Genetic Model for Bladder Dysfunction in Type 2 Diabetes

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Epidemiology

- Type 2 Diabetes affects 7% of US population
- 1.5 million new cases diagnosed annually
- Annual cost: $132 billion dollars per year

Two Forms of Diabetes

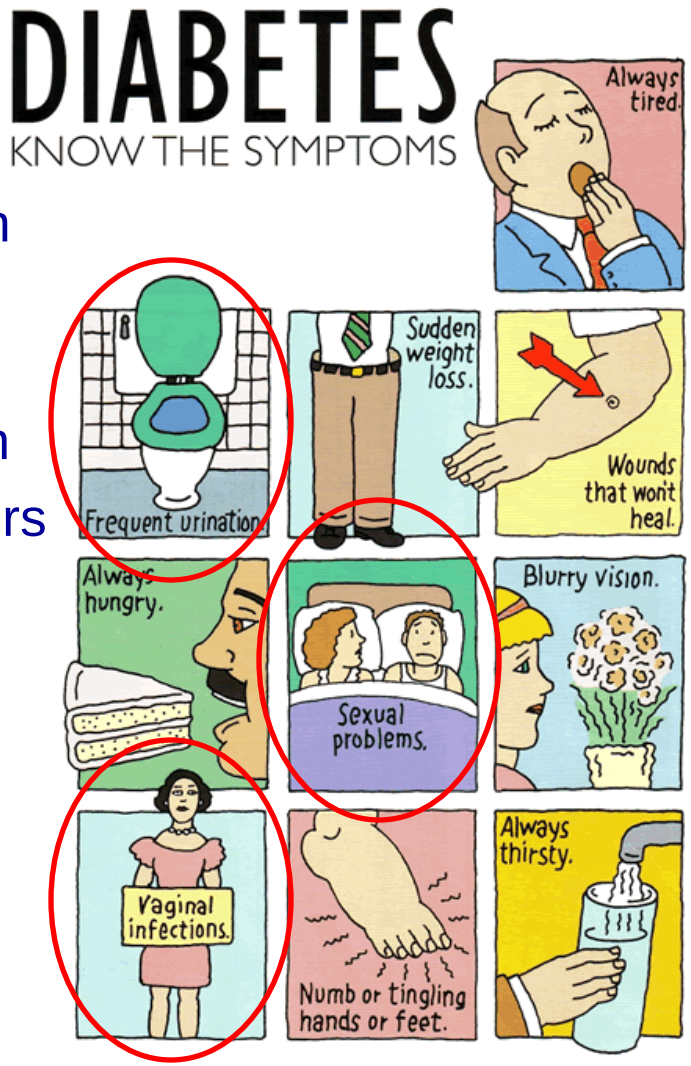
- **Type 1: 10%**
  - Autoimmune destruction of pancreatic beta cells
- **Type 2: 90%**
  - Insulin resistance

Diabetes Related Complications

- Progressive loss of pancreatic B-cell function
- Heart disease
- Stroke
- Hypertension
- Retinopathy
- Neuropathy
- Nephropathy
- Blindness
- Complications with pregnancy

Urologic Complications

- Voiding dysfunction
- Recurrent urinary tract infection
- Erectile dysfunction
- Ejaculatory disorders



www.diabetesandrelatedhealthissues.com

Diabetic Cystopathy

80% of patients with diabetes develop bladder dysfunction

- Decreased bladder sensation
- Increased residual urine in the bladder
- Bladder muscle overactivity

