Mouse Models of Diabetic Nephropathy

Mount Sinai / Jefferson / Einstein / Minnesota

Erwin Böttinger, PI

Kumar Sharma, Co-PI

Group Members

- *Mount Sinai: Phenotyping, Molecular Pathology & Validation*
  - Erwin Bottinger (PI)
  - Sandhya Xavier (Fellow)
  - Liping Yu, Technician
- *Albert Einstein: Strain Generation & Characterization*
  - Maureen Charron (Co-Investigator)
  - Scott Henderson, Technician
  - Katalin Susztak, Co-Investigator
- *Thomas Jefferson: Experimental Diabetes, HPLC Core, Phenotyping*
  - Kumar Sharma (Co-PI)
  - Steve Dunn, Technician and Core Manager
  - Kevin Williams, (Co-Investigator)
  - Peter McCue, Pathology
- *U Minnesota: Phenotyping (Morphometry)*
  - Michael Steffes, Consultant

Progress Report

- *Strain Generation*
  Katalin Susztak (AECOM)/Erwin Bottinger (MSSM)
  - gGT-CD36 tg: ↑ AGE&FFA induced apoptosis in tubules
  - (Sgptl-CD36 tg)

- *Experimental Models*
  Bottinger (MSSM)
  completed
  - db/db C57BLKS longitudinal, standardized phenotyping
  - Ins2-Akita longitudinal, standardized phenotyping
  - flk-1 RAGEtg with low dose STZ to 28 wks
  - Cd2ap+/- with STZ to 28 wks
  - Db/db and Ins2-Akita (glomerular RNA profiles - molecular phenotying: up to 28 wk-of-age)

  ongoing
  - flk-1 RAGEtg x Ins2-Akita CD1xB6 (12 wk-of-age phenotyping completed)
  - Cd2ap+/- x Ins2-Akita CD2xB6 (12 wk-of-age phenotyping completed)

  Kumar Sharma (TJU)
  finalizing
  - Decorin knockout with low dose STZ

- *Phenotyping Cores*
  HPLC creatinine… Kumar Sharma/Steve Dunn (TJU)
  Genomics…. Erwin Bottinger (MSSM)

AMDCC VOTES TO SELECT MODELS (Sept 15, 2005)

Mount Sinai (Bottinger):
We are focusing on the following models and new strains: •C57BL/6J Dcn-/- (lead investigator: K Sharma) •Ins2Akita x Flk-1 RAGE (lead investigator: E Bottinger) •C57BL6/CH3 gGT-CD36 and iL1-sglt2-CD36 transgenic strain (lead investigator: K Susztak/E Bottinger)

I have attached both the voting Excel spreadsheet and the word document with the information given by the PIs for their strains.

ONLY RETURN the Excel spreadsheet.

Instructions :

•All PIs are elegible to vote.

•**Only** select the **6** strains you would like to present to the EAC in the October SC meeting.

For each strain you select, put the NUMBER ONE (1) in the blue Vote box.

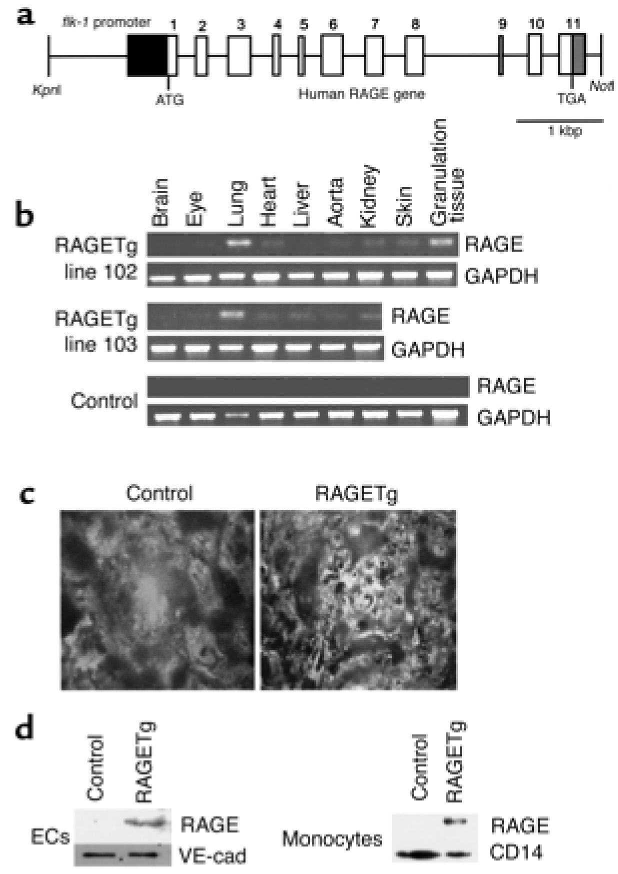
Enter your name in the box provided.

IMPORTANT: Remember **DO NOT VOTE** for the strains in your laboratory.

SAVE THE FILE AND EMAIL IT BACK TO: Rick McIndoe (rmcindoe@mail.mcg.edu)

Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice
Yasuhiko Yamamoto and Hiroshi Yamamoto;
J Clin Invest, 2001



DIABETES INDUCTION

inducible nitric oxide synthase (iNOS) under the control of the insulin promoter (iNOSTg)

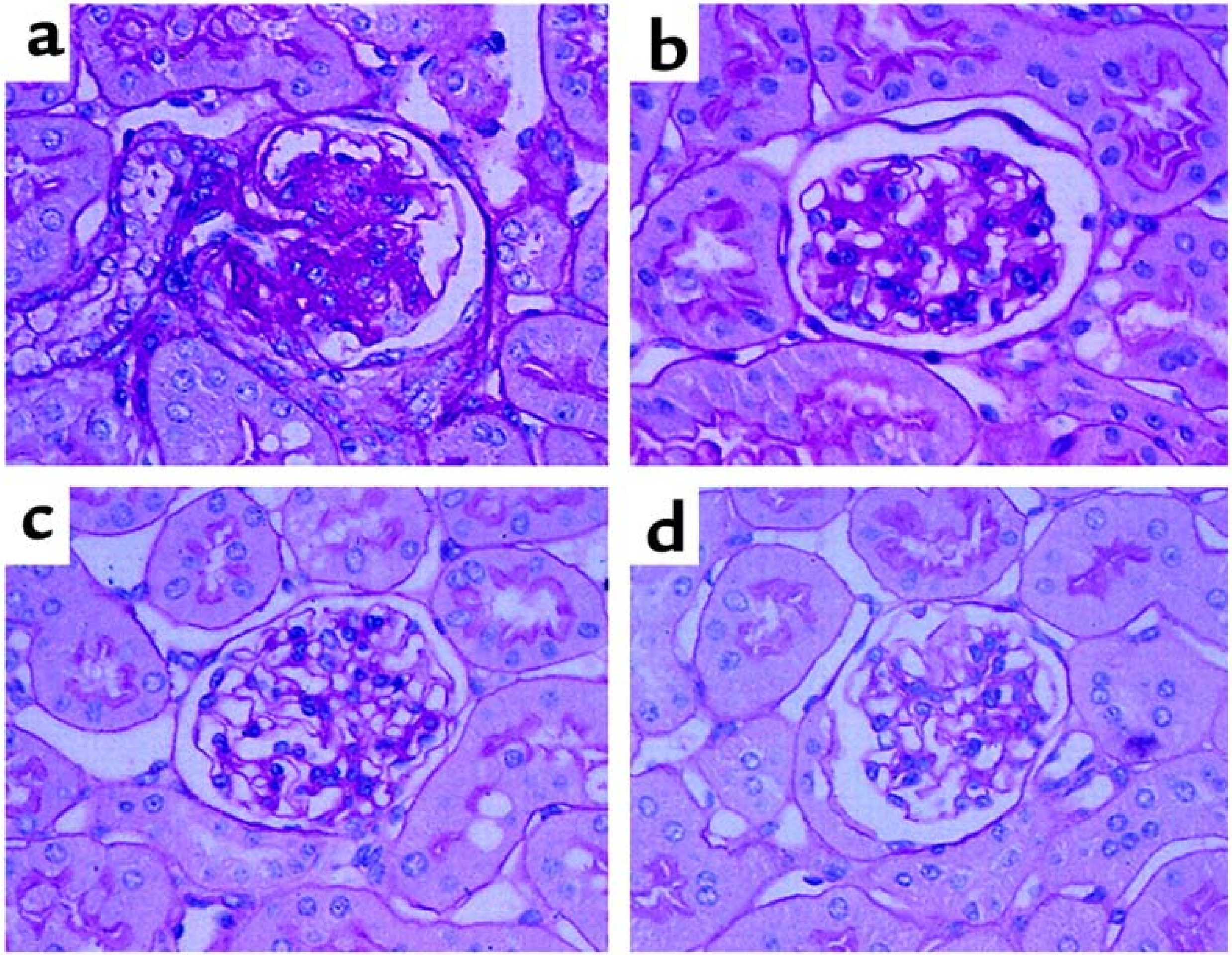
Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice

