# Animal Models of Diabetic Complications Consortium
# (U01 DK076162)

## Annual Report
## (2009)

## Diabetic Uropathy Pathobiology Site
## Case Western Reserve University

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## Progress Report Summary

**Required Questions**-

a) There has been a change in the other support of key personnel since the last reporting period. NO

b) There will not be, in the next budget period, a significant change in the level of effort for key personnel from what was approved for this project.
No.
c) The estimated unobligated balance, including prior year carryover, will not be greater than 25 percent of the current year's total budget.
No.

**Background and Summary**-
Funding received through this grant is intended to support research activities related to participation of the PI in the Animal Models of Diabetic Complications Consortium (AMDCC) as the Diabetic Uropathy Pathobiology Site. Under this grant, we have proposed to create the following mice model of diabetic uropathy. The AMDCC has approved the concept of creation of the $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$. For all the experimental studies, we will use the following groups of mice:

1. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT to activate $CreER^{T2}$ to abolish MnSOD expression.
2. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with OHT and with STZ to induce diabetes.
3. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$, sham treated.
4. $MnSOD^{lox/lox}$, $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ treated with STZ.

### A. Specific Aims

**Our specific aims for the life of grant are as followings.**

**Specific aim #1**: To examine the temporal alterations in the in-vivo bladder function by evaluation of 24 hours micturition habits and conscious cystometry in the above groups of mice at two time points of 8 and 12 weeks after induction of diabetes.

**Specific aim #2**: To examine the temporal course of morphological changes in neurogenic and myogenic components of the bladder remodeling in the above groups of mice by:

Examination the changes of bladder tissue components and their contribution to remodeling of the wall and chamber of the bladder

Examination of the changes in bladder innervations markers.

**Specific aim #3**: To examine the temporal alterations in the contractile function of the detrusor in the above groups of mice by:

Examination of the contractile responses of the detrusor.

Examination of the contractile and regulatory proteins of the detrusor.

Examining the alterations of the L-type $Ca^{2+}$ channel.

Examining the alterations in the capacitive calcium entry (CCE).

Examining the IP3- and RyR-induced calcium release.

Examining the $Ca^{2+}$ sensitivity in permeabilized detrusor strips.

**Specific aim #4**: To examine the temporal alterations induced by STZ in afferent and efferent autonomic pathways innervating the bladder in the in the above groups of mice by:

Assessment of afferent autonomic function by measurement of Current Perception Threshold (CPT)

Examining the relative contribution of cholinergic and purinergic components to the contractile response to transmural electrical stimulation.

Examining the alterations in ATP-P2X3, VR-1 afferent pathway in the bladder.

Examining the alterations in muscarinic receptors (M2, M3) and/or purinergic receptors (P2X1, P2X2).

Examining the connexin 43-containing gap junctions in the bladder.

In addition to scientific aims of the project and via a Minority Supplemental Award, Dr. Adebola Fabiyi has joined our research team. The Minority Award has allowed continued employment of Dr. Fabiyi with the PI and his full participation in activities related to 'Diabetic Uropathy Pathobiology Site'.

In July 2008, the PI moved from Cleveland Clinic to Upstate Medical University (UMU). Our entire research team, including Dr. Fabiyi, also relocated to UMU. Dr. Fabiyi has set up a sophisticated electophysiological laboratory for studies of LUT dysfunction in mice and has generated several lines of preliminary data (see below).

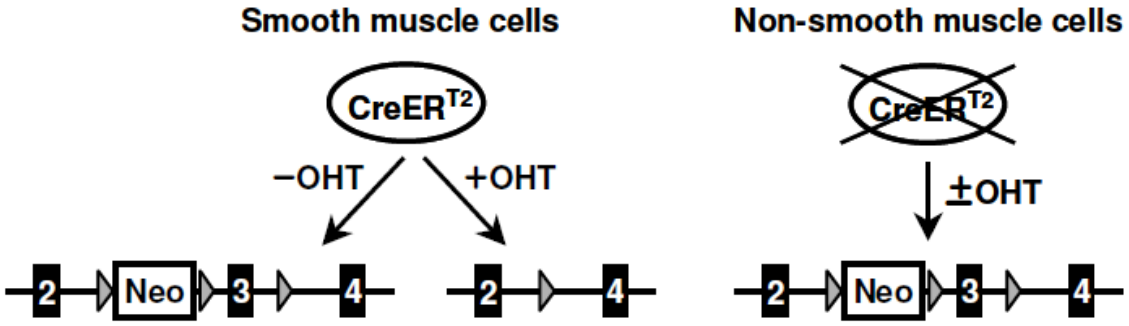
1. *Participation in the Steering Committee and External Advisory Board meeting*– Dr. Fabiyi has attended the AMDCC Steering Committee Meeting in September 2008 and has participated in presentations related to activities of 'Diabetic Uropathy Pathobiology Site'.
2. *NIDDK Sponsored Workshop on 'Urological Complications of Obesity and Diabetes'.* The PI functioned as a co-chair and organized a NIDDK sponsored workshop on '*Urological Complications of Obesity and Diabetes'.* This workshop was attended by over 100 inter/national experts on complications of diabetes. Dr. Fabiyi participated in the workshop and interacted with participants.
3. *Publications*- Dr. Fabiyi's work has led to two peer reviewed publications [1,2] and 5 abstract presentations at national meetings.