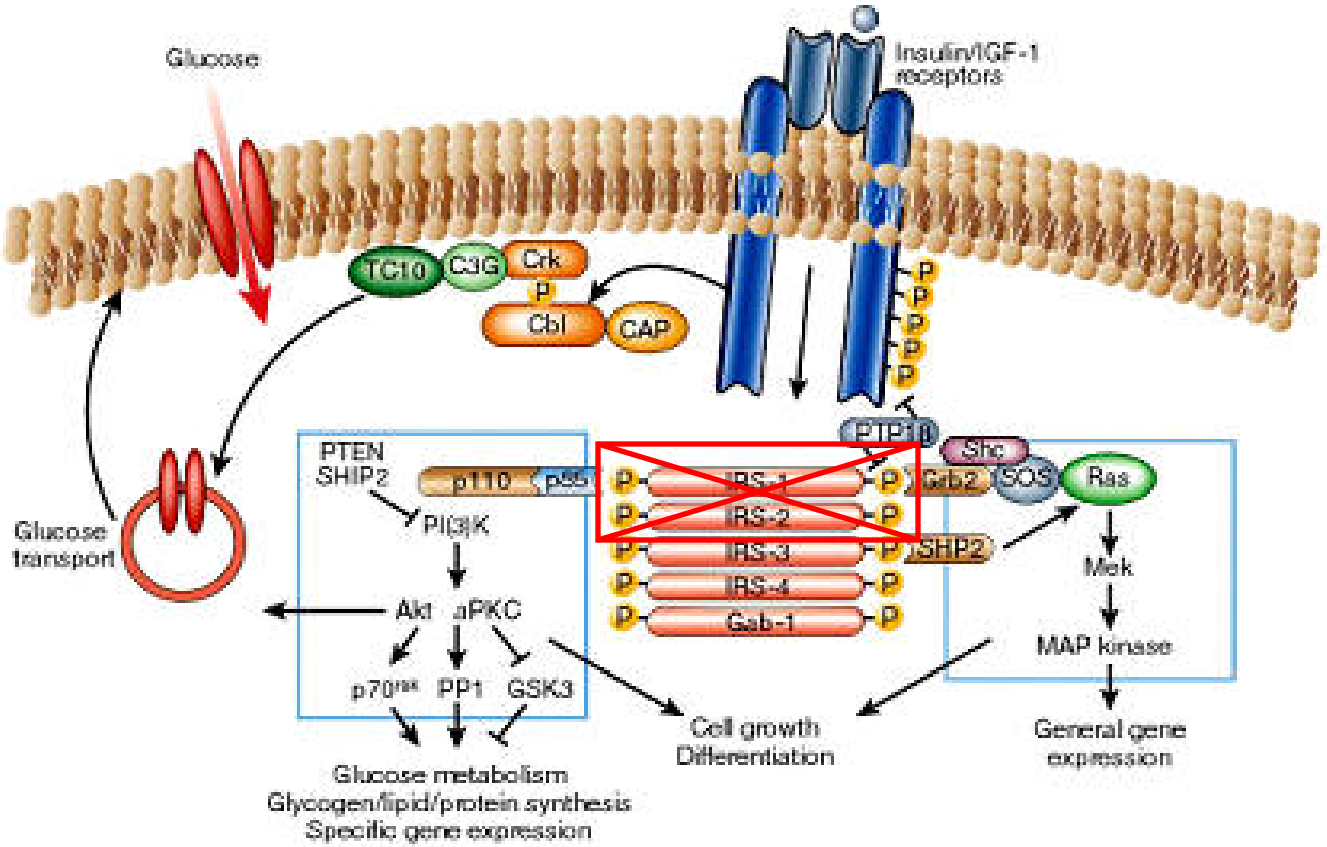
Diabetic Cystopathy

Molecular alterations

- Sacral neuropathy
- Impaired nitric oxide production
- Impaired vascularity
- Alterations of protein kinase C in bladder muscle

Appropriate animal / research models are lacking

Mechanisms of Insulin Action



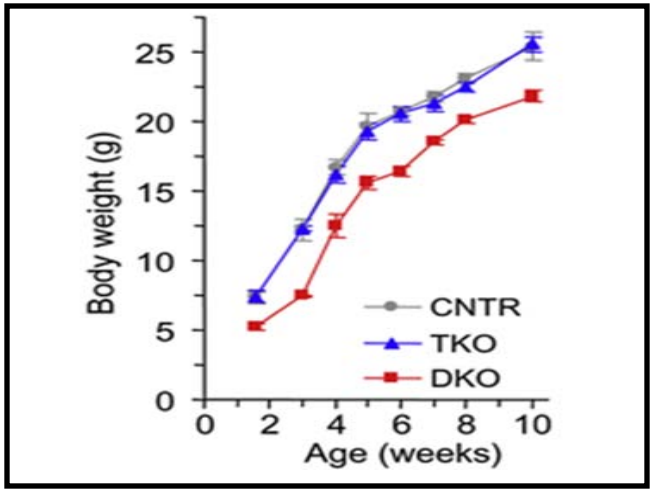
Saltiel and Kahn, Nature 414, 799-806, 2001

