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Diabetic Bladder Dysfunction: Current Translational Knowledge

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Abstract

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

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 future direction of research.

Results—Despite traditional recognition of DBD, as a voiding problem, characterized by poor emptying and overflow incontinence, recent clinical and experimental evidence indicate a presence of storage problems such as urgency, and urge incontinence in DM. Recent experimental evidence from studies of DBD on small animal models of DM, indicate the presence of a temporal effect on DBD: Early phase of DM causes compensated bladder function; and late phase of DM causes decompensated bladder function. The ‘temporal theory’ could plausibly provide the scientific road map for correlation between clinical and experimental findings as well as identification of role of mechanisms such as polyuria, hyperglycemia, oxidative stress, autonomic neuropathy and decompensation of contractile apparatus of the bladder in creation of clinical and experimental manifestations of the DBD.

Conclusions—DBD includes time-dependent manifestations of storage and emptying problems. Identification of mechanistic pathways would lead us to identification of therapeutic intervention.

Keywords

Diabetes; Bladder Dysfunction; Cystopathy; Complications

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INTRODUCTION

About one of every 14 Americans, including almost one of every seven African-Americans and one of every five seniors (≥65 years old), has DM, with roughly 30% of those undiagnosed. Type 1 DM accounts for 5-10% of all diagnosed cases. About one of every 500 children and adolescents has type 1 DM [1]. The incidence of type 2 DM, accounts for 90-95% of cases, increased by 33% between 1990 and 1998 and by 75% among those 30 to 39 years of age [2]. The U.S. Centers for Disease Control and Prevention estimated in 2005 that 20.8 million people in the U.S. (7% of the population) have DM. The total medical and indirect costs of DM and its complications were estimated to be $132 billion in the U.S. in 2005, accounting for about 10% of total health care costs [1]. Continuation of this trend is expected due to the continuing rise in obesity, a major risk factor for type 2 DM. Diabetics live decades with the disease and are susceptible to numerous burdensome and costly complications. It is indeed the complications of DM that render it a debilitating and devastating disease. Lower urinary tract (LUT) complications are among the most common complications of DM. The most common and bothersome LUT complication of DM is diabetic cystopathy, or diabetic bladder dysfunction (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% of patients, respectively [4]. The most common and bothersome LUT complication of DM is DBD. Although DBD is not life threatening, it affects quality of life substantially. Yet, little is known about the natural history and pathophysiology of DBD. The paucity of knowledge has been a barrier to developing the best methods of prevention and treatment.

DBD: storage or voiding problem?

The bladder has two major and distinct functions: urine storage and urine disposal. A simplified categorization of bladder dysfunction into problems of storage or voiding has been widely accepted [5]. Urodynamic studies (UDS) are often used to provide more information on the storage or voiding nature of the bladder dysfunction, as outlined in Table 1. DBD has been described traditionally as a triad of decreased sensation, increased capacity and poor emptying, but many inconsistencies with those “classic” findings have been found. In most of the asymptomatic diabetic patients they studied, Ueda et al. found increased bladder volume at first sensation to void and a decrease in detrusor contractility, with resultant increased post void residual urine volume, but they also found a 25% incidence of detrusor overactivity [6]. A review by Kaplan and coworkers of urodynamic findings in 182 diabetic patients revealed 55% with detrusor overactivity but only 23% with impaired contractility, with 10% of patients areflexic and 11% “indeterminate” [7]. The mixed clinical picture of DBD has also been revealed in recent large-scale studies, in which DM was associated with a 40-80% increased risk of urge incontinence and a 30-80% increased risk for overflow incontinence in controlled multivariate analyses [8]. So, it is now clear that DBD manifestations are a combination of storage and voiding bladder problems.

Temporal theory of DBD: A potential unifying mechanism of the pathogenesis of DBD

In examination of natural history of DBD, we have observed that morphological and functional manifestations of DBD in STZ-induced DM are *time-dependent*. Bladder hypertrophy and remodeling, increased contractility and associated neurogenic changes occur soon after the onset of DM [9,10,11], while the drop of peak voiding pressure in the cystometric measure, develop only at a later stage of DM [12,13]. The time-dependent alterations of DBD served as the basis for our 'temporal hypothesis' of DBD with mixed clinical manifestations, in which we propose that DM causes the bladder to undergo two phases of alterations via two main mechanisms (Table 2): 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 the bladder tissues and function. This temporal hypothesis of the pathophysiology of DBD provides a potentially unifying theory under which the complex interaction between seemingly confusing bladder dysfunction can be explained. Further, it provides a scientific road map under which the timing and specific roles of various components such as detrusor, urothelium, autonomic nerves and urethra can be explored.

PATHOGENESIS

Pathogenesis of DBD could be related to hyperglycemia-induced polyuria and oxidative stress as discussed below:

Polyuria and the early phase of 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 causes rapid and substantial bladder hypertrophy and increased bladder contractility, capacity and compliance that are similar to those 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 role of hyper-osmol polyuria from a normal-osmol polyuria in the mediation of bladder remodeling requires future studies in which separation of role of osmolality vs. increased flow or stretch on sensory elements of the urothelium should be explored.