## B. Studies and Results

**Creation and breeding of healthy MnSOD$^{lox/lox}$ SM-CreER$^{T2}$(ki)$^{Cre/+}$ mice-** Evidence strongly suggests a key role for exaggerated Oxidative Stress (OS) in decompensated phase of diabetic bladder dysfunction (DBD). As the SA#1 of our 'Diabetic Uropathy Pathobiology site" we have aimed to generate a smooth muscle-specific manganese superoxide dismutase (MnSOD) knockout mouse to examine the role of reactive oxidative stress (ROS) in DBD.

**Results:**
We have completed the breeding of MnSOD lox/lox mice with SM-CreERT2(ki) Cre/+ mice and subsequently treated with OHT to activate CreERT2 to delete exon 3 of the MnSOD gene (SOD2) (Figure 1).

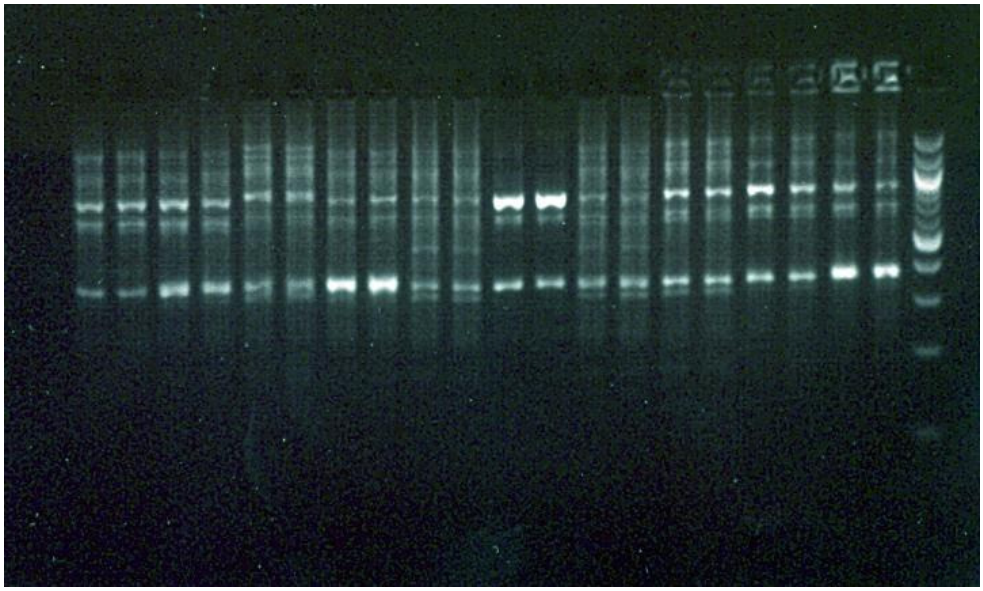
**Figure 1: Cre-lox recombination system with or without OHT activation**




The strategy used for creation of our conditional, smooth muscle-specific MnSOD KO mice [3]. The lines at the bottom show the "floxed" MnSOD gene locus at exon 3. Exons 2-4, loxP sequences and the neomycin resistance gene are indicated by the numbered black boxes, grey triangles and boxed Neo, respectively. The circled CreERT2 indicates the CreERT2 protein. The OHT-dependent CreERT2 is only expressed in smooth muscle cells under control of the 'SM' promoter and only active in the presence of OHT.

To validate our mouse model, mature offspring (8 weeks after birth) were injected with OHT at 40 mg/kg for 5 consecutive days. Three days after the final injection, 31 male mice were sacrificed, and tissues of detrusor of the bladder, urothelium, aorta, heart, liver, skeletal muscle and skin of the tail were examined for MnSOD exon 3 by polymerase chain reaction (PCR). The phenotypical characterization of the created MnSOD lox/lox, SM-CreERT2(ki) Cre/+ mice show normal growth and function with no gross abnormalities. Three days after OHT injection, the PCR of the harvested tissues show deletion of MnSOD exon 3 in the bladder smooth muscle and aorta of the MnSODlox/lox, SM-CreERT2(ki) Cre/+ mice. The MnSOD exon 3 was present in the heart, liver, skeleton muscle, urothelium, and tail of the mice, suggesting a conditional and smooth muscle specific deletion of the MnSOD exon 3 in the created mice
(Figure 2).

Figure 2: PCR result of MnSOD lox/+,SM-CreERT2 Cre/+ treated mice with OHT



Lanes 1-2: tail before OHT
Lanes 3-4: tail after OHT
Lanes 5-6: skeletal muscle
Lanes 7-8: bladder smooth muscle
Lanes 9-10: urothelium
Lanes 11-12: urethra
Lanes 13-14: ureter
Lanes 15-16: liver
Lanes 17-18: heart
Lanes 19-20: aorta
Lane 21: ladder

P1 band: without MnSOD exon 3 excision
P3 band: MnSOD exon 3 was excised

Gene Sequencing of DNA from band @400 bp (P3 band) in above agarose gel after PCR verified the MnSOD gene had been knocked-out. The work was presented at the NIDDK-sponsored workshop in March 2009 in Baltimore, MD titled “Urological Complications of Obesity and Diabetes”, where our research fellow, Dr. Nan Xiao, won the award for Best Basic Science Research poster.

