Case Western Reserve University
School of Medicine

Diabetic Uropathy

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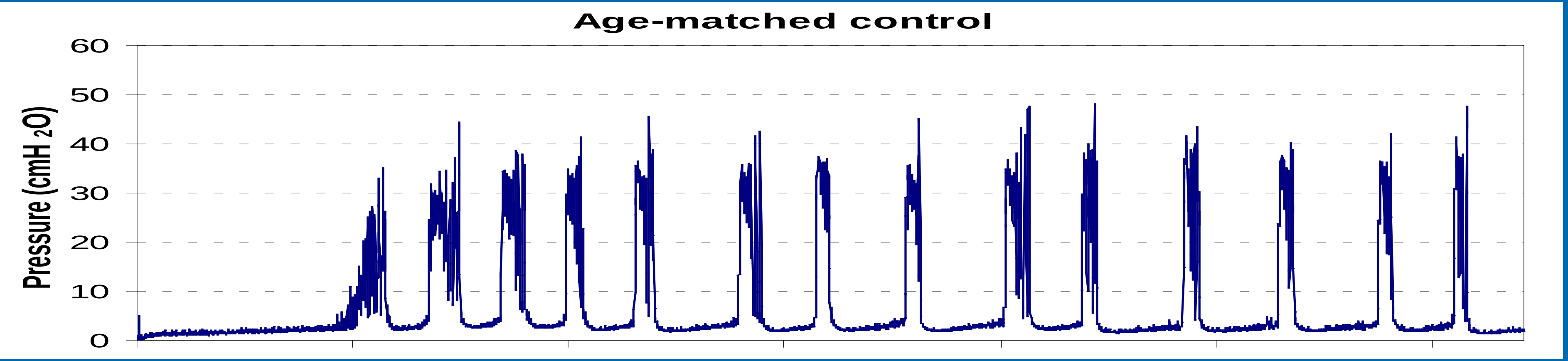
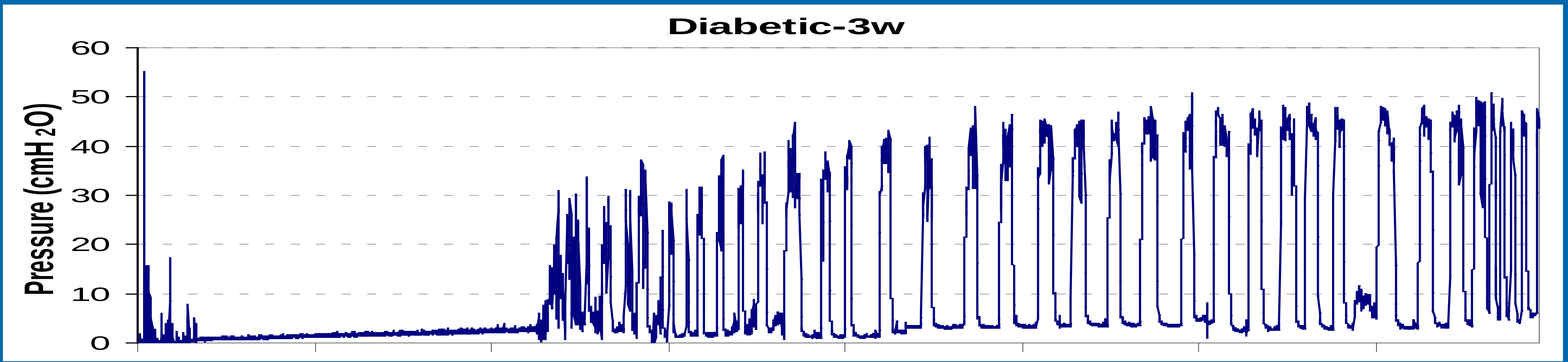
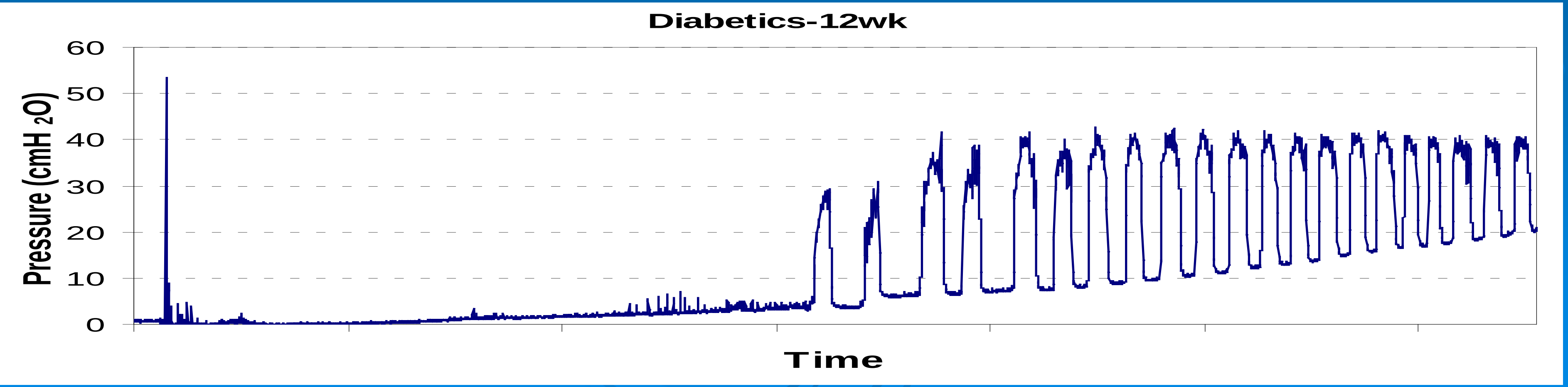
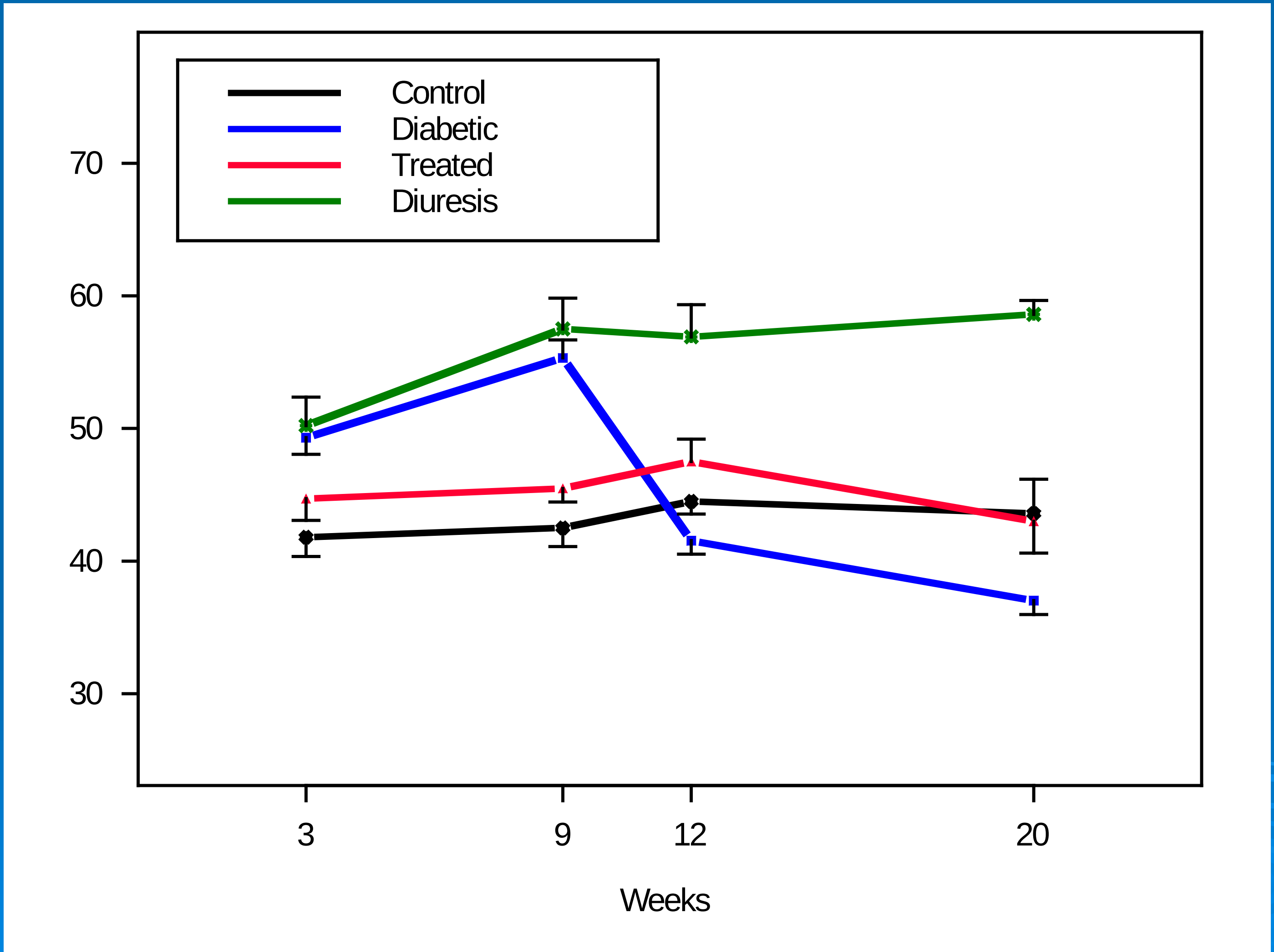