Prolonged hyperglycemia, oxidative stress and late phase DBD

Accumulation of oxidative stress products within most types of cell is a prominent feature of prolonged hyperglycemia [16]. Oxidative stress in diabetes could originate from a variety of mechanisms, which include: oxygen radical production from auto-oxidations of glucose, glycated proteins, stimulations of cytochrome P450–like activity, alterations of NADPH/NADP ratio by excess glucose going through the polyol pathway, increased production of super oxide dismutase and increased production of lipid peroxidation [16,17,18]. Dr. Brownlee has promoted a unifying mechanism that links together all of the seemingly unconnected hyperglycemia-induced pathways, all of which stem from increased mitochondrial production of reactive oxygen species (ROS), primarily superoxide [18]. The excess ROS

cause, in turn, DNA strand breaks, activation of poly (ADP-ribose) polymerase (PARP) and inhibition of glyceraldehyde-3 phosphate dehydrogenase (GAPDH), culminating in activation of the four damaging pathways [18].

While there are numerous studies on the role of oxidative stress on the pathogenesis of diabetic complications in the eye, nervous system, kidney and cardiovascular system, studies on the direct effect of oxidative stress in the urologic complications has not yet been investigated in detail. A few studies on its role in erectile dysfunction [11,19] and on cystopathy [20,21] have indicated the importance of oxidative stress in the pathogenesis of urologic diabetic complications. Using a rabbit model of alloxan-induced diabetes, we showed that the decrease in the contractility of the detrusor smooth muscle (DSM) is associated with increased lipid peroxidation products (Figure 1) and overexpression of aldose reductase [21]. The increased expressions of aldose reductase favor the cycling of elevated glucose through the polyol pathway and produced increased levels of sorbitol [21]. We also have evidence that the exposure of human bladder smooth muscle cells to high glucose also increases the expression of aldose reductase in these cells (unpublished data). Exposure of these cells grown in high glucose to the aldose reductase inhibitor Zopolrestat reverses the overexpression of aldose reductase, which supports the conclusion from our studies on intact muscle from diabetic rabbits that the overexpression of aldose reductase and its over-activity might contribute to the rise in redox and lipid peroxidation (unpublished data).

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

Endogenous antioxidants are able to destroy the ROS and create a balance between antioxidant and free radicals in a normal situation; however, in diabetes the antioxidant defense system is deficient due to the high level of oxidative stress. Intake of antioxidants, such as vitamin E [24] and α-lipoic acid [25], which functions as a cofactor in multi-enzyme complexes, have been successfully used to reverse the oxidative stress produced by hyperglycemia in human diabetics and in STZ-induced diabetic animal models. Oral treatment of α-lipoic acid (600 mg per day orally for five weeks) improved neuropathic deficits in diabetic patients with distal symmetric polyneuropathy in a recent clinical trial [25]. It has not been reported whether urinary bladder function improved in these patients. In our preliminary studies on the effects of antioxidants on oxidative-stress induced by high glucose in cultured hBSMC, we are able to decrease the production of lipid peroxidation (Figure 3).

PATHOPHYSIOLOGY

Pathophysiology of DBD is multifactorial as it involves alterations in detrusor, nerve, urothelium and urethra

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

Myogenic changes in diabetes

In vivo and *in vitro* experimental studies on DSM from animal models of DM do give evidence for myogenic changes. Earlier studies on the effects of diabetes on detrusor contractility show both 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]. Contractility of the DSM was decreased in response to stimulation by KCl and carbachol, and the decrease was associated with not only the duration of the hyperglycemic state but also the level of hyperglycemia (Figure 4). Changes in muscarinic receptor population have also been linked to altered contractility [34]. Unlike the changes in the DSM from an obstructed bladder, we found, both using STZ-induced rat diabetic model and alloxan-induced rabbit model, that there is no change in the myosin isoform composition in the DSM from diabetic animals [35]. Recent physiologic and biochemical studies on the DSM from our group [36,37], as well as others [13,38] show a distinct deficit in the regulation of contraction in the DSM in diabetes.

A major regulatory mechanism for smooth muscle contraction is the myosin-mediated regulation via phosphorylation-dephosphorylation of the regulatory myosin light chain (MLC_{20}) by Ca^{2+}-dependent myosin light chain kinase (MLCK) and the myosin light chain phosphatase (MLCP). The MLCP is inactivated by phosphorylation, catalyzed mainly by Rho-kinase, and also by binding to phosphorylated CPI-17. By lowering the activity of MLCP, these proteins retain the myosin in the phosphorylated state and maintain muscle tone in the absence of an elevation of cytosolic Ca^{2+}. Studies performed on DSM from diabetic animals showed overexpression and overactivity of Rho-kinase and CPI-17 proteins involved in the Ca^{2+}-sensitization in smooth muscle (Figures 5 & 6). Interestingly, we also found high level of basal MLC_{20} phosphorylation in the diabetic detrusor (Figure 7). However, the molecular mechanisms for the diabetes-induced alteration in the expression of

these proteins that regulate the myosin-mediated regulation of DSM contraction are not known.