Next, we began to create the following strains and subject them to the indicated treatments:

| Genotype | OHT Treatment | Time Point after Treatment |
|---|---|---|
| wt/wt wt/wt | Yes | 6 weeks |
| | | 16 weeks |
| | No | 6 weeks |
| | | 16 weeks |
| lox/lox wt/wt | Yes | 6 weeks |
| | | 16 weeks |
| | No | 6 weeks |
| | | 16 weeks |
| wt/wt wt(+)/cre | Yes | 6 weeks |
| | | 16 weeks |
| | No | 6 weeks |
| | | 16 weeks |
| wt/lox wt/cre | Yes | 6 weeks |
| | | 16 weeks |
| | No | 6 weeks |
| | | 16 weeks |
| lox/lox wt/cre | Yes | 6 weeks |
| | | 16 weeks |
| | No | 6 weeks |
| | | 16 weeks |

We have begun to analyze the functional capacity of these mice via 24 hour micturition testing and cystometry. To date, we do not have enough data points to make any conclusive statements about the differences observed among the groups.

In collaboration with Jackson Laboratory, we established two colonies of this mouse model: one kept at JAX and the other in our laboratory. Upon further genotyping of the breeding colony at Jackson Laboratory, our genotyping analysis by PCR revealed the JAX mice showed a fragment at 550bp to represent the lox allele (Figure 3, lanes 4-5), which is in contrast to the published result of 358bp (Ikegami, 2002) which has been seen in our lab's mice both before and after shipment of a sample of our colony to JAX (Figure 4, lanes 12-13). Both sets of mice do exhibit the wt allele at 500bp.

Figure 3:

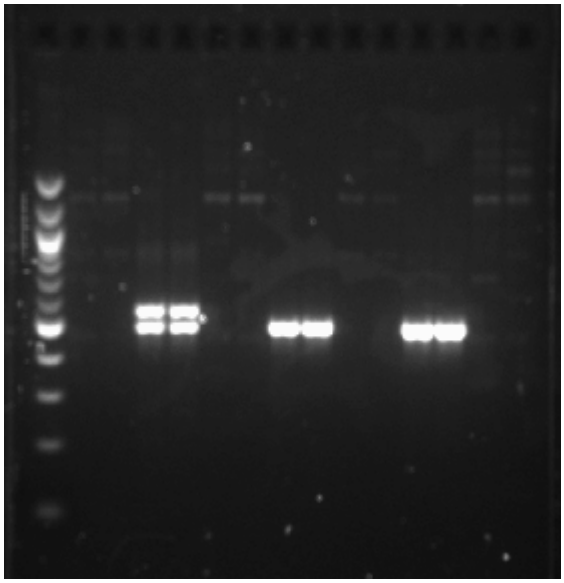
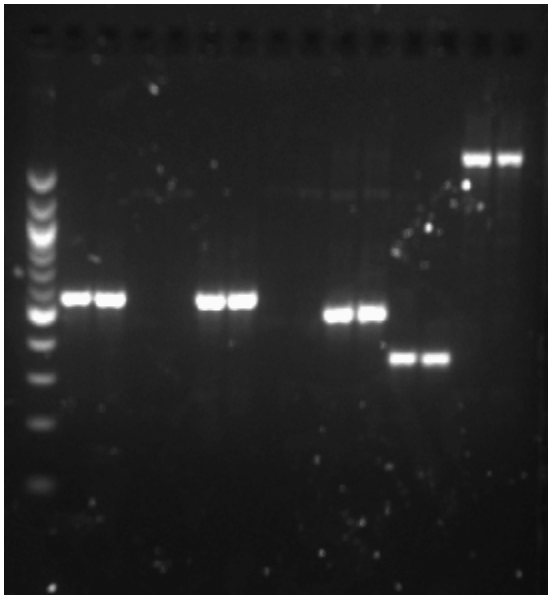


Figure 4:



Jackson Laboratory claims that this difference is not uncommon and that the region of interest should still be intact. However there are no published reports to confirm this.
We recently received a new batch of animals from Jackson Laboratory which were harvested and will be analyzed for SOD activity, which will be compared to tissues extracted from our colony. These results will shed better light on whether in fact the two colonies are functionally similar or not.

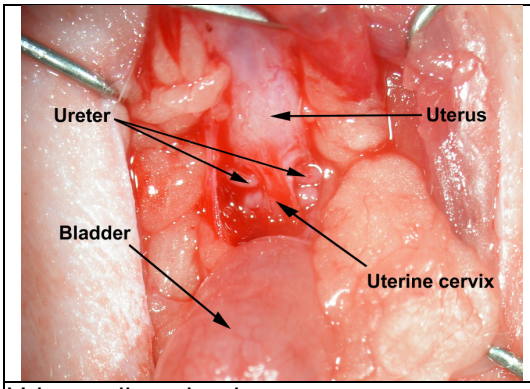
However, with the mix up in genotype of the colony raised at Jackson Laboratory, we have had a set back as we have had to breed more mice from our colony than anticipated and suspended our importation from Jackson until the differences could be sorted out. As a result we did obtain the adequate number of mice sufficient for completion of our experiments.

