Generating mouse mutants with Diabetic Nephropathy

- **Candidate Genes: eNOS,ApoE,LDLR**
  - **db/db BLKS**
- Random mutagenesis: ENU Akita
- Strain dependence: KK and DBA2 STZ

Modifier Genes

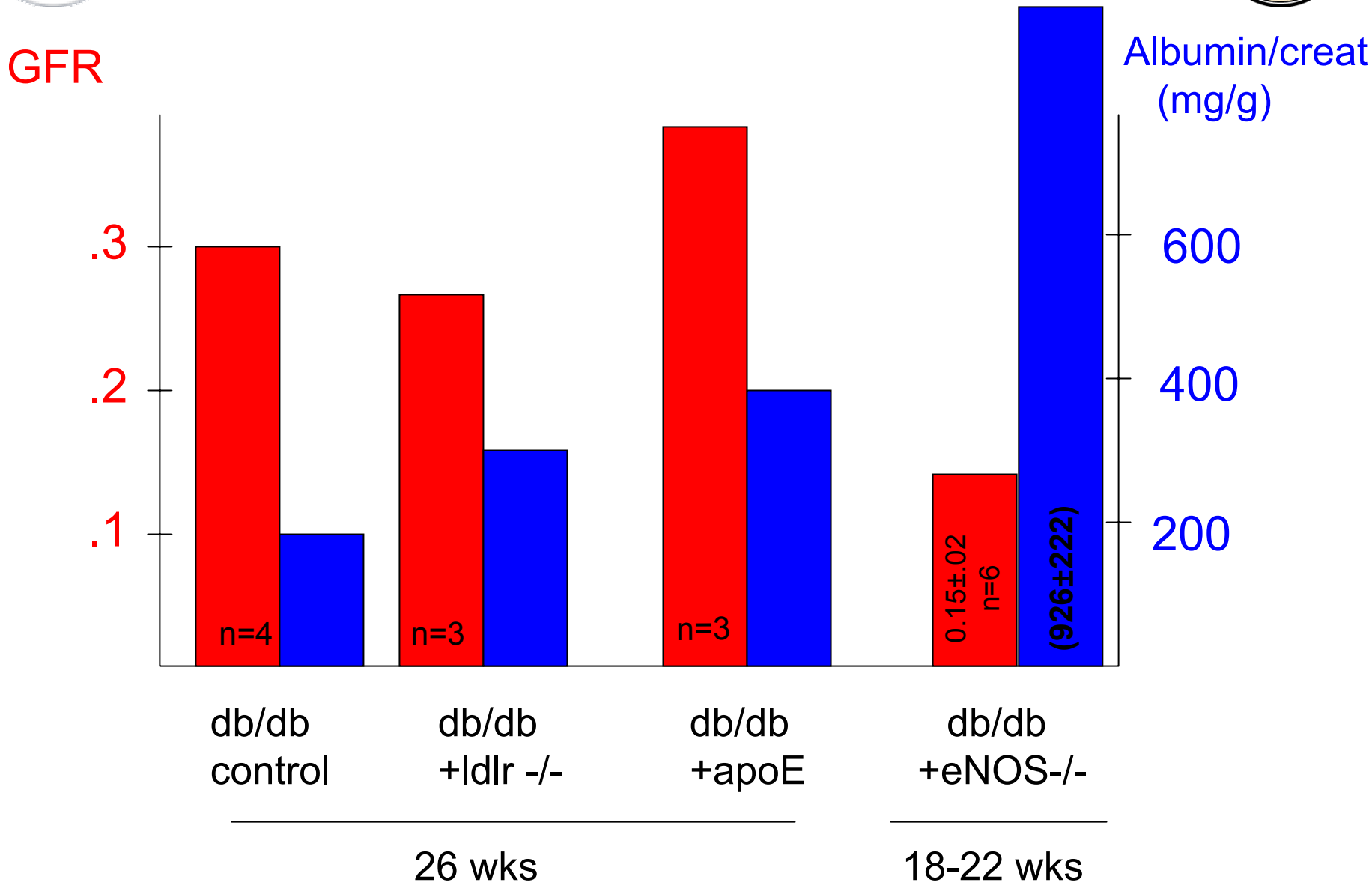