Yasuhiko Yamamoto and
J Clin Invest, 2001

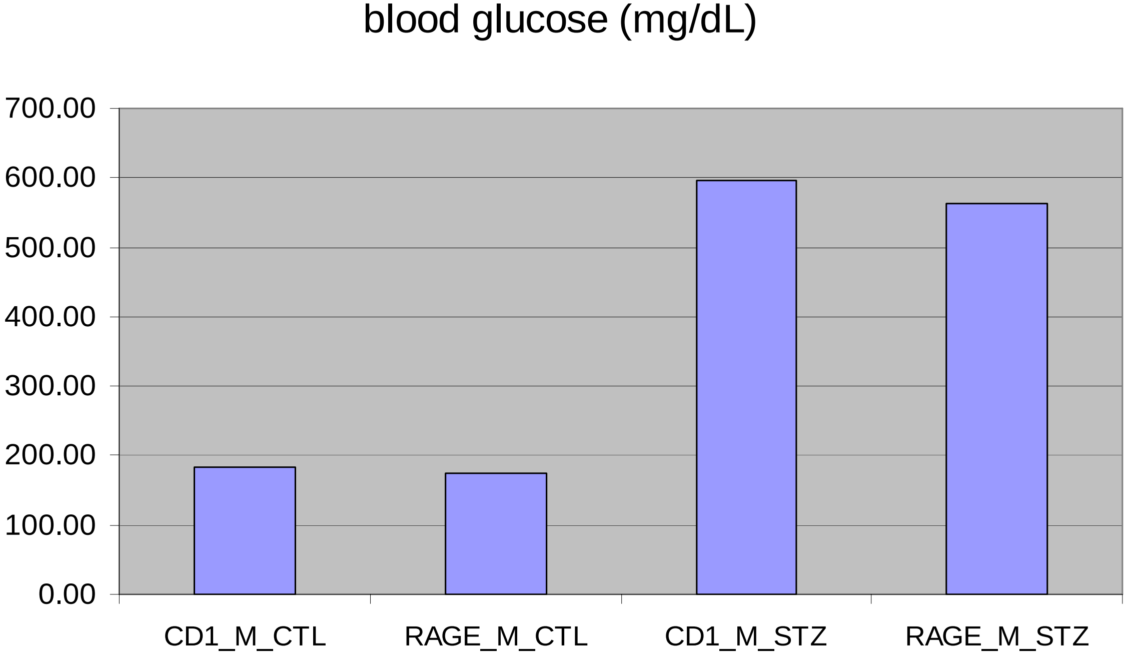
Four months old

a) DM+ RAGE+
b) DM+ RAGE-
c) DM- RAGE+
d) DM- RAGE-



Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Experimental Design
- Female and Male mice
- CD1 inbred
- STZ 8 wk
- Sac 20 wk



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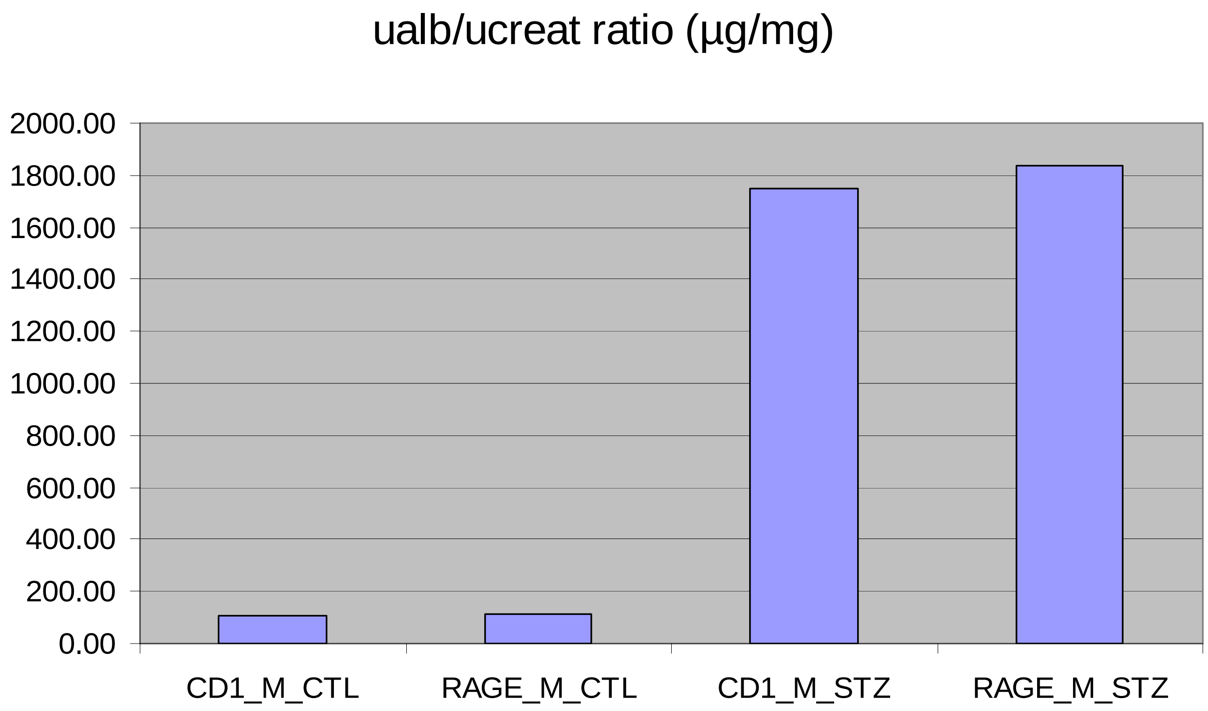
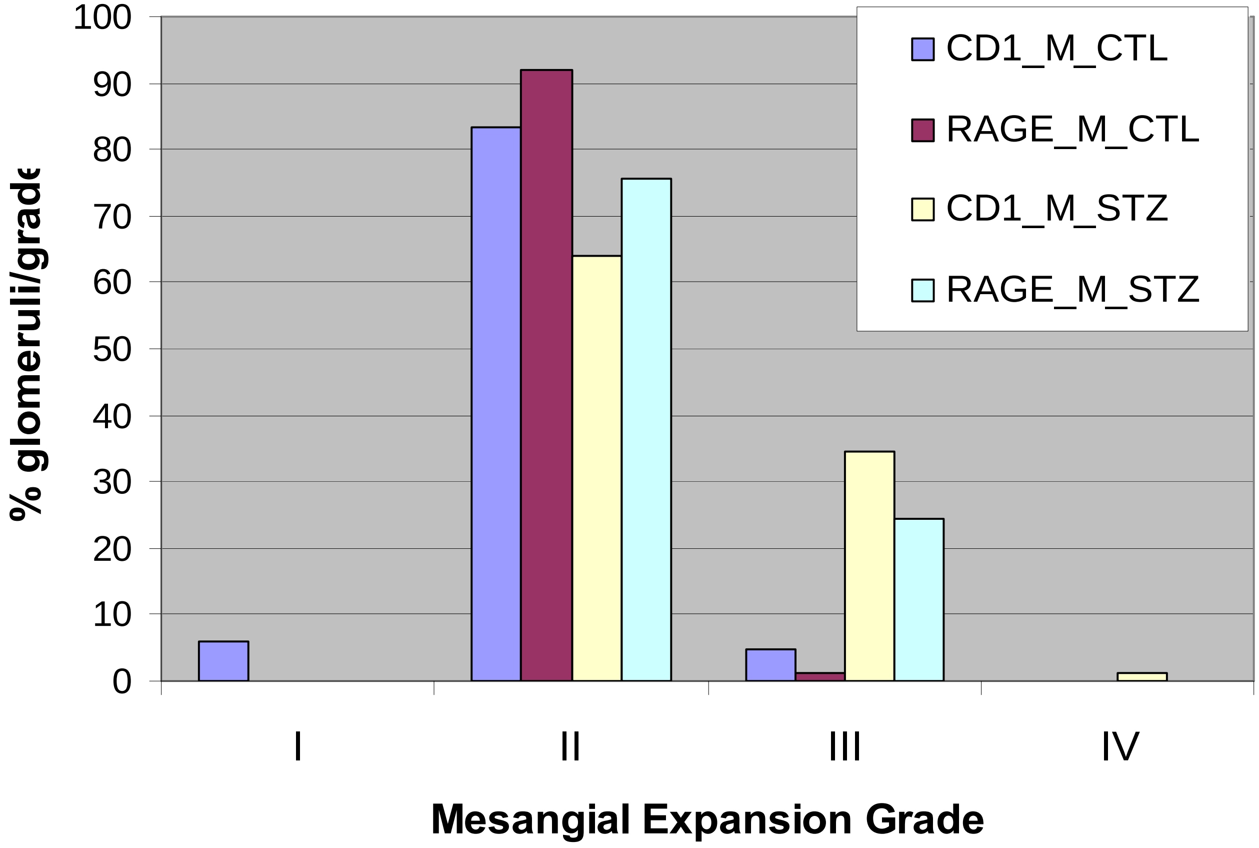


Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

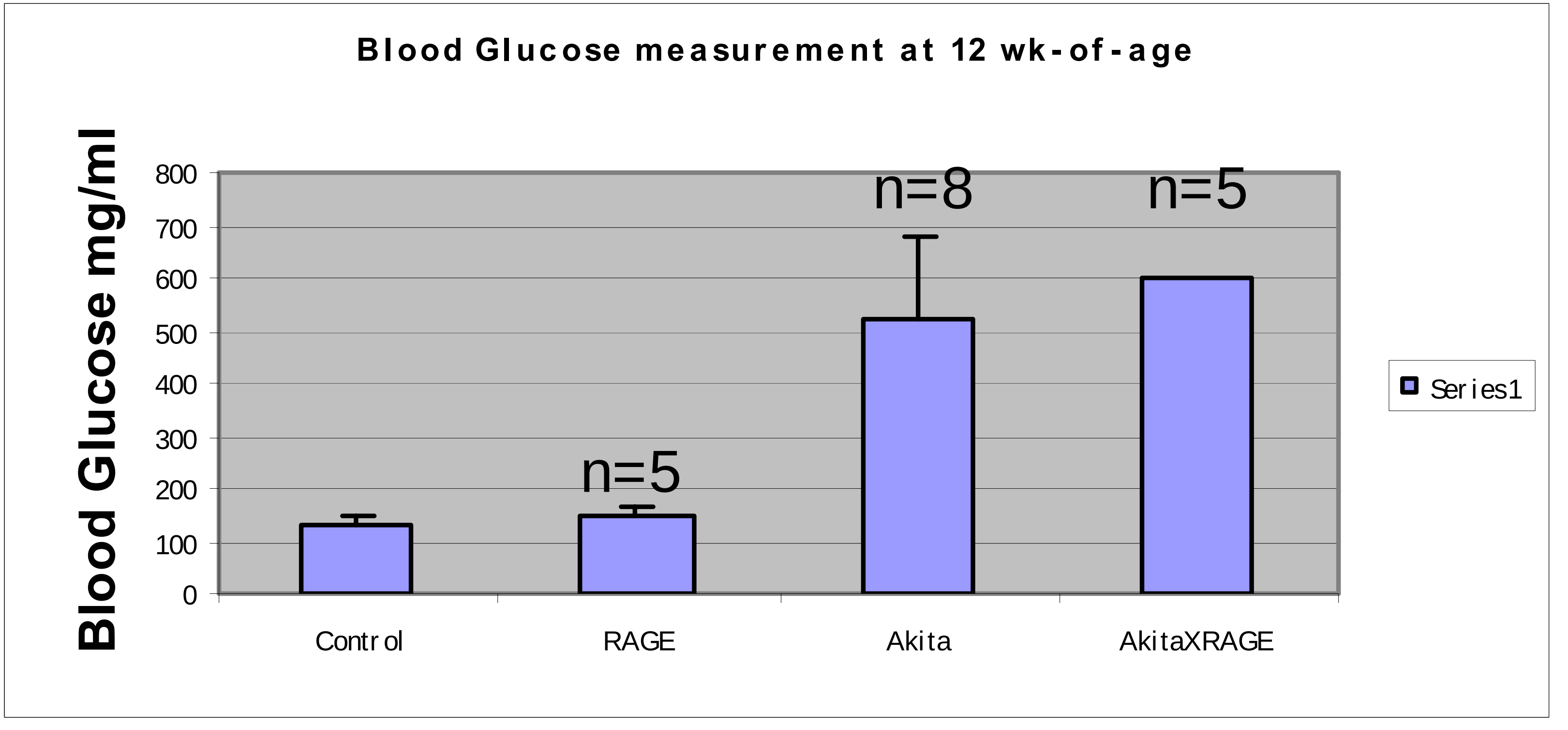
Experimental Design
•Male mice
•CD1 inbred
•STZ 8 wk
•Sac 20 wk

Pathology Report
•No nodular lesion
•No tub.int. lesions
•No vascular leseions



Flk-RAGE tg Plans 2005/2006

- flk-RAGE tg [CD1-tg(Kdr-Ager)]
  - Establish Cohorts for
    - Ins2-Akita x flk-RAGE (B6xCD1)
    - Ins2-Akita x flk-RAGE backcross to CD1
  - follow cohorts to
    - Open end/survival
    - 12, 20, 28, 36 wks, etc.