*Conclusions*: We have successfully deleted MnSOD exon 3 in the detrusor smooth muscle bladder of MnSODlox/lox, SM-CreERT2(ki)Cre/+ mice in a time selective manner by activation of the Cre recombinase system. Upon induction of diabetes in these mice, we will be able to examine the mechanistic role of ROS in remodeling of the bladder in a time specific manner according to the temporal alteration of the DBD previously described by us and other investigators.

**Continuation of major progress on studies of pathophysiology of diabetic bladder dysfunction (DBD).** Our lab continues to be at the forefront of examination of mechanisms of DBD. We have completed the following studies during 2008-2009:

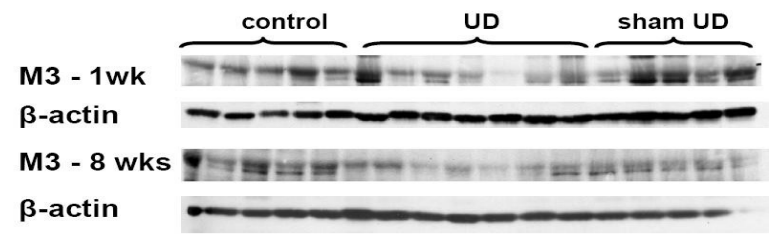
**Creation of a urinary diversion model (*unpublished data*).** To examine the impact of polyuria on LUT, we have developed and maintained a model of urinary diversion (UD) in rats and characterized UD effects on the bladder in non-diabetic (completed experiments) and diabetic (experiments in progress) animals. UD model is created by diverting the ureters to the cervix, which immediately drains the urine into the vagina (Figure 5). The epithelium of the vagina consists of keratinized squamous cells, similar to skin, with less permeability to urine compared to other choices of diversion such as colon or uterus. We have examined the functional (CMG), morphological and molecular profiles of the bladder and the urethra 1 wk and 8 wks after UD in female Sprague-Dawley rats compared to normal and sham diversion controls. Bladder weight in UD group (43.5 ± 2.4) reduced to about half of that in controls (90.4 ± 1.8) 8 wks after UD. Morphological quantification showed detrusor regression, as the percentages of smooth muscle and urothelium in the bladder wall were decreased, while the percentage of collagen was increased after UD (see the preliminary data below). The most obvious changes in CMG of UD rats relative to the sham controls were shortened inter-micturition intervals, reduced voiding volumes and compliance. The expressions of muscarinic receptor-3 (M3), mainly responsible for the micturition contraction, are decreased in bladder after 1-wk and 8 wks UD compared with those of control and sham UD rats (Figure 6). We believe this new animal model will be a very important tool for elucidating the pathogeneses of DBD.

**Figure 5:**




Urinary diversion in rat

**Figure 6:**




Immunoblotting results showed decreased expression of muscarinic receptor-3 (M3) in bladder after 1-week and 8-weeks urinary diversion (UD, n=7) compared with age-matched control (n=5) and sham UD rat (n=5).

**Functional and morphological changes in UD and UD-DM rats (*unpublished data*).** To test the feasibility of this application, we did a pilot experiment to induce DM 10 days after UD in rats. At the time of writing this proposal, we examined CMG and morphology of the bladder 20 wks after DM in some of UD rats. Morphologically (Figure 7), we can see the obvious changes in urethelium, collagen and lumen size in UD and UD+DM rats. UD leads to progressively detrusor regression and relatively increased connective tissues. Interestingly, we found the urothelium deep folds disappeared in 20-wk UD+DM rats, and connective tissues are increased more in 20-wk UD+DM rats compared with those in 20-wk UD only animals. Functionally (Figure 8), both 20-wk UD and 20-wk UD+DM result in significantly reduced bladder capacity, and the bladder capacity in 20-wk UD + DM is significantly smaller than that in 20-wk UD only animals. Obviously, the different manifestations between UD and UD+DM animals resulted from hyperglycemia, but not polyuria.

