March 2005 AMDCC Progress Report

Diabetic Uropathy

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The Cleveland Clinic Foundation

Diabetic Uropathy

Ouline

- Further definition of diabetes- specific changes in the bladder
- Find a phentoyping assay to detect loss of bladder sensation in DBD
- Phenotyping assays in AMDCC animals
- Further definition of Humanoid Condition

Diabetic Bladder Dysfunction

Traditional Findings

- Enlarged Bladder
- Poor Emptying/ Voiding Dysfunction
- Urinary incontinence
- High post void residual (PVR)

DBD in Human

- Bladder Overactivity in Diabetes?
  - 76% of diabetic patients had involuntary contraction as cause of their incontinence (Starer 1990)
  - 25% of diabetic subjects (Ueda 1997)
  - 55% of diabetic patients; 23% had impaired detrusor contractility (Kaplan 1995)

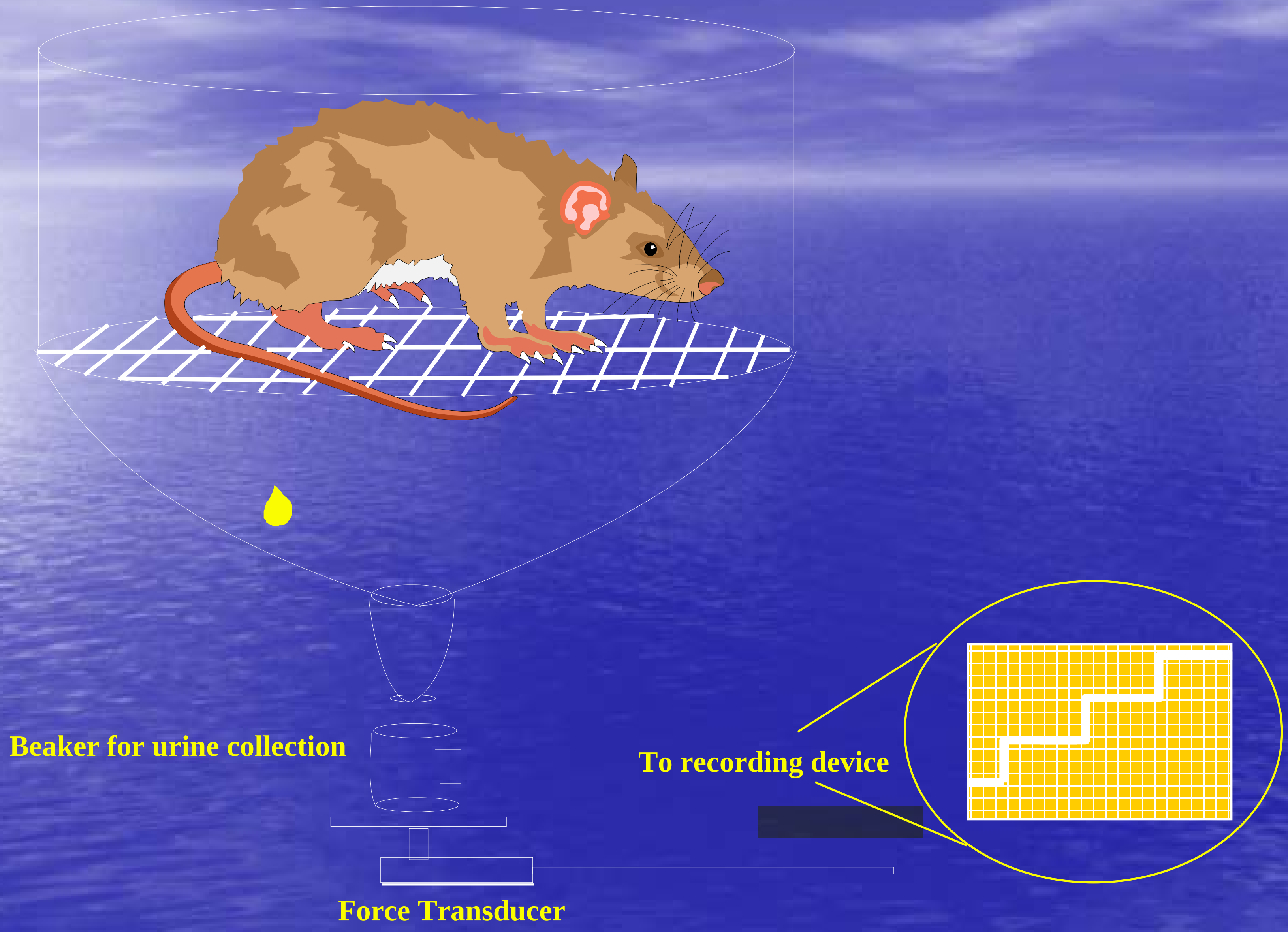
Proposed Natural History of Progression of Diabetic Bladder Dysfunction In Human and Animals

Early Phase ⟶ Late Phase

Time Course/Risk factors ??

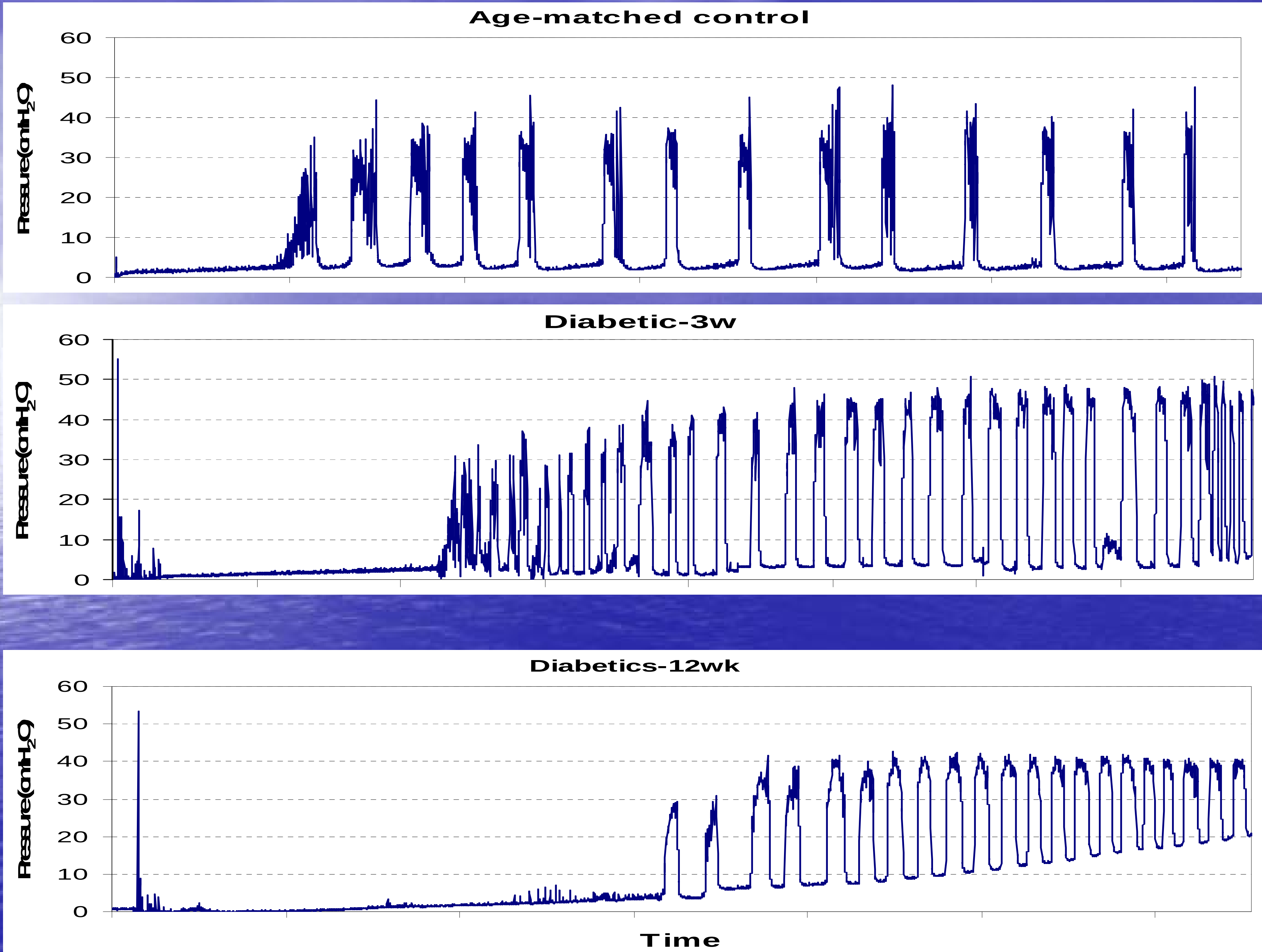
Clinical: Filling problems	**Voiding Problems**
Urodynamics: Overactive Bladder	**Atonic Bladder**
In-vitro: Hypercontractile Detrusor	**Hypocontractile Detrusor**

Daneshgari 2003


Beaker for urine collection
To recording device
Force Transducer

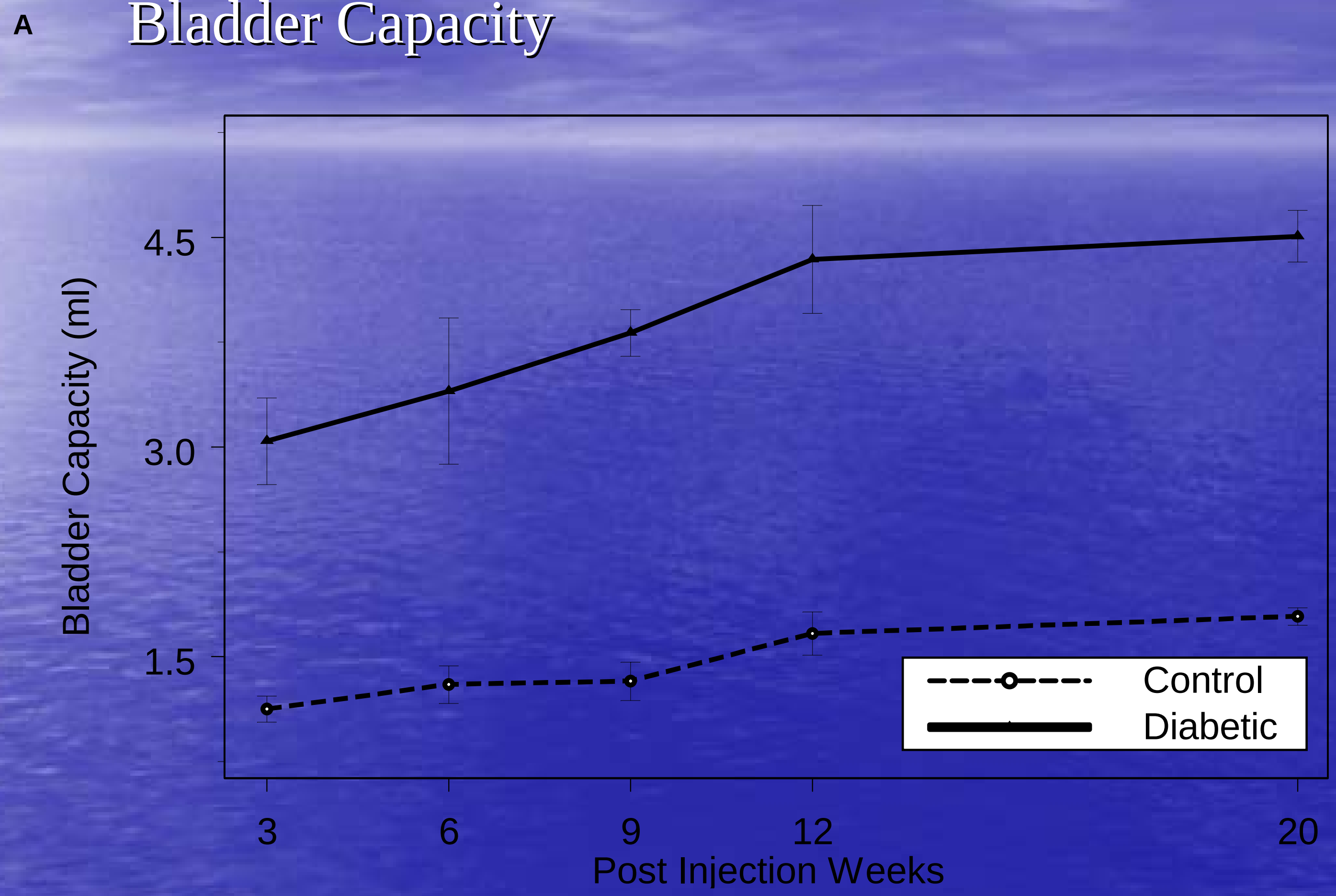
Time-Dependent Alterations in Diabetic Cystopathy

Duration time after diabetes induction: 3wk, 6wk, 9wk 12wk and 20wk


Age-matched control
Pressure(cmH2O)
0
10
20
30
40
50
60
Diabetic-3w
Pressure(cmH2O)
0
10
20
30
40
50
60
Diabetics-12wk
Pressure(cmH2O)
0
10
20
30
40
50
60
Time

