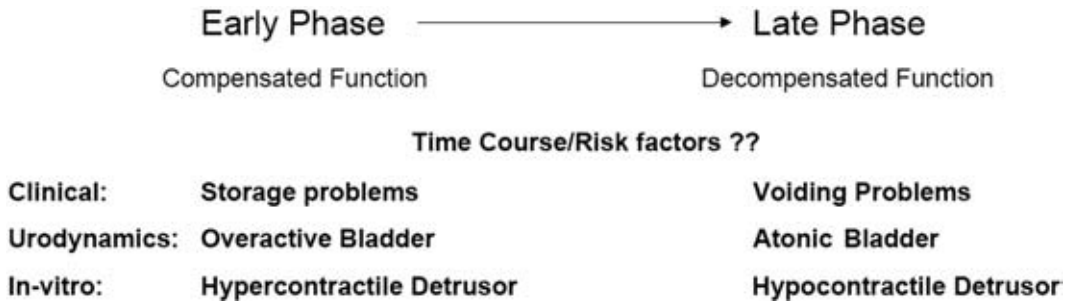

Fig. 1. Proposed natural history of progression of DM bladder dysfunction

PATHOGENESIS

DBD pathogenesis may be related to hyperglycemia induced polyuria and oxidative stress.

Polyuria and Early Phase DBD

Unlike most other organs affected by DM the bladder faces not only hyperglycemia but also an exceptionally high volume of urine output. In experimental models sucrose induced diuresis caused rapid, substantial bladder hypertrophy, and increased bladder contractility, capacity and compliance, similar to changes observed in diabetic rats.[10,14] Those similarities suggest that bladder hypertrophy in diabetic animals may result from a physical adaptation to increased urine production. On the other hand, bladder hypertrophy may also initiate the process of increased oxidative stress.[15] Further separation of the role of hyperosmol polyuria from normal osmol polyuria in the mediation of bladder remodeling requires future studies in which the separation of the role of osmolality vs increased flow or stretch on urothelial sensory elements should be explored.

Prolonged Hyperglycemia, Oxidative Stress and Late Phase DBD

Oxidative stress product accumulation in most cell types is a prominent feature of prolonged hyperglycemia.[16] Diabetic oxidative stress could originate from various mechanisms, including oxygen radical production from auto-oxidation of glucose, glycated proteins, stimulation of cytochrome P450-like activity, alterations in the reduced nicotinamide adenine dinucleotide phosphate-to-nicotinamide adenine dinucleotide phosphate ratio by excess glucose going through the polyol pathway, increased super oxide dismutase production and increased lipid peroxidation production.[16–18] Brownlee promoted a unifying mechanism that links all of the seemingly unconnected hyperglycemia induced pathways stemming from increased mitochondrial production of ROS, primarily superoxide.[18] In turn excess ROS cause DNA strand breaks, poly (adenosine diphosphate-ribose) polymerase activation and glyceraldehyde-3 phosphate dehydrogenase inhibition, culminating in activation of the 4 damaging pathways.[18]

While there are numerous studies of the role of oxidative stress on the pathogenesis of diabetic complications in the eye, nervous system, kidney and cardiovascular system, to our knowledge the direct effect of oxidative stress on urological complications has not yet been investigated in detail. The few studies of its role in erectile dysfunction[11,19] and in cystopathy[20,21] indicate the importance of oxidative stress in the pathogenesis of urological diabetic complications. In a rabbit model of alloxan induced diabetes we noted that decreased DSM contractility is associated with aldose reductase over expression and increased lipid peroxidation products (fig. 2, *A*).[21] Increased aldose reductase expression favors the cycling of increased glucose through the polyol pathway and produces increased sorbitol.[21] We also have evidence that exposing human bladder smooth muscle cells to high glucose increases aldose reductase expression in these cells (unpublished data). Exposing these cells grown in high glucose to the aldose reductase inhibitor zopolrestat reverses aldose reductase over expression, supporting the conclusion from our studies of intact muscle in diabetic rabbits that aldose reductase over expression and