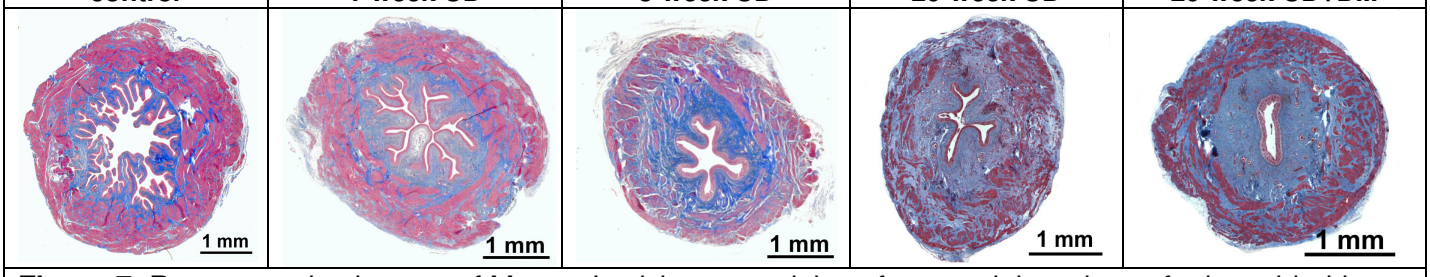



**Figure 7.** Representative images of Masson's trichrome staining of equatorial sections of urinary bladders from control, 1-wk UD, 8-wk UD 20-wk UD and 20-wk UD + DM rats, showing the changes of smooth muscle (magenta), collagen (blue), urothelium (inner light magenta) and lumen area.

**Development of methods of simultaneous studies of cystometrogram and electromyogram of the bladder and external urethral sphincter**- In several pilot studies, we have profiled the urodynamic functions of EAE mice during various stages of neurological deficits. The findings revealed significant distributions of either areflexia and/or hyperreflexia bladder across the various clinical stages of EAE (Figure 8). Figures are representative traces of continuous cystometrogram (**CMG**) in urethane-anaesthetized age-matched naïve control, CFA control and EAE injected mice. For each group n = 3.

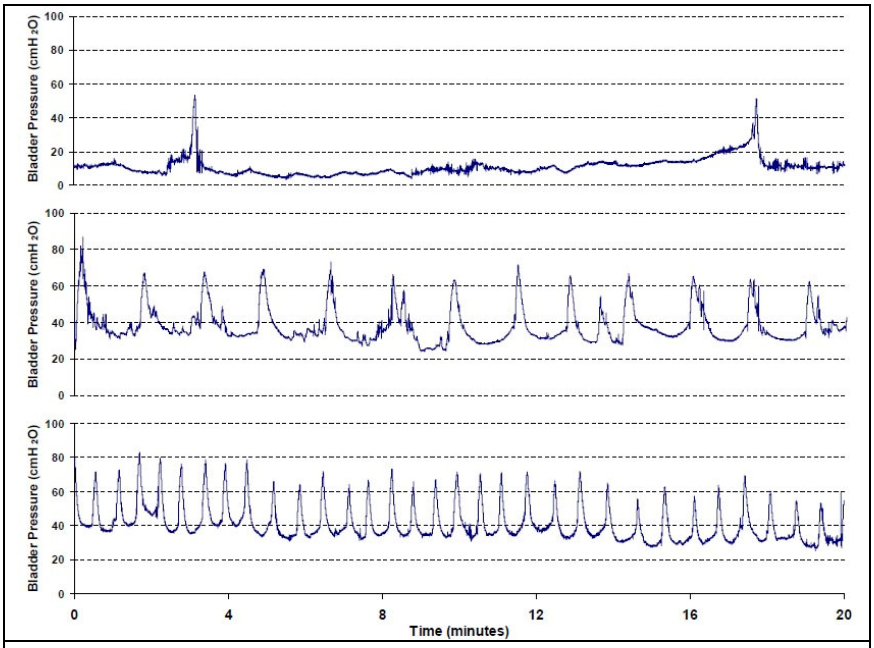



**Figure 8.** Representive tracings of constious Cystometrogram (CMG) from an age-matched control (upper panel), UD-20 wk (middle panel) and UD + DM-20 wk (lower panel) rat.

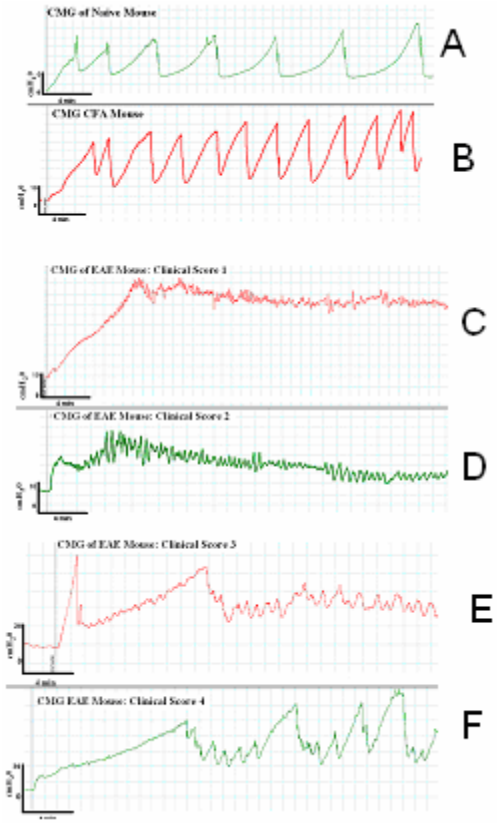



Fig. 9. Representative traces of continuous cystometrogram (CMG) in urethane-anesthetized EAE mice in comparison to controls (age-matched naïve, and age-matched injected only with Freund's adjuvant). CMG profile of **naïve mice (A)** characterized by short filling latency, relatively low baseline pressure, strong voiding contraction preceded by obvious voiding threshold, and consistent inter-voiding intervals with reasonable intervals. **CFA mice (B)** exhibited increase in voiding frequency.