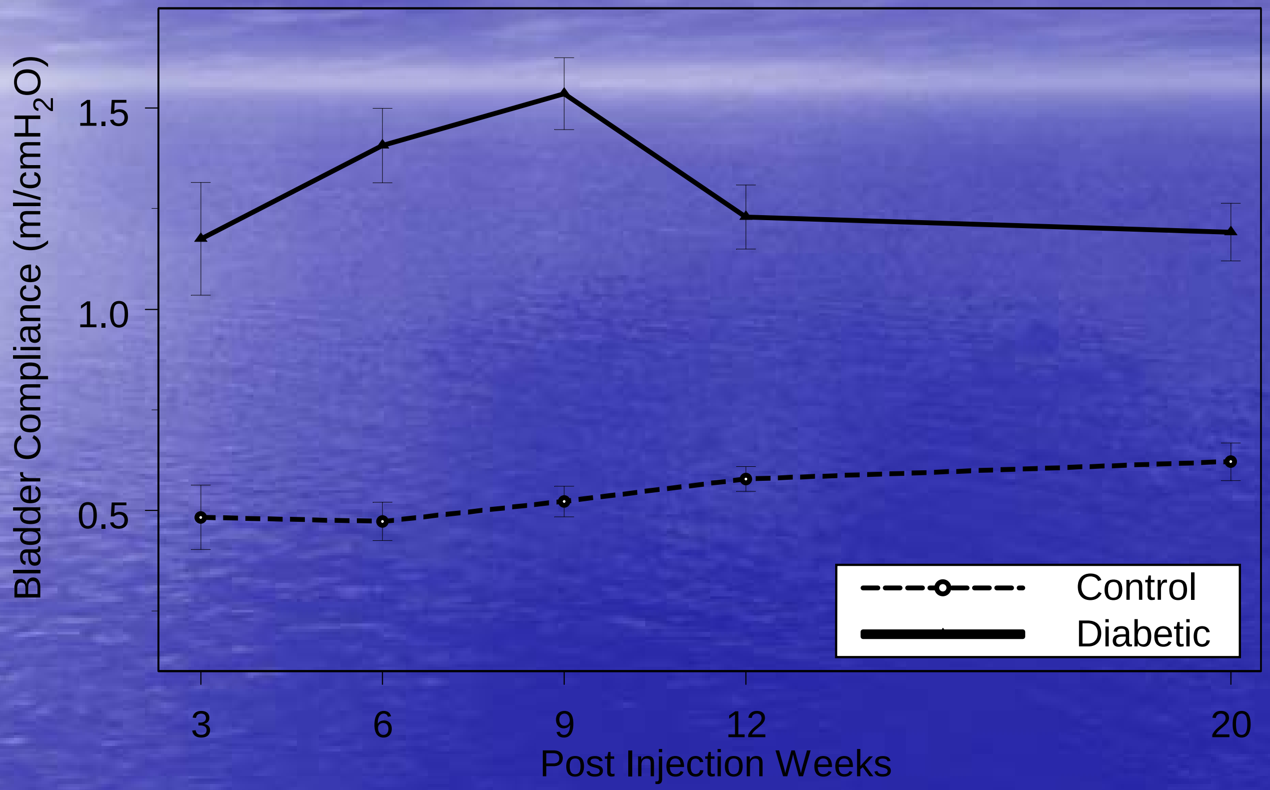
Time course of DBD in SD STZ-Rats

Bladder Capacity

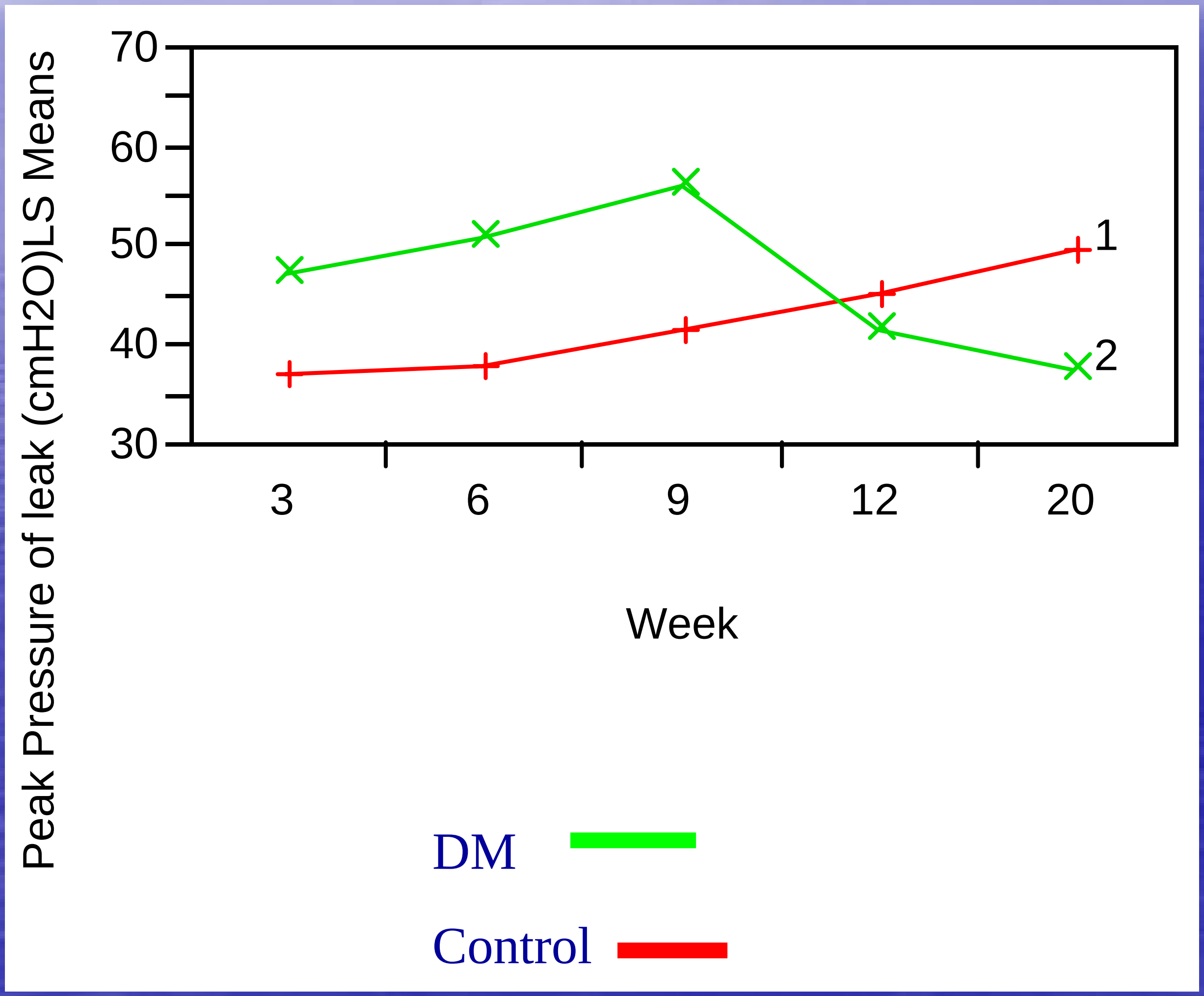


Time course of DBD in SD STZ-Rats

Bladder Compliance



Time course of DBD in SD STZ-Rats
Peak Pressure of Leak: Adjusted Effects of Age and DM



EAC Comments

- Are the changes result of increased diuresis or hyperglycemia?

Time-course of
STZ- induced
Diabetic Bladder Dysfunction in
C57Bl/6 Mouse

Time Course of DBD in C57Bl/6

Groups

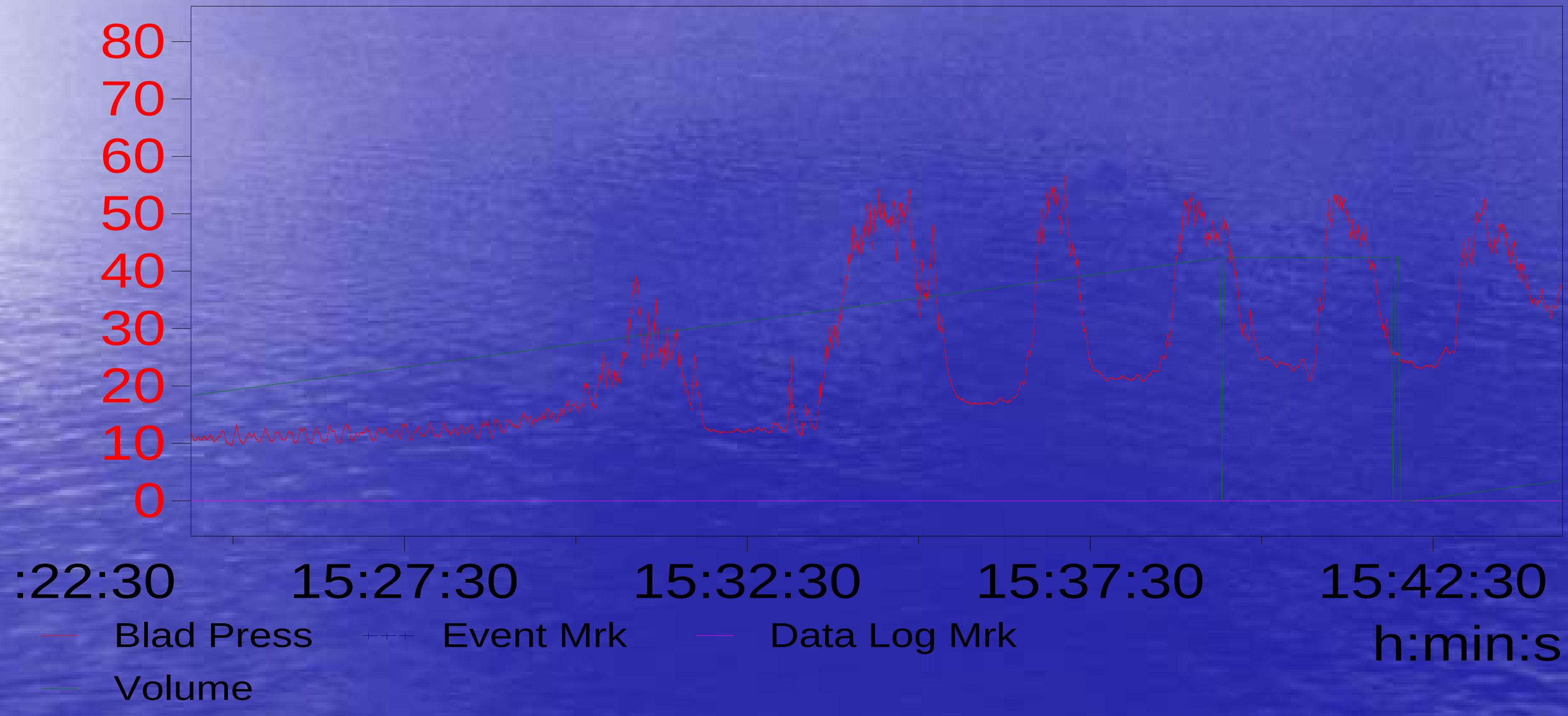
- DM-untreated
- DM-treated
- Diuretic
- Controls