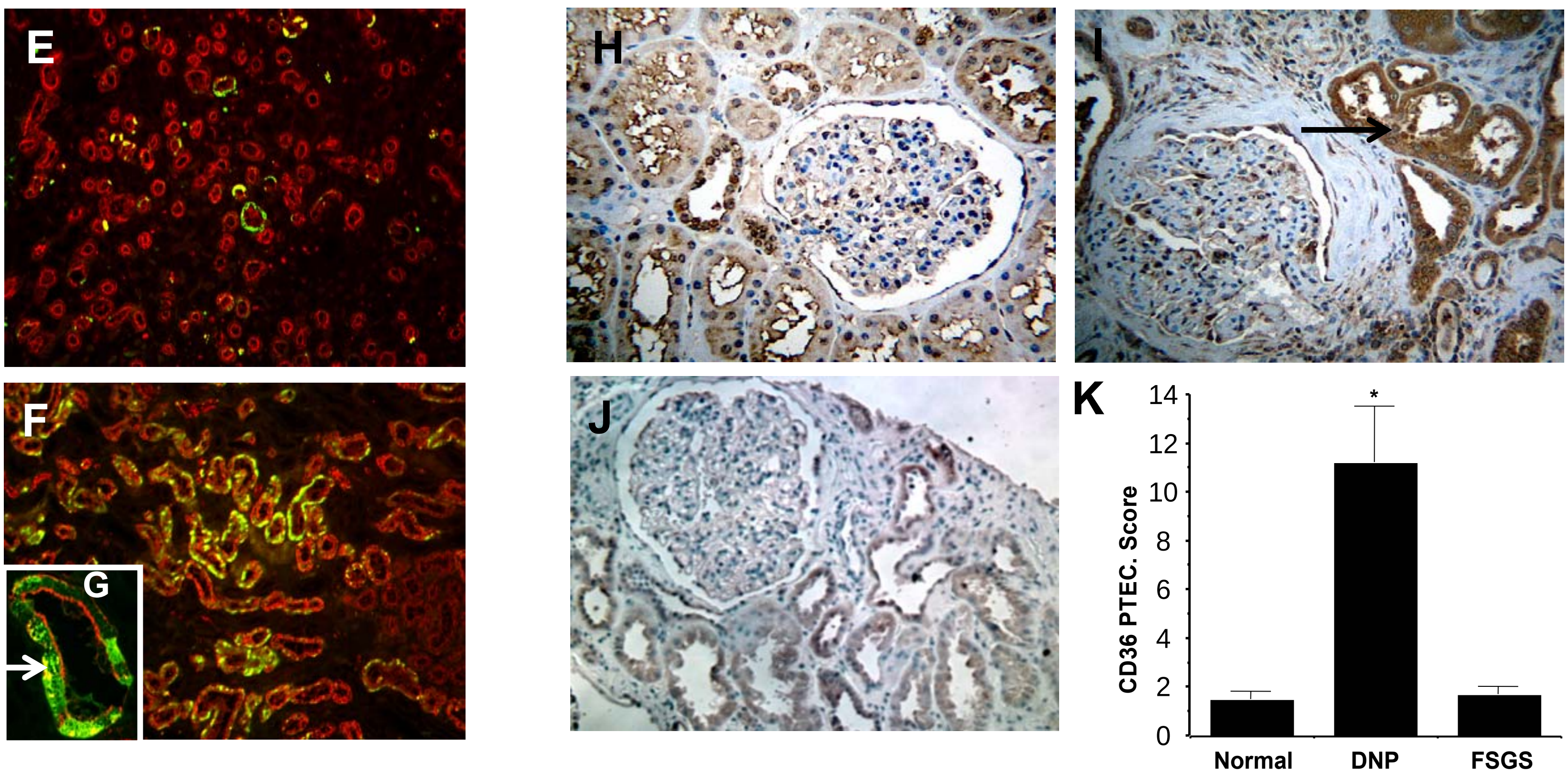

Blood Glucose measurement at 12 wk-of-age
Blood Glucose mg/ml
800
700
600
500
400
300
200
100
0
n=5
n=8
n=5
Control
RAGE
Akita
AkitaXRAGE
Series1

Flk-RAGE tg AMDCC Distribution

- flk-RAGE tg mice
  - shipped to UMich spring 2005
  - Neuropathy phenotyping (Feldman)
- Flk-RAGE X Akita tg bladder
  - Will be shipped to Cleveland Clinic
  - Uropathy phenotyping (Daneshgari)

Multiple Metabolic Hits Converge on CD36 as Novel Mediator of Tubular Epithelial Apoptosis in Diabetic Nephropathy

Katalin Susztak[1,2], Emilio Ciccone[3], Peter McCue[4], Kumar Sharma[3*], Erwin P. Böttinger[1*]



New Candidate Pathomechanisms: Tubular Apoptosis and Tubular Degeneration

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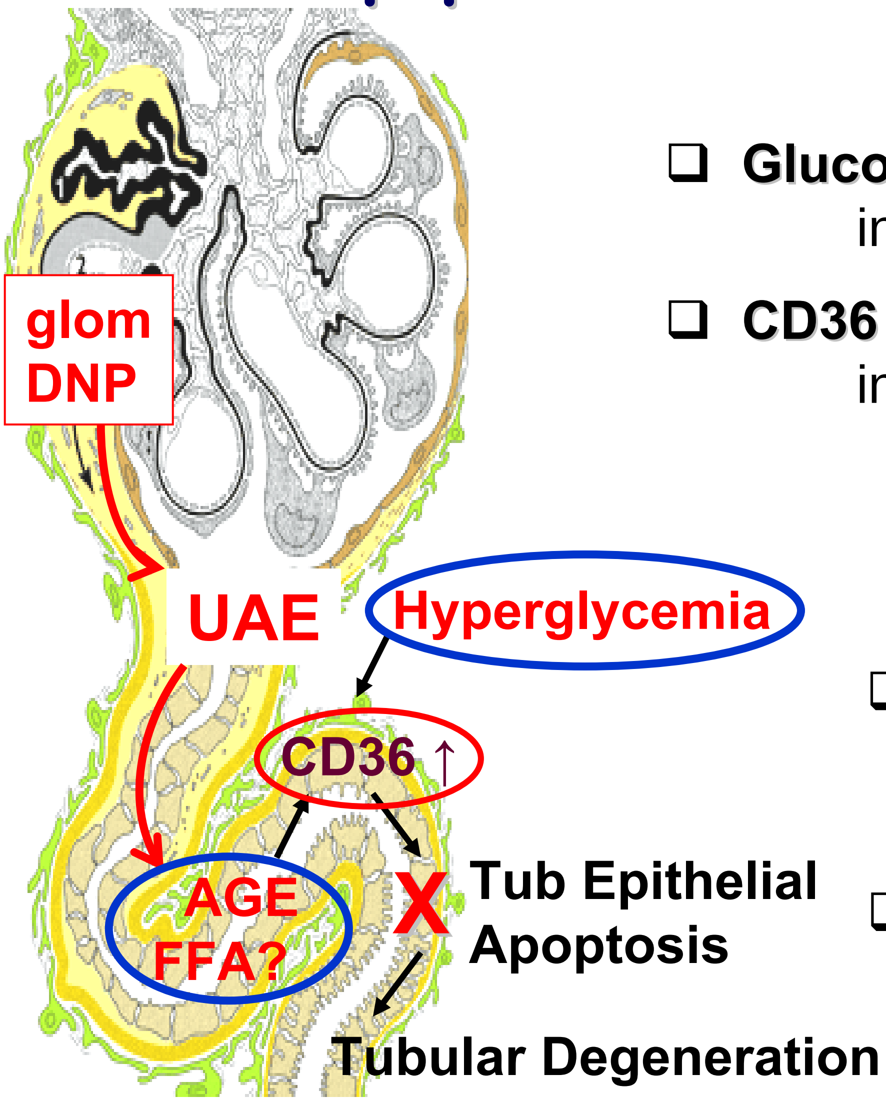
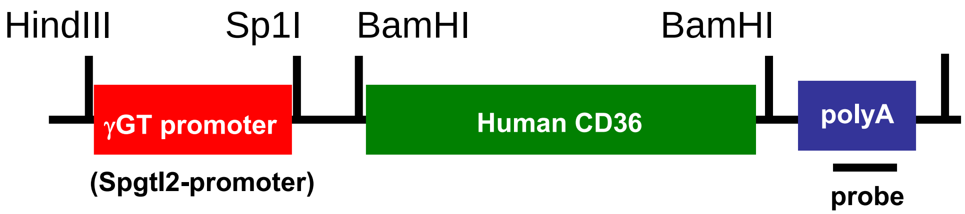


