ANIMAL MODELS OF DIABETIC COMPLICATIONS CONSORTIUM (U01 DK61018)

UPDATE REPORT

(September 2001 – January 2004)

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Project Number and Title: **CORE – "**AMDCC UROPATHY CORE**"**

Progress Report for AMDCC

Rationale of the project and relevance to AMDCC program goals:
Lower urinary tract complications (LUTC) of diabetes mellitus (DM) is one of the more common and costly complications of DM. These complications include bladder dysfunction or diabetic cystopathy, sexual dysfunction and urinary tract infection. Very little if any data exists on basic epidemiological data related to LUTC, including the incidence rate and severity, and the relationship to the type of diabetes or gender. However, the available literature indicates a prevalence of up to 83% of LUTC among diabetic patients (Kaplan 1995). Moreover, the basic science research into the pathophysiology of LUTC lags behind other DM complications, such as macro and micro vascular diseases, nephropathy, and neuropathy, etc. The NIH-NIDDK Bladder Research Progress Review Group's August 2002 report notes: "Because diabetes significantly alters the urinary tract … people who have this disease will develop costly and debilitating urologic complications (Bladder Research Progress Review Group. 2002). These complications include incontinence, infections, loss of sensations, and retention of urine. Unfortunately, the mechanisms involved are poorly understood. Moreover, little is known about the prevalence, natural history of progression, and risk factors of these complications. The paucity of knowledge has been a barrier to developing the best methods of prevention and treatment of urologic complications."

Animal Models of Diabetic Complications Consortium (AMDCC) was established in 2001 to promote the research of diabetic complications through the creation of organ specific animal models. Therefore, studies of LUTC of diabetes (diabetic uropathy) fit well within the goals of AMDCC to provide progress in the understanding of LUTC of DM via characterization and development of animal models. Following this rationale, the P.I., Dr. Firouz Daneshgari, joined the AMDCC in September, 2002 – as a sub-investigator to the Vanderbilt University P.I., Dr. Matthew Breyer – to add his expertise in diabetic uropathy to the AMDCC.

Summary of Accomplishments:
1. *Communication and reporting to the Steering Committee and External Advisory Board* – The P.I. attended his first AMDCC Steering Committee Meeting in March, 2003. That meeting initiated the formation of a diabetic uropathy core/project. The aims of the diabetic nephropathy project are:
   a) to develop assays and protocol for phenotyping characterization of diabetic uropathy;
   b) to conduct validation studies on the mouse models developed by the AMDCC in collaboration with other Centers and Cores.

Following the March, 2003 meeting, a report on assays for phenotyping diabetic bladder dysfunction was submitted to the External Advisory Board (EAB). At that point in time, Drs. Steven Kaplan, from Columbia University in New York, and Kevin McVary, from Northwestern University in Chicago, were identified as EAB members for the uropathy subcommittee. In coordination with Dr. Robert Star and Tom Hoffman, a couple of unsuccessful attempts were made to communicate with EAB members to get their feedback on the submitted protocol and the recommendations for future directions. On a separate occasion, Dr. Kaplan informed the P.I. that he had approved the submitted protocol. However, no official feedback was received from EAB members.

In October 2003, the P.I. presented the continuation of his work on the characterization of assays on diabetic cystopathy to the Steering Committee and the EAB, and also to the NIDDK workshop that preceded the AMDCC steering committee meeting. The workshop was entitled, "Diabetic

Complications Progress Through Animal Models." Following this meeting, a more proactive agenda was set forth by the EAB, which included collaboration among the centers and cores of AMDCC for characterization of created animal models. In January 2003, a revised protocol for phenotyping assays on diabetic uropathy was submitted in response to EAB recommendation.

2. *Scientific Accomplishments* – The overall aims of the diabetic uropathy core within the AMDCC are:
   a) to establish assays for phenotyping characterization of the LUTC;
   b) to perform phenotyping assays on the developed animal models in collaboration with other AMDCC centers.