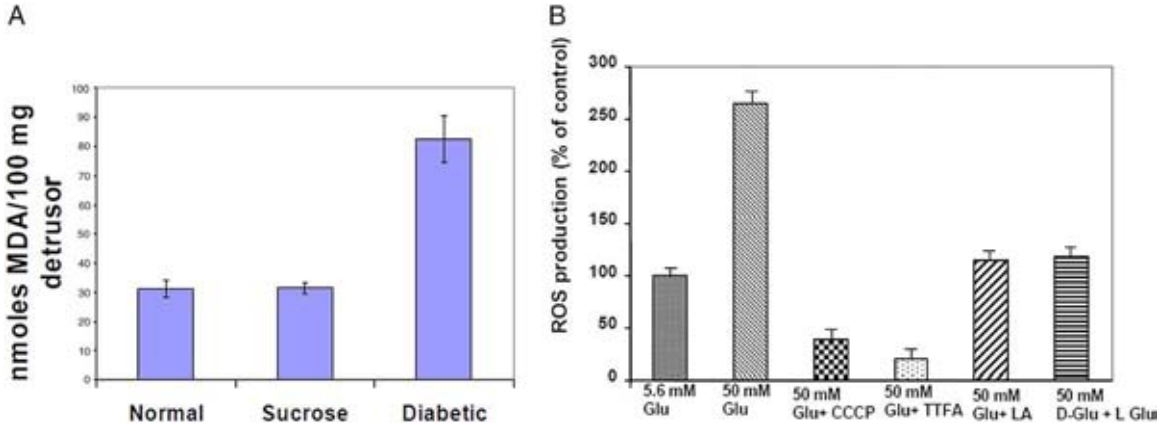

Fig. 2. *A*, mean ± SE increased lipid peroxidation in detrusor of 11 normal and 13 sucrose fed controls, and 5 diabetic rabbits. MDA was significantly higher in detrusor muscle from diabetic rabbits vs normal and sucrose fed controls. *B*, mitochondrial ROS production. Incubating human BSM cells with high glucose (*Glu*) increases ROS generation and is prevented by antioxidant α-lipoic acid. Human BSM cells were grown with 6 and 50 mM glucose plus 10 μM thenoyltrifluoroacetone (*TTFA*), 1 μM carbonyl cyanide m-chlorophenyl hydrazone (*CCCP*), 200 μM α-lipoic acid (*LA*) and 50 mM L-glucose (*L Glu*) for 48 hours. Mitochondrial fractions were assayed for ROS production. ROS concentration was determined from standard curve of 95 to 100 μmol/l H_2O_2 and expressed as percent of ROS incubated at 6 mmol/l glucose.

overactivity may contribute to the increase in redox and lipid peroxidation (unpublished data).

Mitochondria are the major source of superoxide, peroxynitrite and hydroxyl radicals in all cell types.[22] Our preliminary data show that treatment with high glucose increases the mitochondrial membrane potential and ROS in cultured human bladder smooth muscle cells (fig. 2, *B*), in agreement with published reports that mitochondrial dysfunction is a key mechanistic step in diabetes complications.[23] Mitochondrial dysfunction decreases adenosine triphosphate production, affecting the ability of cross bridges to cycle during force generation.

Endogenous antioxidants destroy ROS and create a balance between antioxidant and free radicals in a normal situation. However, in diabetes cases the antioxidant defense system is deficient due to high oxidative stress. Intake of antioxidants, such as vitamin E[24] and α-lipoic acid,[25] which functions as a cofactor in multi-enzyme complexes, has successfully reversed the oxidative stress produced by hyperglycemia in individuals with diabetes and in STZ induced diabetic animal models. Oral treatment with 600 mg α-lipoic acid per day orally for 5 weeks improved neuropathic deficits in patients with diabetes and distal symmetrical polyneuropathy in a recent clinical trial.[25] It was not reported whether bladder function improved in these patients. In our preliminary studies of the antioxidant effect on oxidative stress induced by high glucose in cultured human bladder smooth muscle cells we were able to decrease lipid peroxidation production (fig. 3).