CMG profile of EAE mice with **Clinical Score 1 (C) and 2 (D)** exhibited characteristics of areflexic bladder with overflow incontinence, characterized by elevated baseline pressure, long filling latency with steep increase in detrusor pressure which are an indication of low compliance bladder with weak and poorly sustained contractions.

EAE mice with **Clinical Score 3 (E) and 4 (F)** exhibited characteristics of hyperreflexic bladder with involuntary voiding contractions, characterized by long filling latency, elevated baseline pressure, and non-synchronized voiding pattern.

**Detrusor Sphincter Dyssynergia (DSD) in EAE Mice** [4]- Conscious EAE mice with CS 2-3 (mean peak score 2.5) and 4 (mean peak 4), as well as age-matched controls, underwent conscious cystometry. Mean intercontraction interval (ICI), baseline bladder pressure (BBP), and contraction magnitude (CM) were measured and compared. The results showed that the control mice (n=3) had a mean ICI of 6.6 s (SD 2.2), BBP of 39 cmH2O (SD 7.8), and CM of 4.2 cmH2O (SD 1.2). The mice with moderate disease (CS2; n=2) had a mean ICI of 1.85 s (SD 0.75), BBP of 82.5 cmH2O (SD 3.5), and CM 9.3 cmH2O (SD 4.3) ($p < 0.05$ for all three measures). All contractions correlated with voids in the control mice, whereas those in the moderate disease group did not. Contractions in the control group were peaked and of brief duration, while contractions in the disease group had a plateau-like appearance, suggestive of outlet obstruction. In the severe disease group (CS3, n=2), no contractions were observed, and the mean BBP was 12.5 cmH2O (SD 3.5). We concluded that EAE mice develop bladder dysfunction consistent with outlet obstruction similar to detrusor-sphincter dyssynergia (DSD) [4,5].

**Construction of Tuoyo electrode for** electromyogram **(EMG) recording (unpublished data)-** An ideal model for detection of DSD however, should allow simultaneous assessment of **CMG** and **EMG** of External Urethral Sphincters (**EUS**). **We have successfully developed surgical techniques and created bipolar micro-electrodes named 'Tuoyo'**.. The Tuoyo electrode was constructed in-house from curved suture needle (10-0 Ethicon, CS 160-6 Needle, reduced to approximately 0.38 mm in length, Ethicon, Inc. Somerville, NJ) that was carefully soldered onto a fine Teflon® coated flexible stainless steel wire (0.078 – 0.102 mm, diameter, Cooner Wire Inc, Chatsworth, CA, USA). Copper-braid was soldered to the end of the wire, distal to the electrode for connection to either the stimulator or amplifier. The total length of the electrode is 8 inch. Tuoyo.electrode allow simultaneous recording of mouse CMG and EUS-EMG under continuous transvesical infusion (Figure 10). **To our knowledge, feasibility of this technique in mouse has never been reported.**

A B

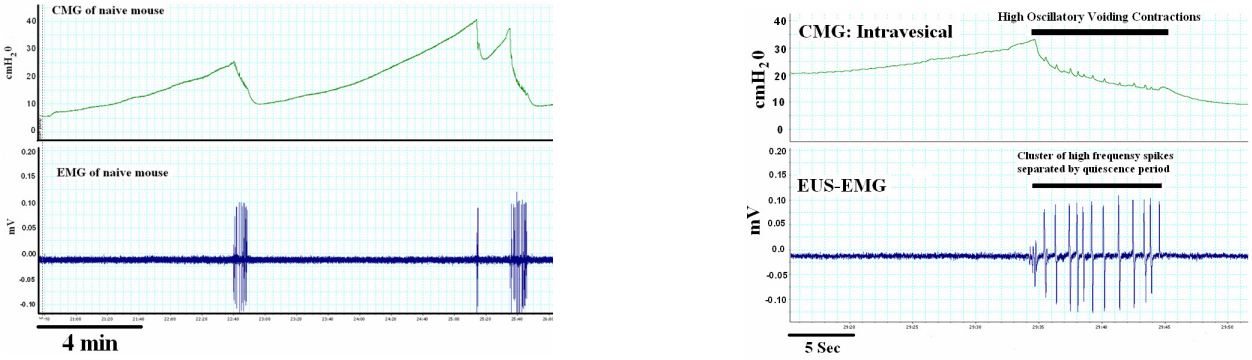



Fig. 10. Trace illustrating simultaneous recording of cystometrogram (top tracing) and EUS/EMG (bottom) in urethane anesthetized female SJWX mouse. EUS exhibited low activity during bladder filling and in between micturition (A). EUS activity was markedly increased in amplitude during bladder contractions. During micturition (B) a bout of long bursting period of phasic EUS activity characterized by clusters of high-frequency spikes separated by a quiescence period were in display.