Age-matched control
Pressure (cmH 2O)
0
10
20
30
40
50
60


Diabetic-3w
Pressure (cmH 2O)
0
10
20
30
40
50
60


Diabetics-12wk
Pressure (cmH 2O)
0
10
20
30
40
50
60
Time

Temporal Differences in Bladder Dysfunction Caused by Diabetes, Diuresis and Treated Diabetes in Mice



Daneshgari AJP 2006

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

Early Phase → Late Phase

Time Course/Risk factors ??

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

Daneshgari 2006

Natural History of Diabetic Bladder Dysfunction

	Early Stage DBD (<9 weeks in rodent)	Late Stage DBD (>12 weeks in rodent)
Symptoms	Storage Problems	Voiding Problems
Main Finding	Compensated Remodeled Bladder	Decompensated Atonic Bladder
Mechanism	**Polyuria**	**Hyperglycemia induced Oxidative Stress**

Hypothesis

In the Early Stage

Polyuria? → Urine Voiding ↑ → Frequently stretch

Compensated?

PI3K/Akt ?
Cn/NFAT?

Bladder hypertrophy, remodeling →

Myogenic change?

Neurogenic change?

Ionic channel contractile proteins regulatory proteins

- number of nerve fiber
- neurotransmitter release and/or receptors

Hypothesis

In the later stage

Consistent hyperglycemia

↓

Reactive oxygen species?
Aldose reductase pathway?
polyol pathway?
AGE?
PKC?

+

Consistent polyuria

→ Neuropathy
- Afferent
  - Sensor molecules release?
  - their receptors?
- Efferent
  - Neurotransmitters release?
  - Muscarinic receptor?
  - Purinergic receptor?