Several issues need to be clarified prior to a discussion of scientific steps taken toward the goals of the diabetic uropathy core:

I. *The variability/unknown nature of LUTC in humans* – Bladder dysfunction in DM, or diabetic cystopathy, is traditionally described as a triad of decreased sensation, increased capacity and poor emptying (Forland 1977, Wyndaele 1999; Frimodt-Moller 1978). Some studies support the "classic" findings in DBD, while others do not. In asymptomatic diabetics, Ueda et al. (Ueda 1997) consistently found increased bladder volume at first sensation to void and a decrease in detrusor contractility with resultant increased PVR. They found a 25% incidence of detrusor instability (DI). An earlier study by Frimoldt-Moller described similar results (Frimodt-Moller 1978). In contrast, Kaplan and co-workers' review of urodynamic findings in 182 diabetic patients (Kaplan 1995) found 55% demonstrated DI, but only 23% showed impaired contractility. Findings were "indeterminate" in 11% and areflexic in 10% of patients. Starer's study of elderly diabetic patients (Starer 1990) similarly showed that the majority (76%) of the elderly diabetic subjects presenting with incontinence had involuntary (equivalent to DI) bladder contractions. Starer's conclusion was that: "It can not be assumed that all elderly diabetic patients presenting with urinary symptoms have poorly contracting bladders."

II. *An appropriate animal model for LUTC studies* – Many investigators in the field of urology believe that mouse is not an appropriate model for studies of LUTC (Urologic Complications of Diabetes 2003). The scientific rationale for this belief arises from the differences in micturition cycles of mice. Therefore, a majority of investigators in the field of urology have stayed away from using mice in studies of LUT diseases by choosing rat or rabbit as a superior animal model. Thus, very little previous track record exists for the use of mice in studies of LUTC.

Faced with the above challenges, the diabetic uropathy core has accomplished the following steps from September 2002 to the time of this writing:

1. *Defining the time course of changes in the diabetic cystopathy* – Given the variability reported in the clinical findings of diabetic cystopathy, and the P.I.'s earlier experience in studies of diabetic cystopathy in a transgenic rat model (see appendix), and in order to determine the time course of changes in DBD, the P.I. completed a series of experiments in which the changes in the CMG and contractility of detrusor muscle in Streptozosin (STZ)-induced diabetes were studied in rats 3, 6, 9, 12 and 20 weeks after the establishment of diabetes. The diabetic animals had increased compliance and gradually increased capacity compared to controls (Figure 1). Micturition peak pressure increased in 3, 6 and 9-week diabetic rats, and subsequently decreased in 12 and 20-week

diabetic animals (Figure 2). Early review of data on the response of detrusor muscle to various stimuli (Electrical Field Stimulation, Carbachol, ATP) showed:

a) a gradual rise in the force of contraction in the 3 and 6 weeks diabetic rats compared to controls; and,
b) a sharp drop in the contraction force after 9 weeks of diabetes.

In summary, this data showed that the manifestations of diabetic bladder dysfunction include an increased bladder emptying force during the early stages of DM with its decompensation at a later stage in the STZ animal model of DM. These findings may explain the paradoxical findings in the human where both overactive and atonic bladder function has been reported.

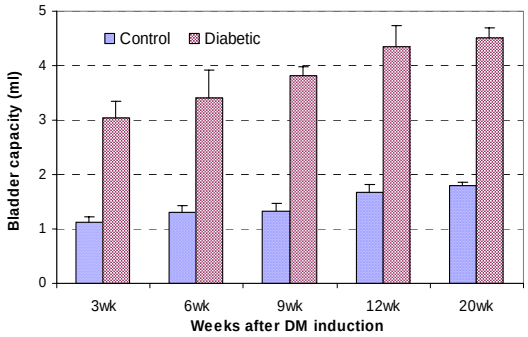

Figure 1. Changes in bladder capacity with age and time after diabetes induction. The bladder capacity in DM animals increases in a continuous, upward and significant level beyond that in Wild type animals

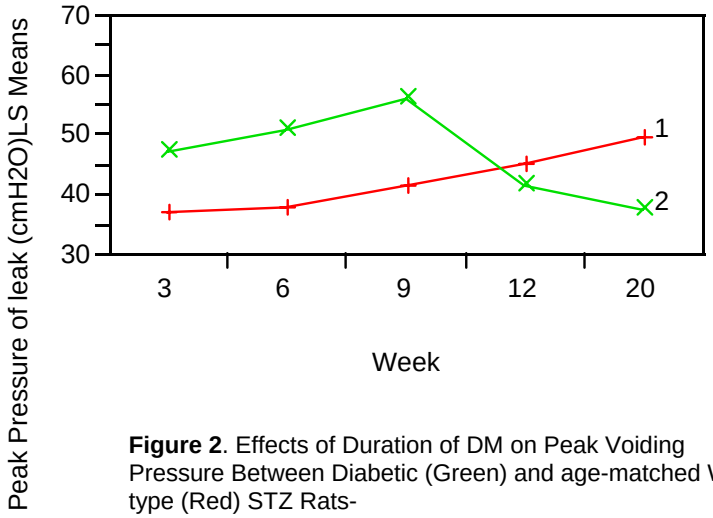

Figure 2. Effects of Duration of DM on Peak Voiding Pressure Between Diabetic (Green) and age-matched Wild type (Red) STZ Rats-

This data indicates a statistically significant (P= 0.0001) decompensation in the peak voiding pressure between ages of 9 and 12 weeks after initiation of DM.