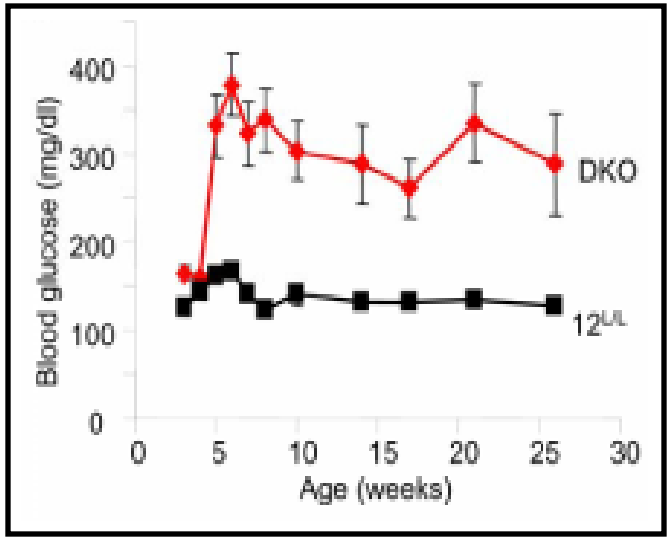
DKO mice: An ideal system for DM2 without obesity

- DKO: Genetically engineered with conditional knockout of Irs1 and Irs2 genes in the hepatocytes
- Consistent with the lower urinary tract signs and symptoms of human patients with DM2.
- The metabolic disturbances of DM2 in DKO mice are *reversible*.

Profile of DKO mice

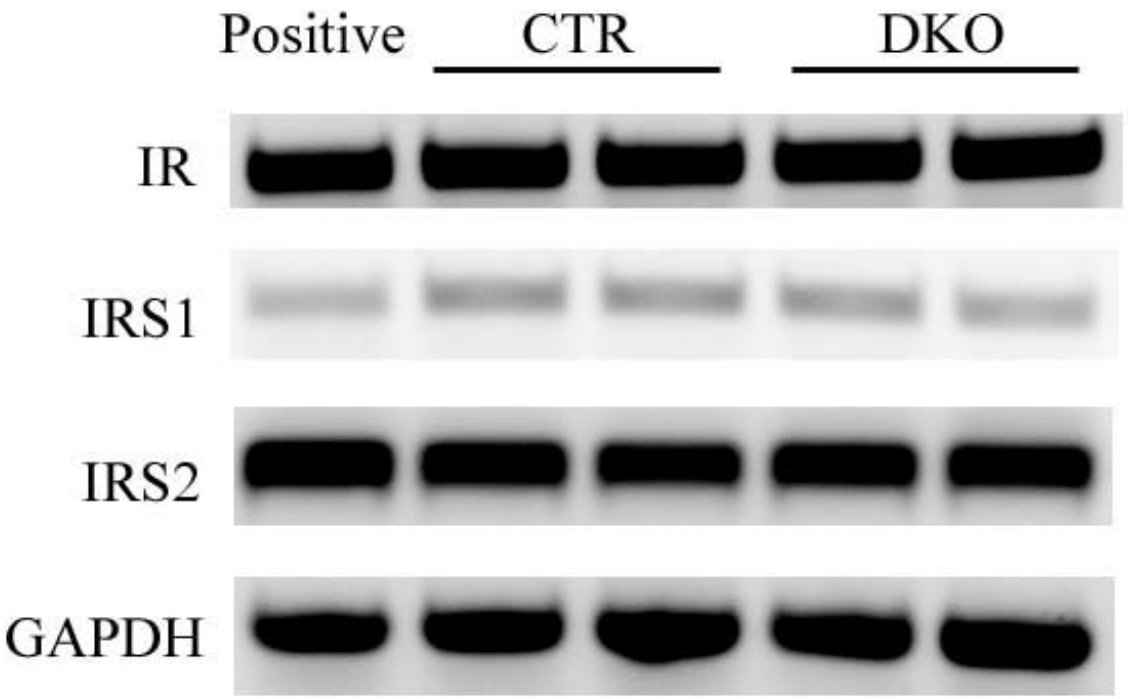
- DKO mice are not obese – therefore, can uncouple molecular alterations of obesity and type 2 diabetes
- IRS1/IRS2 DKO mice have a slight growth retardation.
- Hyperglycemia persists in DKO mice – beginning at 5 wks of life





Dong X, et al. Cell metabolism 2008;8(1):65-76.