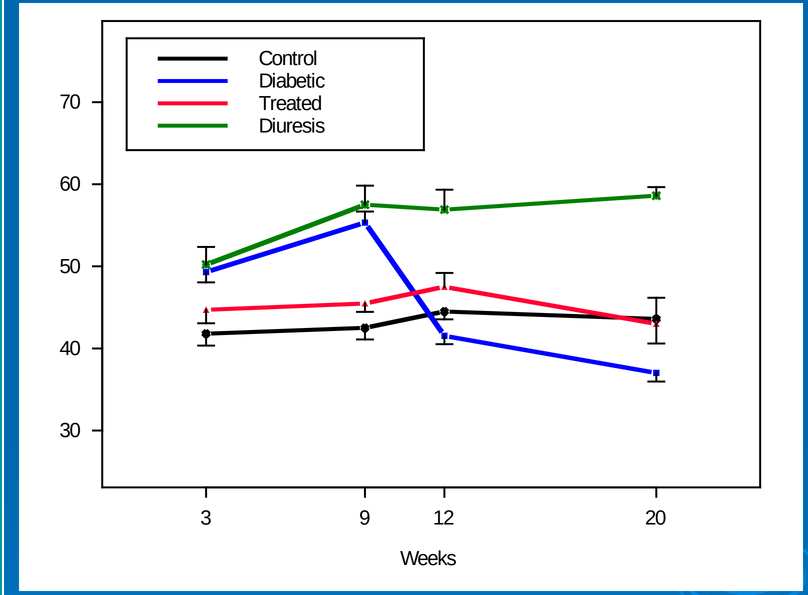
→ Myopathy
- ionic channel contractile proteins
- regulatory proteins

- - - → Decompensated? → Atonic bladder

Mechanisms of Bladder Remodeling in DM

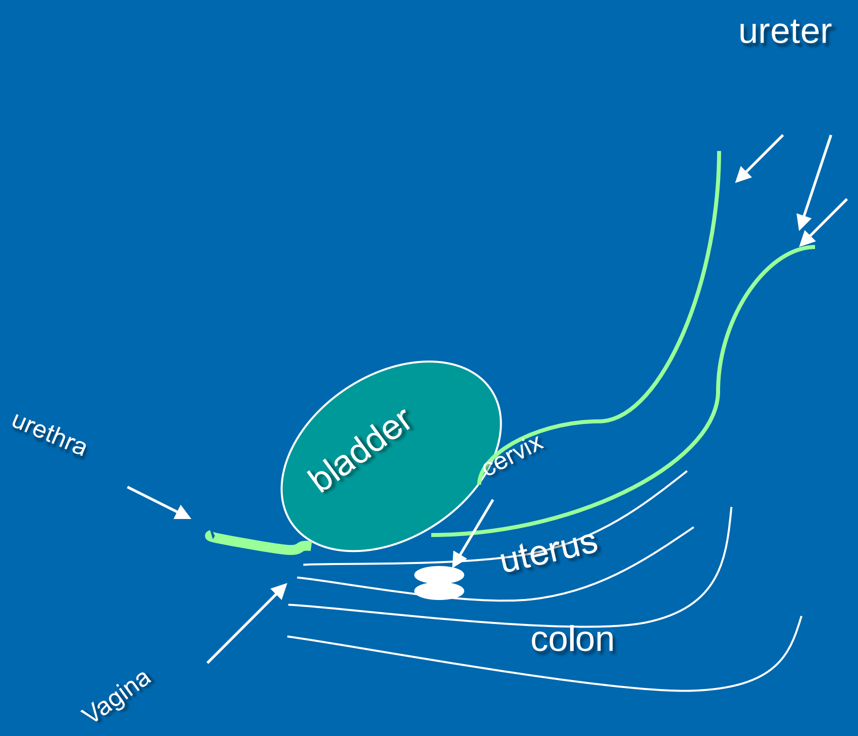
Early DM (<9 Weeks in rat/mice):
Polyuria

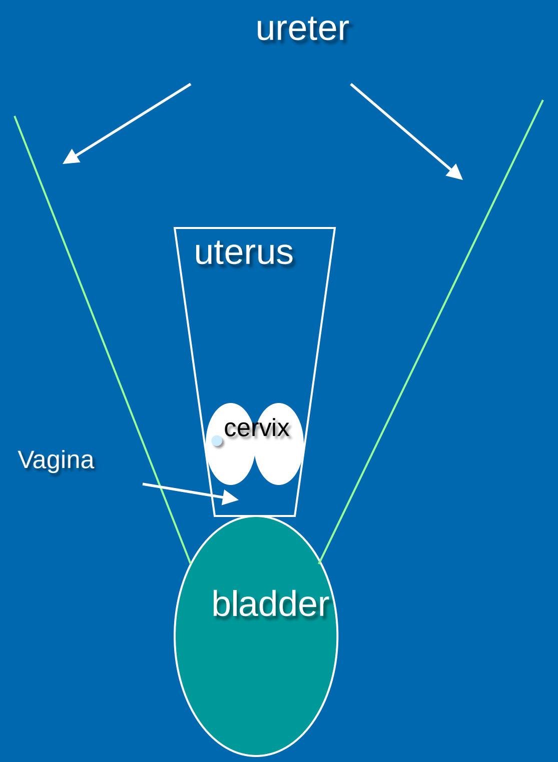
Late DM (>12 weeks in rat/mice)
Oxidative Stress



