Animal Models of Diabetic Complications Consortium

Part A:

Principal Investigator's Summary

Diabetic Uropathy

Firouz Daneshgari, MD.

The Cleveland Clinic Foundation

1. Program Accomplishments:

The overall goals of the existing diabetic uropathy core within the AMDCC are:

a) To establish assays for phenotyping characterization of the lower urinary tract complications of diabetes, and
b) To perform phenotyping assays on the developed and selected animal models in collaboration with other AMDCC laboratories.

Our main strategy is to:

1) Define the elements of diabetic uropathy in small animals that are representative of the humanoid condition. The challenge for diabetic uropathy is not so much finding the animals in which complications of diabetic uropathy occur (see EAC comments below), as it appears that the majority of animals that become diabetic develop diabetic uropathy regardless of their inter-species differences. The challenge, therefore, is to discover what really constitutes the changes of diabetic uropathy that are accurate representations of the humanoid condition. No single theory explains the variation in the clinical presentation of diabetic cystopathy. From the reported literature, it appears that diabetic cystopathy can present as a range of filling or voiding bladder dysfunction. Portions of such presentations are shared with other conditions such as diuresis induced bladder dysfunction. During 2005, we have confirmed the presence of diabetic cystopathy in a number of mice used in the AMDCC, including male C57BL/6, Glut-4 knock out, KLS, db\db on C57BL/6, ob\ob on C57BL/6 and ob\ob and LDLR -\- on C57BL\6 mice.
2) Continue to systematically explore, in active communication with the EAC, the findings in small animals until the full extent of diabetes induced specific pathologies representative of human conditions are identified in the animal models.
3) Continuously search and expand the development of assays that would identify diabetic cystopathy beyond the existing methods. An example of this strategy is the exploration of the assessment of afferent autonomic neuropathy in diabetic animals by measurement of the Current Perception Threshold (see project #5 below).
4) Expand the collaboration between our laboratory and other laboratories (inside and outside AMDCC) by conducting in-vitro assays on the bladder tissues of animals sacrificed in other laboratories and in-vivo assays on animals that are tested or developed in other laboratories.

Major achievements have been:

Following projects have been conducted and/or completed during 2005

Project 1: **Daneshgari F, Huang X, Liu G, Bena J, Saffore L, Powell C: Temporal Differences in Bladder Dysfunction Caused by Diabetes, Diuresis, and Treated Diabetes in Mice.** American Journal of Physiology- Regulatory, Integrative and Comparative Physiology. Am J Physiol Regul Integr Comp Physiol. 2006 Jan 26; [Epub ahead of print]

In follow up of EAC recommendation and in this study we examined the specific role of DM on temporal changes in bladder function in streptozotocin (STZ)-induced diabetes 3, 9, 12 and 20 weeks after DM in male C57BL/6 mice in comparison to age-matched DM treated with insulin, 5% sucrose-induced diuretic and normal control mice. Conscious cystometrograms (CMG) of mice were examined in addition to the measurement of micturition and 24 hour urine volumes, post void volumes, and body and bladder weight of the animals. Diabetes resulted in decreased body weight, which reversed with treatment. Bladder weight and urine output increased in DM and diuretic mice. Bladder capacity and compliance increased in the DM and diuretic groups, correlating with increased urine production. Peak micturition pressure (PMP) increased initially in both DM and diuretic mice. However, in DM mice, PMP dropped dramatically at and after 12 weeks to a lower level compared with all other groups. These data suggest that similar changes in bladder capacity, compliance and voiding ability are seen only during the first 9 weeks of diabetes or diuresis, whereas significant decline in the voiding ability of the bladder is seen in diabetes only after 12 weeks of disease in the mice. This data suggest that that bladder undergoes a transition from a compensated to a decompensated dysfunction 9-12 weeks after STZ-induced DM in mice.