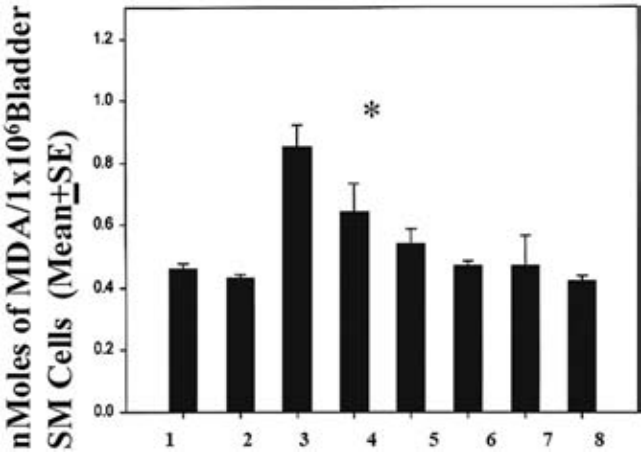

Fig. 3. Increased lipid peroxidation products in BSM cells treated with 50 mM (high) glucose. Group treated with 50 mM mannitol (*7*) served as control for osmotic shock. High glucose alone (*3*) induced lipid peroxidation, which was inhibited by 50 (*4*) and 100 (*5*) μM α-lipoic acid. Normal 6 mM glucose (*1*) and mannitol induced no lipid peroxidation changes. *2*, vehicle. *6*, 200 μM α-lipoic acid. *8*, 6 mM glucose and 200 μM α-lipoic acid. Asterisk indicates $p < 0.05$ vs 6 mM glucose.

PATHOPHYSIOLOGY

Multifactorial Detrusor, Nerve, Urothelial and Urethral Alterations

The traditional view recognized autonomic neuropathy as the only pathophysiological cause of DBD.[26] That view would consider decreased bladder sensation the primary event with patients unaware of bladder filling and lacking the desire to empty. It is presumed to result from autonomic neuropathy and it results in high post-void residual and overflow incontinence. To our knowledge details of how autonomic neuropathy or sensation loss leads to the mixed clinical manifestations of DBD are unknown. Evolution of that view is represented by the notion of most contemporary investigators that DBD is multifactorial, including disturbances of the bladder detrusor, urethra, autonomic nerves and perhaps the urothelium.[27] We and others observed that upon DM induction in rodents by destruction of pancreatic β-cells with STZ the bladder and urethra undergo morphometric and functional changes in myogenic and neurogenic components.[9–13,28–30] Another study revealed the potentially obstructive effects on urethral sphincteric mechanisms in DBD cases.[31]

DM Changes

Myogenic. In vivo and in vitro experimental studies of DSM in DM animal models provide evidence for myogenic changes. Earlier studies of the effects of diabetes on detrusor contractility showed decreased[32] and increased[33] force production in rat DSM strips. We investigated the effects of a long-term diabetic state on DSM contractility and associated oxidative stress changes.[21] DSM contractility was decreased in response to stimulation by KCl and carbachol, and the decrease was associated with the duration of the hyperglycemic state as well as the level of hyperglycemia (fig. 4). Changes in muscarinic receptor population are also linked to altered contractility.[34] Unlike DSM changes due to an obstructed bladder, we found in an STZ induced rat diabetic model and an alloxan induced rabbit model that there was no change in DSM myosin isoform composition in diabetic animals.[35] Recent physiological and biochemical studies of DSM from our group[36,37] and others[13,38] show a distinct deficit in the regulation of DSM contraction in diabetic cases.

A major regulatory mechanism of smooth muscle contraction is myosin mediated regulation via phosphorylation-dephosphorylation of regulatory MLC_{20} by Ca^{2+} dependent MLC kinase and MLCP. MLCP is inactivated by phosphorylation, catalyzed mainly by Rho-kinase and by binding to phosphorylated CPI-17. By lowering MLCP activity these proteins retain myosin in the phosphorylated state and maintain muscle tone in the absence of increased cytosolic Ca^{2+}. Studies in DSM from diabetic animals showed over expression and overactivity of Rho-ki-