Urothelial changes in diabetes

An important, but not well understood, function of epithelial cells is their ability to sense changes in their extracellular environment and then communicate these changes to the underlying nervous, connective and muscular tissues [39]. This communication is likely to be important for tube- and sac-shaped organs such as blood vessels, the gut, and the bladder, whose normal function can be modulated by stimuli initiated within the epithelium. Though alterations in smooth muscle and nerve innervation have been shown in diabetic patients [4], there is little information regarding the involvement of the urothelium in the pathophysiology of DBD.

The small number of studies on the effects of DM on bladder urothelium, using the STZ-induced diabetic rat model, report an increase in urothelium proliferation [40,41] without an increase in the thickness of the urothelial lining itself [41]. This increase in proliferation may divert the physiology of the urothelium cells from their normal inter-communication/two-way communication with the underlying bladder tissue, by modifying both 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 observed in DM. It has been reported that urothelial cell prostaglandin release is impaired, in STZ-DM rats [40]. This might affect the barrier function of the urothelium. Prostaglandins are known to play an important role in the maintenance of musical integrity in the gut [42]. In addition it has been proposed that the common occurrence of urinary tract infections seen in DM and attributed in part to the bladder stasis seen in the pathology, may be as a result of altered expression of adherence receptors for bacteria by urothelial cells [43].

Abnormalities in bladder urothelium could impact the LUT function by altering release of mediators as well as excitability of sensory fibers in the bladder. In addition, because many of these urothelial functions may be altered in diabetes, defects in urothelial cells may underlie, in part, changes such as detrusor instability and/or changes in bladder capacity. Thus, the urothelium is an active participant in the normal function of the bladder and 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 functions. This communication is made possible by the input and output pathways of the urothelium, which allow it to respond to its chemical and physical environment and to engage in multi-directional communication with neighboring cells in subadjacent tissues. Defects in the urothelial expression of receptors or aberrant release of mediators may contribute to diabetes-associated bladder complications.

Neuronal changes in diabetes

The neuronal control of bladder function involves a sophisticated and complex interaction between the autonomic and somatic afferent and efferent pathways. One group reported an association of DBD with autonomic neuropathy detected by the sympathetic skin response

in diabetic patients [44]. Steers et al showed significant abnormalities in afferent pathways innervating the bladder in STZ induced diabetic rats [45]. Adult rats treated with capsaicin, a C-fiber afferent neurotoxin, exhibit a number of similarities to diabetic rats [46]. Since capsaicin is known to affect predominately small myelinated and unmyelinated afferents, it is temping to speculate that DM affects a similar afferent neuron population. On the other hand, it has also been suggested that DBD is initiated by neuropathy in the efferent limb of the micturition reflex [47].

Neurotrophic factors derived from target tissues can support the growth and survival of peripheral neurons. Rats with STZ-induced DM, 12 weeks after induction, showed significantly decreased levels of nerve growth factor (NGF), 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 have prompted studies to investigate the efficacy of neurotrophic factor supplementation on nerve disorders of diabetic rats. Unfortunately, NGF was not shown to be effective in a recently completed phase III trial [49]. In addition, the use of exogenous neurotrophic factors as a therapy is limited by the need for non-oral delivery, the fiber selectivity of individual neurotrophins, limited delivery to the nervous system and concerns about harmful systemic actions of growth factors. The alternative approaches warrant further investigation.

CONCLUSIONS

The review of current literature indicates the following state of knowledge on DBD:

- Temporal manifestation of DBD may explain the spectrum of bladder dysfunction seen in patients affected by type I or II DM.
- Early phase of DBD is manifested by storage problems; such as urgency, urge incontinence manifested at the clinical level, whereas hypercontractility of the detrusor muscle, and neuronal changes are manifested at the experimental level. Early evidence indicates that polyuria plays a major role in pathogenesis of changes seen in the early phase of DBD.
- The late phase of DBD is manifested by voiding problems leading to inability of the bladder to empty causing high post-void residual and overflow incontinence, at the clinical level and decompesated detrusor function at the experimental level. Accumulating evidence indicate a role for oxidative stress in pathogenesis of changes seen in the late phase of DBD.

Thus, it appears that the future investigative work should address the following issues:

- Studies of time course of alterations in the function of urothelium, including impact of urine hyperosmolarity on urothelial remodeling.
- Studies of afferent sensitivity during the early phase of DBD; and whether the observed experimental alterations translate to presence of overactive bladder manifested at the clinical level.

- Mechanistic studies related to role of oxidative stress in DBD. Availability of genetically manipulated animals may facilitate such mechanistic studies.
- Studies of time course of DBD in type II animal models, and whether type I and II mimic each other in DBD.