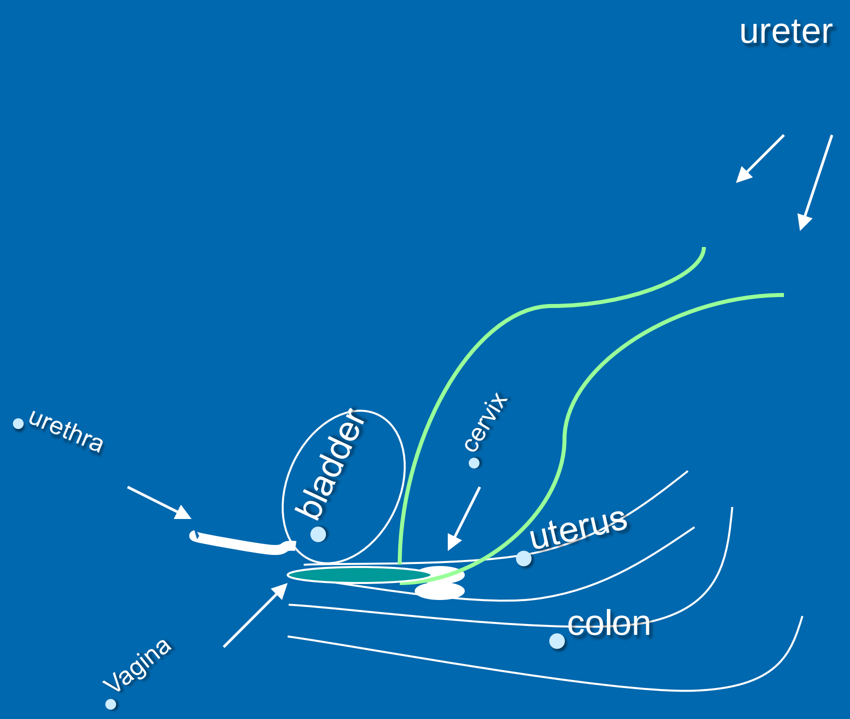
Methods

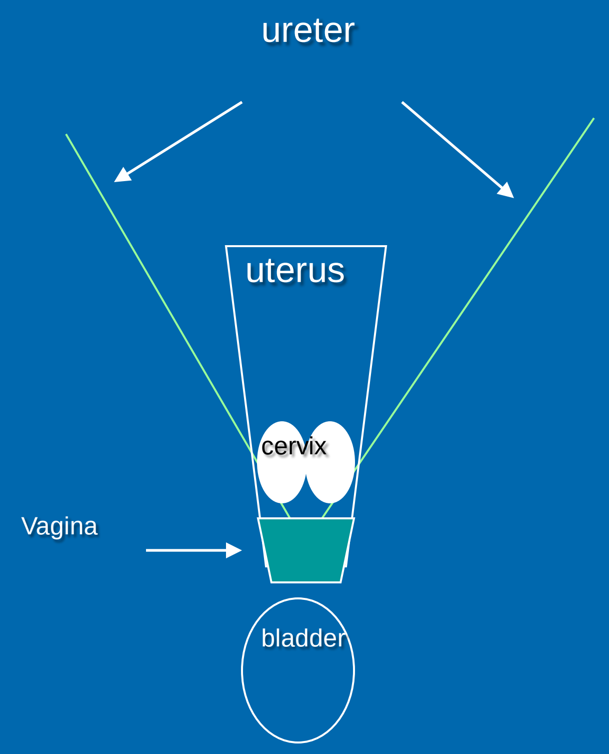
- Urinary diversion (UD) / Sham UD
- inject STZ 1week After UD / Sham UD surgery
- Test blood glucose 3 days after STZ injection
- DM rats > 350 mg/dl
- Sham+Diuresis group drink 5% sucrose water
- Study after 20 weeks of DM





Before Urinary diversion





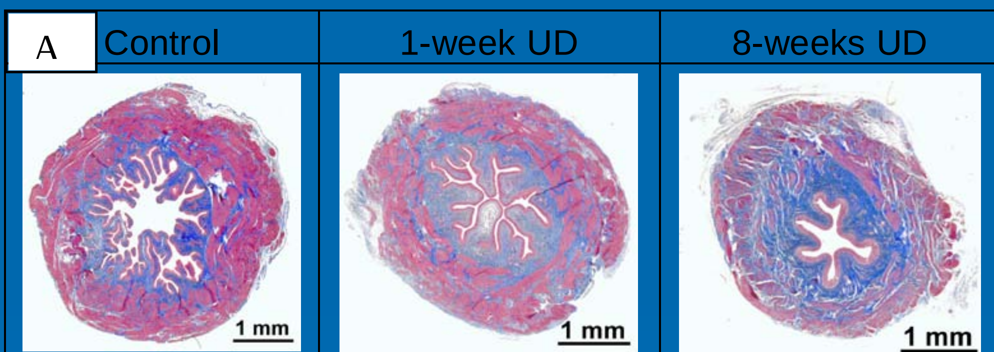
After Urinary diversion

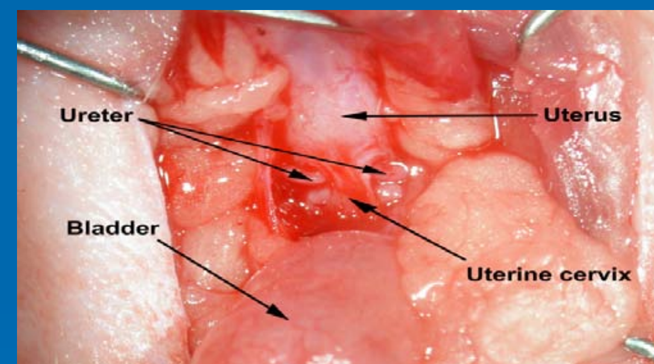
Methods

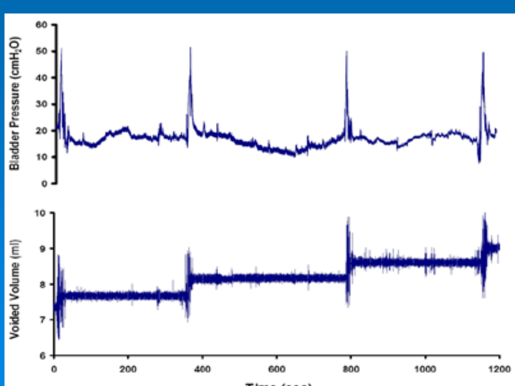
- Cystometrogram *CMG*
- Harvest bladder, MPG (major pelvic ganglion), DRG (dorsal root ganglion)
- *Dye-labled* MPG and DRG to show the morphological change
- Detect NGF and p75 level in these tissues by *Western blot and ELISA*
- Detect NGF LNGFR receptor p75 by *RT- PCR*
- Detect total *antioxidant capacity* and *SOD activity level*

Hypothesis: polyuria causes bladder remodeling

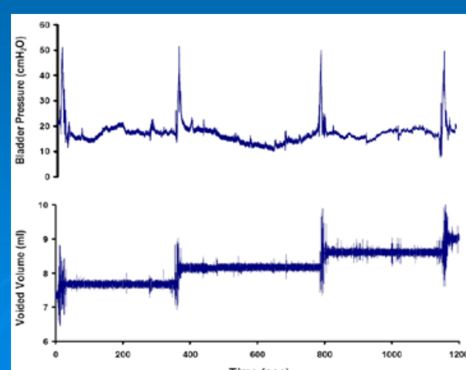
Creation of DM without polyuria
Or
Bypassing the bladder from polyuria impact