- **Glucose stimulates CD36** expression in PTECs in vitro
- **CD36** expression is increased in in **prox tubules** in **human DNP**
- **AGE** and **FFA** may induce **tubular epithelial apoptosis** through **CD36**-dependent signaling
- **Tubular epithelial apoptosis** may underlie **tubular degeneration**

Transgenic expression of CD36 in proximal tubules

- CD36 is upregulated in prox tubule in human DNP, but not diabetic mouse
- CD36 mediates prox tubule apoptosis induced by AGE and FFA
- gGT and Sgptl2 promoters are used to target CD36 expression to prox tubule



Schematic representation of GGT/CD36 targeting vector

- gGT-CD36
  - 11 founders with germline transmission
  - CD36 transgene level not significantly above endogenous CD36 by Western, QRT-PCR, and IHC

- Spgtl2-CD36 oocyte injections x3 completed week of Oct 10

Reduced Podocyte Number in Glomerular Diseases

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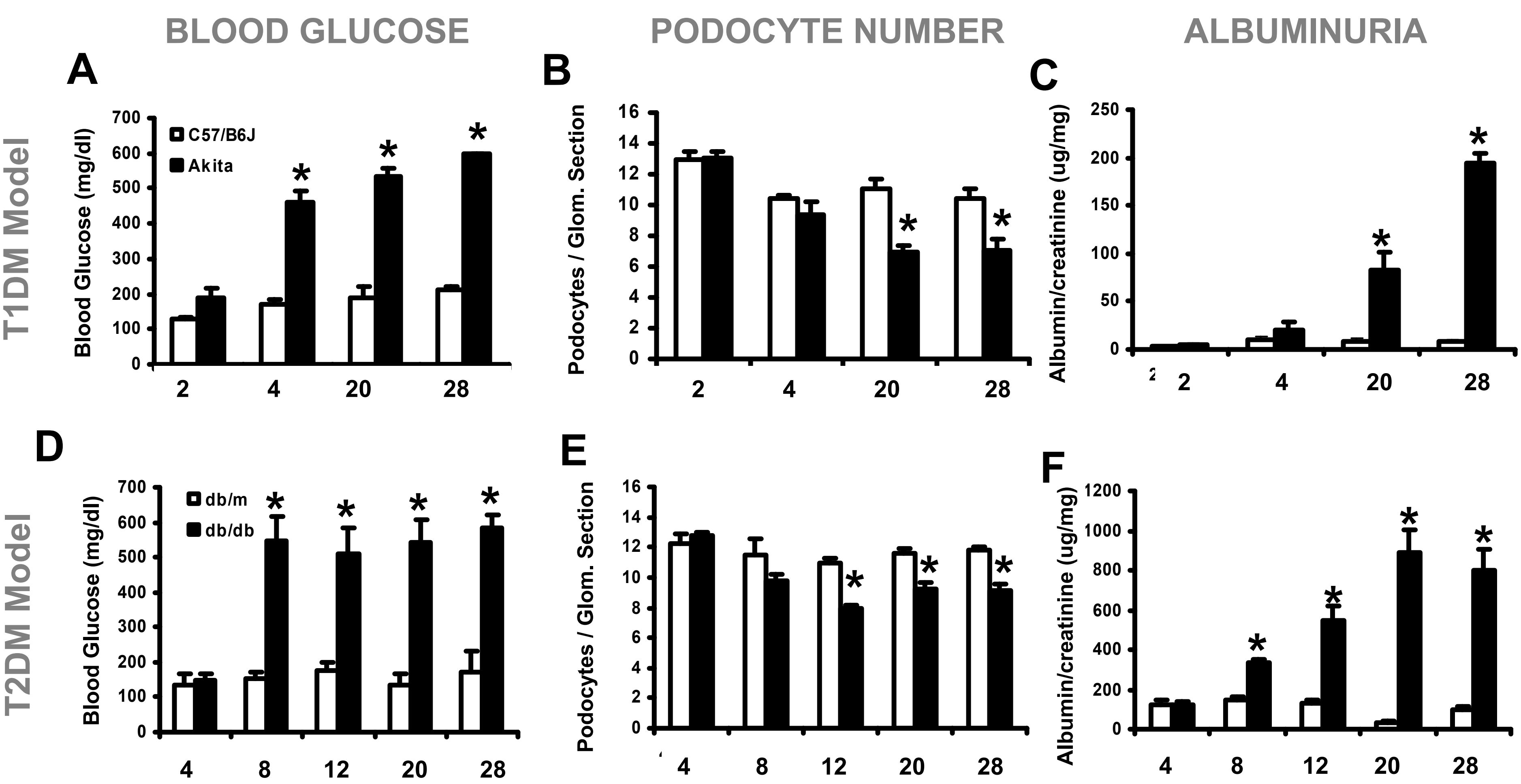
- Type 2 Diabetes Mellitus [Pagtalunan et al., JCI, 1997]
- Type 1 Diabetes Mellitus [Steffes et al., Kidney Int, 2001]
- IgA Nephropathy [Lemley et al., Kidney Int, 2002]
- Focal Segmental Glomerulosclerosis

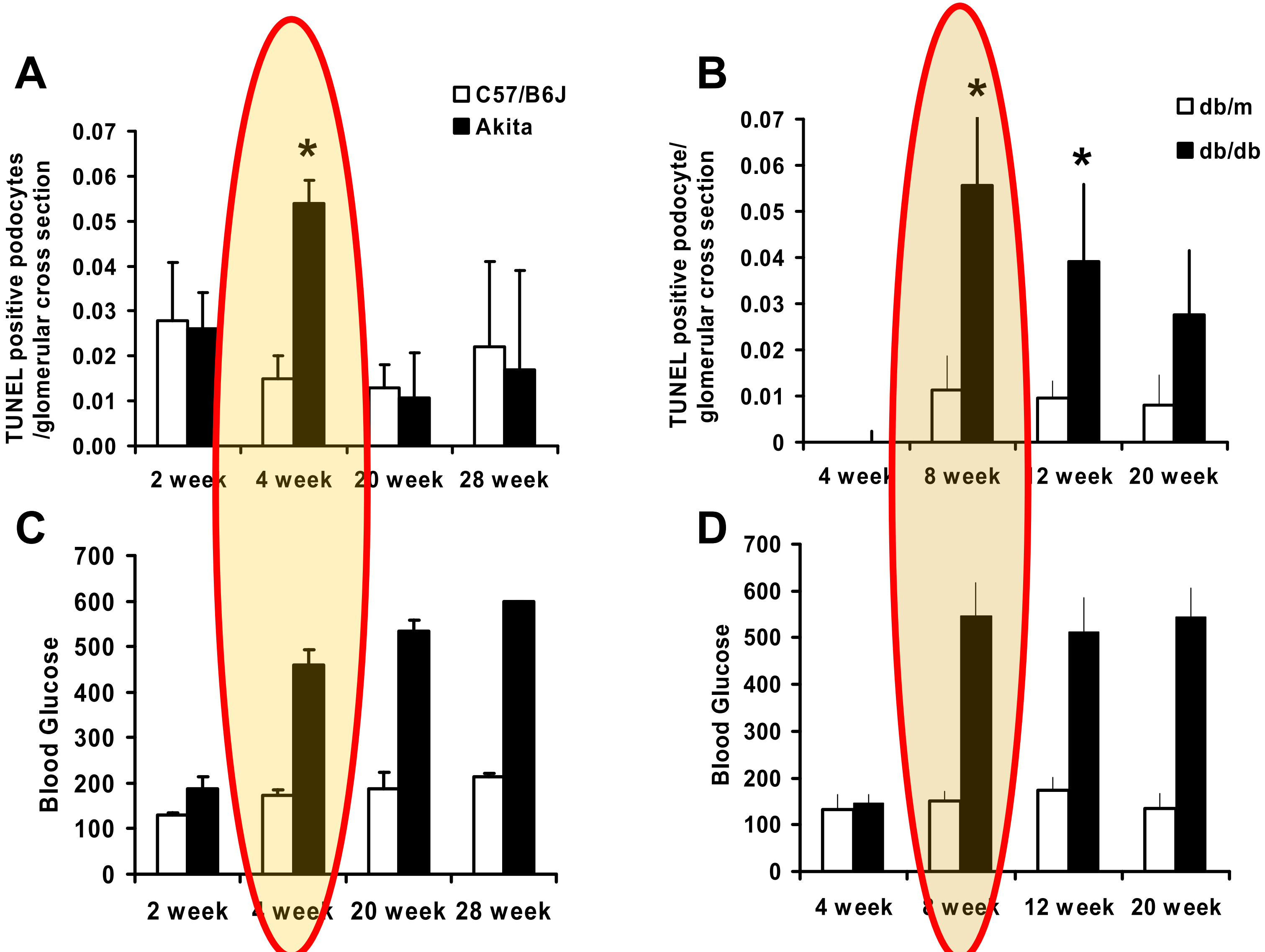
- Experimental Models
  - Puromycin aminonucleoside model [Kim et al., JASN 2001]
  - TGF-β1 transgenic mice [Schiffer et al., JCI 2001]
  - Cd2ap knockout mice [Schiffer et al., JBC 2004]