Project 2: Daneshgari F, Liu G, Imrey PB: Time course changes in diabetic cystopathy in rats may include bladder over-and under activity. Journal of Urology June 2006

Diabetic bladder dysfunction (DBD) is among the most common and bothersome complications of this disease. While both filling and voiding problems have been reported, the precise functional changes in the diabetic bladder remain unclear. To examine the role of diabetes duration on bladder function, 60 male Sprague-Dawley rats were compared to age-matched controls at 3, 6, 9, 12 and 20 weeks after diabetes induction with streptozotocin. In cystometrograms, under urethane anesthesia, peak leak pressure was elevated in diabetes and increased gradually during weeks 3-9 in both diabetic and control animals. However, at 12-20 weeks, the diabetic rats deviated strongly from this trend, with peak leak pressures decreasing and resting pressures after 45 minutes post-void increasing from 9-week levels and compared to controls ($p<0.0001$ for interactions). In organ bath studies, elevated contractile force responses of diabetic animals to stimulation by carbamylcholine chloride (carbachol, CCh), potassium chloride (KCl), adenosine 5'- triphosphate (ATP), and electric field stimulation (EFS) peaked at 6 or 9 weeks, but at 12-20 weeks generally reverted towards those of controls (interaction $p=0.0022$ for CCh, $p=0.01$ for EFS). In combination, these findings suggest that diabetic

bladders may undergo a transition from a compensated to a decompensated state, and that transition in the streptozotocin rat model may begin 9-12 weeks after induction.

Project 3: Liu G, Daneshgari F: Alterations in Neurogenically Mediated Contraction Caused by Diabetes and Diuresis. Am J Physiol Renal Physiol. 2005 Jun;288(6):F1220-6. Epub 2005 Feb 1

Diabetic bladder dysfunction (DBD) is among the most common and bothersome complications of diabetes mellitus (DM). Autonomic neuropathy has been counted as the cause of DBD. In this study, we compared the alterations in the neurogenically-mediated contractile responses of the urinary bladder in rats with streptozotocin -induced diabetes, 5% sucrose-induced diuresis, and age-matched controls. Male Sprague-Dawley rats were divided into 3 groups: 9-week diabetics, diuretics and age-matched controls. Micturition and morphometric characteristics were evaluated using metabolic cage and gross examination of the bladder. Bladder detrusor muscle strips were exposed to either periodic electrical field stimulation (EFS) or to EFS in the presence of atropine, alpha,beta-methylene adrenasine 5'-triphosphate, or tetrodotoxin. The proportions of cholinergic, purinergic and residual nonadrenergic-noncholinergic (NANC) components of the contractile response were compared among the three groups of animals. Diabetes caused significant reduction of body weight compared to diuresis and controls, although the bladders of diabetic and diuretic rats weighed more than the controls. Both diabetes and diuresis caused significant increase in fluid intake, urine output, and bladder size. Diabetes and diuresis caused similarly increased response to EFS, and reduced response to the cholinergic component compared to controls. However, the purinergic response was significantly smaller in diuretic bladder strips compared with controls, but not in diabetics. A residual NANC of unknown origin increased significantly, but differently in diabetics and diuretics compared with controls. In conclusion, neurogenically-mediated bladder contraction is altered in the diabetic rat. Diabetic-related changes do not parallel diuretic induced changes, indicating that the pathogenesis of DBD needs further exploration.

Project 4: Liu G, Daneshgari F: Temporal Diabetes and Diuresis-Induced Remodeling of the Urinary Bladder In rat (submitted to Am J of Physiology December 2005)

Diabetes mellitus cause remodeling of the urinary bladder and bladder dysfunction. The natural history of this remodeling and its resultant bladder dysfunction is poorly understood. In this study, we examined the time-dependent remodeling of bladder in diabetic rats. Male SD rats were divided into 3 groups: streptozotocin-induced diabetics, 5% sucrose-induced diuretics, and age-matched controls. Micturition and morphometric characteristics were evaluated using metabolic cage and gross examination of the bladder by light microscopy in 4 days, 1 week, 2 weeks, 3 weeks or 9 weeks after induction. Digital image analysis was used to quantify equatorial cross-sectional areas of bladder tissue and lumen, as well as relative content of the three primary tissue components: smooth muscle, urothelium, and collagen. Diabetes caused significant reduction of body weight, although the bladders of diabetic and diuretic rats weighed more than the controls. Both diabetes and diuresis caused significant increase in fluid intake and urine

output. Progressive increases in lumen area were observed since 4 days after induction, and the wall area increased at 2 weeks in the diabetic and diuretic animals. The wall thickness decreased within the first 2 weeks, but returned to its base at 3 and 9 weeks. Time-dependent smooth muscle increase, no change in relative amounts of urothelium and reduction in collagen density were observed in diabetic and diuretic rats. In conclusion, diabetic induced remodeling of the rat bladder within 4 days after induction of the disease, with significant subsequent changes occuring within the first 3 weeks. The altered micturition characteristics of diabetic animals might induce the time-dependent remodeling of the bladder.