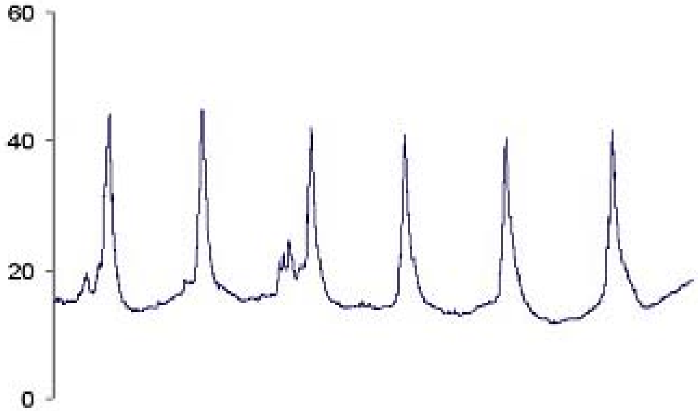
Time points

- 3 weeks
- 9 weeks
- 12 week
- 20 weeks

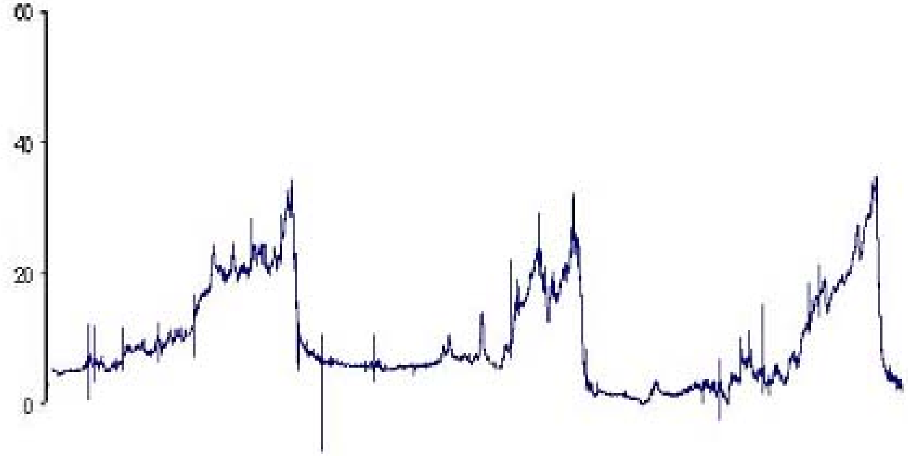
Time Course of DBD in C57BL/6

Conscious Cystometrogram

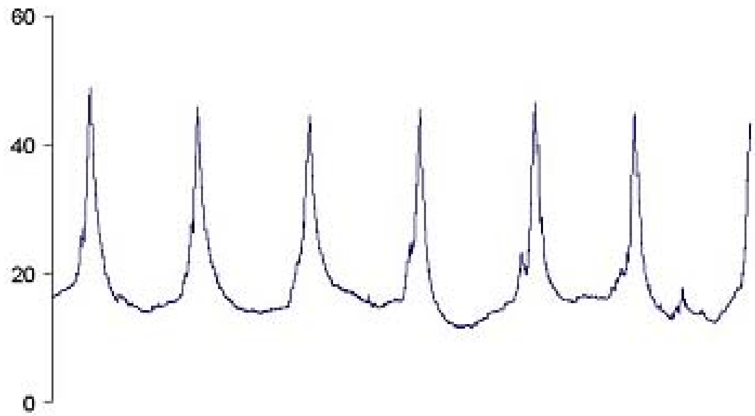




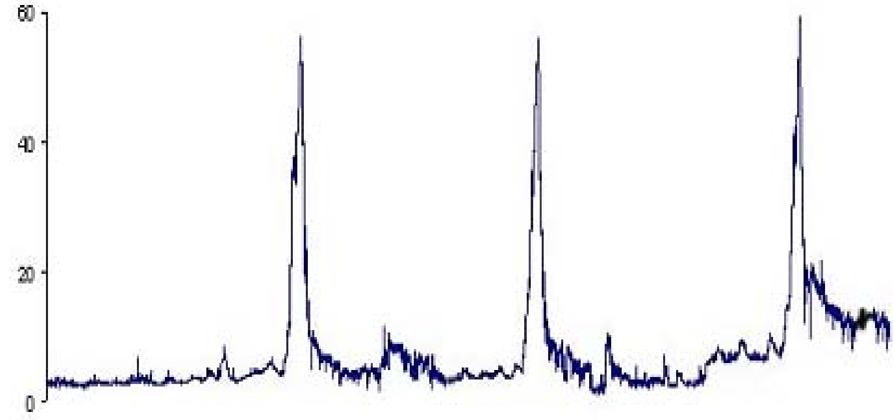
A Control



B DM

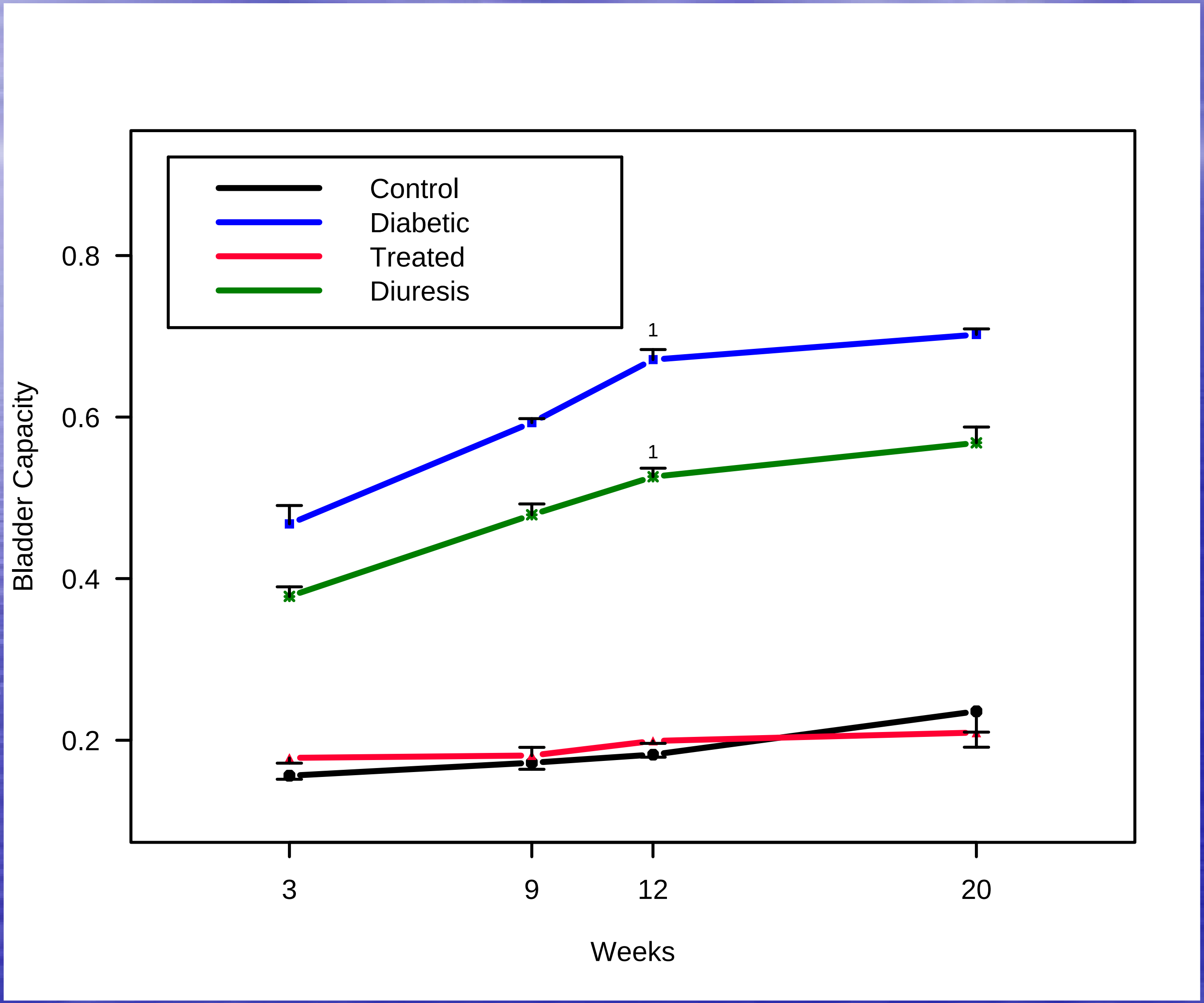


C DM-Treated



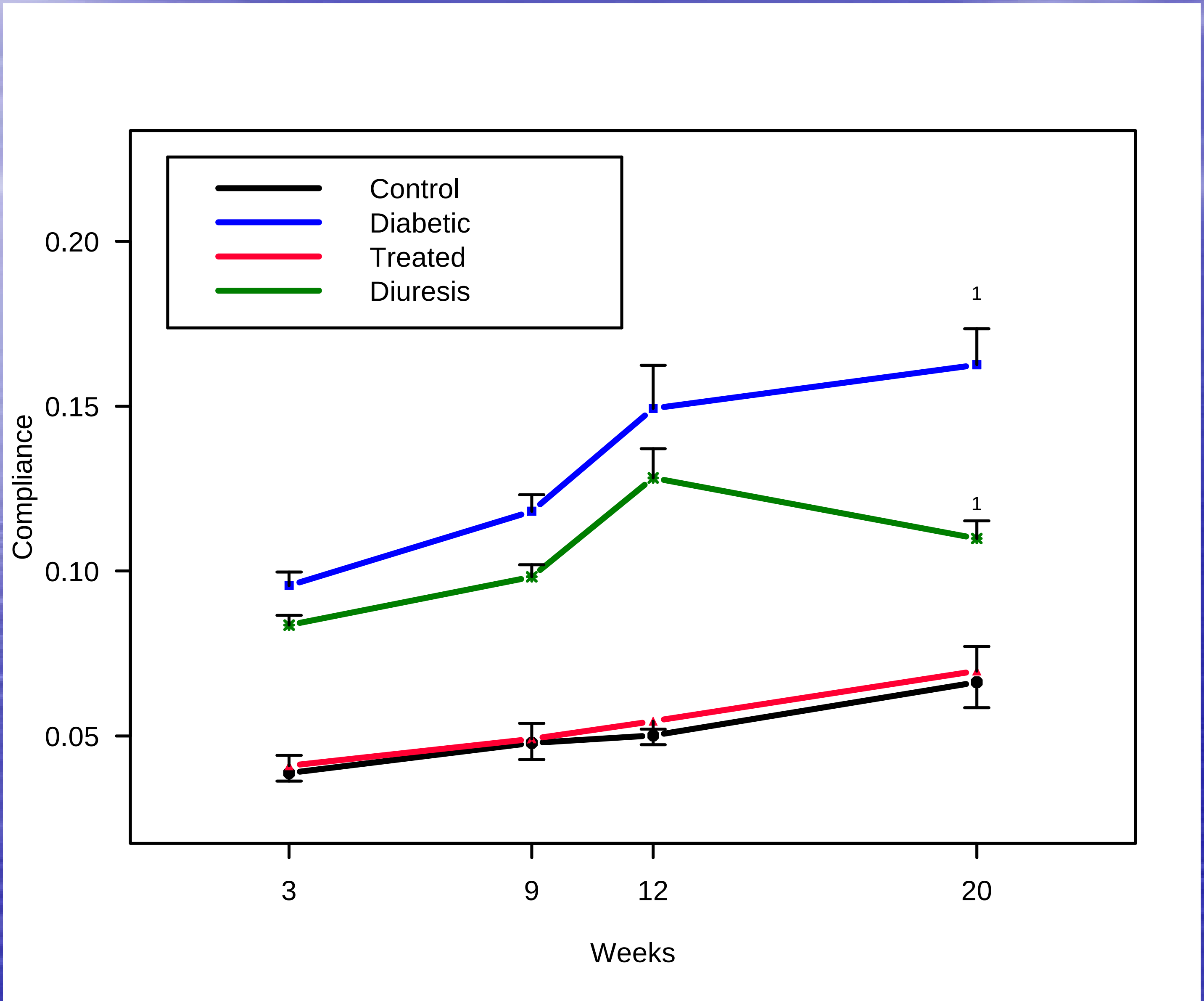
D Diuretic

Time Course of DBD in C57BL/6
Bladder Capacity



Daneshgari 2005

Time Course of DBD in C57BL/6
Bladder Compliance



Time Course of DBD in C57BL/6
Micturition Volume per void

Measurement	Group	3	6	12	20
Micturation volume (mL)	Control	0.13±0.00	0.13±0.00	0.13±0.00	0.18±0.02
	DM	0.22±0.01	0.20±0.02	0.19±0.02	0.21±0.02
	Treated DM	0.14±0.00	0.12±0.01	0.13±0.00	0.14±0.01
	Diuretic	0.30±0.02	0.32±0.01	0.35±0.01	0.36±0.02

Time Course of DBD in C57BL/6

24-Hour urine output

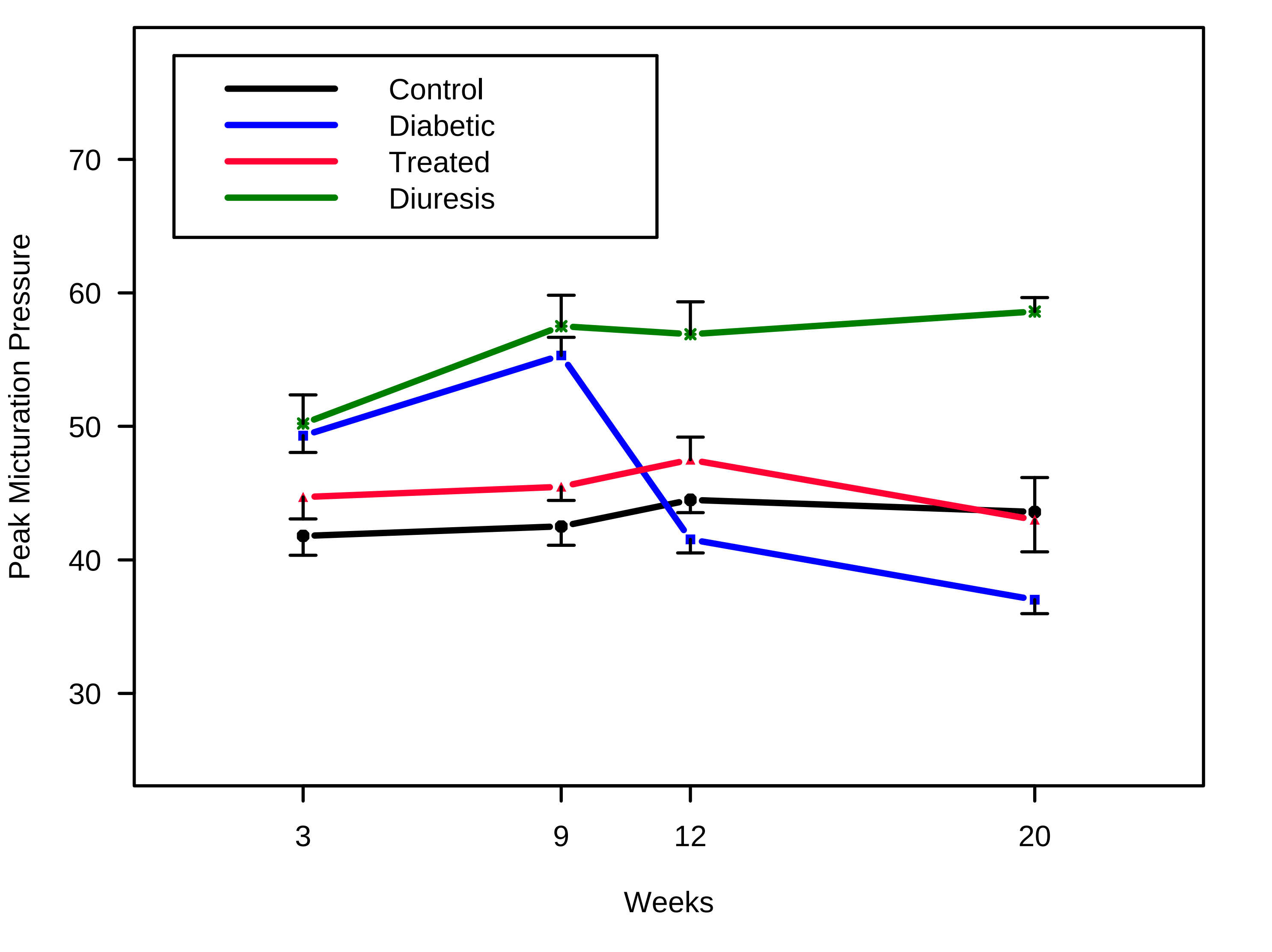
Measurement	Group	3	6	12	20
24-Hour urine output	Control	1.05±0.06	1.65±0.19	2.60±0.21	2.67±0.03
	DM	9.53±0.69	12.50±0.51	22.40±1.29	26.70±1.74
	Treated DM	3.20±0.21	3.18±0.30	5.19±0.28	7.21±0.48
	Diuretic	7.31±0.33	15.90±0.99	16.50±0.73	14.50±0.95

Time Course of DBD in C57BL/6
Residual Urine

Measurement	Group	3	6	12	20
Residual Urine (mL)	Control	0.03±0.00	0.04±0.00	0.05±0.0	0.06±0.01
	DM	0.35±0.02	0.39±0.01	0.46±0.03	0.49±0.02
	Treated DM	0.04±0.00	0.06±0.00	0.07±0.00	0.07±0.01
	Diuretic	0.09±0.01	0.17±0.02	0.18±0.01	0.21±0.02