  - **T1DM (Ins2^{Akita}) and T2DM (Lepr$^{db/db}$) mice [Susztak et al., Diabetes (In Press)]**

Diabetes, Podocyte Number, and Albuminuria in Murine T1DM and T2DM



Podocyte Apoptosis Coincides With Onset of Hyperglycemia in Murine T1DM and T2DM



Glucose Stimulates Podocyte Apoptosis

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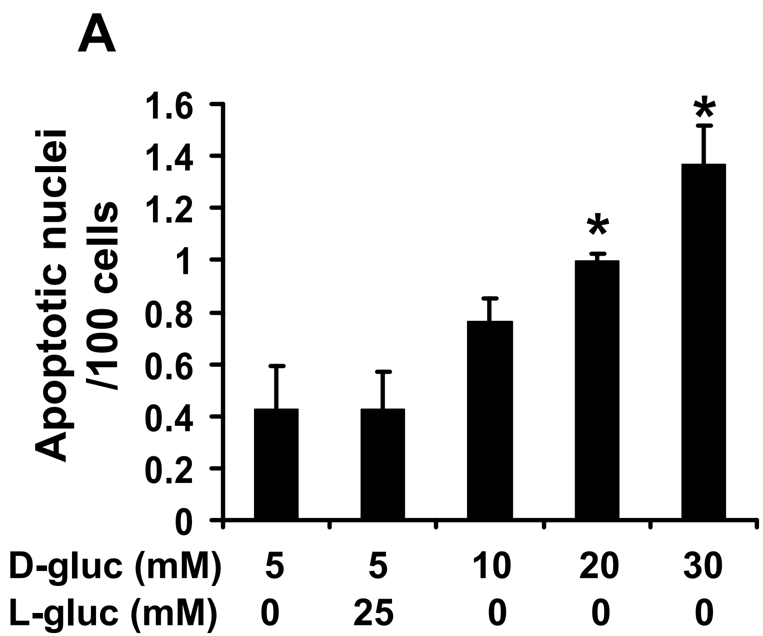

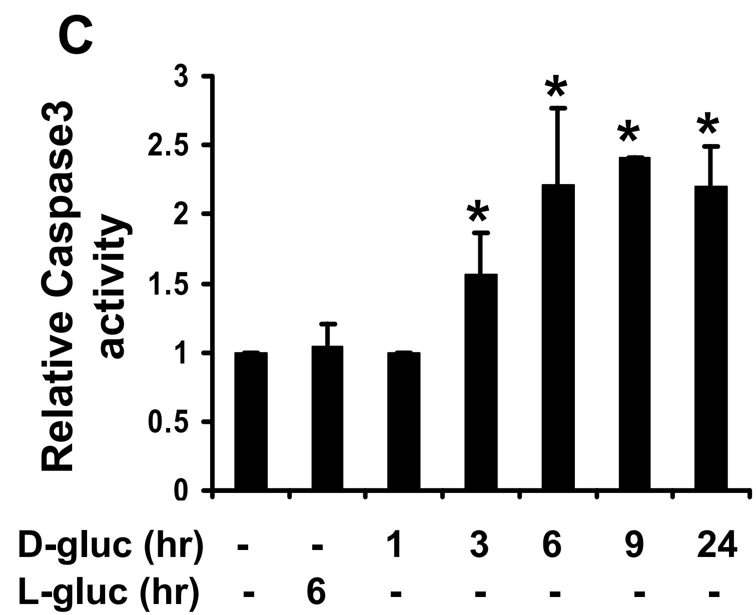

Glucose Stimulates Podocyte Apoptosis Via ROS Production

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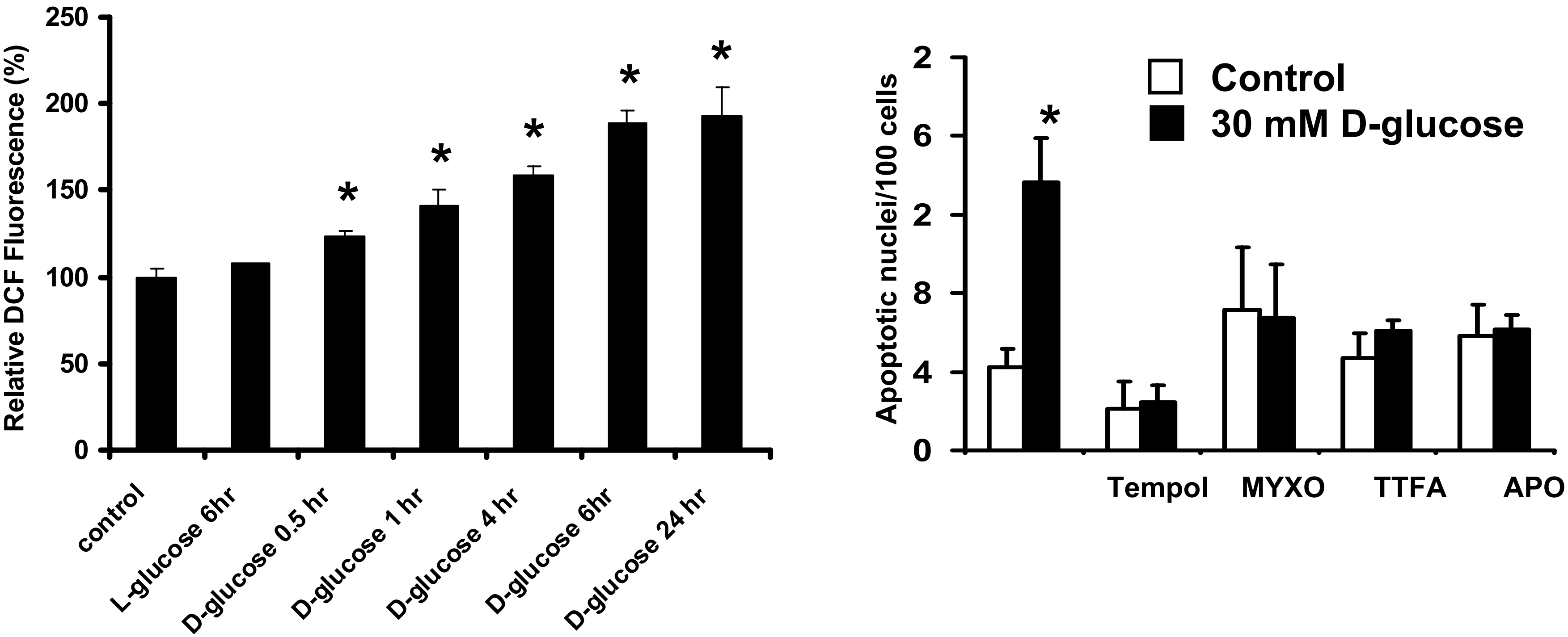