Hepatic conditional knockout of Insulin Receptor Substrate (IRS)1/2 does not affect IR and IRS1/2 in bladder



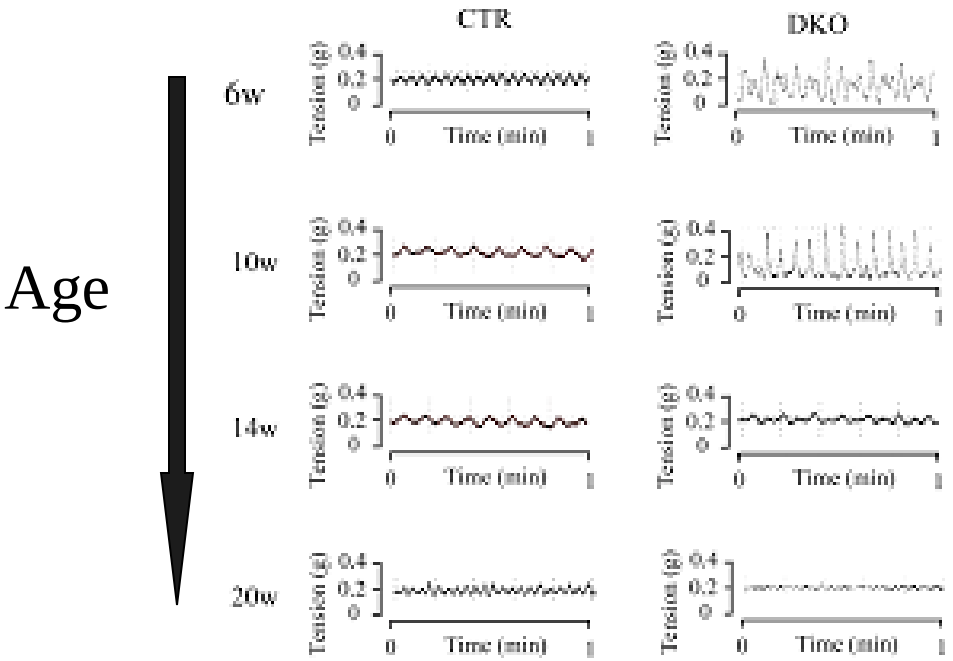
Bladder and Body weight ratios are comparable in DKO mice

Age (wk)	Group	Body Weight (g)	Bladder Weight (mg)	Ratio of Bladder Weight / Body weight (mg/g)
4	Control	14.2±1.2	12.0±1.5	0.85
	DKO	10.3±0.8*	9.0±1.4*	0.87
6	Control	17.6±1.6	12.0±1.4	0.68
	DKO	12.8±1.5*	10.0±1.1	0.79
10	Control	24.9±3.3	23.0±9.8	1.02
	DKO	20.4±1.1*	14.3±2.1**	0.71
14	Control	27.5±3.2	24.8±1.8	0.9
	DKO	22.3±1.4*	23.3±2.6	1.04
20	Control	30.6±1.9	27.0±3.1	0.88
	DKO	23.7±1.6**	25.1±1.8	1.05
25	Control	31.2±1.3	22.0±1.2	0.71
	DKO	25.2±1.3*	23.5±0.7	0.94

n=4~5/group, Compared with control group, *P<0.05, **P<0.01

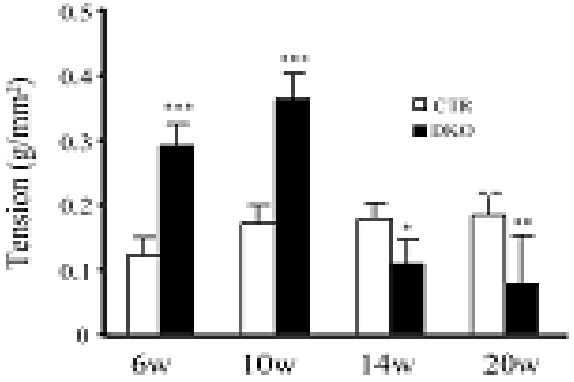
Bladder contractions in DKO mice

- Abnormal spontaneous bladder contractions
  - High amplitude (hyperactive) in early life
  - Low amplitude (hypoactive) in late life