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Key of Definitions for Abbreviations

DBD	diabetic bladder dysfunction
DM	diabetes mellitus
DSM	detrusor smooth muscle
LUT	lower urinary tract
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
NGF	nerve growth factor
ROS	reactive oxygen species
STZ	streptozotocin

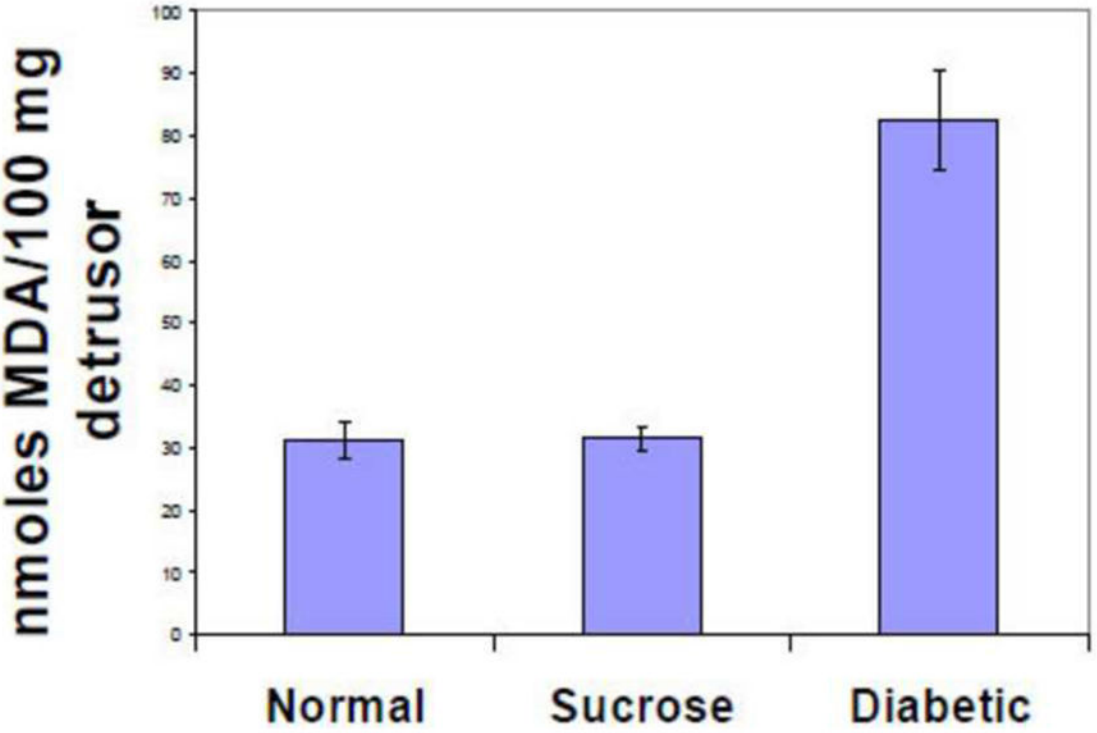

Figure 1. Increase in Lipid Peroxidation

Products in Detrusor MDA levels are given for detrusor from normal (N=11), sucrose fed controls (N=13) and diabetic rabbits (N=5). MDA levels were significantly higher in detrusor muscle from diabetic rabbits compared to normal and sucrose feed controls. Data are given as mean±S.E. *, $p<0.05$ compared to normal sucrose.