Projects 1-4 have been accomplished as direct results of follow up of EAC recommendations and have shown the followings in regard to diabetic uropathy:

a) **the manifestations of bladder dysfunction are time dependent with bladder storage dysfunction (or overactivitiy) during the early phase of diabetes and bladder voiding dysfunction (decompensation) during the late stage of diabetes-**
b) **the early bladder dysfunction including bladder remodeling is caused by diuretic effects of diabetes**
c) **the differences between diabetes and diuresis may be seen at the in-vitro (contractility) or molecular levels**

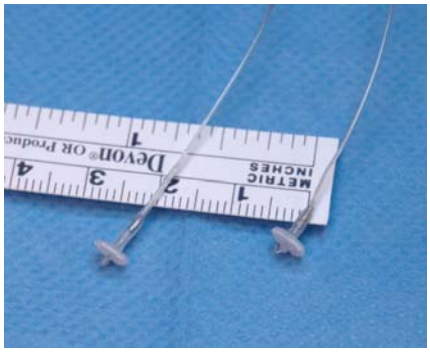
The above findings will guide us on the future studies of diabetic uropathy including the phenotyping work within AMDCC.

Project 5: Assessment of afferent autonomic function of the bladder in small animal models of diabetes (NIH-NIDDK- R41 HD050684-01- Daneshgari (PI).

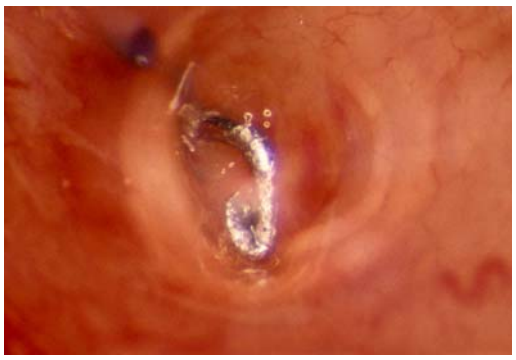
This project is the direct result of the work of PI with the EAC of the AMDCC in exploring the methods by which the afferent sensation of the bladder can be assessed. DBD could affect both the filling and voiding function of the bladder leading to urinary incontinence, poor emptying, high post void residual, and urinary tract infection. Dysfunction of the autonomic nervous system (ANS) innervating the bladder is suspected to play a major role in the pathogenesis of DBD. Yet, no reliable diagnostic test exists to assist in the detection of disturbances in the ANS of the bladder. During late 2004 and early 2005, we submitted and received funding for a STTR proposal that is collaboration with Neurotron Inc. to develop and test a user friendly measurement tool to enable laboratory scientists and clinicians to assess the disturbances of the ANS affecting the lower urinary tract of animals and humans with DBD.

(*R41 -HD-04-018- Daneshgari F (PI)- Transurethral Assessment of Altered Autonomic Function in Diabetic Bladder- July 2005-2007*). The products and results of this newly funded project will be used for assessment of autonomic bladder function in the developed mice models of AMDCC for the current proposal. The ultimate goal of this project is to able scientists (and investigators of the AMDCC) to assess the neuroselective disturbances of the afferent autonomic innervation of the bladder. Presently, we have developed a suprapubic-like device and have obtained preliminary data on stimulation of myleinated and non-mylineated fibers of the bladder (Figure 1)

A



C



B

Figure – above shows the size, configuration (B) and position of the prototype catheter in the bladder (C) developed to assess the neuroselective autonomic innervation of the bladder in small animal models using a Neurometer® (A). Table- below demonstrates the values and reproducibility of obtaining Current Perception Threshold values (CPTv) obtained 1-3 days after implantation of the catheter in rat (n=8): Frequency of 5-Hz stimulates C-unmyelinated , 250-Aδ, and 2000 Hz Aβ myelinated fibers of the afferent autonomic innervation of the bladder respectively. (Proprietary data).

Frequency	Day 1- CPTv (mean±SD)	Day 2- CPTv (mean±SD)	Day 3- CPTv (mean±SD)
5 Hz	29.4 ± 10	24.3 ± 6.9	20.4 ± 9.6
250 Hz	35.5 ± 12	34.6 ± 14	28.2 ± 12
2000 Hz	75.4 ± 18	67.8 ± 18	67.1 ± 21

The results of this work is under preparation for submission to the Journal of Urology

Project #6- Phenotyping of other AMDCC animals Collaboration with AMDCC sites – in continuation of our active collaboration between regional (Neuropathy and Nephropathy cores at the University of Michigan, at the retinopathy at Case Western Reserve University) and other AMDCC investigators, we have completed a first round of phenotyping assays on the below models.