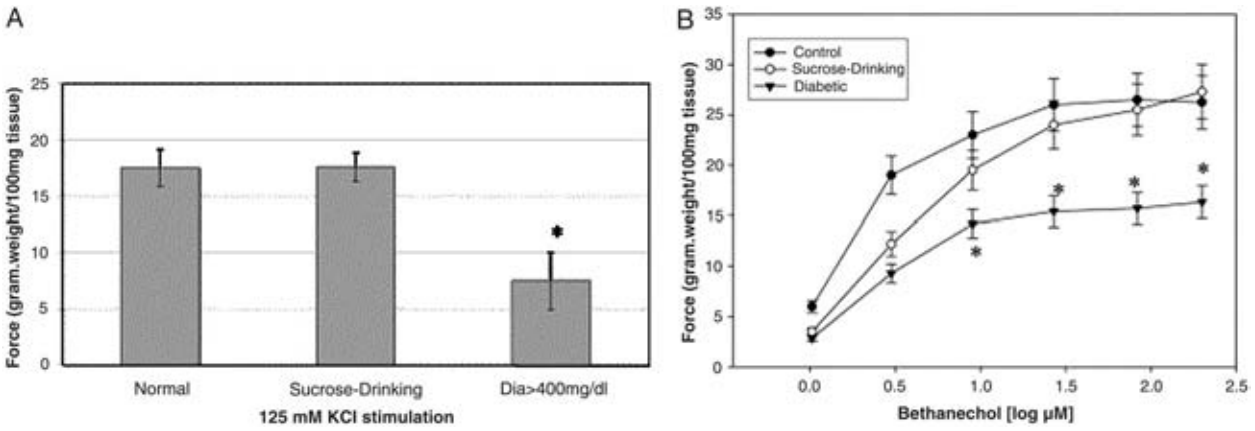

Fig. 4. Mean ± SEM detrusor muscle strip force generation in normal, sucrose drinking and diabetic rabbits. *A*, 125 mM KCl effects show significantly decreased force in diabetic (greater than 400 mg/dl) rabbits. *B*, bethanechol dose response curve shows significantly decreased force in diabetic rabbits Asterisk indicates $p < 0.05$. Reproduced with permission.[21]

nase and CPI-17 proteins involved in Ca^{2+} sensitization in smooth muscle (figs. 5 and 6). Interestingly we also found high baseline MLC_{20} phosphorylation in the diabetic detrusor (fig. 7). However, to our knowledge the molecular mechanisms of the diabetes induced alteration in the expression of these proteins that regulate myosin mediated regulation of DSM contraction are unknown.

Urothelial. An important but poorly understood function of epithelial cells is the ability to sense changes in the extracellular environment and communicate these changes to underlying nervous, connective and muscular tissues.[39] This communication is likely to be important for tube-shaped and sac-shaped organs such as blood vessels, gut and bladder, of which normal function may be modulated by stimuli initiated in