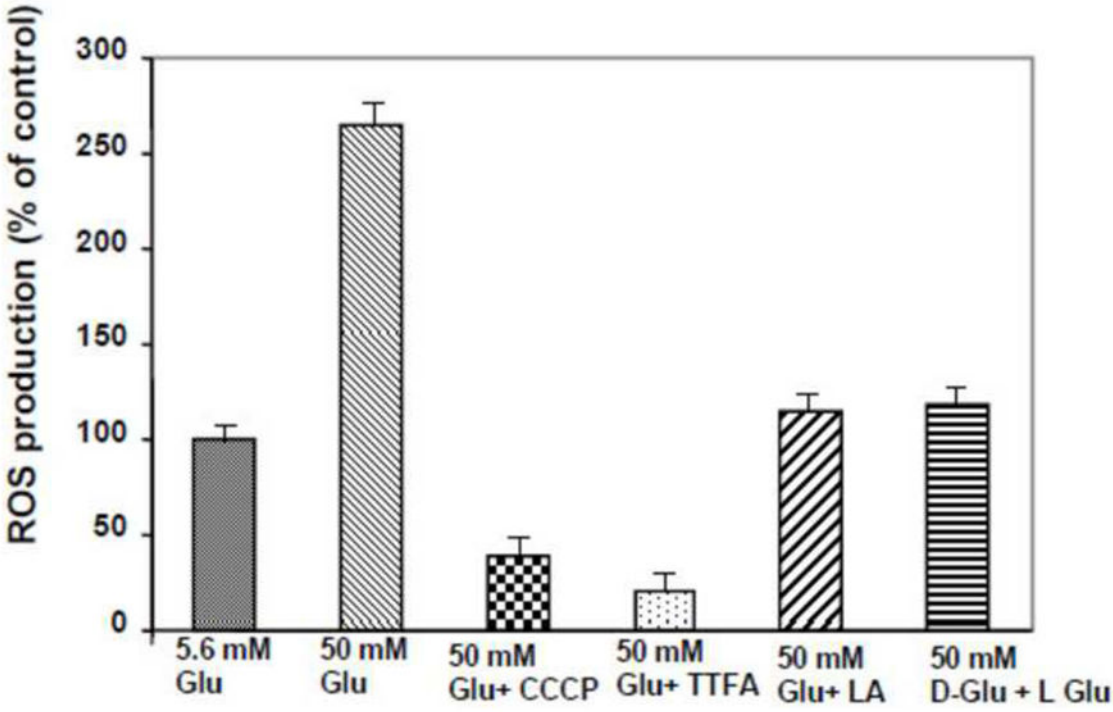

Figure 2. Mitochondrial ROS production

Incubation of human BSM cells with high glucose increases ROS generation and is prevented by the antioxidants alpha lipoic acid. Human BSM cells were grown with low (6mM) and high (50 mM) glucose plus 10 uM TTFA, 1uM CCCP, 200 uM of alpha lipoic acid and 50 mM of L-glucose for 48 hrs. The mitochondrial fractions were assayed for the ROS production. The ROS concentration was determined from a standard curve of H2O2 (95-100 umol/l) and was expressed as a percentage of ROS incubated at 6 mmol/l glucose.

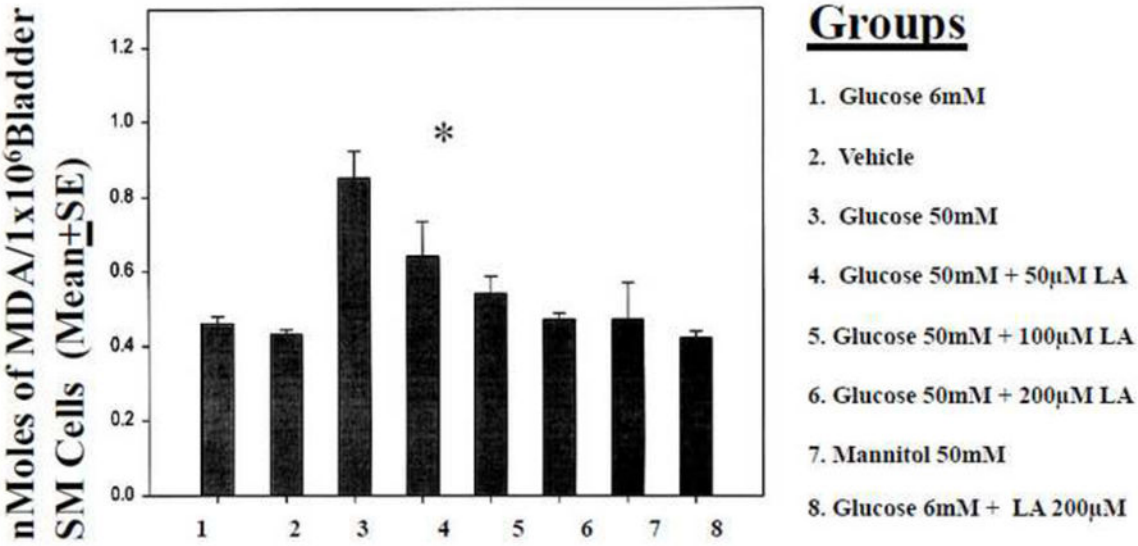

Figure 3. Lipid peroxidation induced by high glucose

Increase in lipid peroxidation products in BSM cells treated with high glucose (50 mM). Mannitol 50 mM treated group served as control for osmotic shock. High glucose (50 mM) induced lipid peroxidation, was inhibited by lipoic acid treatment (4&5). Normal (6 mM) glucose treatment and mannitol treatment did not induce any changes in lipid peroxidation. *$p<0.05$ compared to Glucose 6 mM.