**The results of the above studies were submitted as preliminary data toward a R03 proposal. We are told that the R03 will be funded for fiscal year of 2009-2010.**

**Examination of role of the autonomic nervous system in the pathogenesis of diabetes neuropathy bladder dysfunction in diabetes mellitus mice -** Good knowledge of the functions of the afferent fiber receptors (transient potential vanilloid receptor 1; TRPV1) in diabetes mice may provide a biological target for either drug or neuromodulation treatments
To achieve the above goals, we are currently conducting the following experiments:

Ex-vivo cystometry: Isolated Whole Bladder Preparation
A functional study experiment that compares nerve mediated response to capsaicin (**TRPV1** agonist) of normal isolated whole bladder vs. isolated whole bladder extracted from diabetes mellitus mice at different time points (4, 8 & 12 weeks) after induction of diabetes

- Whole bladder will be suspended in organ bath and connected to pressure transducer
- Through platinum wire, electrical field stimulation at parameters sensitive to tetradotoxin (to ensure that bladder response is solely mediated by neuronal activity and not muscle) will be applied

Measured Parameters
Intravesical pressure response of isolated whole bladder to transmural nerve stimulations will be measured

- Baseline intravesical pressure will be acquired
- Intravesical pressure either in the presence of agonist (capsaicin) or antagonist (capsaizepine) would also be acquired
- Magnitude of contraction will computed as a function of biological activity. Baseline contractility responses will be compared with contractility responses generated in the presence of agents.
- Difference in response between baseline intravesical pressure and pressure in the presence test agents will be computed

**Assessment of bladder function in T2D mice.** (Data not shown) We have recently completed pilot studies of LUT dysfunction in monogenic mice models of T2D and obesity in relation to our work within

the AMDCC and related to studies of animal models of urinary incontinence. The studies have included 24 hour micturition habits, CMG, measurements of leak point pressure (LPP). The models have included C57Bl6/db/db and C57Bl6/ob/ob mice with their respective age and sex-matched controls. The initial aim of these experiments was to assess the presence or absence of urinary incontinence in these animals as measured by LPP. Further, the animals underwent survival surgery (vaginal distension and implantation of suprapubic tube) for 20 days [6].

**Neuroselectivitiy of Bladder Sensory Threshold Testing** (submitted: Journal of Urology- Data not shown). The neuroselectivity of Neurometer® electrostimulation of bladder afferent pathways was assessed using expression in different spinal cord regions of the protooncogene c-Fos, known to be induced by increased neuronal activity, as a marker. Using the Neurometer® with our newly developed BST device, sine-wave electrical stimulation was applied for 90 minutes to the bladder in rats. Following Neurometer® stimulation at 5 Hz with a current of 2.0 mA, the distribution of immunocytochemically-detected c-Fos positive cells in the spinal cord segments (L1 to S1) that contribute axons to the pelvic and hypogastric nerves was measured (Fig. 2). The distribution of a major peak of c-Fos expression in L6 and minor peaks in L1 and S1 was very similar to that found in rats that received a 30 minute intravesical injection of capsaicin instead of Neurometer® stimulation. Since capsaicin stimulates predominantly C-fibers that experiment provides evidence for C-fiber selectivity of Neurometer® stimulation at 5 Hz.

**D. Plans**-

Our plans for the next year are along two parallel pathways: a) continue our investigation of pathophysiology of diabetic bladder dysfunction; b) phenotype and genotype characterization of our created $MnSOD^{lox/lox}$, SM-$CreER^{T2}$ mice, begin the experiments related to the following specific aims. For all the experimental studies, we will use the following groups of mice:

1. $MnSOD^{lox/lox}$, SM-$CreER^{T2}(ki)^{Cre/+}$ treated with OHT to activate $CreER^{T2}$ to abolish MnSOD expression.
2. $MnSOD^{lox/lox}$, SM-$CreER^{T2}(ki)^{Cre/+}$ treated with OHT and with STZ to induce diabetes.
3. $MnSOD^{lox/lox}$, SM-$CreER^{T2}(ki)^{Cre/+}$, sham treated.
4. $MnSOD^{lox/lox}$, SM-$CreER^{T2}(ki)^{Cre/+}$ treated with STZ.

Specific aim #1: To examine the temporal alterations in the in-vivo bladder function by evaluation of 24 hours micturition habits and conscious cystometry in the above groups of mice at two time points of 8 and 12 weeks after induction of diabetes.

We plan to further test $MnSOD^{lox/lox}$, SM-$CreER^{T2}(ki)^{Cre/+}$ mice for blood pressure (via tail cuff) and perform smooth muscle contractility testing. We also will examine the oxidative and antioxidant status in bladder smooth muscle, urothelium, and other control organs (heart, brain, aorta, liver, skeletal muscle, small intestine, tail). In addition we will measure total antioxidant ability, SOD activity, and Cu-ZnSOD and MnSOD immunoblotting.

Once these tests are complete, our next goal is to induce diabetes in these same mice and examine the additive effect upon diabetic bladder dysfunction.

Specific aim #2: To examine the temporal course of morphological changes in neurogenic and myogenic components of the bladder remodeling in the above groups of mice by:

1. Examination the changes of bladder tissue components and their contribution to remodeling of the wall and chamber of the bladder
2. Examination of the changes in bladder innervations markers.