2. *Gross characteristics of diabetic cystopathy* – As part of our investigation of DBD, we have conducted a quantitative analysis of the gross changes seen in the bladder smooth muscle of STZ and WT rats. In summary, the following changes were seen:
   a) increased tissue mass;
   b) expanded extracelluar matrix;
   c) increased collagen deposits;
   d) appearance of cytoplasmic and nuclear vacuoles; and,
   e) appearance of amyloid-appearing globules in the cytoplasms of the detrusor smooth muscle.

3. *Biochemical basis for diabetic cystopathy* – In collaboration with Dr. Vincent Monnier from Case Western Reserve University, we have investigated the presence of Advanced Glycation End Products in the soluble and insoluble elements of detrusor muscle of STZ-diabetic rats. Glycated lysine, a marker of glucose concentration, and CML, a glycoxidation product, were increased 220 – 250% in both soluble and insoluble fractions from diabetic rats ($p< 0.001$). Insoluble proteins were 5-6x more modified than soluble ones ($p<0.001$), suggesting slower turnover rate. CEL, a marker of methylglyoxal levels, was 176% ($p<0.001$) and 167% ($p<0.01$) increased in soluble and insoluble fractions respectively. However, in contrast to furosine and CML, levels were higher in the soluble than the insoluble fraction ($p<0.01$, and <0.001, respectively), suggesting highly elevated intracellular methyglyoxal concentrations. Pentosidine, another glycoxidation marker was also elevated in the insoluble protein fraction ($p< 0.05$). These data provide the first evidence for a broad increase in carbonyl stress to bladder proteins in experimental diabetes, which may in part participate in bladder dysfunction by impairing protein function.

4. *Further characterization of LUTC in Humans* – to further understand the variability in the manifestation of DMD in humans, the P.I. has initiated comprehensive clinical investigations in which the symptomatic and asymptomatic diabetic patients are recruited to a three stage study. During the first stage, the prevalence of LUT complications is assessed by the administration of three validated questionnaires for bladder control problems, pelvic organ prolapse and sexual dysfunction (Barber 2001). Subsequently, patients undergo uroflowmetry and ultrasonic measurement of postvoid residual urine volume. HbA1c values recorded within 3 years were collected. If patients have evidence of LUT complications, they are asked to undergo multichannel urodynamic studies, cystoscopy and biopsy of their bladder tissue. At the time of this writing, 70 adult DM patients (31 male and 39 female; 11 type 1, mean age 49.9; 59 type 2, mean age 59.9) have been recruited into this study.

   In summary, <u>68% of women and 29% of men demonstrate evidence of LUT dysfunction</u> (Daneshgari 2003b). Abnormal voiding patterns were not significantly associated with neurological DM complications (OR = 1.05, $P = 0.93$), other individual DM complications, or number of DM complications. Thus, DBD appears to have a greater prevalence among women than traditionally thought – and its cause does not appear to be related to neuropathy or other complications. Collection and analysis of data on urodynamic parameters of this study is ongoing.

III. *Follow up on EAB recommendation* – In October 2003, EAB suggested that we:
   a) perform awake CMG on the animals; and,
   b) add a group of animals with increased diureses to our validation studies.

Following these recommendations, we have invested and begun the set up for awake CMG in our laboratory. As part of our plans for the next year, we will investigate the effects of time course of changes in diabetic cystopathy in both mouse and rat models of DM using awake cystometrogram. In addition, in a new protocol submitted to IACUA, we will have a parallel arm of animals who will have increased urine output from a feeding of 5% sucrose.

IV. *Collaborations* – Toward achieving the goals of a diabetic uropathy subcommittee of the AMDCC, the P.I. has initiated collaborations with two groups of investigators: a) other core laboratories of the AMDCC; and b) investigators with an interest in LUTC of DM.