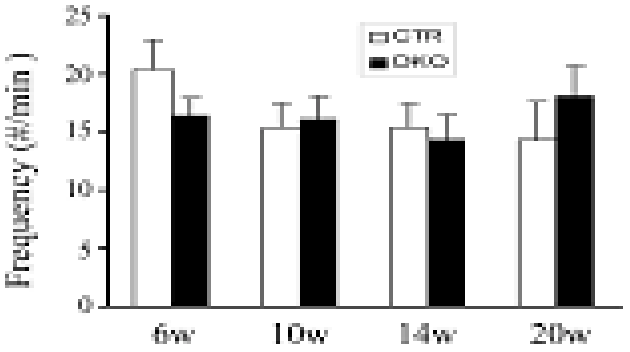


Bladder contractions in DKO mice

Alterations in amplitude of contractions.



Frequency of bladder contractions is not affected.



Isometric contractile response of bladder smooth muscle strips after EFS

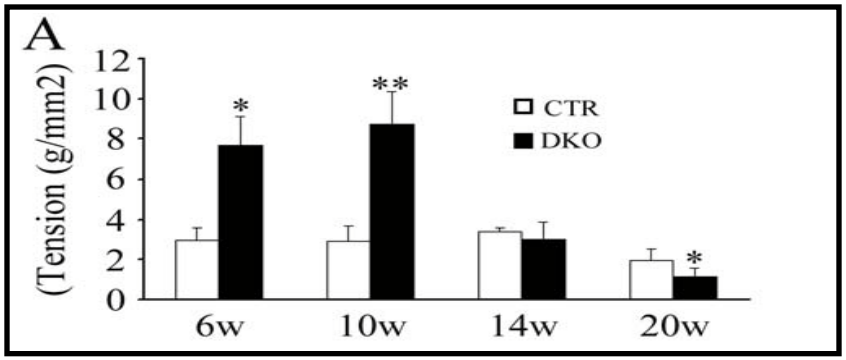
Frequency of EFS

Age (wk)	Group	2Hz	4Hz	8 Hz	16 Hz	32 Hz	64 Hz
6	Control	0.13±0.02	0.25±0.05	0.46±0.09	0.72±0.06	0.97±0.08	1.02±0.24
	DKO	0.20±0.05*	0.32±0.04	0.67±0.26*	1.18±0.30*	1.44±0.11*	1.64±0.26*
10	Control	0.33±0.03	0.59±0.07	1.17±0.14	2.46±0.35	3.22±0.36	3.44±0.41
	DKO	1.05±0.14**	2.16±0.09***	4.74±0.46***	6.13±0.87***	6.70±0.46***	6.72±0.87***
14	Control	0.21±0.03	0.37±0.06	0.85±0.07	1.24±0.31	2.63±0.31	2.93±0.25
	DKO	0.27±0.04	0.46±0.07	0.98±0.10	1.40±0.24	1.77±0.13*	1.89±0.17*
20	Control	0.27±0.06	0.42±0.10	1.04±0.21	1.58±0.22	1.92±0.25	2.02±0.19
	DKO	0.20±0.03	0.35±0.08	0.73±0.20	1.02±0.16*	1.20±0.20*	1.27±0.26*

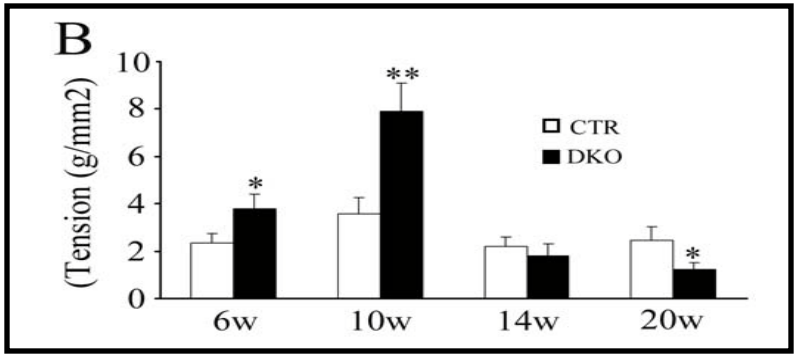
- Young/middle aged mice have hypersensitivity (red)
- Older mice with hyposensitivity (blue)