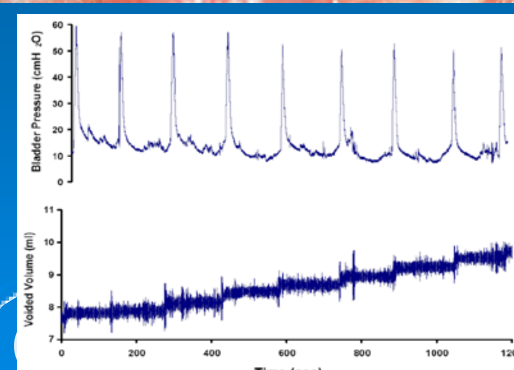




- Control



- **UD-1 week**

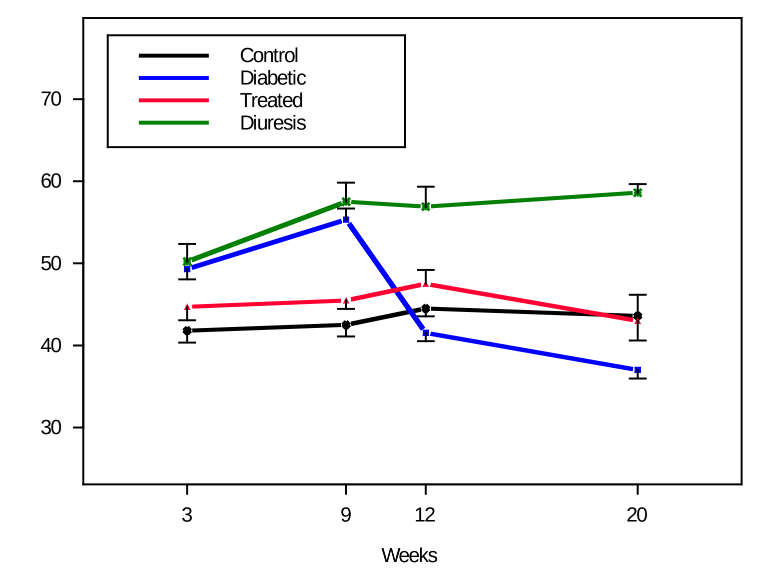


- **UD-8 week**

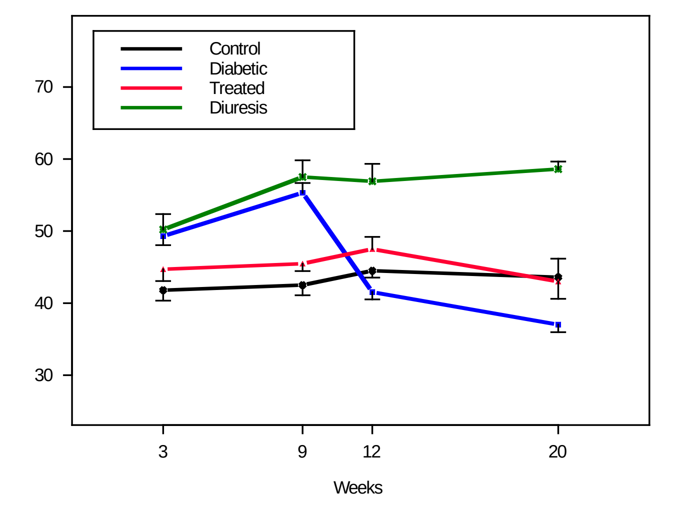
Mechanisms of Bladder Remodeling in DM

Early DM (<9 Weeks in rat/mice):
Polyuria

Late DM (>12 weeks in rat/mice)
Oxidative Stress

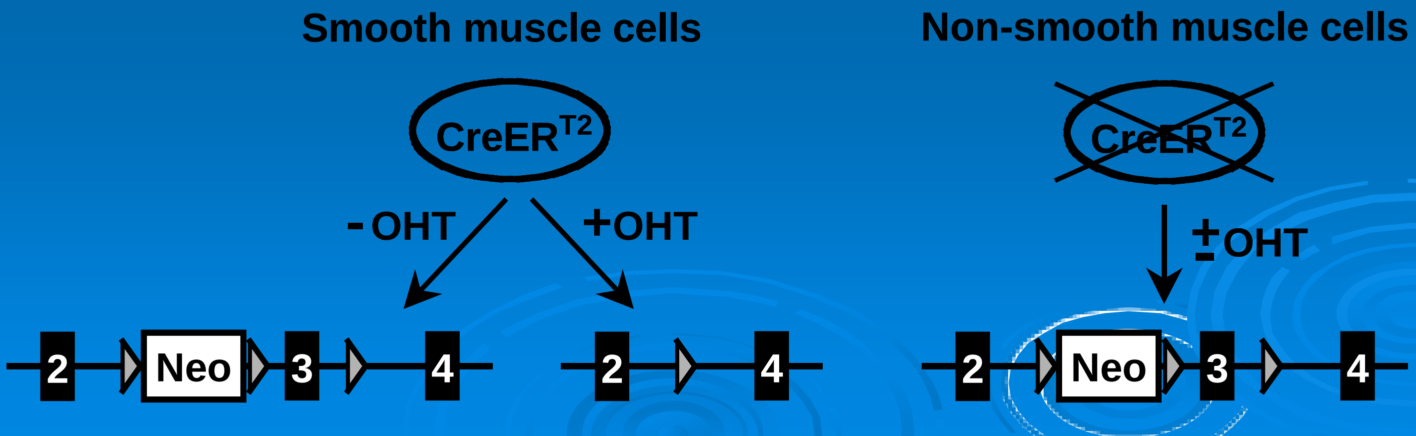


Hypothesis: Increased ROS causes bladder function failure in late DM



A mouse model with conditional, smooth muscle-specific excision of

MnSOD exon 3



Breeding Mice

- Premature death

- Genetic knock out MnSOD

- MnSODlox/lox

- MnSODlox/+ SM-CreERT2(ki) Cre/+

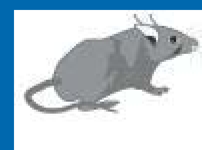
- A

- SM-CreERT2(ki) Cre/+

Breeding Mice

- MnSODlox/+ SM-CreERT2(ki) Cre/+

- MnSODlox/+ SM-CreERT2(ki) Cre/+



- MnSODlox/lox SM-CreERT2(ki) Cre/+

- B

Experimental Design

	Description	# Mice
1	Wild type C57BL/6 mice, sham treated	6
2	MnSOD$^{lox/lox}$, sham treated	6
3	SM-CreER$^{T2\ Cre/+}$, sham treated	6
4	MnSOD$^{lox/+}$, SM-CreER$^{T2\ Cre/+}$ sham treated	6
5	MnSOD$^{lox/lox}$, SM-CreER$^{T2\ Cre/+}$ sham treated	6
6	Wild type C57BL/6 mice, treated with OHT	6
7	SM-CreER$^{T2\ Cre/+}$ treated with OHT	8
8	MnSOD$^{lox/+}$, SM-CreER$^{T2\ Cre/+}$ treated with OHT	10
9	MnSOD$^{lox/lox}$, SM-CreER$^{T2\ Cre/+}$ treated with OHT	10

Characterization the smooth muscle specific knock out mice model

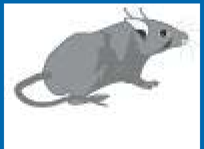