A) *AMDCC investigators* – Over the last year, we have shared diabetic animals and experiments with Dr. Tim Kern from Case Western Reserve University. In addition, we have initiated a communication with Dr. Eva Freeman, from the University of Michigan, to conduct joint phenotyping studies on the mouse models of DM. From this collaboration, a trend is emerging which indicates that due to the geographical proximity of three core laboratories of AMDCC (Dr. Freeman in Ann Arbor, Michigan; Drs. Kern and Daneshgari, in Cleveland, Ohio), phenotyping assays for diabetic neuropathy, uropathy, and retinopathy can be performed in the same animal by the three core laboratories with relative logistical ease. If this trend is fruitful, it would be a significant achievement for the core laboratories of AMDCC.

B) *Non-AMDCC investigators of LUTC in DM* – Over the last year, we have also established collaboration with Dr. George Christ from Albert Einstein School of Medicine and Dr. Karen McVary from Northwestern University in Chicago. The aims of this collaboration are:
   a. to develop relationships with other laboratories interested in studies of LUTC of DM for future interaction within and outside AMDCC goals.
   b. to use additional expertise in other areas of diabetic uropathy, such as sexual/erectile dysfunction in diabetes.

Plans for the Coming Year:

As the role and function of the diabetic uropathy component of the AMDCC is becoming clearer, our plans for the coming year will be to:

a) validate the elements of awake cystometrogram in mouse models of diabetes;
b) add a diuretic animal model as a control to the diabetic and non-diabetic animals in the validation assays;
c) build on the collaboration between the core laboratories of AMDCC;
d) expand the services of our laboratory to other investigators of the AMDCC; and,
e) make the methodology of the assays and our preliminary data available on the AMDCC web site for access by other investigators.

The final goal of our core is to be a fully functional laboratory within AMDCC to screen the developed or available animals for the presence of diabetic uropathy.

Significant Achievement:

In general, the focus of the first 14-16 months of activities of the diabetic uropathy subcommittee has been on:

a) defining the targeted human conditions that need to be demonstrated in the animal models of DM;
b) development of assays for characterization of LUTC in DM that are consistent with published literature, the recommendations of the EAB, and the views of the urologic scientific community at large;
c) establishment of collaboration with both AMDCC core laboratories and investigators and non-AMDCC investigators active in the field of diabetic uropathy; and, finally,
d) initiate investigations at the clinical level to further characterize diabetic uropathy in Humans.

In more specific terms, the most significant achievement of our core laboratory has been:
a) the identification of the time course of changes in the bladder of diabetic animals; and,
b) the establishment of collaboration with two other core laboratories of AMDCC.

A significant achievement for next year would be a collaboration with existing AMDCC members and other investigators in the field of urology to standardize the methods for phenotyping assays in diabetic animals – both in rats and mice. This step, in itself, will encourage the standardization of the methodology among investigators, and will hopefully facilitate and encourage the interest of other investigators to study the lower urinary tract complications of diabetes. In addition, this step may foster interest in the next level of investigation into the pathogenesis of LUTC in diabetes – which is the identification of the cellular and molecular pathways responsible for LUTC complications. It would be at this stage that the studies of lower urinary complications could be related to studies of other complications, such as kidney, heart, or vascular disease.

Publications – the work of our core laboratory has led to the preparation of the following manuscripts:

1. Weber, A.M., G. Buchsbaum, B. Chen, A. Clark, M.S. Damaser, **F. Daneshgari**, G. Davis, J.O.L. DeLancey, K. Kenton, A. Weidner, R.A. Word (2003) Basic Science and Translational Research in Female Pelvic Floor Disorders: Proceedings of an NIH-Sponsored Meeting. *Accepted for publication at Neurourology and Urodynamics September 2003*
2. **Daneshgari F**, Liu G, Lemack GE, Esser V , Wyne KL: A New Genetic Rat Model of Diabetes Mellitus for Studies of Lower Urinary Tract (LUT) Dysfunction. (Submitted to Urology, Nov 2003).
3. **Daneshgari F**, Saffore L, Wyne KL, Powell CT: Profile and alterations of Protein Kinase C in the Bladder tissues of Diabetic Rat. Submitted to Neurourology and Urodynamics Oct 2003

In addition, the following publications are at the final stages of preparation:
C) Daneshgari F, Liu G, Saffore L, Imrey: Time course of changes in the diabetic cystopathy includes hypo and hyper active bladder.
D) Daneshgari F, Huang X, Brainard J: Gross changes in the diabetic cystopathy changes with duration of the disease in streptozosin DM rats.