NADPH Oxidase Inhibitor Apocynin Prevents Podocyte Apoptosis and Improves Diabetic Glomerulopathy

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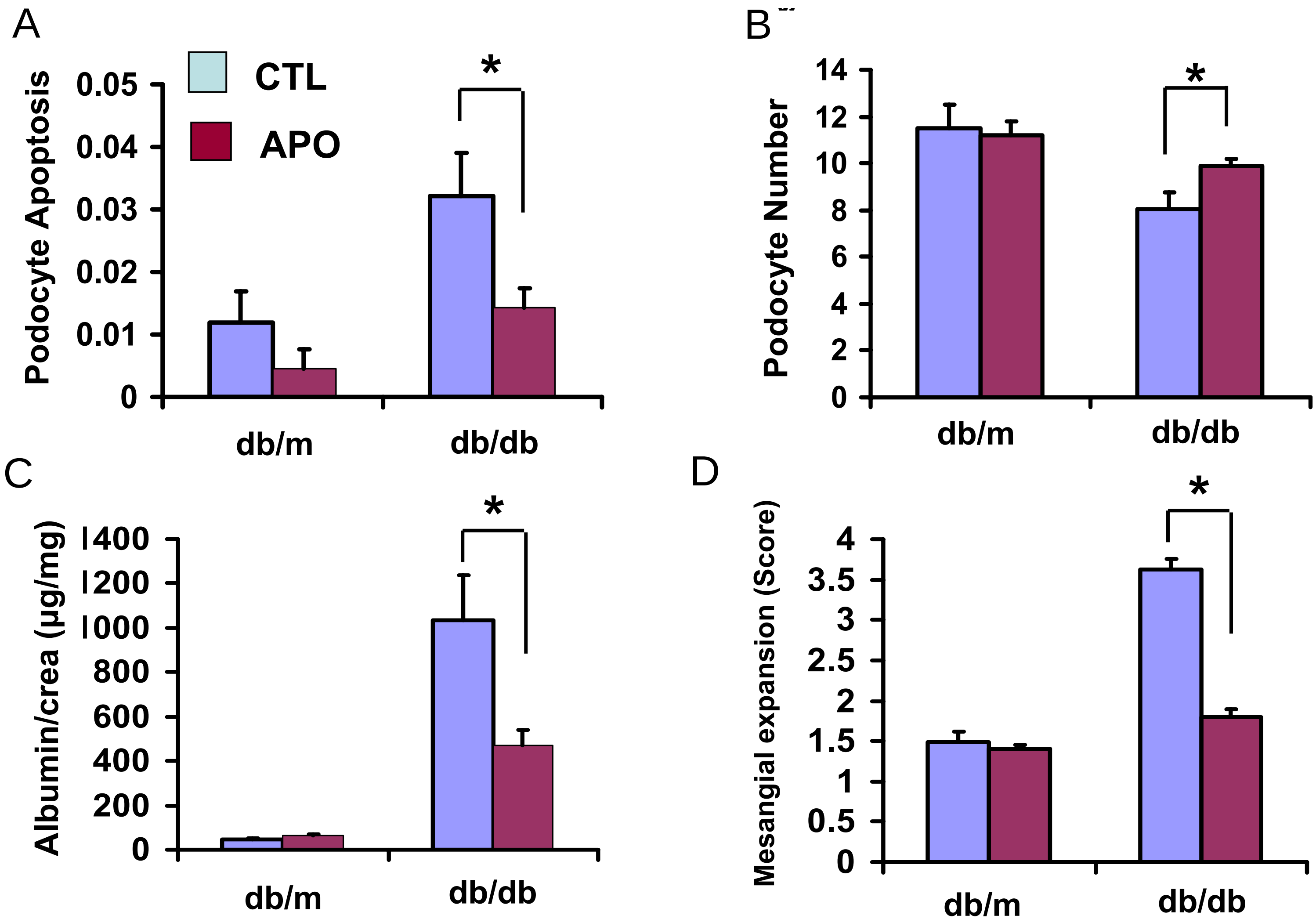

Podocyte Apoptosis -
An Early Lesion in Diabetic Nephropathy

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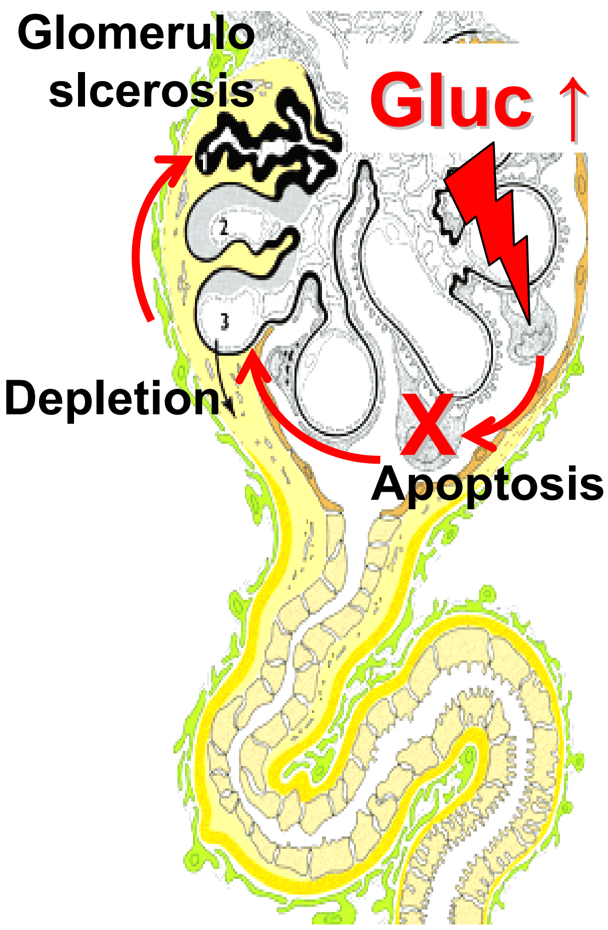


- **Glucose** is sufficient to cause apoptosis via ROS production in cultured podocytes
- **Podocyte apoptosis in vivo**
  - coincides with onset of hyperglycemia in T1DM and T2DM mice
  - causes loss of podocytes (depletion)
  - If podocyte depletion exceeds threshold, it may initiate glomerulosclerosis
- **NADPH oxidase blockade prevents**
  - podocyte apoptosis
  - podoycty depletion
  - albuminuria/proteinuria
  - mesangial expansion