1. PCR test:
   - to show the specific gene band was knock out. (DONE)
2. Gene sequence:
   - sequence the specific band after gene knock out to make sure we knock out the MnSOD gene. (DONE)
3. Western blot:
   - compare protein MnSOD/Actin /GAPDH in gene knock out mice tissue
4. SOD activity assay:
   - test mice tissue SOD activity change by acompetitive inhibition assay

Genotyping

- newborn pups were determined on tail DNA by PCR amplification
- OHT injection at 8 weeks old,
- ip 40 mg/kg 5 days,
- Bladder smooth muscle, urothelium, and other control tissue (heart, brain, aorta, liver, skeleton muscle and tail)

- MnSODlox/+ SM-CreERT2(ki) Cre/+
- MnSOD-/+ SM-CreERT2(ki) Cre/+

- OHT
- (4-hydroxytamoxifen)



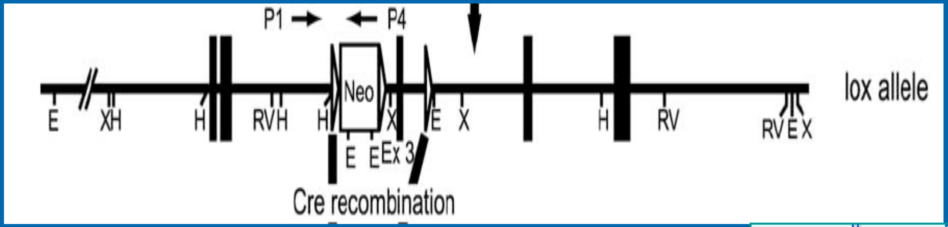
- MnSODlox/lox SM-CreERT2(ki) Cre/+
- MnSOD-/- SM-CreERT2(ki) Cre/+

Dr. Firouz Daneshgari

Project: To combine the floxed *Sod2* allele with the transgene transgelin-Cre/Esr (Tagln-creER) on a C57BL/6 background to explore the impact of increased ROS production in smooth muscle, specifically diabetic bladder dysfunction

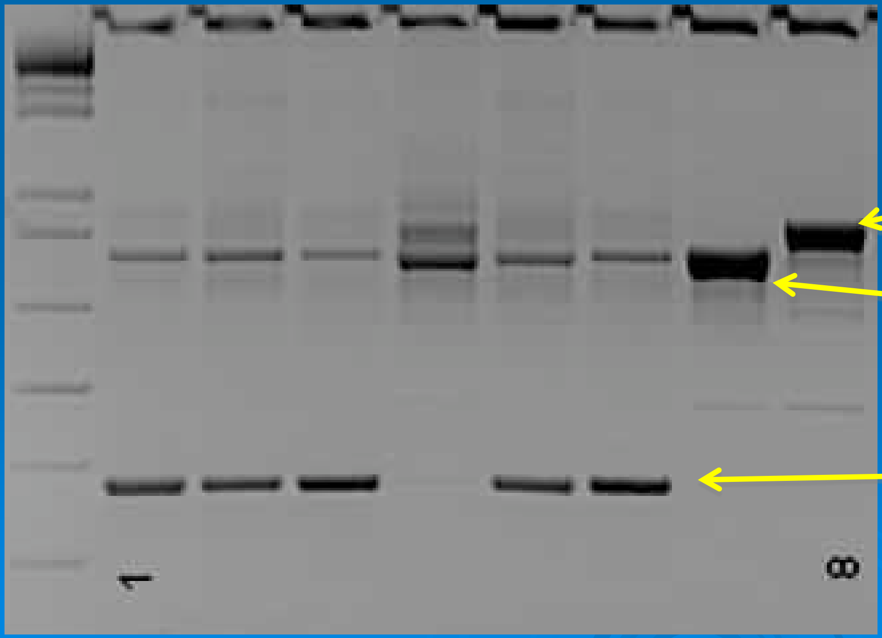
1. Imported/rederived B6. *SOD2floxed* and B6-Tagln-cre/Esr stocks
2. Bred the 2 alleles together.
3. Distributed heterozygous and homozygous B6. *SOD2floxed* mice also heterozygous for Tagln-cre/Esr
4. Discovered that the imported *SOD2floxed* allele had lost its *neo* cassette.