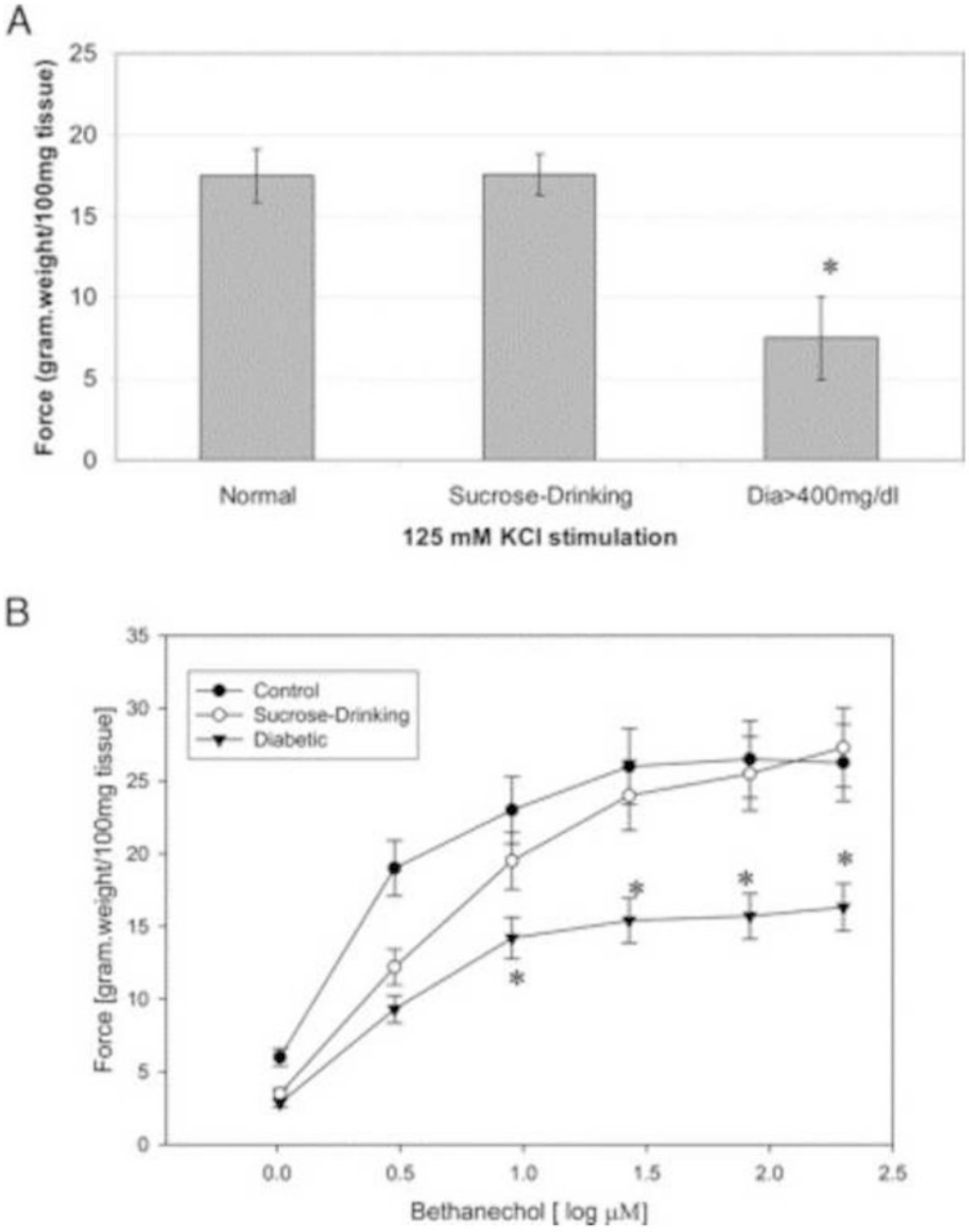

Figure 4. Force generation by detrusor muscle strips

A, effects of 125 mM KCl on force generation by detrusor muscle from normal, sucrose drinking and diabetic (Dia>400mg/dl) rabbits. Force was significantly decreased for diabetes. Data are shown as mean ± SEM. Asterisk indicates $p < 0.05$. B, bethanechol dose response curve of force generation by detrusor muscle from normal, sucrose drinking and diabetic rabbits. Force was significantly decreased for diabetes. Data are shown as mean ± SEM. Asterisk indicates $p < 0.05$. (Reproduced from Changolkar et al., 2005 with permission.)