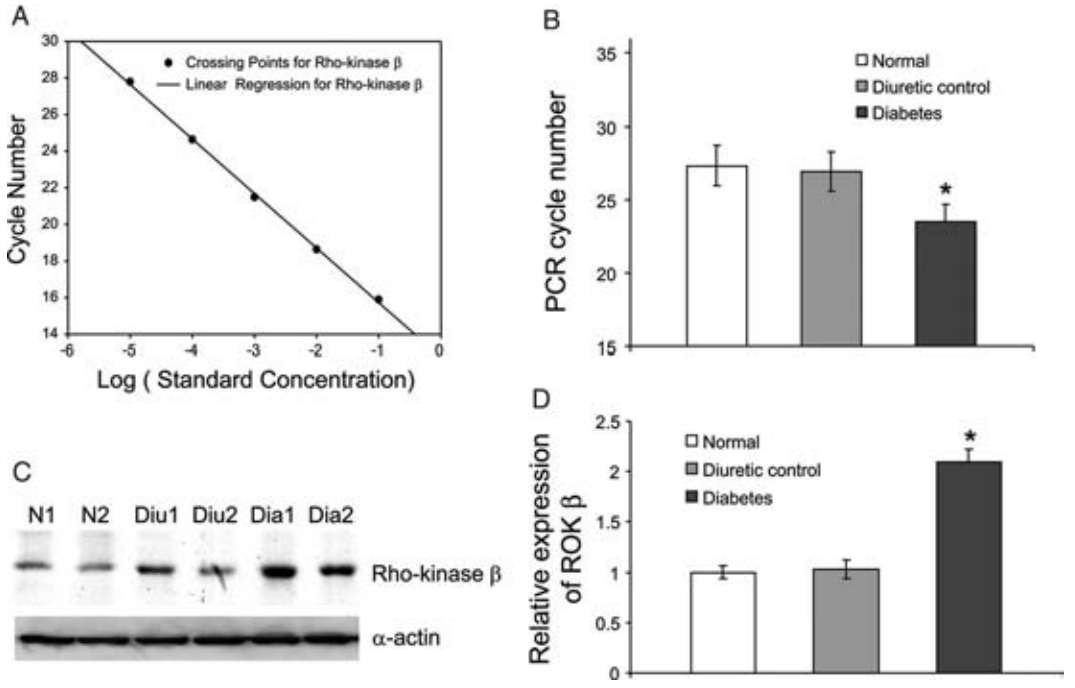

Fig. 5. Rho-kinase expression at mRNA and protein levels. *A*, rho-kinase β real-time PCR standard curve. *B*, average number of required PCR cycles to attain crossing threshold was 27.3 in normal and 26.9 in diuretic control samples, and 23.5 in diabetic samples. Significantly fewer PCR cycles in diabetic sample indicated more Rho-kinase transcript copies in diabetic DSM. Asterisk indicates significant difference between 4 samples each ($p < 0.01$). *C*, Rho-kinase βand smooth muscle actin Western blot. *N*, normal. *Diu*, diuretic. *Dia*, diabetes. D, average relative protein expression. Rho-kinase β (*ROK β*) was almost 2.1-fold higher in diabetic vs normal and diuretic detrusor samples. Asterisk indicates significant difference between 4 samples each ($p < 0.01$). Reprinted with permission from Chang S: Am J Physiol Renal Physiol 2006; **290**: F650.

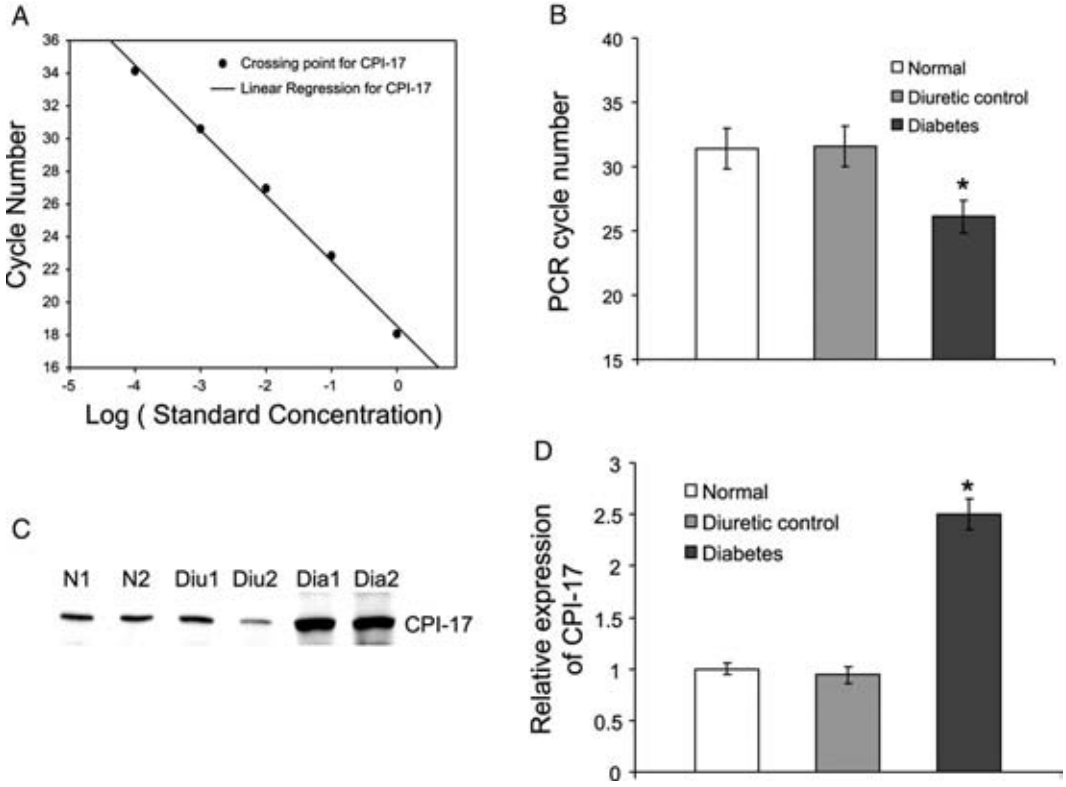

Fig. 6. CPI-17 expression at mRNA and protein levels. *A*, CPI-17 real-time PCR standard curve. *B*, average number of required PCR cycles was 31.4 in normal, 31.6 in diuretic and 26.1 in diabetic DSM samples, that is significantly decreased in diabetic samples. *C*, CPI-17 Western blot. Asterisk indicates significant difference between 4 samples each ($p < 0.01$). *D*, relative CPI-17 expression at protein level was almost 2.5-fold higher in diabetic vs normal and diuretic detrusor samples. Asterisk indicates significant difference between 4 samples each ($p < 0.01$). Reproduced with permission from Chang S: Am J Physiol Renal Physiol 2006; **290**: F650.

the epithelium. Although alterations in smooth muscle and nerve innervation were noted in patients with diabetes,[4] there is little information on urothelial involvement in DBD pathophysiology.