Sod2^{flox} allele



- Ikegama, BBRC 296, 729-736

- *Sod2flox* cut by Tg(CMV-Cre)



- *Sod2Flox/Sod2Flox*, uncut by Cre
- WT allele (CMV-cre)
- *Sod2Flox* allele cut by CMV-Cre

Result- DU Pathobiology site

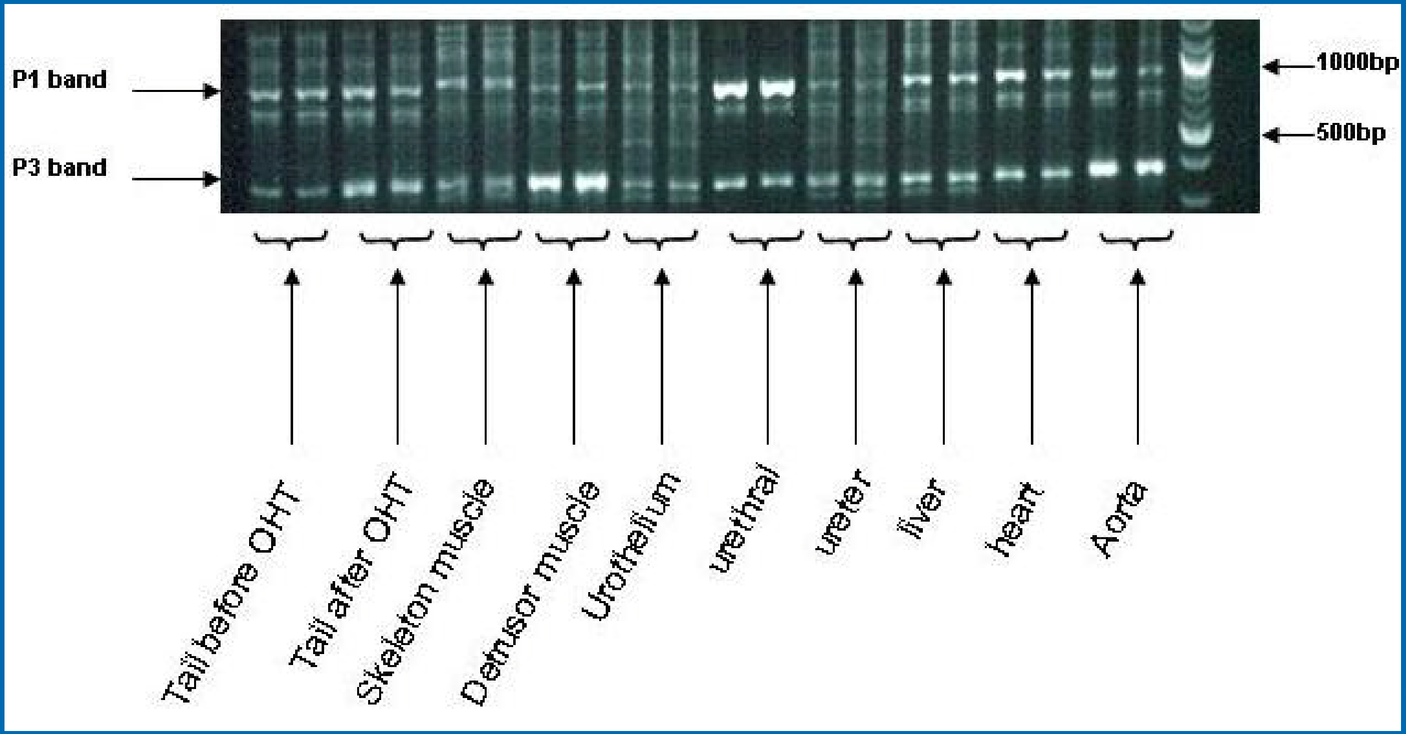


Figure. PCR result from a MnSOD$^{lox/+}$, SM-CreERT2 $^{Cre/+}$ treated with OHT.

Gene Sequencing, extract DNA From band @400 bp in agarose gel after running PCR (p1',p3') verified the MnSOD gene been knock out

In February 2009, we were informed that we should be seeing a 378bp PCR product with the primer pairs specified rather than the 562 bp product observed at JAX.