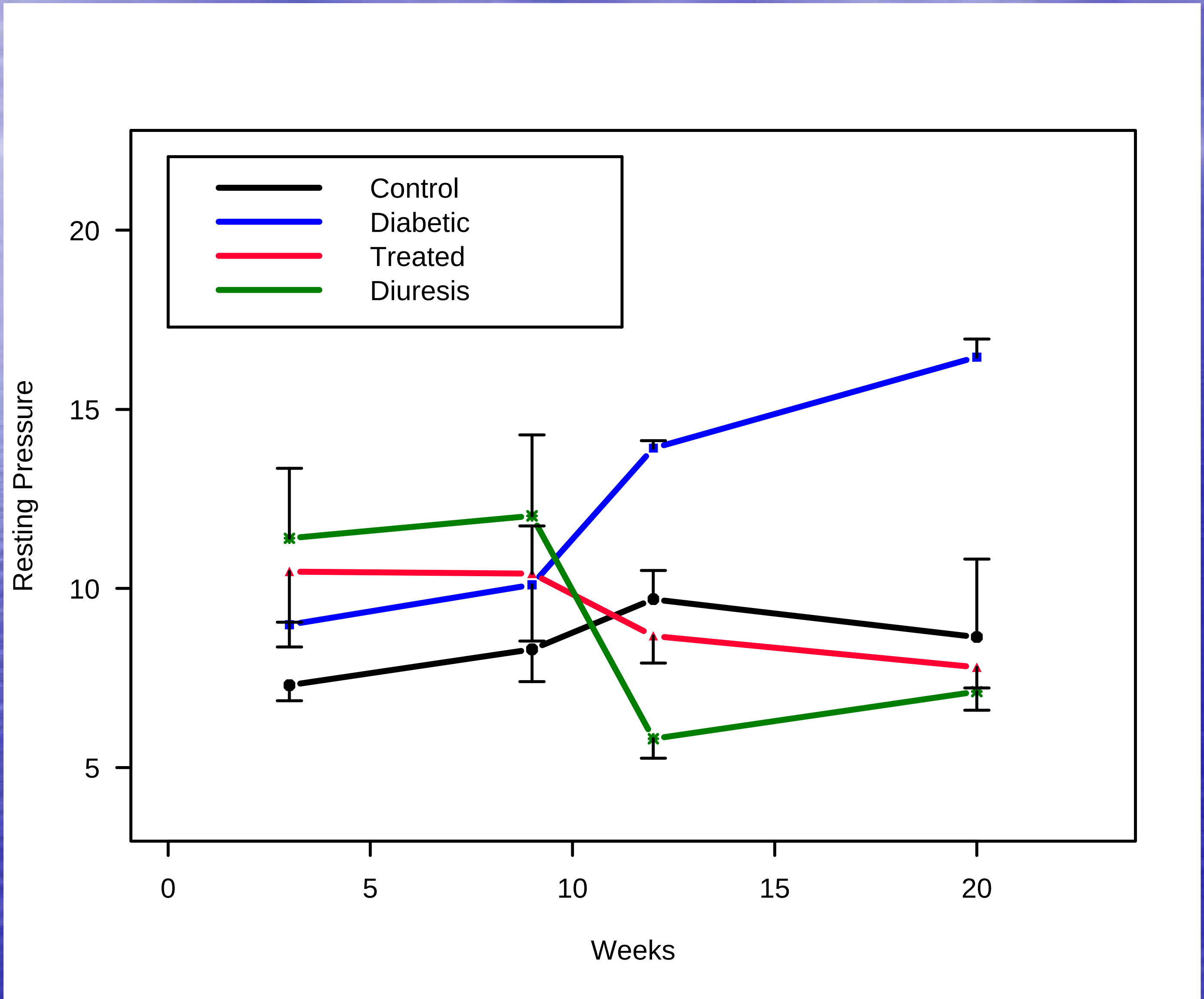
Time Course of DBD in C57BL/6

Peak Micturition Pressure of the Bladder

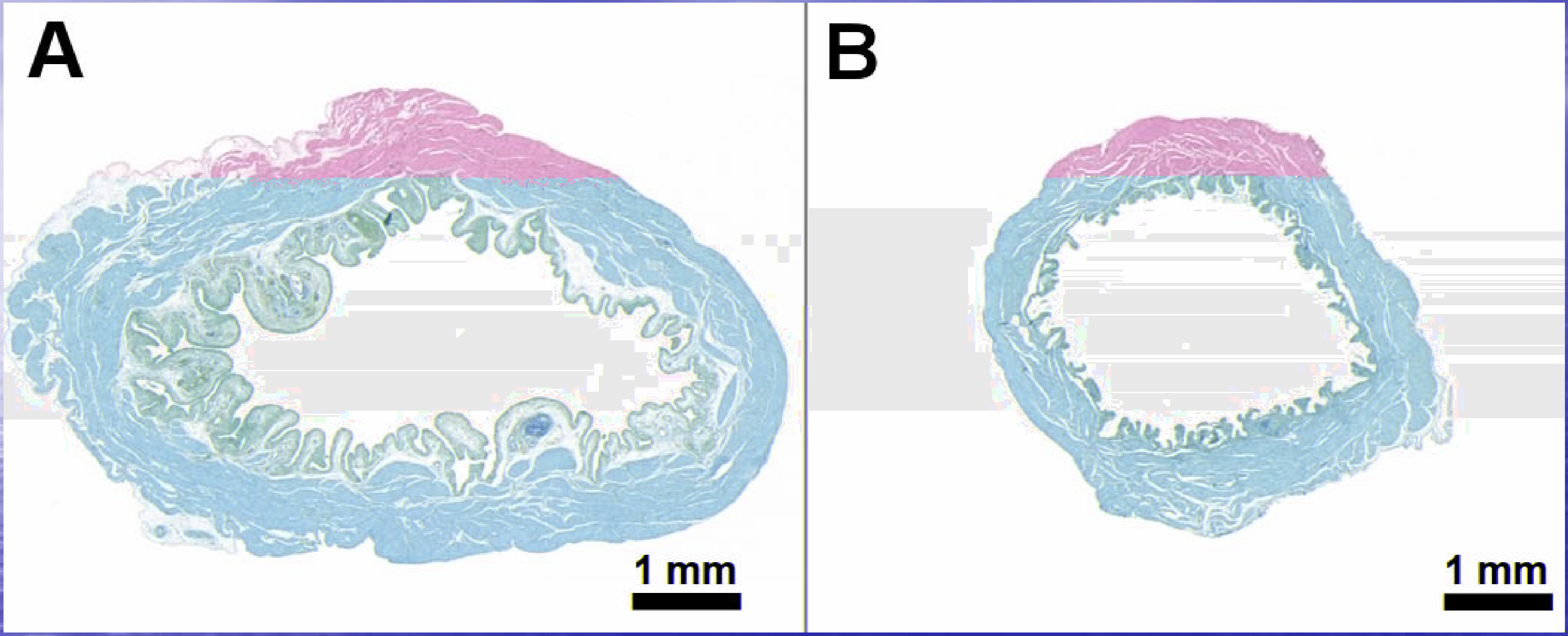


Time Course of DBD in C57BL/6

Resting Pressure of the Bladder



Morphometric Alterations in DBD

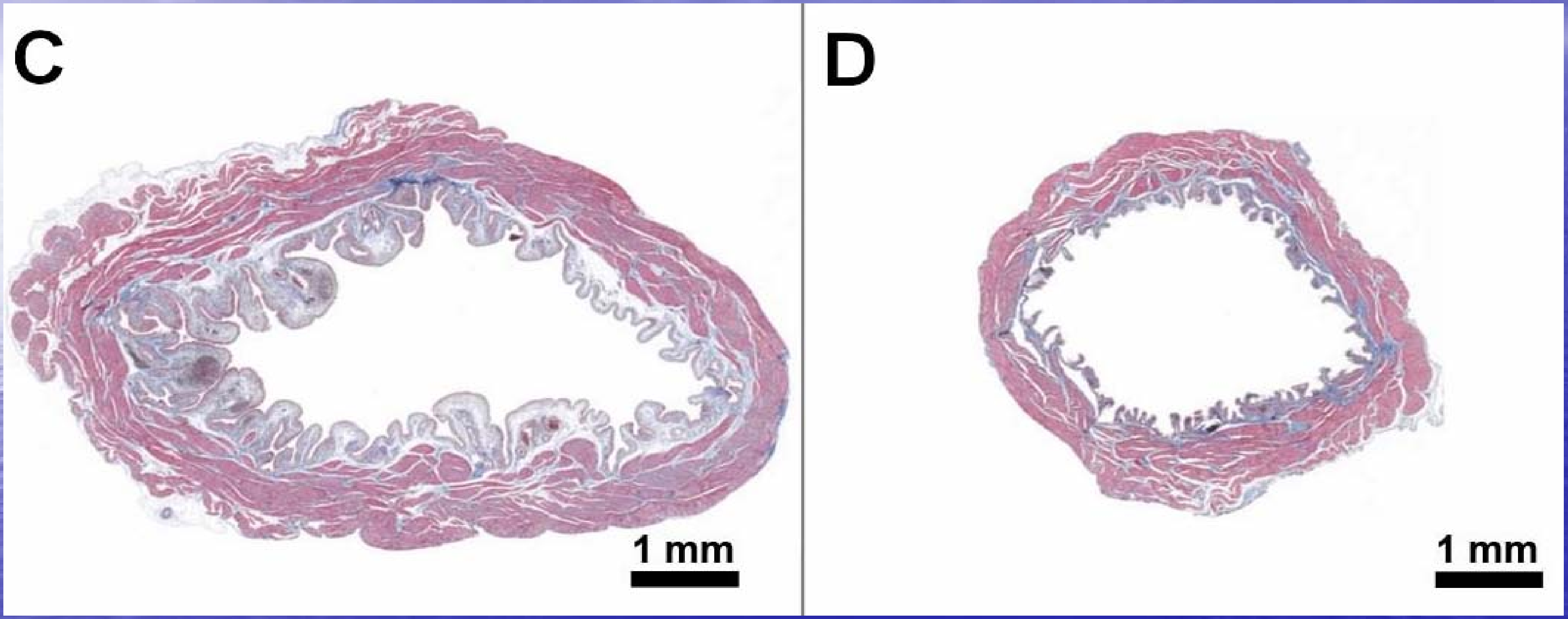


Diabetic

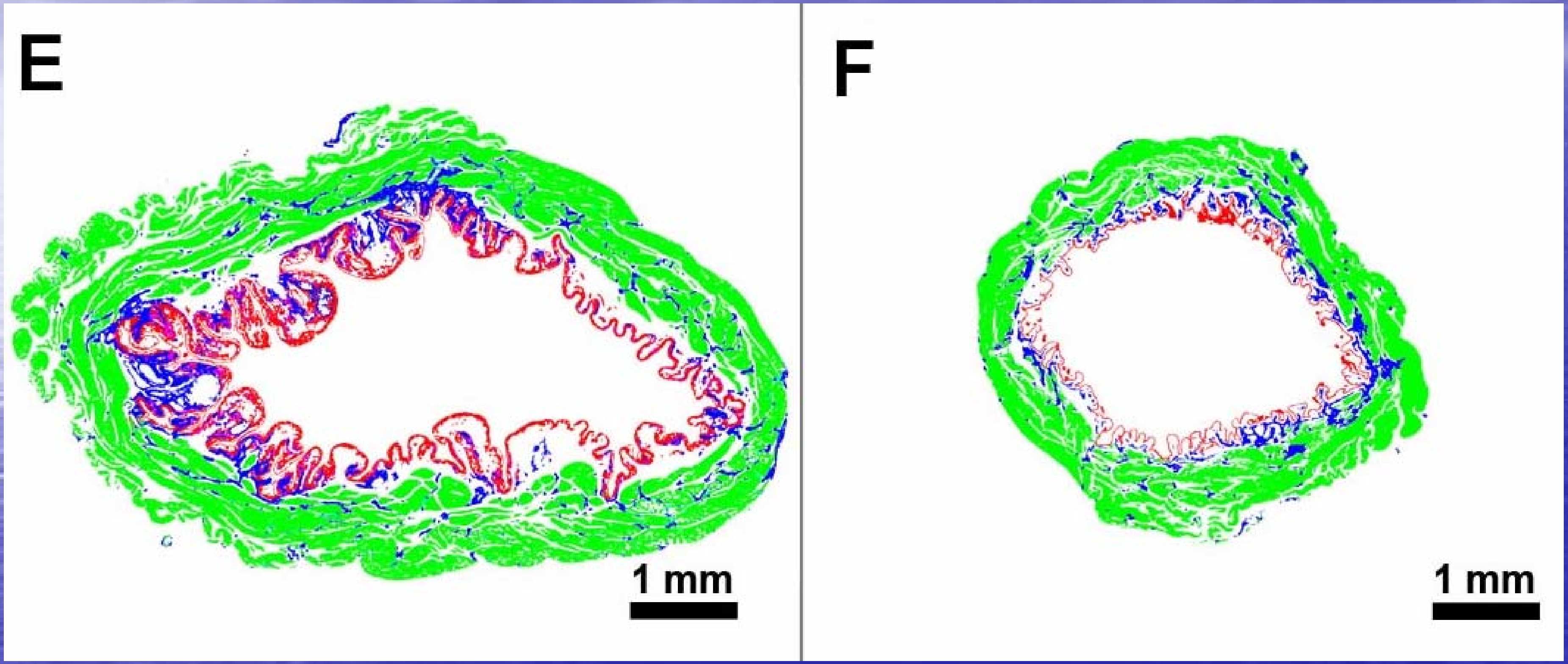
Control

H&E

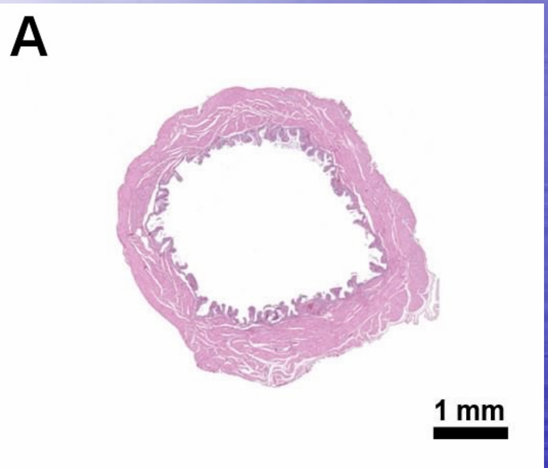
Morphometric Alterations in DBD

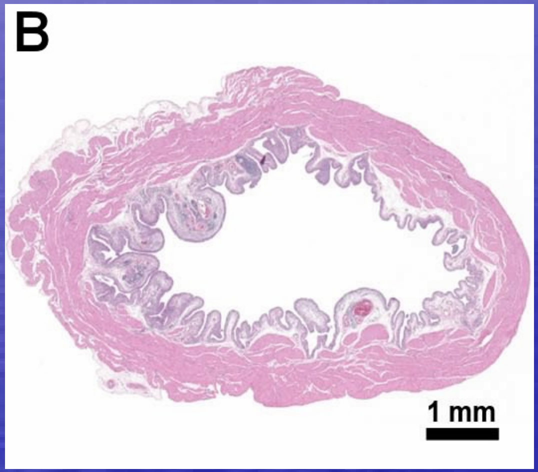


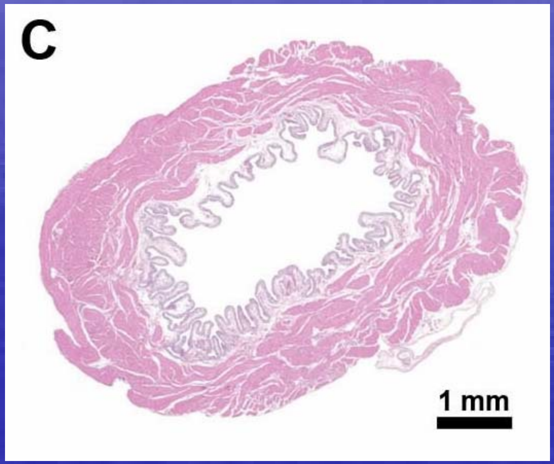
Morphometric Alterations in DBD



Morphometric Alterations in DBD







Control

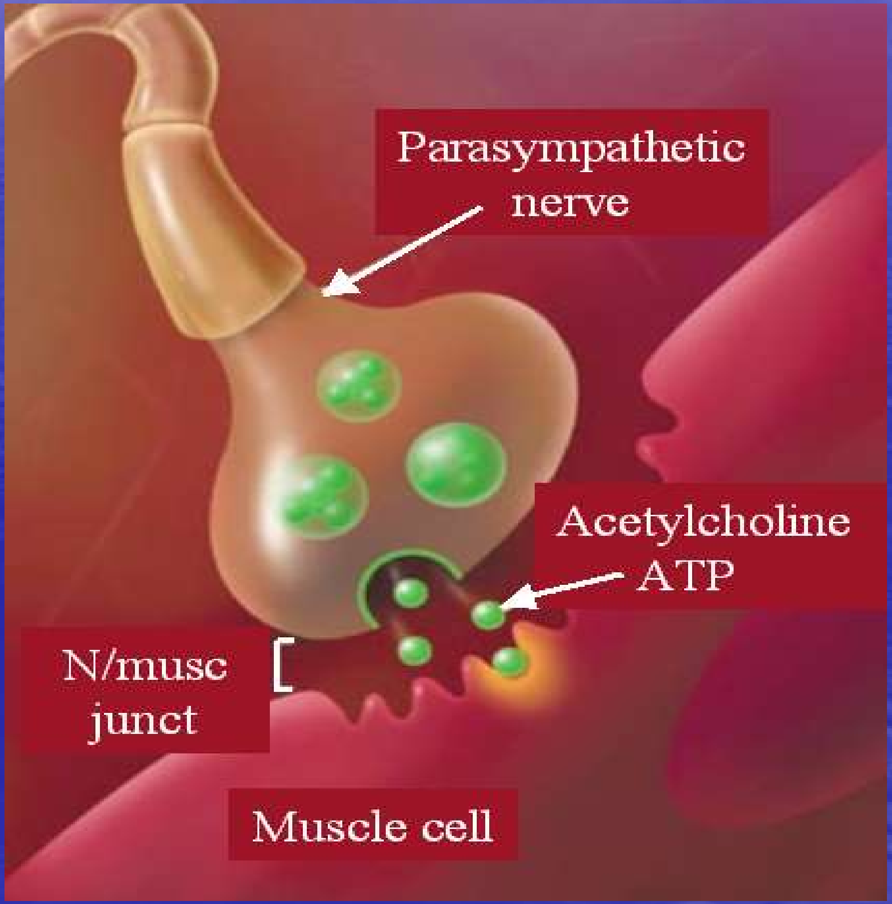
Diabetic

Diuretic

Liu & Daneshgari: AJP 2005

Bladder Contractility

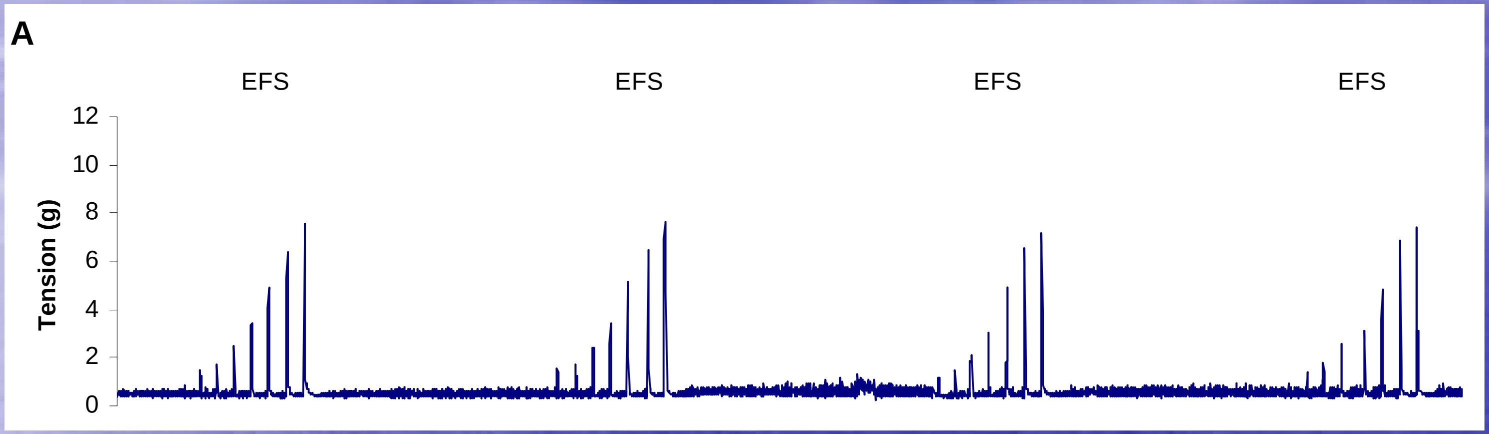
- Neurogenic Mediated:
  - Cholinergic
  - Purenergic
  - NANC
- Myogenic



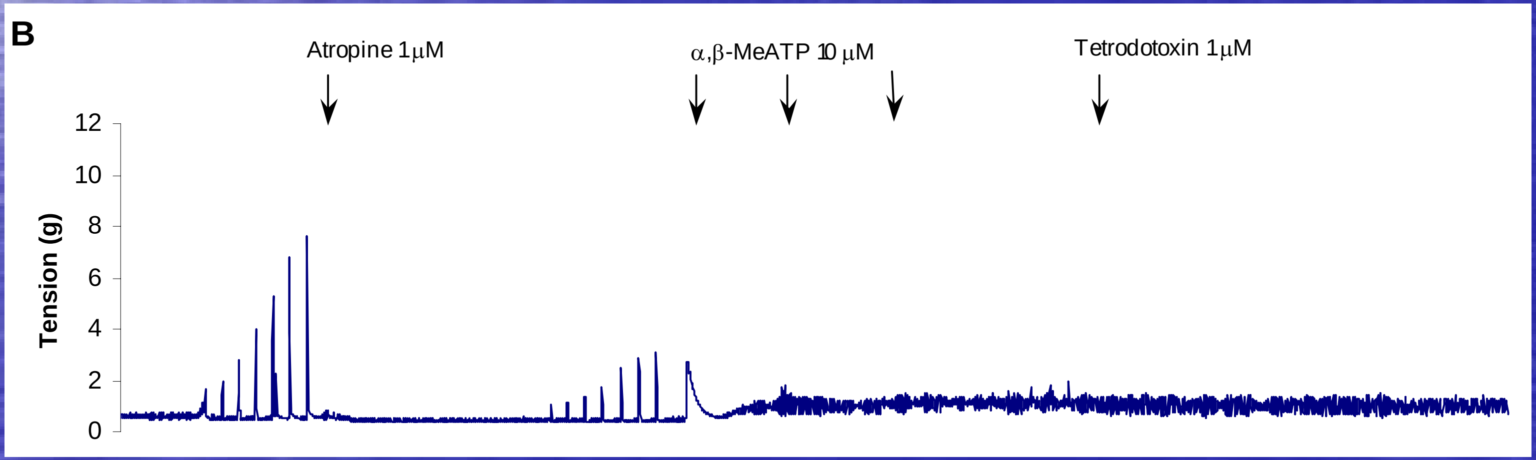
Bladder Contractility

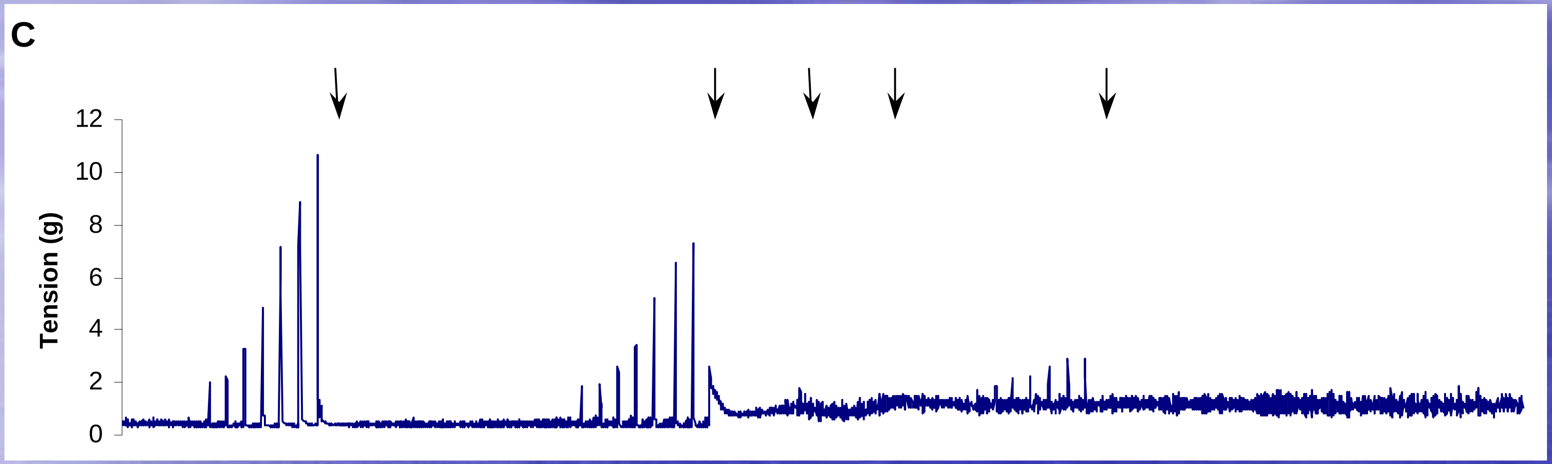
- Neurogenic Mediated:
  - Cholinergic
  - Purenergic
  - NANC
- Myogenic