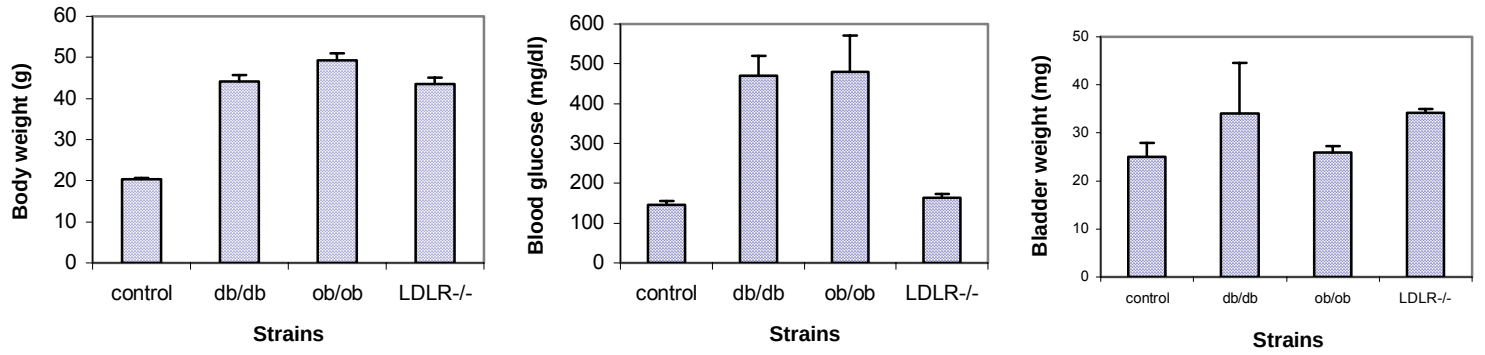


Figure – General characteristics of mice used for AMDCC phenotyping of diabetic uropathy: db\db; ob\ob and LDLR-\- on C57BL\6 mice (n=8

Glut-4 Deficient Protects Mice Against Diabetic Bladder Dysfunction. Presented at 2005 Annual Meeting of the American Urological Association.

Glut-4, a muscle cell-membrane protein involved in the transmembrane transportation of glucose has been used as the therapeutic target for treatment of the complications of diabetic mellitus. Our aim was to study the effects of diabetes duration on bladder function in the Glut-4 knock-out mouse. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) in wild type (WT) and age matched male Glut-4k/o/ C57Bl/6 mice. An equal number of age, sex, and weight matched C57Bl/6 and Glut-4k/o/ C57Bl/6 mice were used as controls. One pair from each diabetic and non-diabetic group was housed together with free access to water and food. 34, 36 and 42 weeks after the induction of diabetes, the animals underwent anesthesia by urethane (1.5 g/kg, i.p.) and cystometrogram through a P50 tube placed transurethraly. After sacrifice by decapitation, the whole bladder was removed and weighed. Distribution, mean±SE of the data on animal weight, glucose level, bladder weight, capacity, compliance and CMG pressure parameters were compared among the three aged groups using a two sided t-test, with a p value of <0.05 considered significant.

Results – A total of 12 animals were used (n=4 in each group). Diabetes caused a significant reduction of the body weight and an increase in the bladder weight of 34 week-WT animals. The changes in the body and bladder weight of the 36 and 42 week age groups of diabetic Glut-4k/o animals were not statistically different from their non-diabetic controls. Although all diabetic animals showed a significant increase in their bladder capacity compared to non-diabetic controls, the diabetic 34 week-WT mice's increase in bladder capacity was twice as much as either age group of Glut-4k/o animals.

Most importantly, none of the diabetic Glut-4k/o groups showed reduction in their peak micturition pressure as seen in 34 week old WT mice.

Conclusions – These findings suggest that Glut-4 deficiency has protective effects on the decay of bladder contractility that is seen in STZ-induced diabetes in C57Bl/6 mice. This data supports that notion that diabetic bladder dysfunction could be prevented by manipulation of a glucose transport system.