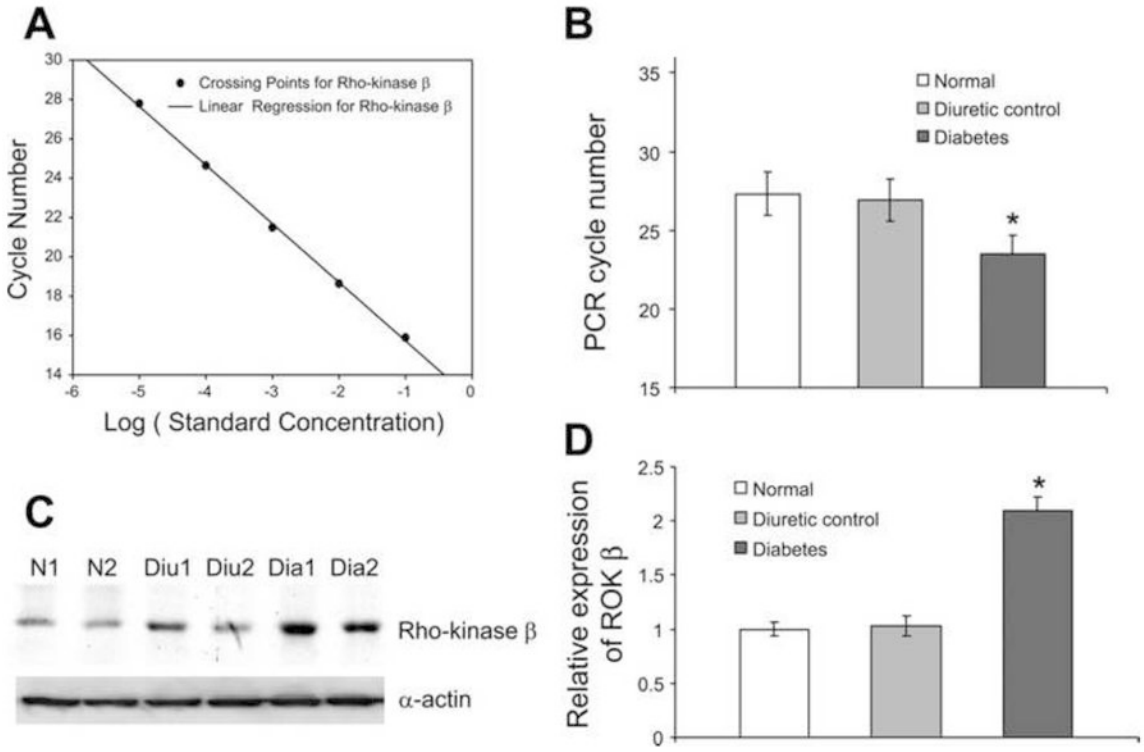

Figure 5. Expression of Rho-kinase at both mRNA and protein level

A: real-time PCR standard curve for Rho-kinase **B:** average of required PCR cycle numbers to reach crossing threshold. The required PCR cycle numbers was 27.3 for normal samples, 26.9 for diuretic controls, and 23.5 for diabetic samples. A significantly lower number of PCR cycles for the diabetic sample indicated that diabetic DSM sample had more copies of Rho-kinase transcript. **C:** Western blot for Rho-kinase and smooth muscle -actin. **D:** bar graph showing the average of relative protein expression level. There was almost a 2.1-fold higher protein expression of Rho-kinase in diabetic detrusor compared with normal and diuretic samples. *Significant difference between samples ($n = 4$, $P < 0.01$). (Reproduced with permission from Chang et al, 2006.).

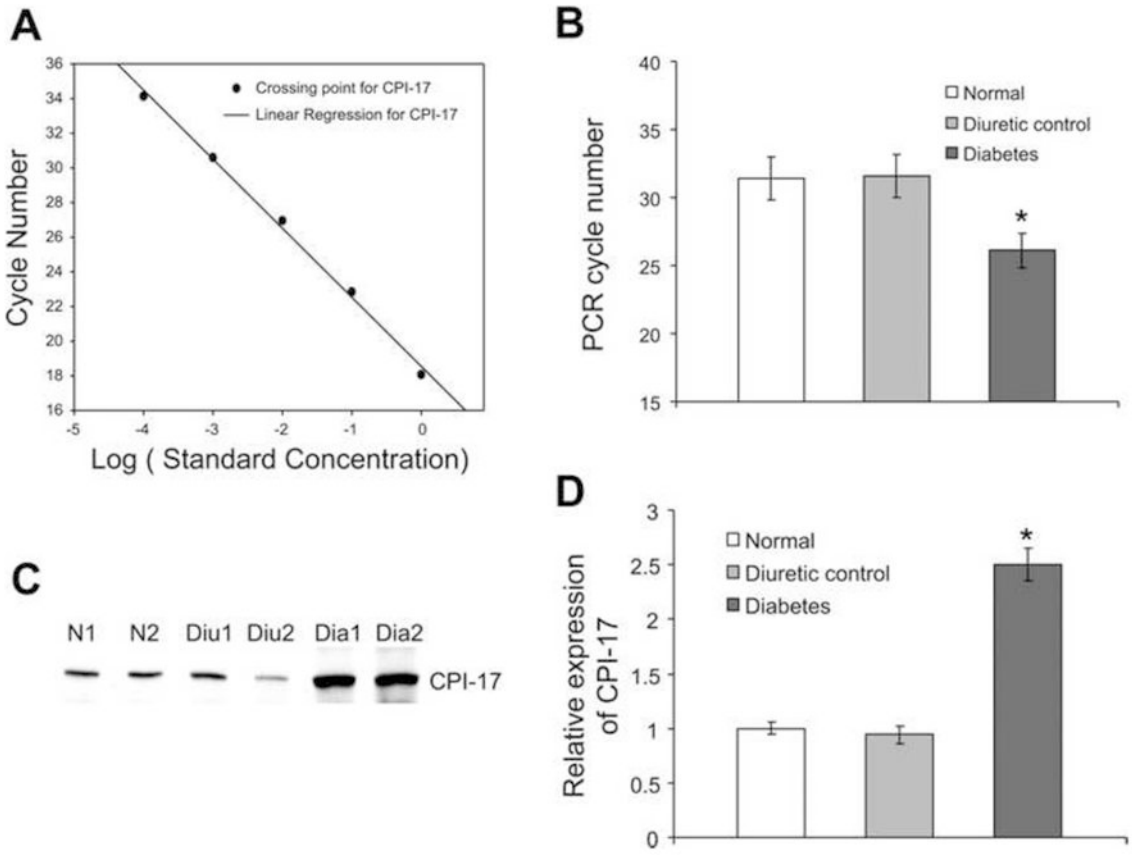

Figure 6. Expression of CPI-17 at both mRNA and protein level

A: standard curve of real-time PCR for CPI-17. B: average of the PCR results. The required PCR cycle numbers was significantly decreased in diabetic DSM samples compared with normal or diuretic controls. It was 31.4 cycles for normal sample, 31.6 cycles for diuretic controls, and 26.1 cycles for diabetic samples. C: Western blot for CPI-17. D: bar graph showing the relative expression of CPI-17 at the protein level. There was almost a 2.5-fold higher expression of CPI-17 at the protein level in diabetic detrusor compared with normal and diuretic samples. *Significant difference between samples ($n = 4$, $P < 0.01$). (Reproduced with permission from Chang et al, 2006.).