- Electrical Field Stimulation
  - Carbachol
  - ATP
- KCL

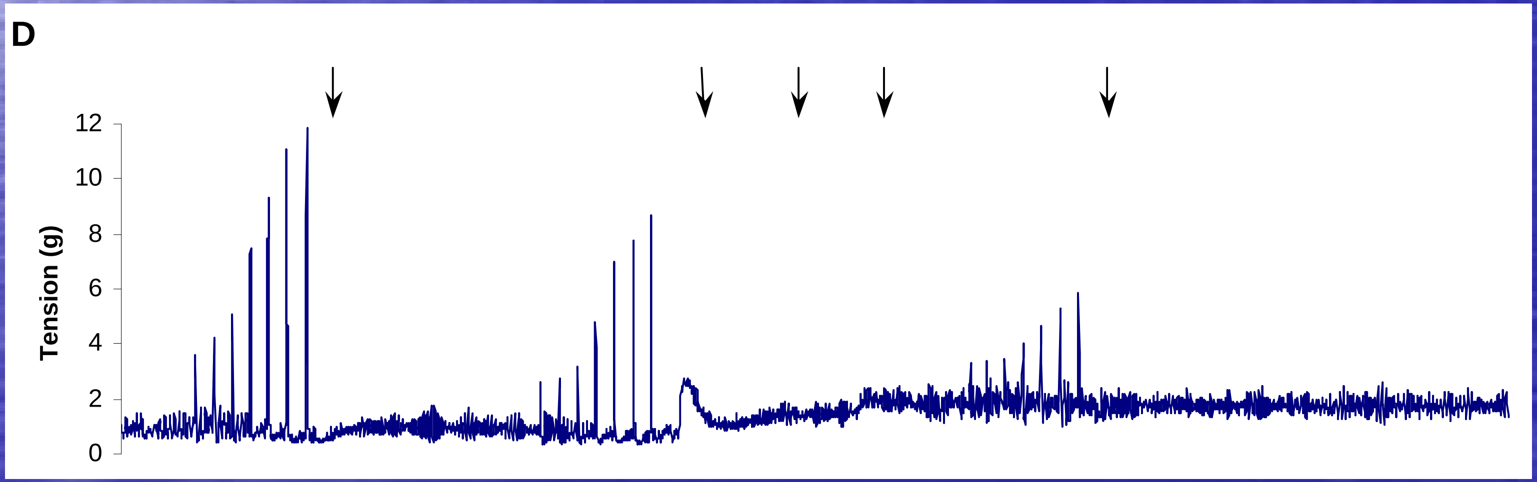

A
EFS
EFS
EFS
EFS
Tension (g)
12
10
8
6
4
2
0

Controls


B
Atropine 1μM
α,β-MeATP 10 μM
Tetrodotoxin 1μM
Tension (g)
12
10
8
6
4
2
0

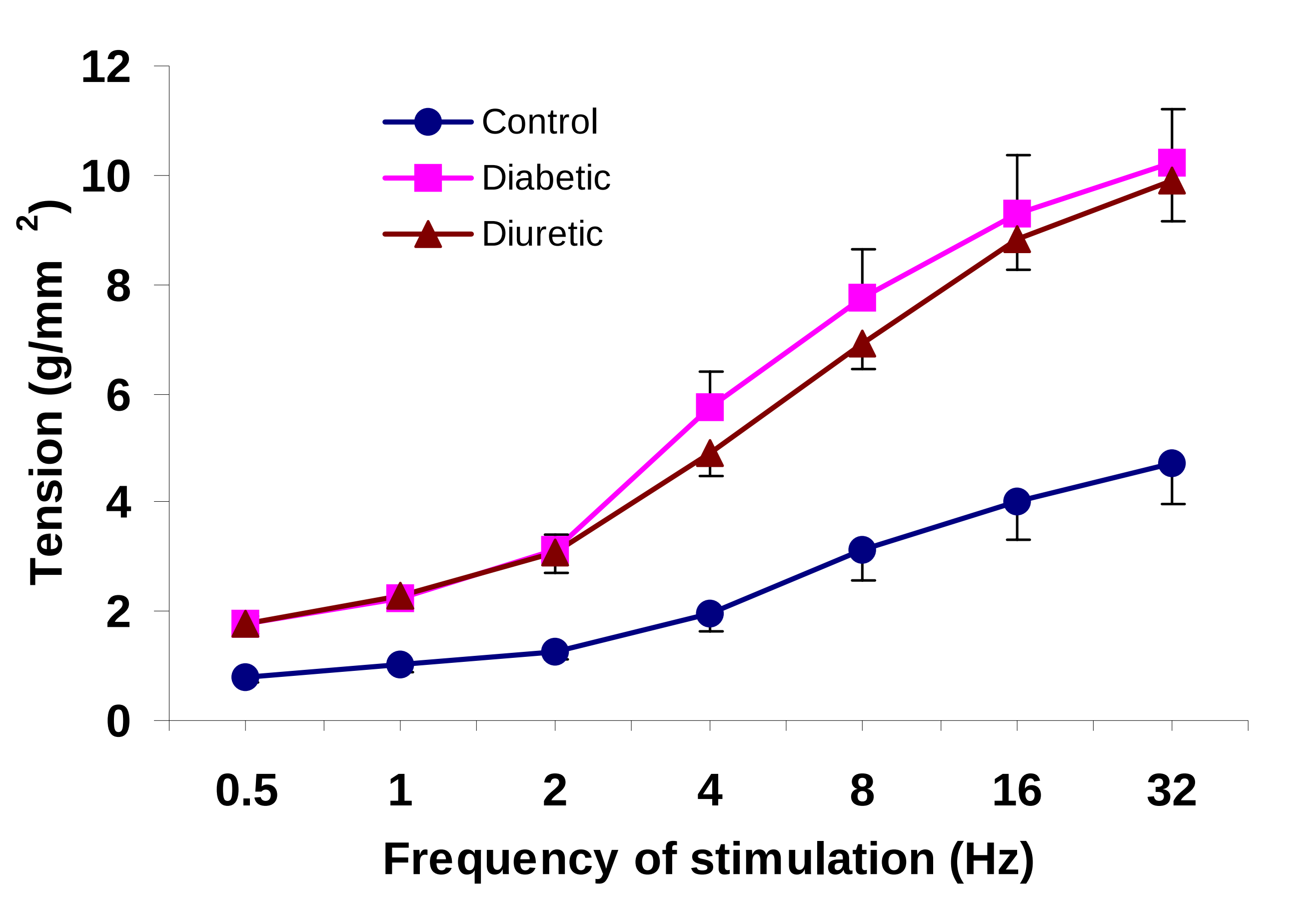


Diabetic

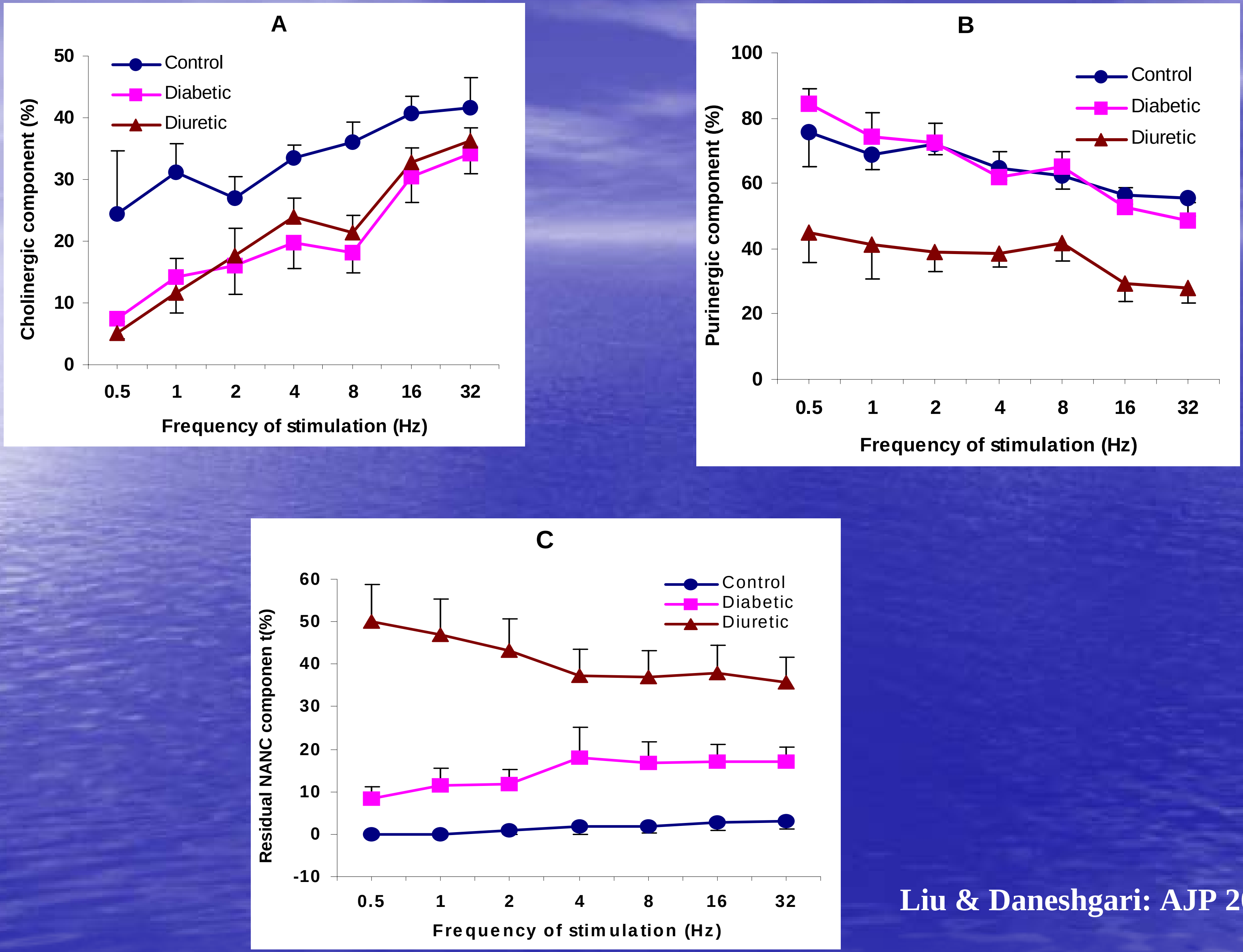


Diuretic

ALTERATIONS IN NEUROGENICALLY MEDIATED CONTRACTILE RESPONSES OF URINARY BLADDER IN RAT WITH DIABETES



Liu & Daneshgari: AJP 2005



Liu & Daneshgari: AJP 2005

Diabetic Cystopathy
Conclusions

- Time-dependent alterations in diabetic cystopathy are consistent in small animal models of DM
- Bladder undergoes compensatory and decompensatory changes
- Compensation is partially related to increased diuresis
- Decompensation is related to hyperglycemia and its disease specific changes in detrusor contractility

Diabetic Cystopathy

Conclusions

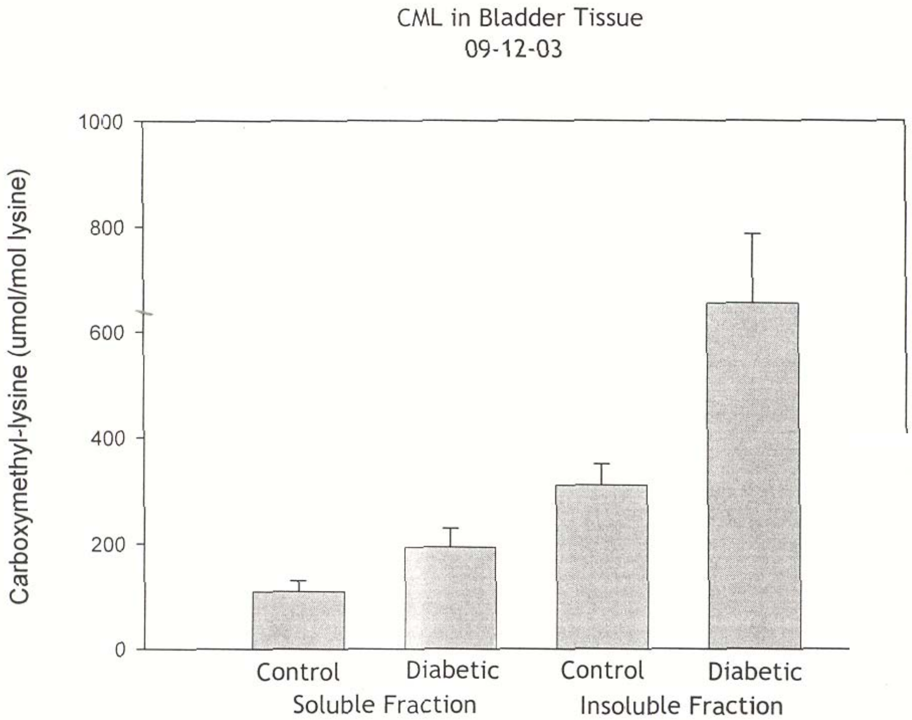
- Identification of mechanism of detruosr decompensation in the late stage of DM is needed
- Mechanisms of detrusor decompensation may involve ROS pathways

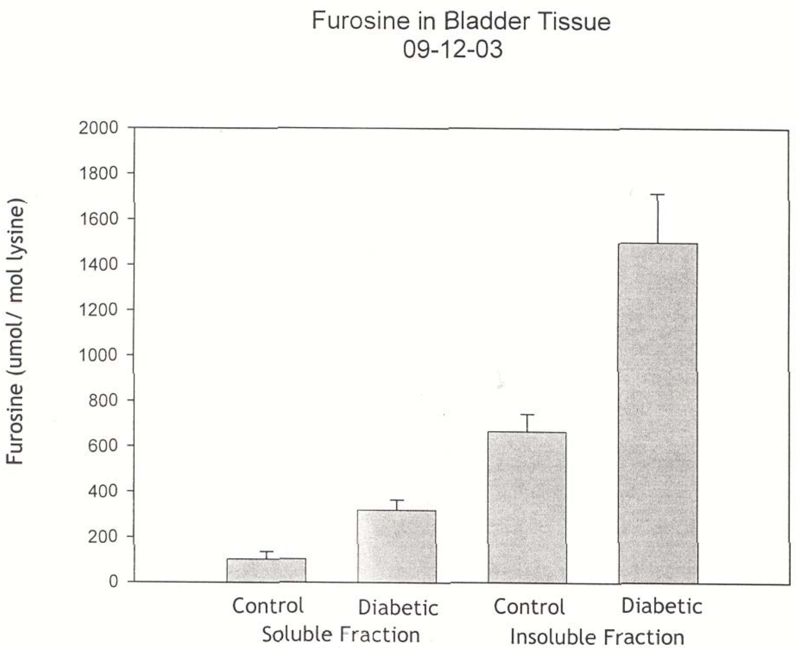
EAC Comments: Downstream alterations?