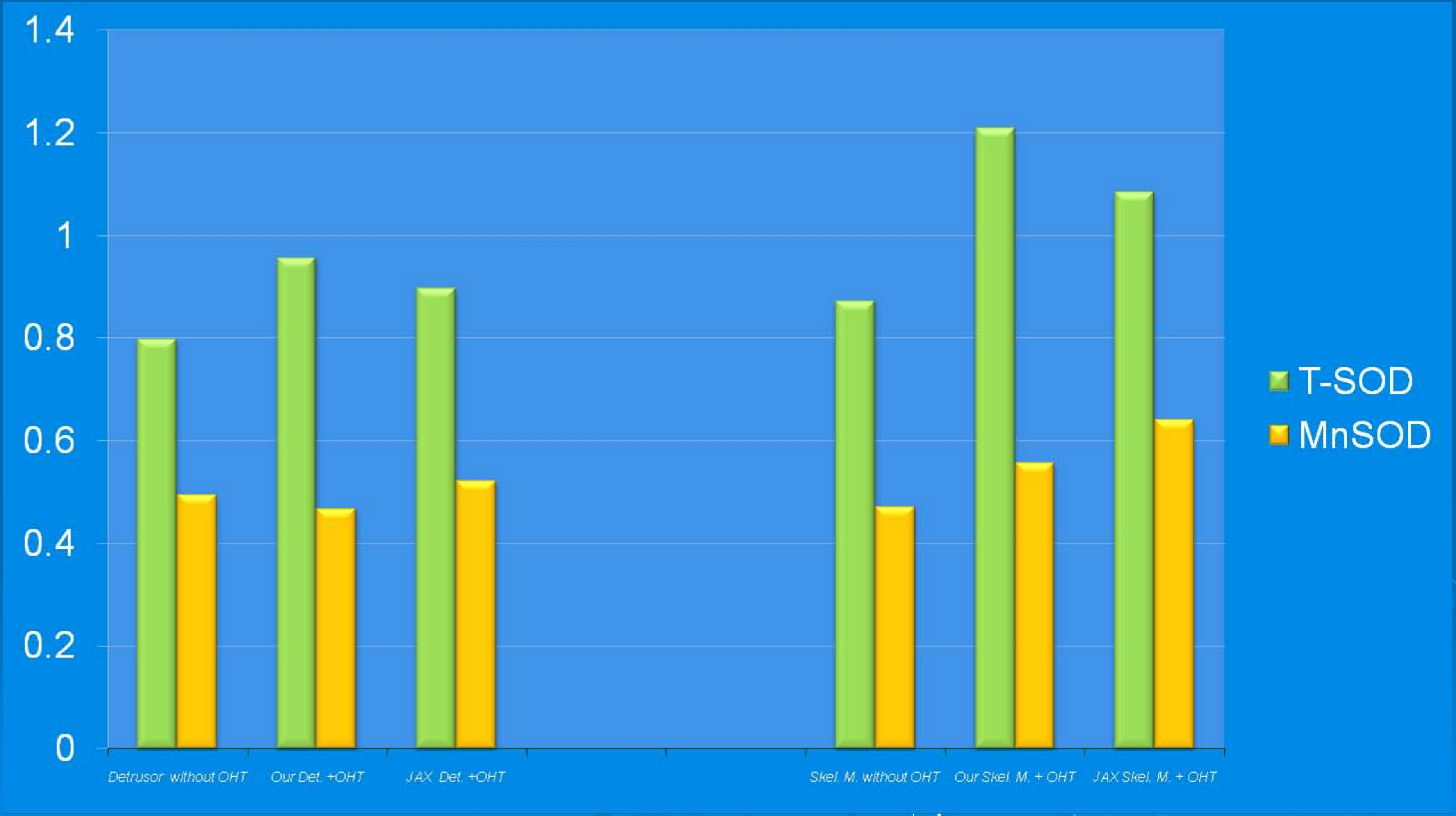
Mice Genotype Change

- JAX strain Lost Neo gene from original sequence
  - Possibility of unknown phenotype change

MnSOD Activity Assay

- Tested the mice with/without OHT injection
- Tissue :bladder detrusor muscle and skeleton muscle
- Three group mice
  - Jax wt/lox wt/cre OHT
  - Jax wt/lox wt/cre No OHT
  - Our lox/lox wt/cre OHT
- Tested both total SOD and MnSOD

MnSOD Activity Assay



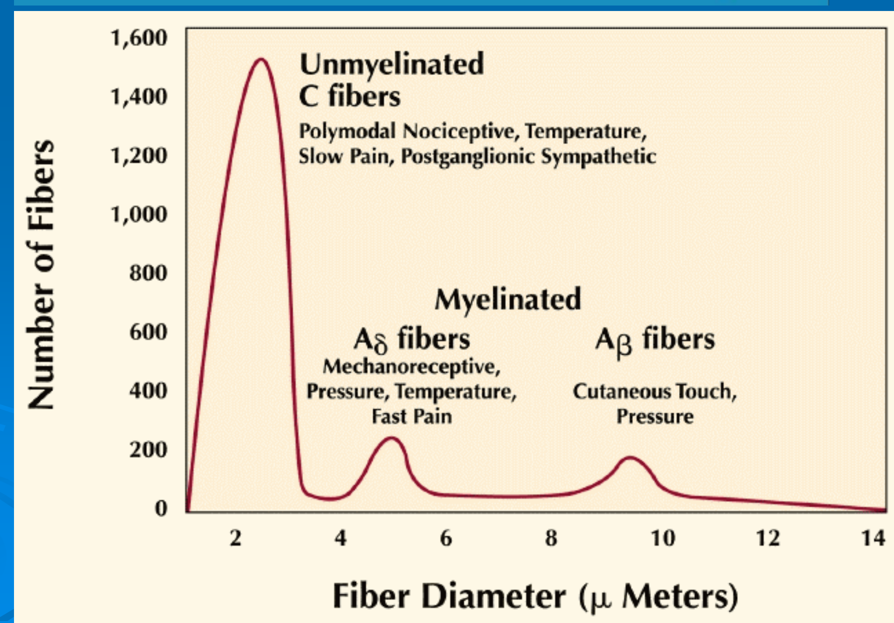
Characterization the smooth muscle specific knock out mice model

5. Histology
6. Functional experiments:

   - cardiovascular system, bladder function,
7. Induce DM
8. Functional test after DM (FVC / CMG)
9. Lipid peroxidation, , and other ROS products:
10. Measurement of Bladder Sensory Threshold (BST)*

Bladder Sensory Test (BST) Device for assessment of altered afferent sensation

EFFICACY OF A NOVEL DEVICE
FOR
ASSESSMENT OF
AFFERENT AUTONOMIC SENSORY FUNCTION
IN THE RAT BLADDER
Abouassaly et al: J Urology 2008



Diabetic Uropathy in
Type II DM Mouse Models
(+/- Obesity)

#	Polygenic obesity	Monogenic obesity	#
1	NONcNZ010/ LtJ (diabetogenic obesity)	FVB ob/ob (diabetogenic obesity)	4
2	NONcNZ05/ LtJ (non-diabetogenic obesity)	B6 ob/ob (non-diabetogenic obesity)	5
3	NON/ShiLtJ (non-obese non-diabetic parental strain)	FVB/NJ (non-obese non-diabetic parental strain)	6
		C57BL/6 (non-obese non-diabetic parental strain)	7
		FVB OVE26 (non-obese diabetic)	8

National Programs

- **NIDDK Urologic Complications of Obesity & Diabetes: March 10-11, 2009; Baltimore, MD**
  http://www3.niddk.nih.gov/fund/other/urologiccomplic/

- **Urologic Complications of Diabetes and Obesity: Journal of Urology, November 2009**

- **Society for Urodynamcis and Female Urology (SUFU)**
  Urologic Complications of Diabetes and Obesity

Thank you!