Abstracts. the following abstracts were accepted and presented during March 2003 to February 2004:

1. Biochemical Basis for Bladder Dysfunction in Experimental Diabetes: Glycoxidative and Oxoaldehyde Stress are Markedly Enhanced in Soluble and Insoluble Bladder Proteins. Xiao Huang, Chris Strauch, Lateef Saffore, Ashwini Viswanathan, Vincent M. Monnier, Firouz

Daneshgari Society for Urodynamics and Female Urology. Scottsdale, Arizona. February 26 – 29, 2004

2. Gross Structural Changes in the Diabetic Bladder Corresponds to Changes in the Cystometrogram. Xiao Huang[1], Jennifer Brainard[2], Guiming Liu[1], Firouz Daneshgari. American Diabetic Association. Orlando, Florida. June 4 – 8, 2004
3. Time-Course of Changes in the Diabetic Bladder Dysfunction. Urological Complications of Diabetes. Guiming Liu, Firouz Daneshgari. Urologic Complications of Diabetes NIH-NIDDK Sponsored symposium Bethesda, MD. December 3-4, 2003
   And Society for Urodynamics and Female Urology. Scottsdale, Arizona. February 26 – 29, 2004
4. Use of Bladder Needle Biopsy Prototype to Obtain Selective Samples of Urothelium and Detrusor Muscle. Firouz Daneshgari, Louis Moy, William Rabbitt, John Hall. Annual Meeting of North Central Section of the AUA. Vancouver, BC. September 2003
5. Prevalence Survey of Voiding Dysfunction in Diabetic Patients. Firouz Daneshgari, Louis Moy, Jeannette Potts, Ahmed Elazab, Raymond Rackley, Sandip Vasavada, Peter Imrey. Annual Meeting of North Central Section of the AUA. Vancouver, BC. September 2003
6. Use of Urodynamic Studies in Diagnosis of Urinary Incontinence and Voiding Dysfunction. Nivedita Bhatta Dhar, Nadir Babekir, Peter Imrey, Firouz Daneshgari. Annual Meeting of North Central Section of the AUA. Vancouver, BC. September 2003
7. Changes in Contractility of the Bladder associated with Diabetes Mellitus- 24th Annual Meeting of the American Urogynecological Society. Hollywood, FL. September 2003
8. Use of Urodynamic Studies in Diagnosis of Urinary Incontinence and Voiding Dysfunction. Annual meeting of the Society for Urodynamics and Female Urology. Chicago, Illinois. April 2003.
9. Alterations in the profile of protein kinase C isozymes in the urothelium and detrusor of diabetic rat. Annual meeting of the Society for Urodynamics and Female Urology. Chicago, Illinois. April 2003
10. Impact of Dissection Methodology on the Amount of Urothelium and Detrusor removed from the Bladder of Rat. Lateef Saffore, Guiming Liu, Firouz Daneshgari. International Bladder Symposium. Washington, D.C. March 6 – 9, 2003
11. Protein Kinase C Isozyme Expression is Altered in the Bladder of Diabetic Patients. Firouz Daneshgari, Lateef Saffore, Nadir Babekir, Venkatesh Kirshnamurthy, Raymond Rackley, Sandip Vasavada, David Goldfarb. International Bladder Symposium. Washington, D.C. March 6 – 9, 2003

In all the publications and presentations, credit has been given to the source of funding as NIH-NIDDK-K08DK02631.

Reference:

1. Urologic Complications of Diabetes Mellitus in Overcoming Bladder Disease: A Strategic Plan for Research. A Report of the Bladder Research Progress Review Group: National Institute of Diabetes and Digestive and Kidney Diseases. National Institutes of Health. August 2002, pp 133-143.
2. Wyndaele JJ. Normality in urodynamics studied in healthy adults. J Urol 1999; 161(3):899-902.
3. Frimodt-Moller C. Diabetic cystopathy. A review of the urodynamic and clinical features of neurogenic bladder dysfunction in diabetes mellitus. Dan Med Bull 1978;25(2):49-60.

4. Kaplan SA, Te AE, Blaivas JG. Urodynamic findings in patients with diabetic cystopathy. J Urol 1995;153(2):342-344.
5. Starer P, Libow L. Cystometric evaluation of bladder dysfunction in elderly diabetic patients. Arch Intern Med 1990;150(4):810-813.
6. Forland M, Thomas V, Shelokov A. Urinary tract infections in patients with diabetes mellitus. JAMA 1977;238(18):1924-1926.
7. Ueda T, Yoshimura N, Yoshida O. Diabetic cystopathy: relationship to autonomic neuropathy detected by sympathetic skin response. J Urol 1997;157(2):580-584.