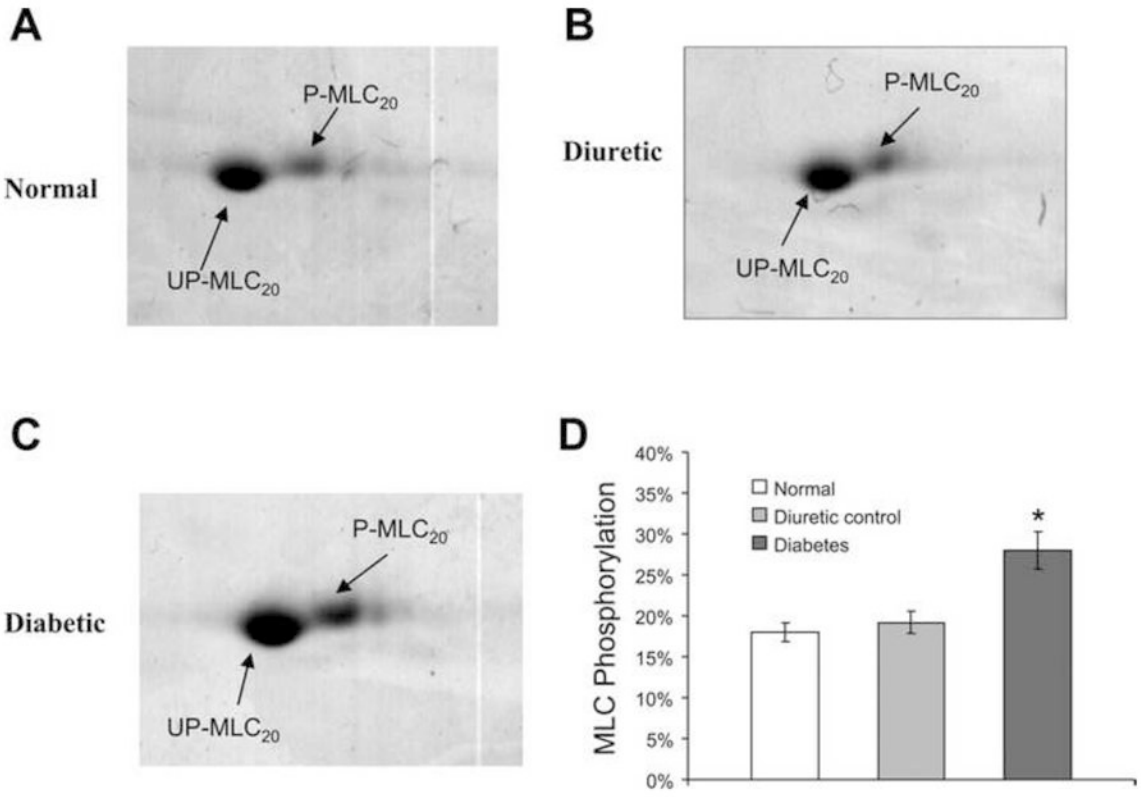

Figure 7. Selected areas from 2-dimensional (2D) gel showing basal MLC_{20} phosphorylation in normal, diuretic, and diabetic detrusor smooth muscle (DSM)

A: normal. B: diuretic. C: diabetic. D: bar graph showing the average values of myosin light chain (MLC) phosphorylation. Phosphorylated MLC_{20} (P-MLC_{20}) runs slightly higher and more toward the acidic side than unphosphorylated MLC_{20} (UP-MLC_{20}) in the gel. The basal phosphorylation of MLC_{20} was 18% in normal DSM. Diuretic control had a very similar level (19.2%) of MLC_{20} phosphorylation. However, the phosphorylation level was significantly increased to 28% in diabetic detrusor. *Significant difference between samples ($n = 3$, $P < 0.05$). (Reproduced with permission from Chang et al, 2006.).

Table 1

Types of Bladder Dysfunction

	Storage problems	**Voiding problems**
Symptoms	urgency, urge incontinence	hesitancy, slow urine stream
Urodynamics results	sensory urgency, detrusor overactivity	slow flow, high detrusor pressure, post-void residual

Table 2

Proposed natural history of progression of diabetic bladder dysfunction

	Early Phase →	Late Phase
	Compensated Function	Decompensated Function
	Time Course/Risk factors ??	
Clinical:	**Storage problems**	**Voiding Problems**
Urodynamics:	**Overactive Bladder**	**Atonic Bladder**
In-vitro:	**Hypercontractile Detrusor**	**Hypocontractile Detrusor**