Carbachol and KCl bladder stimulation in DKO and Control mice

Carbachol Stimulation



KCl Stimulation



- Young/middle aged mice have hypersensitivity
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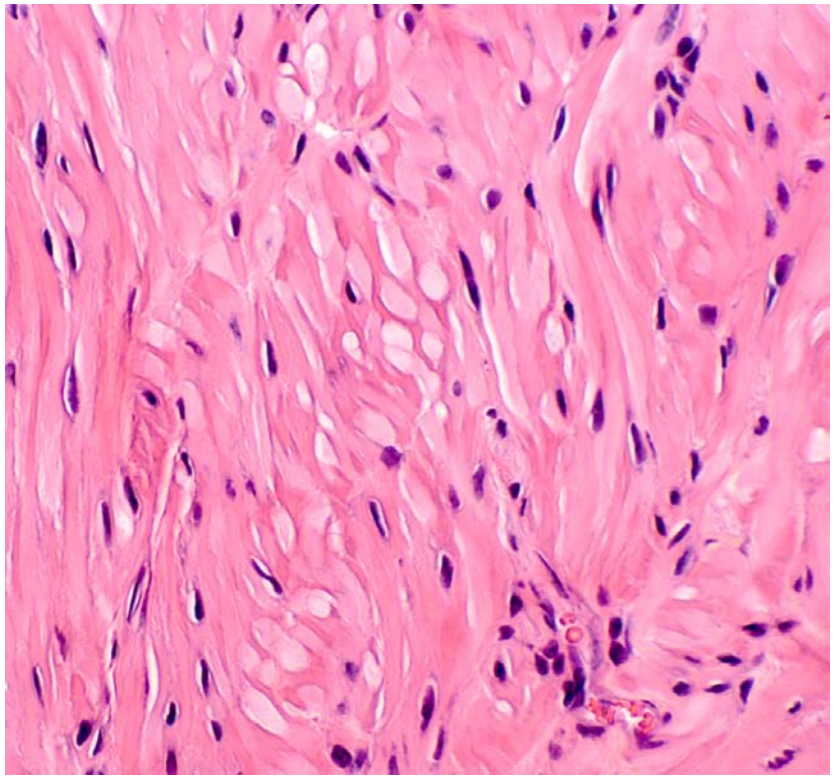
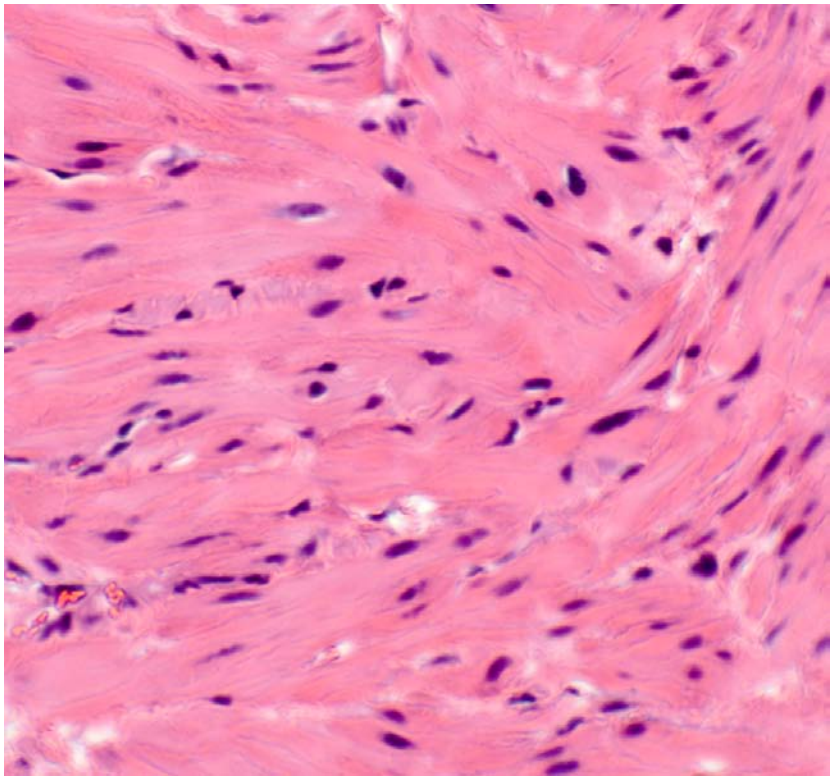
Bladder Smooth Muscle Histologic Evaluation