Assessment of AGEs
in the diabetic bladder

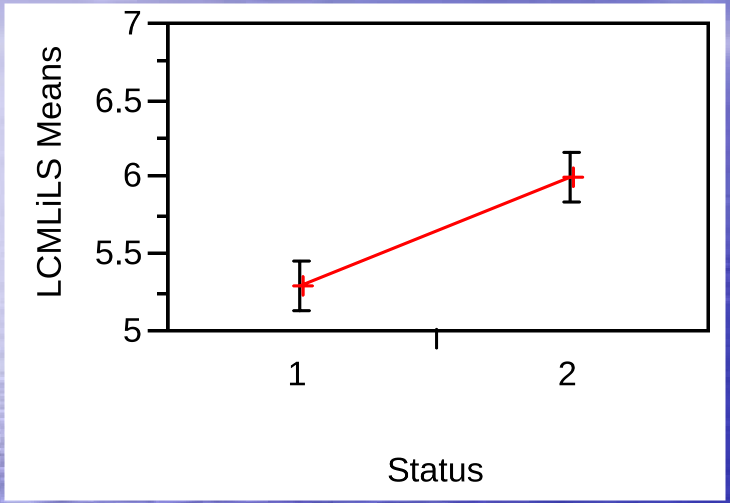
In Collaboration with

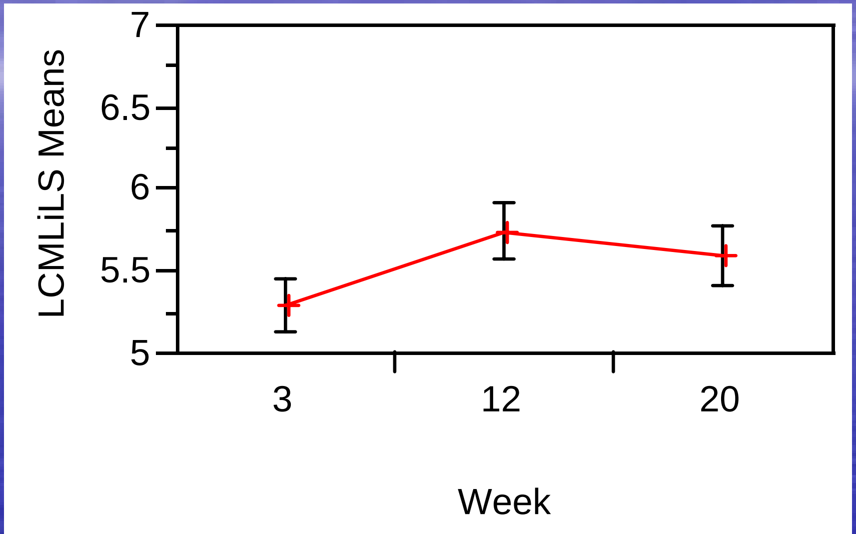
Vincent Monnier, M.D.


CML in Bladder Tissue
09-12-03
Carboxymethyl-lysine (umol/mol lysine)
1000
800
600
400
200
0
Control
Diabetic
Control
Diabetic
Soluble Fraction
Insoluble Fraction

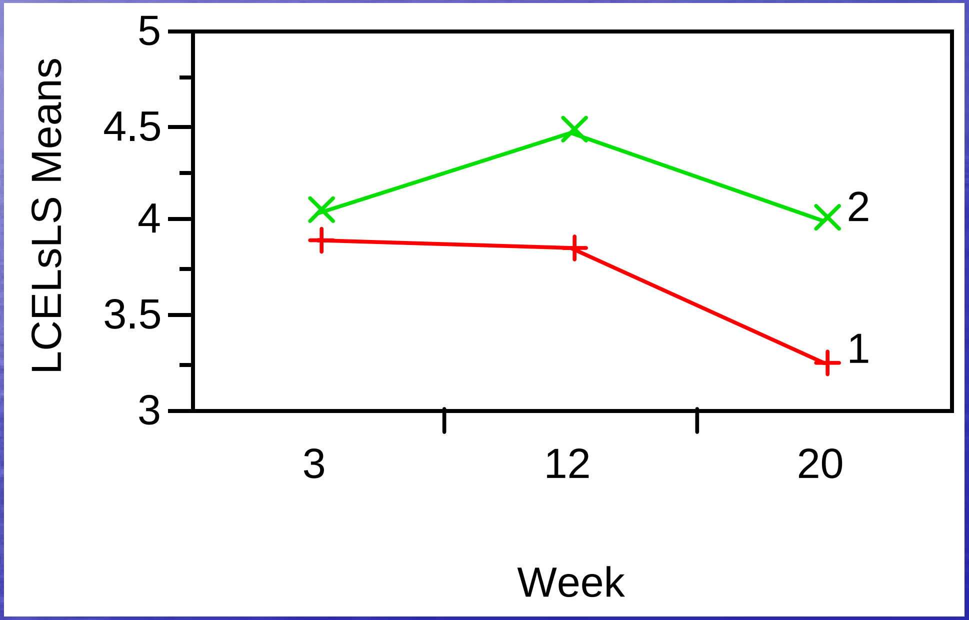

Furosine in Bladder Tissue
09-12-03
Furosine (umol/ mol lysine)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Control
Diabetic
Control
Diabetic
Soluble Fraction
Insoluble Fraction

Carboxymethyl Lysine (CMLi)
Adjusted Effects of Age and DM (Natural Log Scale)





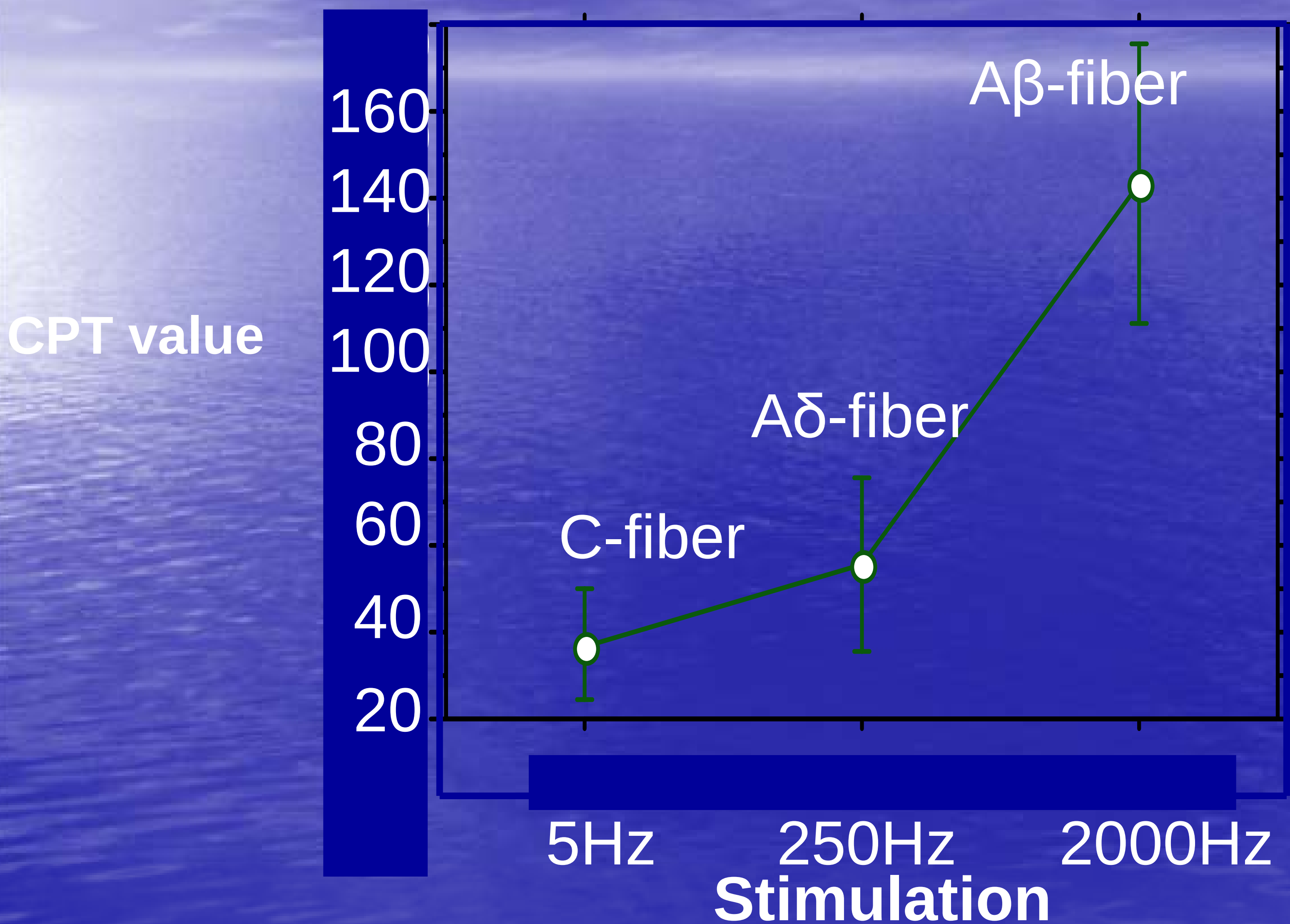
Carboxyethyl Lysine (CELs, Natural Log Scale)



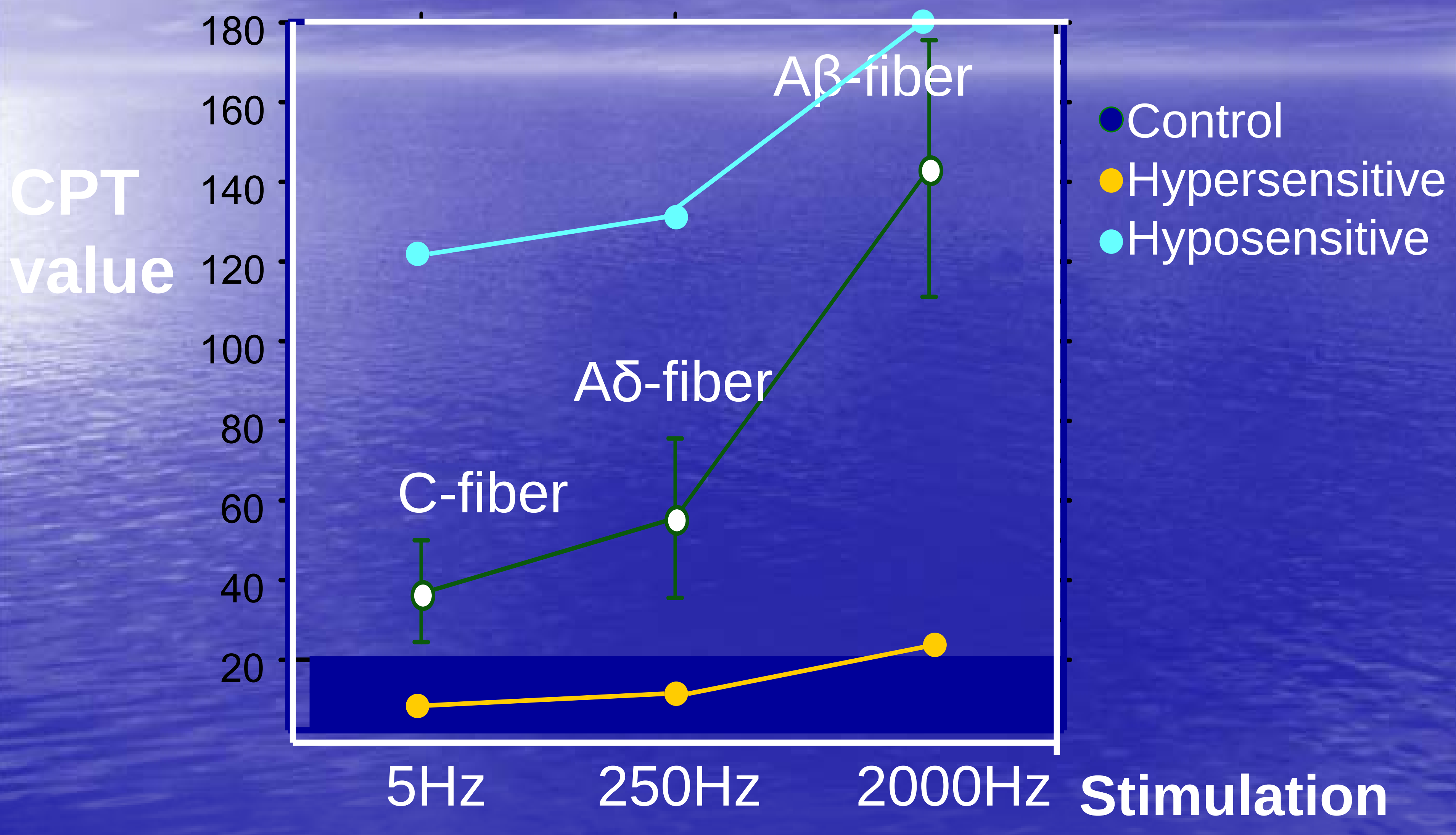
EAC Comments

- Find a phentoyping assay to detect loss of bladder sensation in DBD

Preliminary Results of Quantitative Measurement of Urinary Sensory Function <u>in Normal Bladder</u>