## E. Publications-

The following publications have been completed by the PI and his collaborators during 2008-2009 funding period:

1. Abouassaly R, Liu G, Yamada Y, Ukimura O and **Daneshgari F**: Efficacy of a novel device for assessment of autonomic sensory function in the rat bladder. J Urol 2008; **179:** 1167.

2. Altuntas CZ, **Daneshgari F**, Liu G, Fabiyi AC, Kavran M, Johnson J et al: Bladder dysfunction in mice with experimental autoimmune encephalomyelitis. J Neuroimmunol 2008; **203:** 58.

3. Barber MD, Spino C, Janz NK, Brubaker L, Nygaard I, Nager CW et al: The minimum important differences for the urinary scales of the Pelvic Floor Distress Inventory and Pelvic Floor Impact Questionnaire. Am J Obstet Gynecol 2009; **200:** 580.e1.

4. Chen CC, Hijaz A, Drazba JA, Damaser MS and **Daneshgari F**: Collagen remodeling and suburethral inflammation might account for preserved anti-incontinence effects of cut polypropylene sling in rat model. Urology 2009; **73:** 415.

5. **Daneshgari F**: Words of wisdom. Re: FDA public health notification: serious complications associated with transvaginal placement of surgical mesh in repair of pelvic organ prolapse and stress urinary incontinence. Eur Urol 2009; **55:** 1235.

6. **Daneshgari F**: Editorial comment on: The current status of laparoscopic sacrocolpopexy: a review. Eur Urol 2009; **55:** 1103.

7. **Daneshgari F**, Imrey PB, Risendal B, Dwyer A, Barber MD and Byers T: Differences in urinary incontinence between Hispanic and non-Hispanic white women: a population-based study. BJU Int 2008; **101:** 575.

8. **Daneshgari F**, Kong W and Swartz M: Complications of mid urethral slings: important outcomes for future clinical trials. J Urol 2008; **180:** 1890.

**9. Daneshgari F**, Liu G, Birder C, Chacko S: Diabetic Bladder Dysfunction – Current Translational Knowledge. Journal of Urology (in press) December 2009.

**10. Daneshgari F**, Leiter E, Liu G, Reeder J: Animal Models of Diabetes for Studies of Lower Urinary Tract Dysfunction. Journal of Urology (in press).

**11. Daneshgari F**, Kenton K: Bladder Sensation Testing – Where do we stand? Current Bladder Dysfunction Reports 2009, 4:65–70

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## F. Project Generated Resources-

Based on preliminary data developed from the current grant, we have developed a collaboration with Lori Birder, Ph.D. and Anthony Kanai, Ph.D. from Departments of Medicine and Pharmacology of the University of Pittsburgh- Our collaboration stated from studies of role of urothelium and reactive oxidative stress products in mechanisms of diabetic bladder dysfunction and led to our joint project funded by JDRF for 2006-2009.

In addition, Dr. Guiming Liu has submitted an application to American Diabetic Association for a Junior Investigator Award. This application has received a favorable score and is currently under review for funding determination.

## G. Research Development-

As above

### H. Other Activities/Achievements-

We have developed an extensive ***collaboration*** within and outside AMDCC as the followings:

We obtained the $MnSOD^{lox/lox}$ from the laboratory of Dr. Frank Brusios at the University of Michigan.

With Jackson Lab- We held couple of meetings with JAX scientists and personnel during the February 2008 SC meeting in Vancouver and since then have communicated with Dr.

Lietter at Jax in regard to our breeding strategy. During the 2008 we shipped our healthy $MnSOD^{lox/lox}$ mice and $SM\text{-}CreER^{T2}(ki)^{Cre/+}$ mice to Jackson lab for cross-breeding the two species.

With other non-AMDCC PIs- We continue to have active collaboration with internal and external investigators in the Cleveland Area. The followings are some of our active collaborators:

1. Lori Birder, Ph.D. and Anthony Kanai, Ph.D. from Departments of Medicine and Pharmacology of the University of Pittsburgh- Our collaboration stated from studies of role of urothelium and reactive oxidative stress products in mechanisms of diabetic bladder dysfunction and led to our joint project funded by JDRF for 2006-2008.
2. Margot Damaser Ph.D.- Lerner Research Institute (LRI) of the Cleveland Clinic- Dept of BME-we have the most extensive collaboration with Dr. Damaser's research team. Our collaboration extents from sharing joint lab space, joint experiments, joint mentoring of trainees; joint weekly lab meetings; and submission of several research proposals.
3. Timothy Kern, Ph.D.- Case- Department of Medicine and Ophthalmology- We have extensive collaboration with Dr. Kern extending from sharing animals for joint experiments to monthly joint lab meetings that are alternatively held at Case or CCF campus.
4. Vincent Monnier., M.D.- Case- Department of Pathology.- to study the role of Advanced Glycation Endproduct in Diabetic Bladder Dysfunction.
5. Fernando Casas, Ph.D.- LRI- BME- to study the integration of vocalization of animal models into the assessment of afferent function of the bladder.

## I. Research Development and other activities planned for the next year.

See above

Respectfully submitted,

Firouz Daneshgari, M.D.

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