DKO

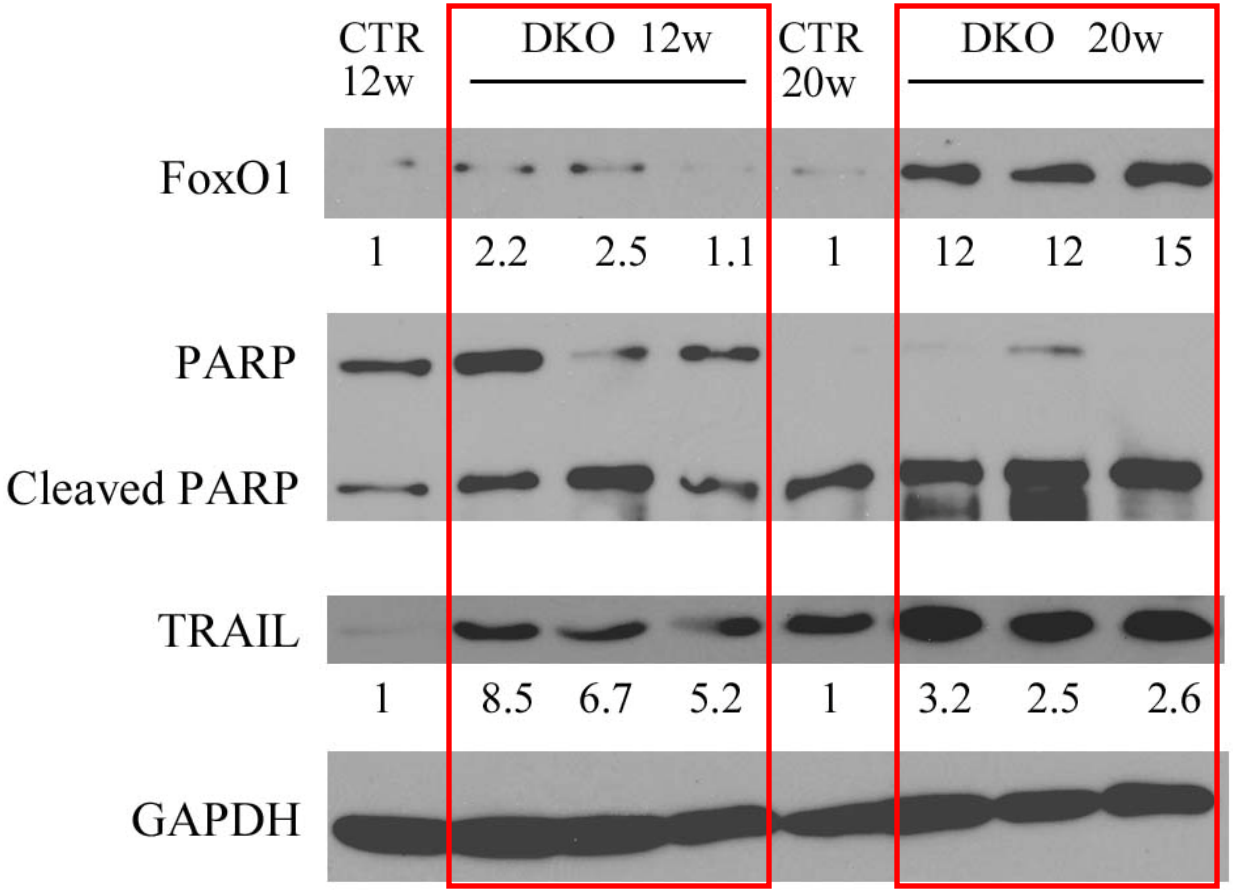


Control

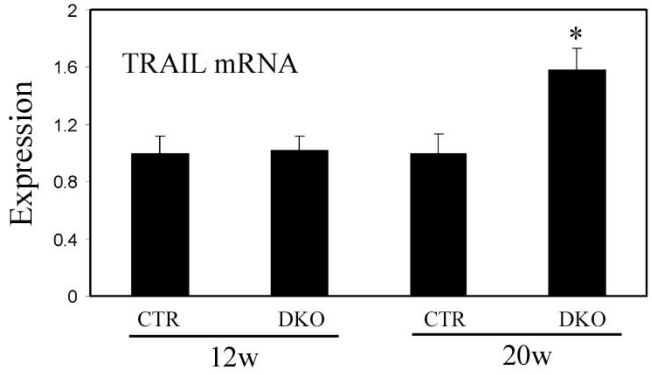


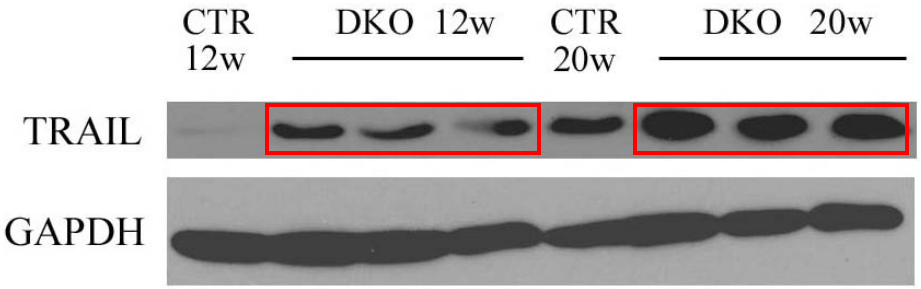
No detectable difference in histologic appearance.

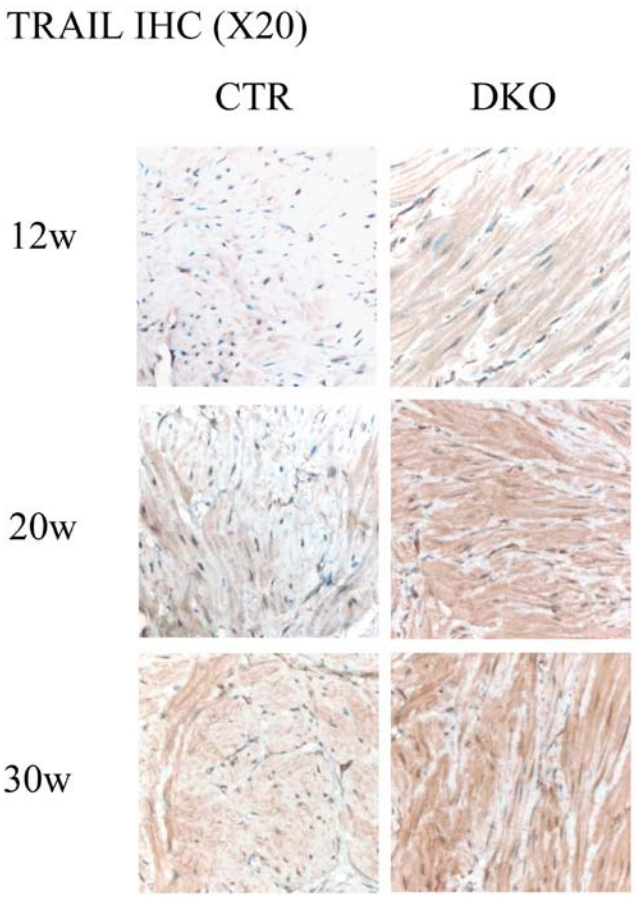
Apoptosis and Diabetic Cystopathy



Apoptosis in Bladder of DKO Mice







Bladder smooth muscle of DKO mice express higher levels of TRAIL

Hypothesis

- Diabetic cystopathy is biphasic:
  - Hyperactive in early phase
  - Hypoactive in late phase

- Cytokines and apoptotic mediators may regulate diabetic cystopathy.
  - Increased expression of some cytokines may precede hyperglycemia

Conclusions

- Urologic complications associated with type 2 diabetes are common, and affect 80% of patients.
- The IRS1/IRS2 knockout mice (DKO) is an appropriate animal model to evaluate diabetic cystopathy
  - Bladder dysfunction
    - Hyperactive in early life
    - Hypoactive in late life
- Cytokines and apoptotic mediators are upregulated in the bladder smooth muscle of DKO mice
- Chronic exposure to apoptotic mediators may account for one mechanism of diabetic cystopathy.

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