The few studies of the effects of DM on bladder urothelium in the STZ induced diabetic rat model show increased urothelial proliferation[40,41] without an increase in the thickness of the urothelial lining.[41] This increase in proliferation may divert urothelial cell physiology from the normal intercommunication/2-way communication with underlying bladder tissue by modifying urothelial cell receptor expression and the release of signaling molecules such as neurotransmitters. This in turn could impact/modify activity in underlying smooth muscle and nerve endings, and contribute to the bladder function modification in DM cases. Urothelial cell prostaglandin release is impaired in STZ-DM rats,[40] which may affect the urothelial barrier function. Prostaglandins have an important role in maintaining gut mucosal integrity.[42] It was also proposed that the common occurrence of urinary tract infections in patients with DM, which are attributable in part to bladder stasis due to the pathological condition, may be the result of altered expression of adherence receptors for bacteria by urothelial cells.[43]

Bladder urothelial abnormalities may impact LUT function by altering the release of mediators and sensory fiber excitability in the bladder. Also, because many of these urothelial functions may be altered in diabetes cases, defects in urothelial cells may in part underlie changes such as detrusor instability and/or changes in bladder capacity. Thus, the urothelium is an active participant in normal bladder function. It exists as an integral part of a sensory web, in which it communicates the degree of bladder filling to the underlying nervous and muscular tissues, and affects their function. This communication is made possible by the urothelial input and output pathways, which allow it to respond to its chemical and physical environment, and engage in multidirectional communication with neighboring cells in subadjacent tissues. Defects in urothelial receptor expression or aberrant release of mediators may contribute to diabetes associated bladder complications.

Neuronal. Neuronal control of bladder function involves a sophisticated, complex interaction between