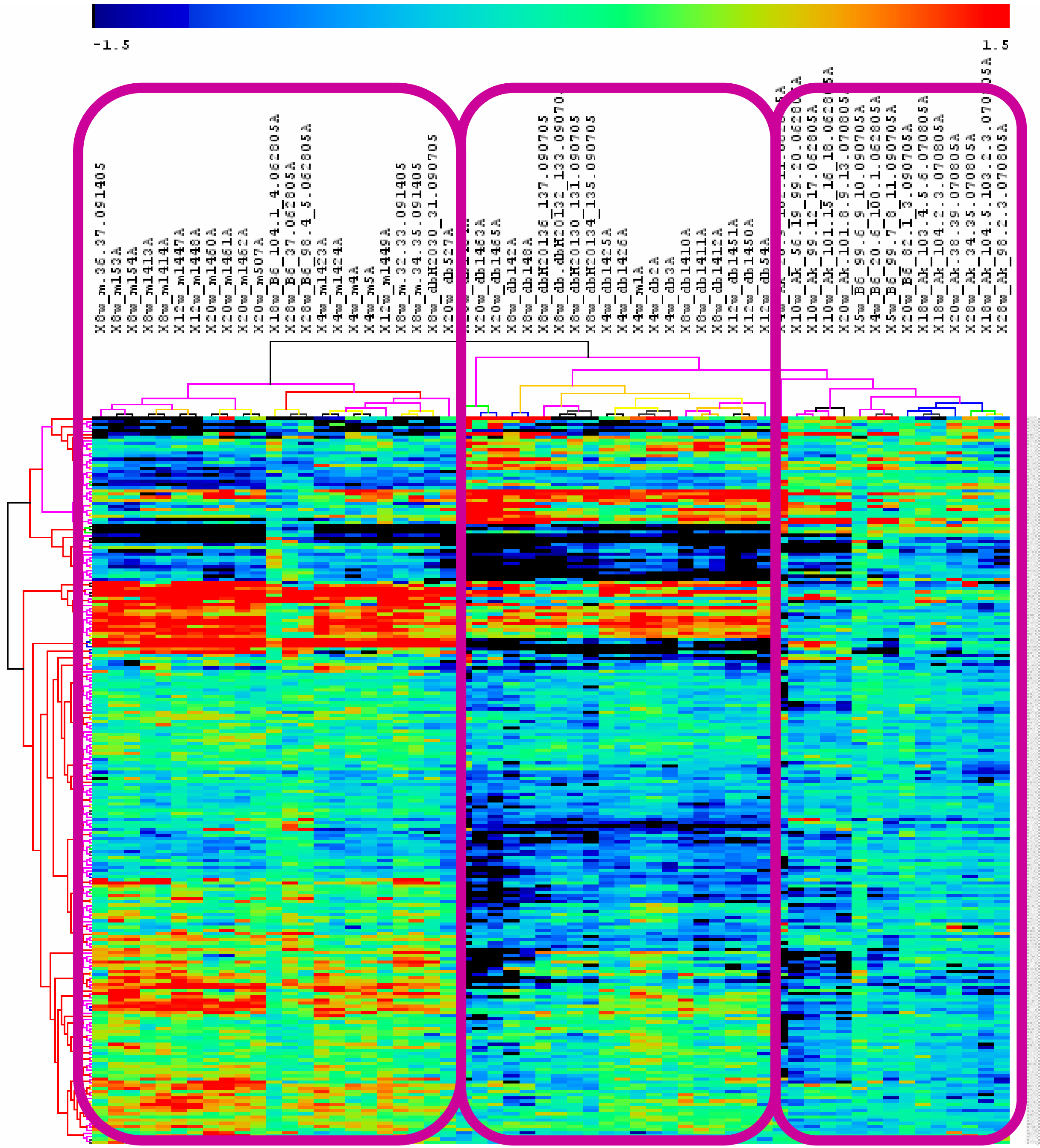
Glucose-Induced Reactive Oxygen Species Cause Apoptosis of Podocytes and Podocyte Depletion at the Onset of Diabetic Nephropathy

Katalin Susztak[1], Amanda C. Raff[1], Mario Schiffer[1], Erwin P. Böttinger[2]

[1]Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461
[2]Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, One Gustave L. Levy Pl., New York, NY 10029

Summary of Glomerular Gene Expression Patterns in Db/db and Ins2-Akita:

Model	Age (wk)	Sample# Db/db&Akita	Sample# Db/m&B6	Up	Down	Total	Method
Db	4	5	4	3	0	3	SAM 2-class <10%FDR
Db	8	7	10	39	503	542	SAM 2-class <10%FDR
Db	12	3	3	23	240	243	SAM 2-class <10%FDR
Db	20	4	4	311	989	1300	SAM 2-class<10%FDR
Db	**All**	**24**	**21**	**248**	**921**	**1169**	**SAM 2-class 5%FDR**
Akita	4-5	1	3	99	83	182	Ratio (4x)
Akita	10	3	0	7	58	65	SAM, 2-class 5% (wk4 control)
Akita	18-20	5	2	226	726	952	SAM 1-class 5%FDR
Akita	28	2	2	101	238	339	Ratio 1.5x
Akita	**All**	**11**	**7**	**163**	**620**	**783**	**SAM 2-class 5% FDR**
Db-Akita	**All**	**35**	**28**	**41**	**189**	**230**	**SAM 2-class 5% FDR**



-1.5
1.5

System	*Category*	*LH*	*LT*	*PH*	*PT*	*EASE Score*	*Fisher Exact*
biological_process	response to heat	**4**	132	20	7522	0.00474	0.000353
biological_process	response to temperature	**4**	132	23	7522	0.00709	0.000619
biological_process	acyl-CoA metabolism	**3**	132	9	7522	0.01	0.000411
biological_process	lipid metabolism	**12**	132	292	7522	0.0127	0.00508
biological_process	electron transport	**10**	132	218	7522	0.0136	0.00487
biological_process	sulfur metabolism	**4**	132	40	7522	0.0319	0.00508

System	*Category*	*LH*	*LT*	*PH*	*PT*	*EASE Score*	*Fisher Exact*
cellular_component	extracellular space	55	141	2105	7775	0.00185	0.00125
cellular_component	endoplasmic reticulum	14	141	308	7775	0.00358	0.00134
cellular_component	membrane fraction	11	141	205	7775	0.00381	0.0012
cellular_component	extracellular	56	141	2237	7775	0.00447	0.00315
cellular_component	cell fraction	11	141	225	7775	0.00728	0.00253
cellular_component	vesicular fraction	6	141	82	7775	0.0159	0.00362
cellular_component	microsome	6	141	82	7775	0.0159	0.00362
cellular_component	integral to membrane	57	141	2558	7775	0.0449	0.0352
cellular_component	lytic vacuole	5	141	75	7775	0.0459	0.0114
cellular_component	lysosome	5	141	75	7775	0.0459	0.0114

System	Category	LH	LT	PH	PT	EASE Score	Fisher Exact
molecular_function	oxidoreductase activity	25	151	472	8304	3.44E-06	1.12E-06
molecular_function	catalytic activity	79	151	3133	8304	0.000232	0.000164
molecular_function	monooxygenase activity	8	151	87	8304	0.000953	0.000172
molecular_function	heat shock protein activity	5	151	32	8304	0.00248	0.000252
molecular_function	chaperone activity	8	151	112	8304	0.00407	0.000957
molecular_function	acyl-CoA thioesterase activity	3	151	6	8304	0.00464	0.000113
molecular_function	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	6	151	62	8304	0.00509	0.00087
molecular_function	glutathione transferase activity	4	151	20	8304	0.00526	0.000406
molecular_function	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	6	151	69	8304	0.008	0.00153
molecular_function	oxidoreductase activity, acting on CH-OH group of donors	6	151	73	8304	0.0101	0.00204
molecular_function	CoA hydrolase activity	3	151	9	8304	0.0107	0.000457
molecular_function	isomerase activity	6	151	87	8304	0.0203	0.00493
molecular_function	disulfide oxidoreductase activity	3	151	15	8304	0.0292	0.00228
molecular_function	lyase activity	6	151	100	8304	0.0345	0.00961
molecular_function	heparin binding	4	151	42	8304	0.0397	0.00688
molecular_function	transferase activity, transferring alkyl or aryl (other than methyl) groups	4	151	42	8304	0.0397	0.00688

Thanks to

Advisors
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JDRF
Consortium