2. Collaboration within your group:

Our laboratory is the only group studying the DM complications of the lower urinary tract. We have collaborated closely with Drs. Tim Kern (Retinopathy) and Eva Feldman (Neuropathy), Brusios (Nephropathy) and Huseh (CV) during 2005. Based on the results of our work, a collaborative application was submitted to form a Microvascular Complication Center in response to RFA-DK-05-008 "Mouse Metabolic Phenotyping Centers Consortium" in November 2005.

3. Collaboration with other AMDCC groups:

AMDCC investigators – Over the last year, we have shared diabetic animals and experiments with Dr. Tim Kern from Case Western Reserve University, Dr. Eva Feldman, from the University of Michigan, and Dr. Willa Huseh, from the UCLA. During 2005, with the CCF's IACUS approval we have begun shipping the animals from other AMDCC centers for doing in-house phenotyping assessment. At the time of this writing, we have done the primary round of phenotyping on male C57BL/6, Glut-4 knock out, KLS, db\db on C57BL/6, ob\ob on C57BL/6 and ob\ob and LDLR -\- on C57BL\6 mice.

4. Pertinent non-AMDCC Collaboration:

Over the last year, we have established collaboration with the following investigators:

a- Maureen J. Charron, Ph. D.- Professor, Dept. of Biochemistry and Ob/Gyn & Women's Health Co-Director, Institute for Obesity Research- Albert Einstein College of Medicine of Yeshiva University- we have started our collaboration on mechanism of Glut-4 diabetic uropathy.

b- Manju Bhat, Ph. D.- Assistant Staff, Pain Research Laboratory Center for Anesthesiology Research, The Cleveland Clinic Foundation. We have started our collaboration on molecular mechanisms related to diabetic neuropathy in the lower urinary tract.

c- Kevin McVary, M.D. – Professor of Urology, Northwestern University – this collaboration entails the establishment of in-vivo and in-vitro measurement of sexual dysfunction in mice.

d- Margot Damaser, Ph.D. – this collaboration has evolved around the development of a model for urinary incontinence in female diabetes. The collaboration has resulted in a number of joint projects, two of which have received funding (R21 DK-071143-01).

e- Osamu Ukimura, M.D. Ph.D. from Kyoto University in Japan – this collaboration has resulted in formation of a research projects and in the submission of a R-41 application for assessment of afferent autonomic neuropathy in diabetic animals (project #3).

f- Jefferson Katims, M.D.- Neurotron Inc. this collaboration has focused on development and testing of the device for assessment of afferent autonomic neuropathy in small animal models.

g- Robert Shields, M.D. Director of Autonomic Neuropathy Laboratory of the Cleveland Clinic Foundation. In continuation of the PI's effort in translating the most pertinent work to clinical work, we have begun the process of assessment of autonomic neuropathy in 2 clinical studies currently under review by the CCF IRB

5. Address previous EAC comments:

During 2005, the EAC comments have converted to 'Model Oriented' format. In close communication with Dr.Wade Bushman, the Urology Expert member of the EAC, we have addressed the previous EAC comments as below:

- "His (Daneshgari) presentations are important because he is more or less working alone, which is unlike the other groups. It is important for Dr. Daneshgari to continue to demonstrate the significance of uropathic pathology in diabetes, and to clearly communicate how individuals collect bladders for further study. This was an excellent presentation.
- The human condition of diabetic cystopathy is still imprecisely defined. There are no universally accepted criteria for diagnosing this condition and distinguishing it from other age-related voiding dysfunctions. Without this starting point, it is difficult to assess the validity of any animal model. Continued efforts *must* be made in this area.
- On reviewing the conscious CMG in control and diabetic mice (slide 13), it would appear that the diabetic mice have a later onset of uninhibited contractions and that this increases with duration of diabetes. Is this real? If so, it could point to an alteration in afferent function as well as detrusor overactivity.
- Slide 18 clearly suggests that diuresis plays a role in increasing bladder capacity. This is a very important and clinically relevant observation that may tease out one element of the complex picture that is diabetic uropathy.
- There's an interesting divergence in peak pressure (slide 19) in the diabetic and diuretic mice. This suggests that the diabetic condition interferes with the detrusor compensation to the demand of diuresis. This could have real import for the combination of DM and BPH.
- The effort to measure and quantitate changes in bladder sensation are an important and clinically relevant addition."

Respectfully Submitted,

Firouz Daneshgari, M.D.