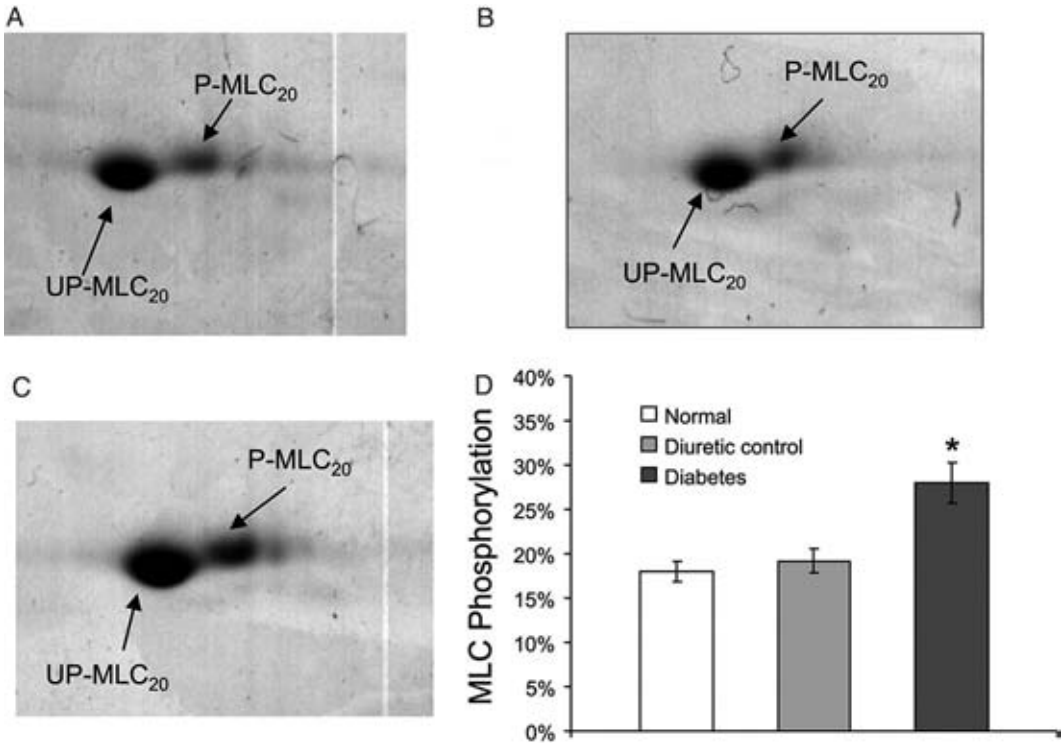

Fig. 7. Baseline MLC_{20} phosphorylation in DSM. *A* to *C*, 2-dimensional gel shows that phosphorylated MLC_{20} (*P-MLC$_{20}$*) ran slightly higher and more toward acidic side than unphosphorylated MLC_{20} (*UP-MLC$_{20}$*). *A*, normal. *B*, diuretic. *C*, diabetic. *D*, average phosphorylation was 18% in normal DSM. Diuretic control had similar 19.2% level but level was significantly increased to 28% in diabetic detrusor. Asterisk indicates significant difference between 3 samples each ($p < 0.05$). Reproduced with permission from Chang S: Am J Physiol Renal Physiol 2006; **290**: F650.

autonomic and somatic afferent and efferent pathways. A group reported an association of DBD with autonomic neuropathy detected by the sympathetic skin response in patients with diabetes.[44] Steers et al noted significant abnormalities in afferent pathways innervating the bladder in STZ induced diabetic rats.[45] Adult rats treated with the C-fiber afferent neurotoxin capsaicin have a number of similarities to diabetic rats.[46] Since capsaicin affects predominately small myelinated and unmyelinated afferents, it is temping to speculate that DM affects a similar afferent neuron population. On the other hand, it was also suggested that DBD is initiated by neuropathy in the efferent limb of the micturition reflex.[47]

Neurotrophic factors derived from target tissues can support peripheral neuron growth and survival. Rats with STZ induced DM 12 weeks after induction showed significantly decreased nerve growth factor, a member of the neurotrophin family, in the bladder and in L6 to S1 dorsal root ganglia, which contain bladder afferent neurons.[48] Reports that diabetic rodents show loss of neurotrophic support to peripheral nerves prompted studies to investigate the efficacy of neurotrophic factor supplementation on nerve disorders in diabetic rats. Unfortunately nerve growth factor was effective in a recently completed phase III trial.[49] Also, using exogenous neurotrophic factors as therapy is limited by the need for nonoral delivery, the fiber selectivity of individual neurotrophins, limited delivery to the nervous system and concerns about harmful systemic actions of growth factors. Alternative approaches warrant further investigation.

CONCLUSIONS

The review of the current literature indicates the state of knowledge on DBD. 1) DBD temporal manifestation may explain the spectrum of bladder dysfunction in patients with type I or II DM. 2) Early phase DBD manifests as storage problems such as urgency and urge incontinence at the clinical level, whereas detrusor muscle hypercontractility and neuronal changes manifest at the experimental level. Early evidence indicates that polyuria has a major role in the pathogenesis of changes at early phase DBD. 3) Late phase DBD manifests as voiding problems, leading to bladder inability to empty, which causes high post-void residual urine and overflow incontinence at the clinical level, and decompensated detrusor function at the experimental level. Accumulating evidence indicates a role for

oxidative stress in the pathogenesis of changes at the late phase of DBD.

Thus, it appears that in the future 4 issues should be addressed, including 1) studies of the time course of alterations in urothelial function, such as the impact of urine hyperosmolarity on urothelial remodeling; 2) studies of afferent sensitivity during early phase DBD and whether observed experimental alterations translate to overactive bladder at the clinical level; 3) mechanistic studies of the role of oxidative stress in DBD cases (the availability of genetically manipulated animals may facilitate such mechanistic studies); and 4) studies of the DBD time course in type II animal models, and whether types I and II mimic each other in DBD cases.

APPENDIX

Types of Bladder Dysfunction

	Storage Problems	Voiding Problems
Symptoms	Urgency, urge incontinence	Hesitancy, slow urine stream
Urodynamic results	Sensory urgency, detrusor overactivity	Slow flow, high detrusor pressure, post-void residual urine

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