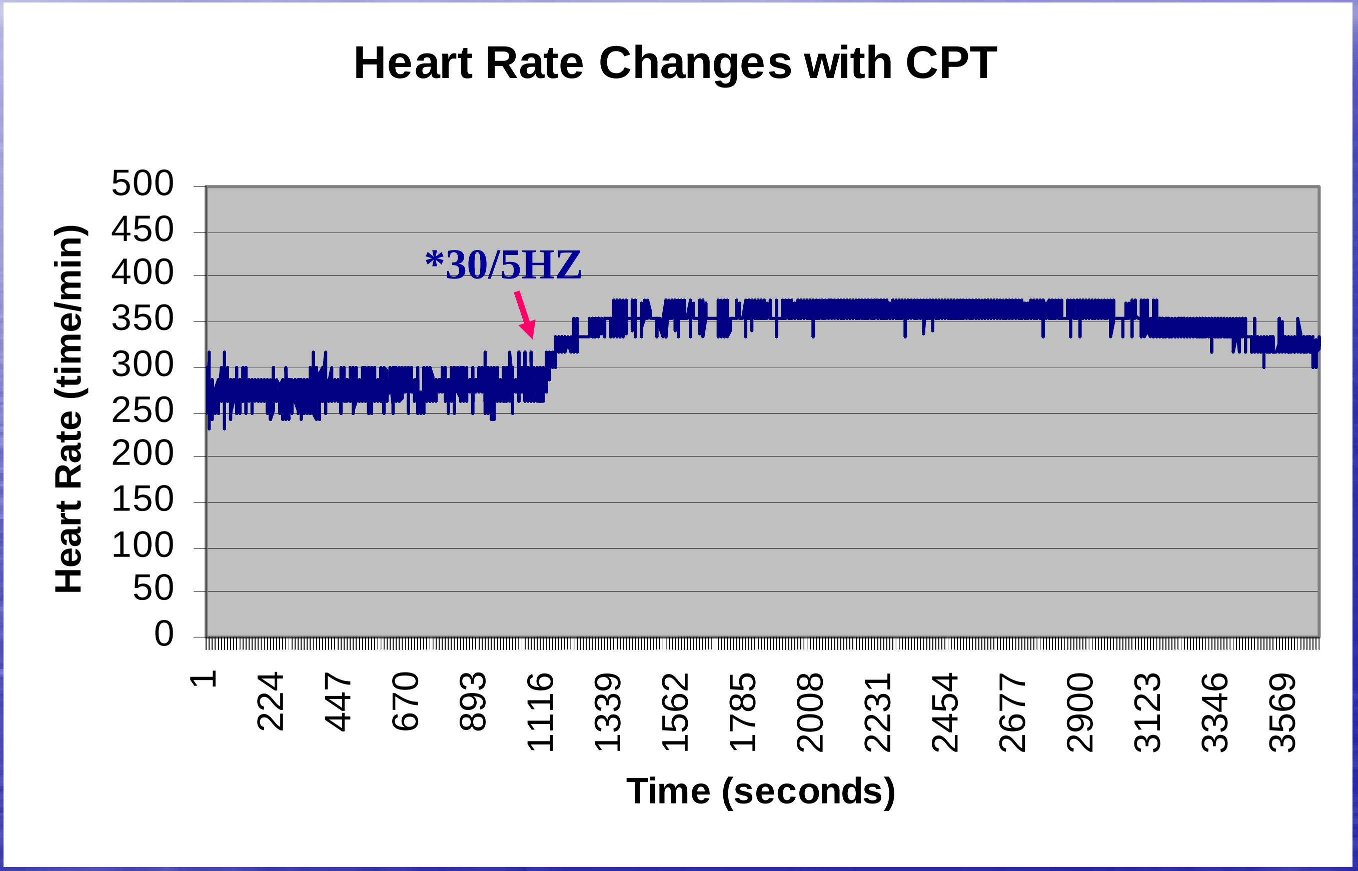


Results of CPT values in individual cases comparing to the values in control



TransUrethral Afferent Current Perception Threshold (TUR-ACT) in rat/mouse

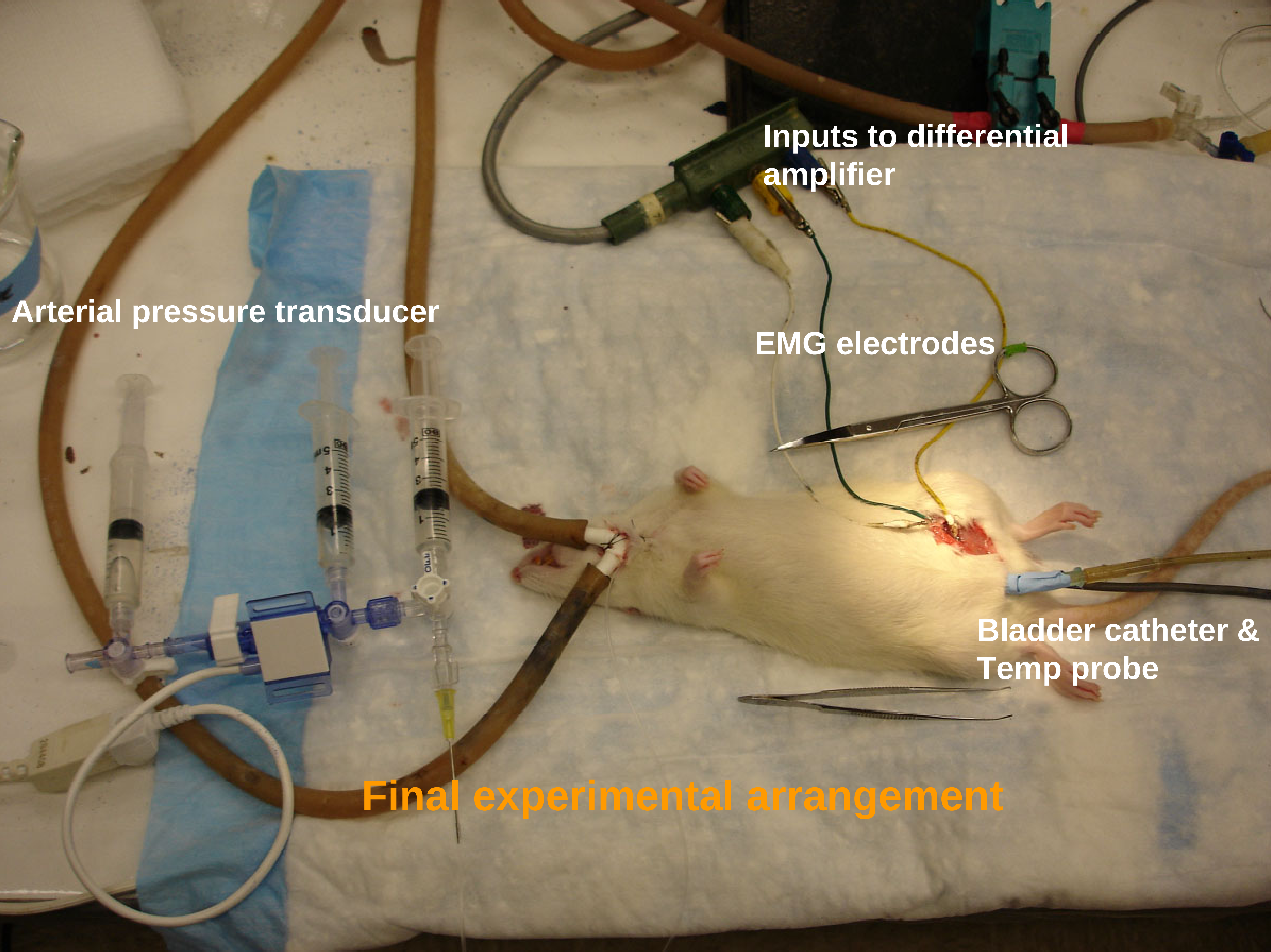
Daneshgari 2004


Heart Rate Changes with CPT
*30/5HZ
Heart Rate (time/min)
500
450
400
350
300
250
200
150
100
50
0
1
224
447
670
893
1116
1339
1562
1785
2008
2231
2454
2677
2900
3123
3346
3569
Time (seconds)

Measurement of CPT values in small animal models of diabetes

- Measurement of BP and HR as the ANS response
- Measurement of abdominal rectus muscle as a somatic pain response independent of ANS (Timothy Ness, M.D., Ph.D. UAB)

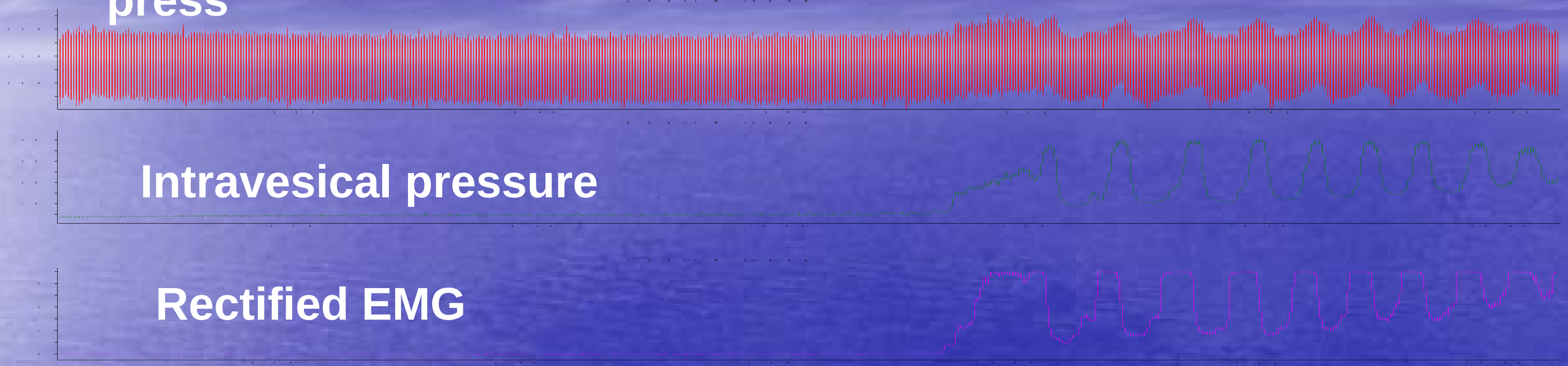
Assessment of CPT with Vesicomotor response


Inputs to differential amplifier
Arterial pressure transducer
EMG electrodes
Bladder catheter & Temp probe
Final experimental arrangement

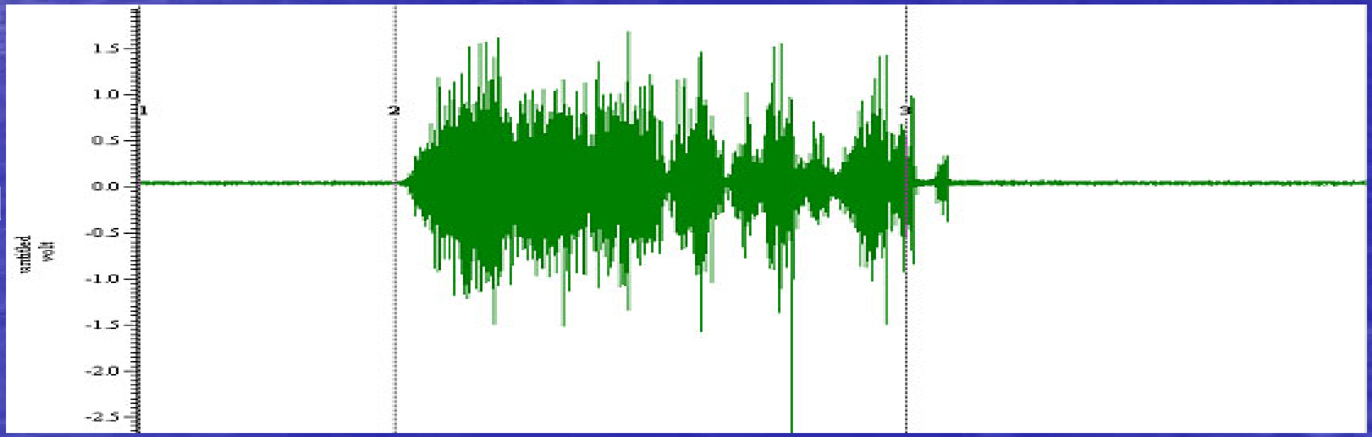
Final experimental output

Arterial press

For CMG (slow fill)



EMG measu
For phasic UBD



Diabetic Uropathy

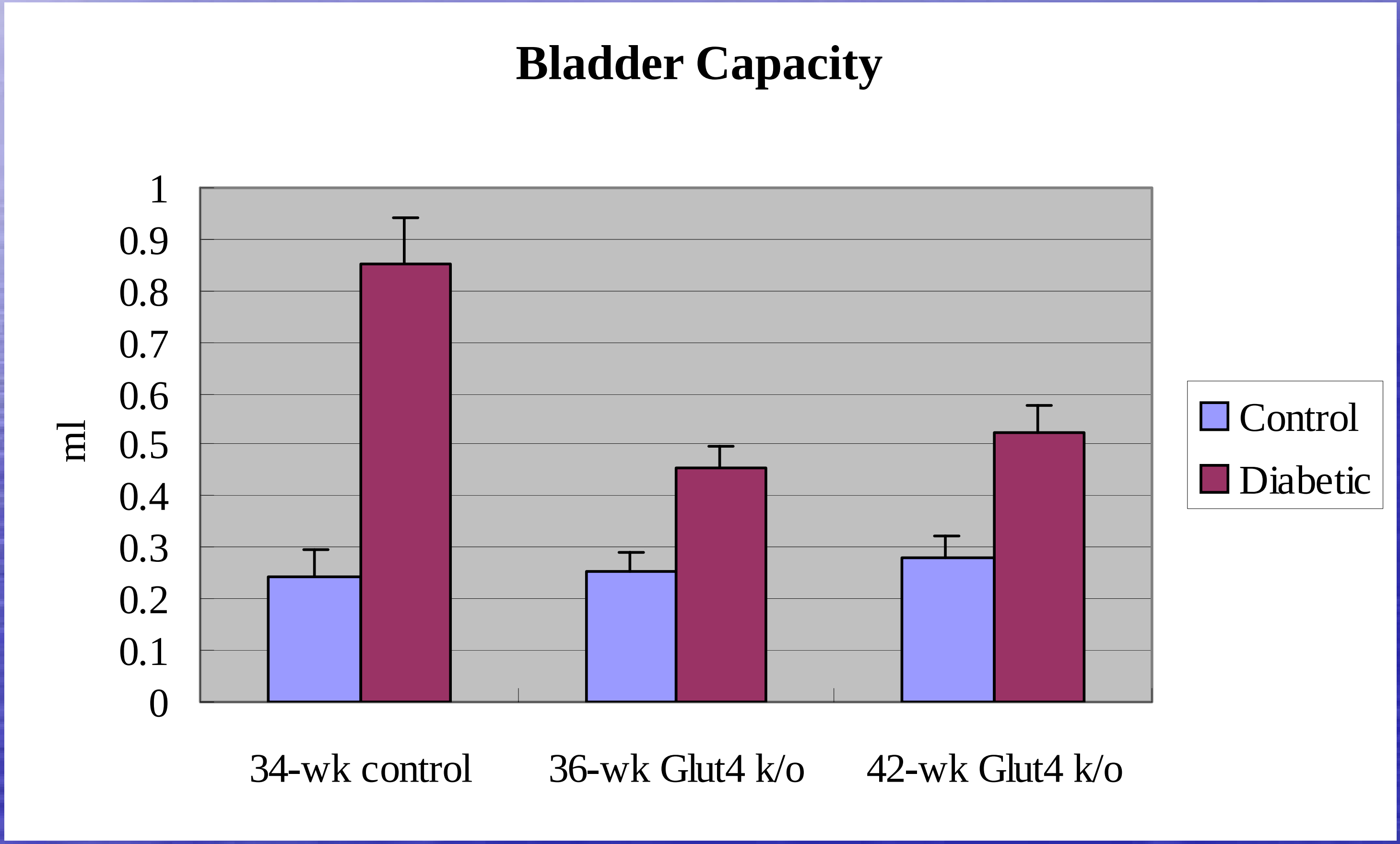
2004 Work

- 4 manuscripts
- JDRF post-doc fellowship award
- STTR application
- Phenotyping of AMDCC animals

AMDCC Collaborative studies

Total Body Glut4/ko-C57Bl/6- University of MI

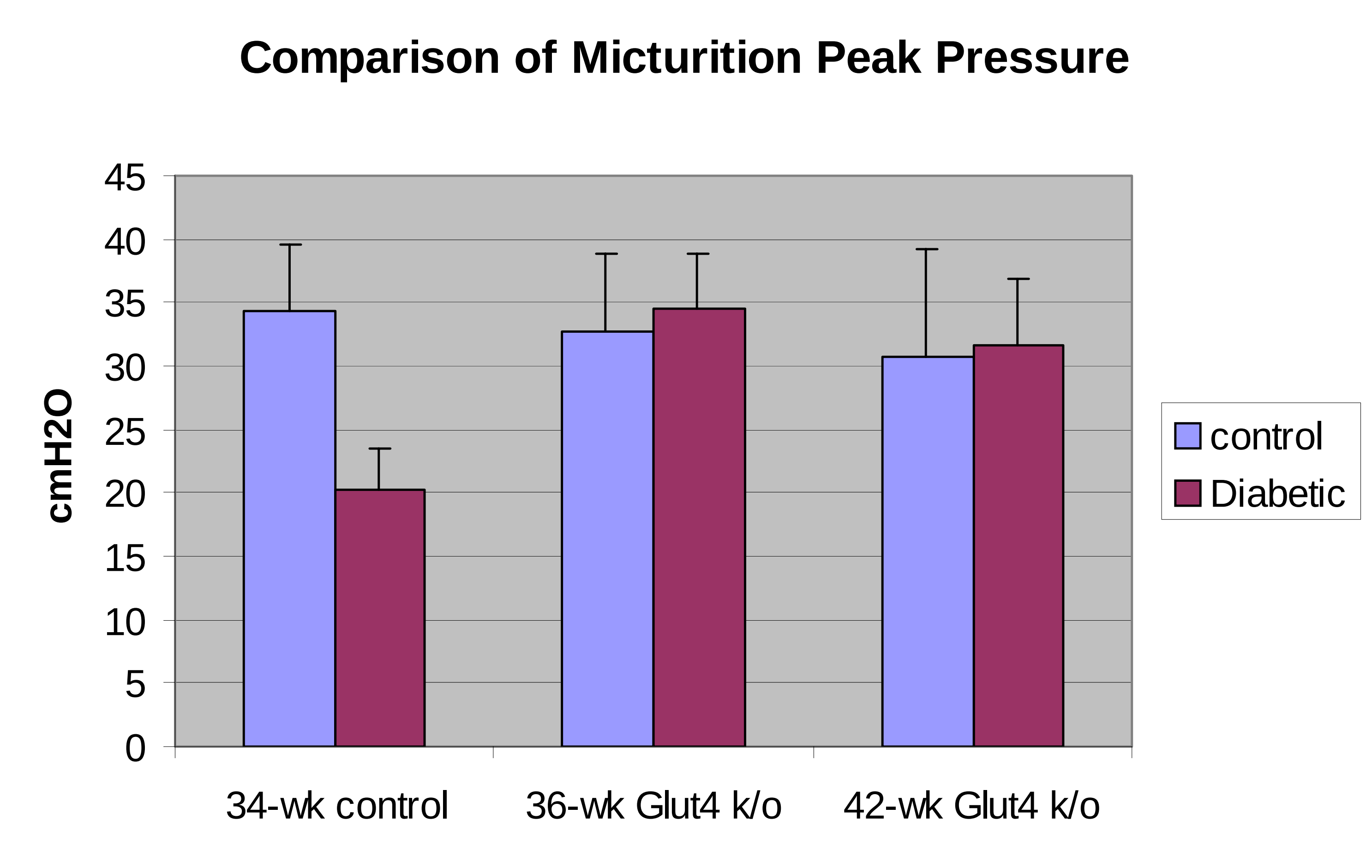
Bladder Capacity



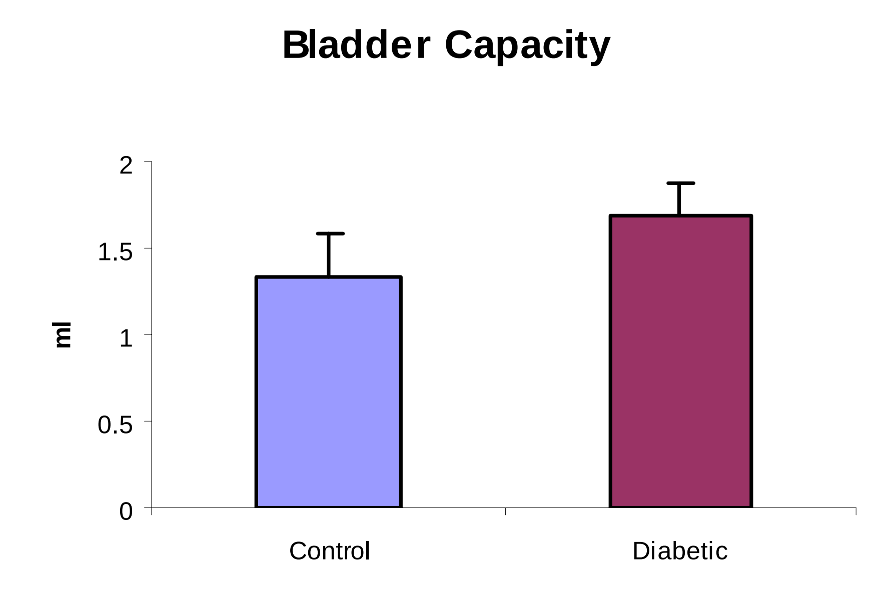
AMDCC Collaborative studies

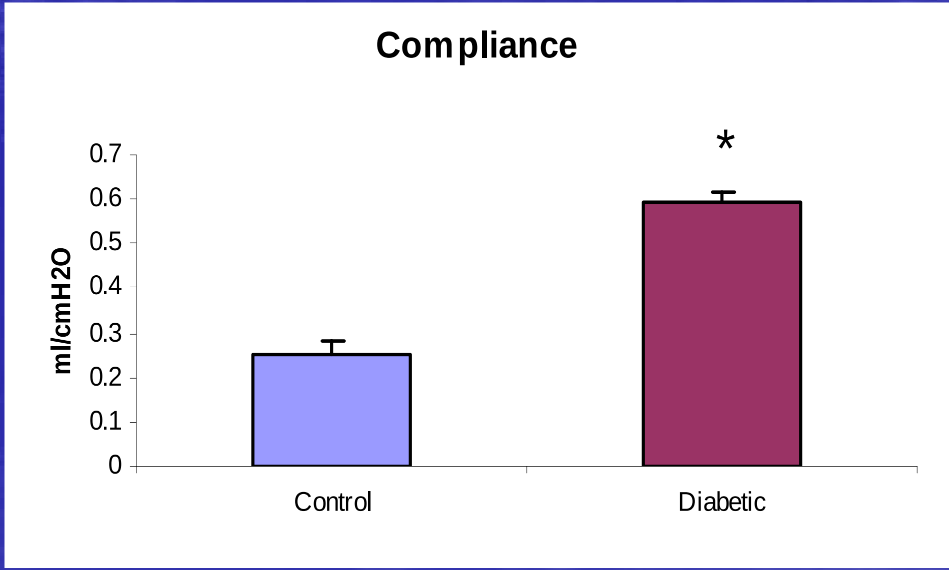
Total Body Glut4/ko-C57Bl/6- University of MI

Voiding Pressure

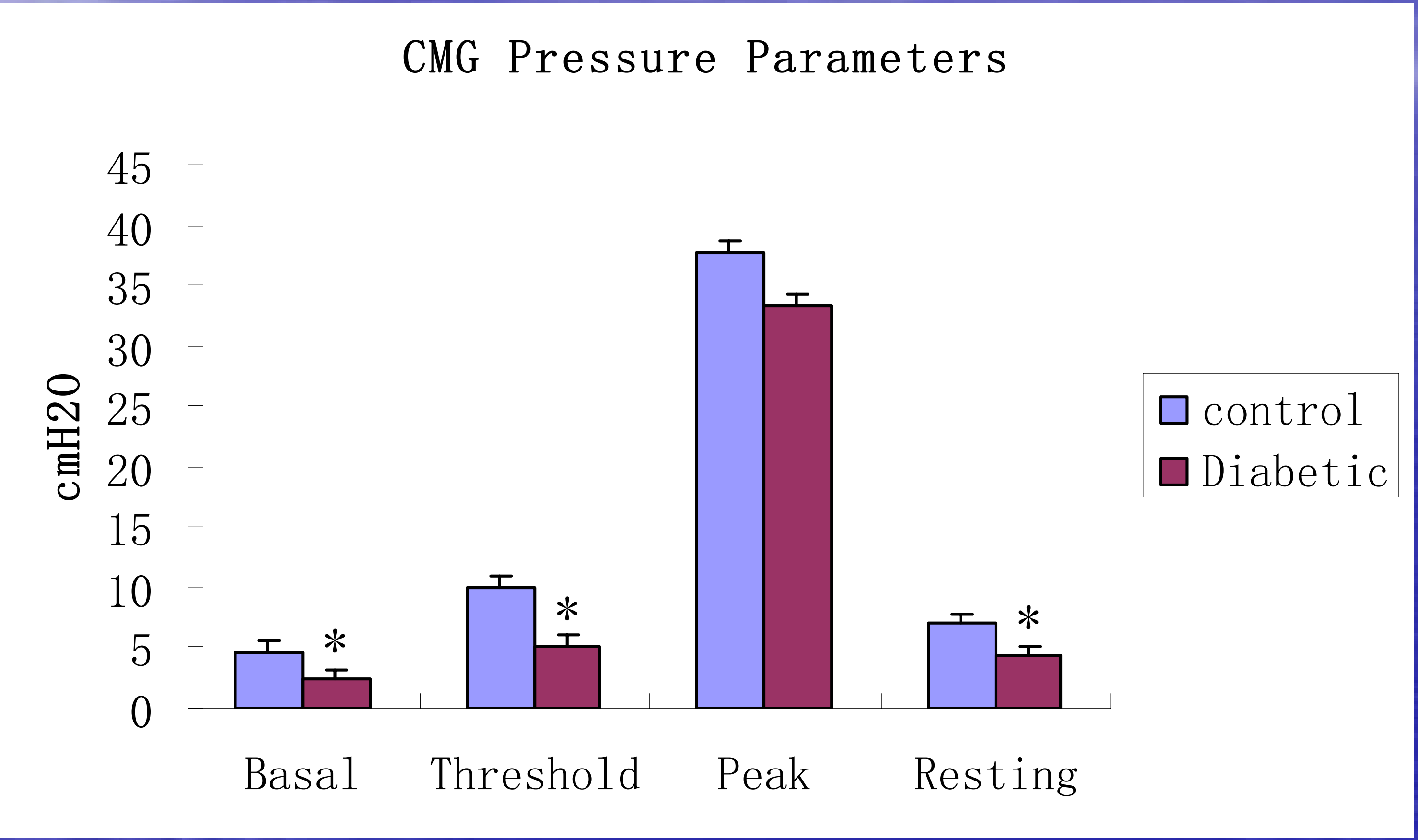


KLS Mice- U of MI





KLS Mice- U of MI



METHODS

A/symptomatic Patient with Type I or Type II Diabetes

Informed Consent Obtained

Medical & Diabetic History Obtained

Complete Self-Administered Questionnaires

1. Diabetes Lower Urinary Tract Screening Questionnaire
2. Pelvic Floor Disorder Screening Questionnaire

A1c Hemoglobin (HgA1c)

Uroflowmetry & Determination of Post-Void Residual

CPT measurement+ Autonomic Neuropathy Assessment

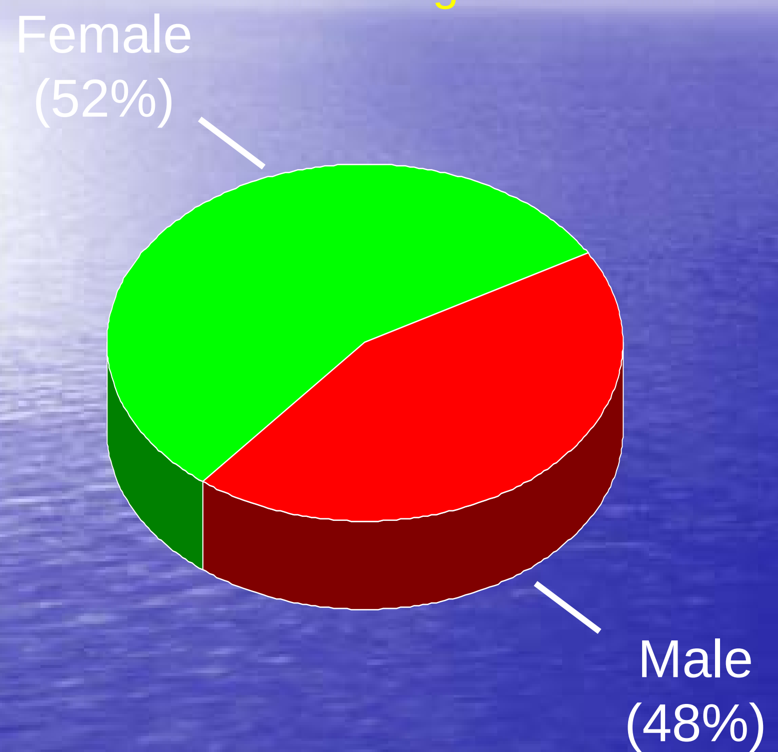
Urodynamics

RESULTS

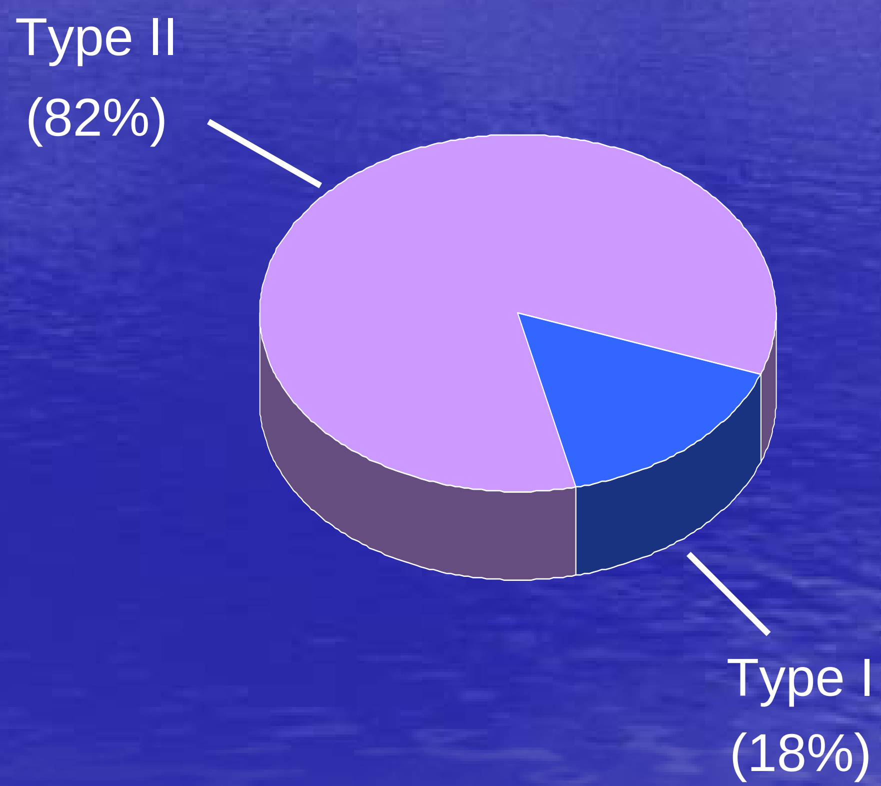
n = 70

Gender

Mean Age = 56.7



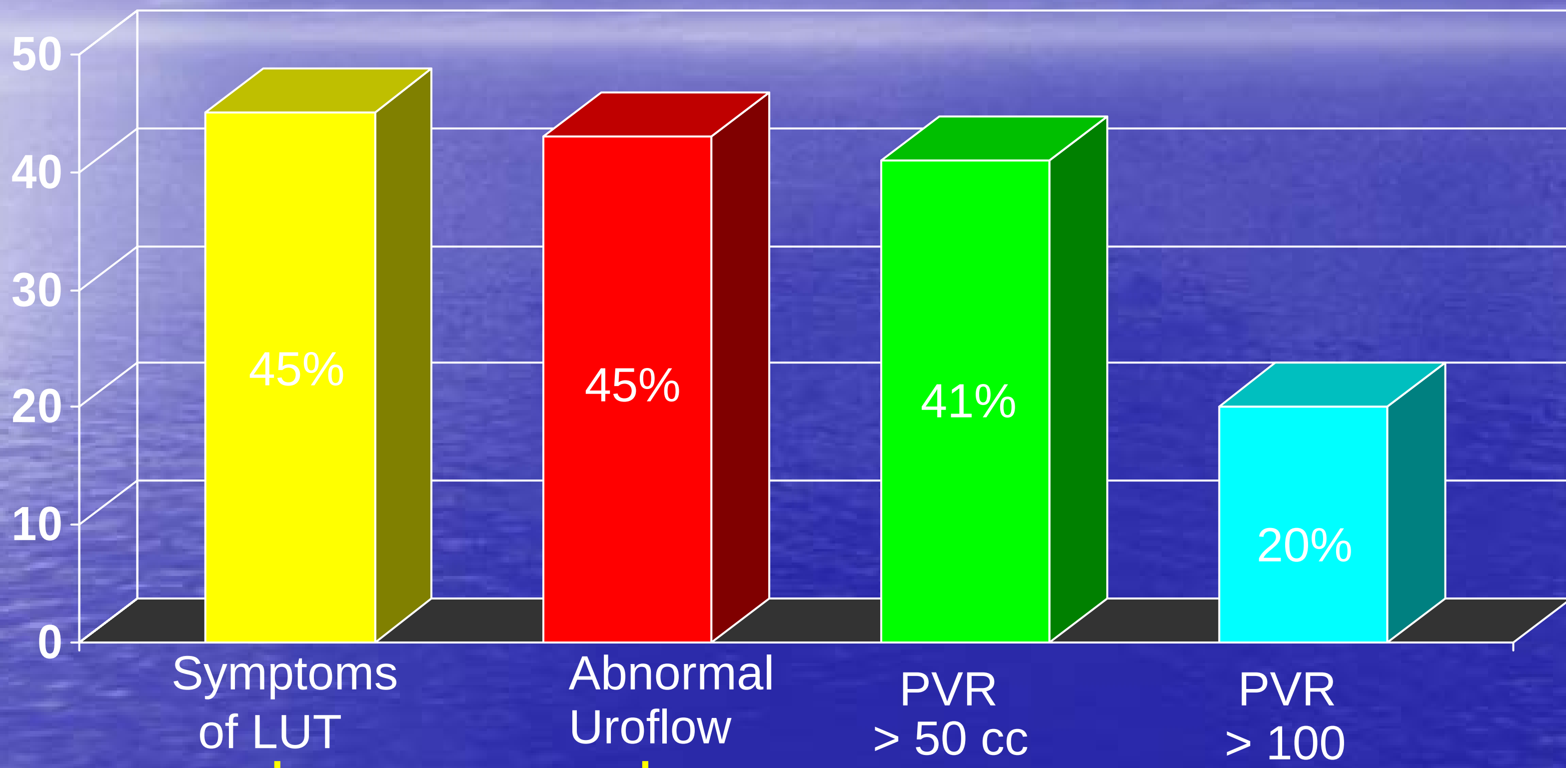
Diabetic Type



DM LUT Screening Questionnaire		
	Percent Responders	
Voiding Frequency > 8/day	Female	Male
Voiding Frequency >2/night	54	52
Urgency	61	42
Urge accidents	62	42
Wear pads for urgency	44	13
Leak on cough/laugh/lift	24	0
Post-void dribbling	58	0
Wear Pads for leaks	44	23
UTI within year	30	0
Three or more questions positive	32	3
	68	29

RESULTS

LUT Complications



Urinary incontinence
Voiding dysfunction

Maximum flow rate of <15 mL/cc

Diabetic Uropathy

Feldman Table

Animals	Micturition Cycles	CMG	Contractility	AGEs CPT
C57BL / 6	√	√	√	
Glut4/ko-C57Bl/6	-	√	-	
KLS	-	√	-	

2005-2006 Work

- Role of AGEs and other ROS in decompensation of detrusor function
- Assessment of Afferent autonomic neuropathy in the bladder
- Better definition the humanoid condition
- Phenotyping of AMDCC animals

DBD Phenotyping of AMDCC animals

- 49 Strains in the AMDCC
- Identify 2-3 models from each complication
- Do on-site or off-site phenotying of top models within the next 6-12 months

Model/Strain	Complication	24 hr MC	CMG	Contractility
Ins2^{Akita} Bdkr2-/-	Duke-Nephropathy			
D2B6F1Ins2 ^{Akita}	Duke-Nephropathy			
129-Ins2 ^{Akita}	Duke-Nephropathy			
C57BL/6JLDLR-/- Pdx-1+/+	Rockefeller-CV			
C57BL/6JLDLR-/- Pdx-1+/-	Rockefeller-CV			
C57BL/6JLDLR-/-	Rockefeller-CV			
C57BL/6JLDLR-/- Ob/Ob	Rockefeller-CV			
C57BL/6J Akita	Utah- Cardiac			
FVB Dominent Neg P13 Kinase	Utah- Cardiac			
ACS-CIRKO	Utah- Cardiac			

Model/Strain	Complication	24 hr MC	CMG	Contractility
C57BL/6J	Vanderbilt-Nephropathy			
DBA/2J	Vanderbilt-Nephropathy			
FVB/NJ	Vanderbilt-Nephropathy			
C57BLKS db/db	Michigan-Neuropathy			
GCLC+/-db/db High fat diet	Michigan-Neuropathy			
GLUT1 tg db/db High fat diet	Michigan-Neuropathy			
Elderly LDLR-/-	UCLA-Macrovascular			
Hyplip Cross	UCLA-Macrovascular			
SM PPAKg-/-	UCLA-Macrovascular