Lipid metabolism

1) apoE -/-
2) ldlr -/-

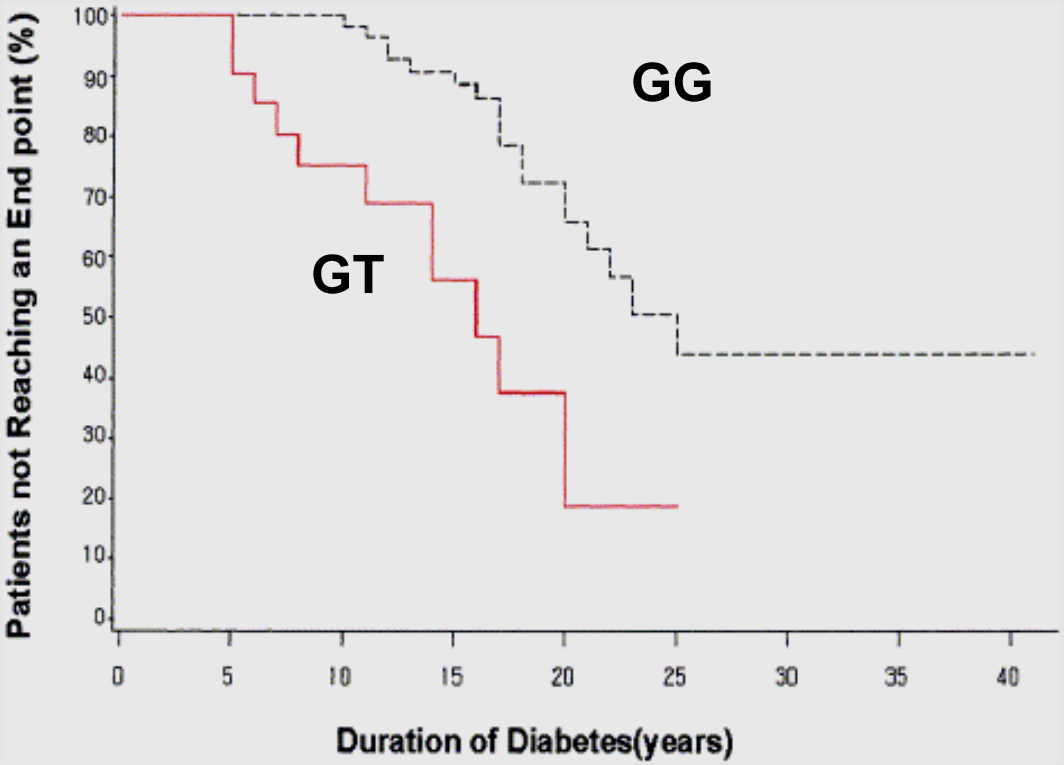
Vascular Reactivity

eNOS -/-

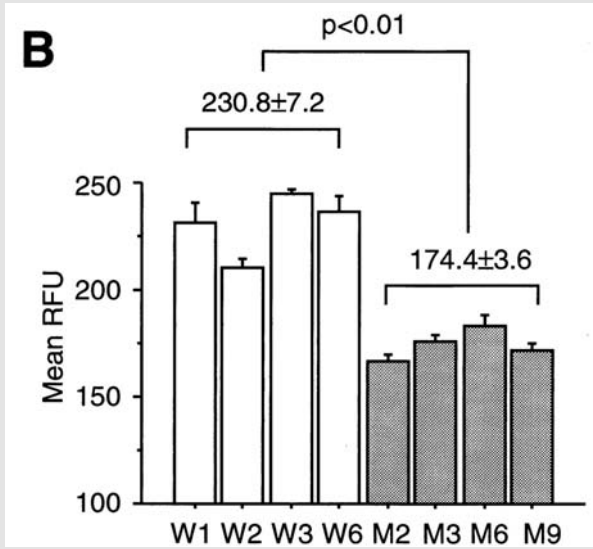
db/db BLKS Background



Renal survival in type 2 diabetics by eNOS Genotype Glu298Asp

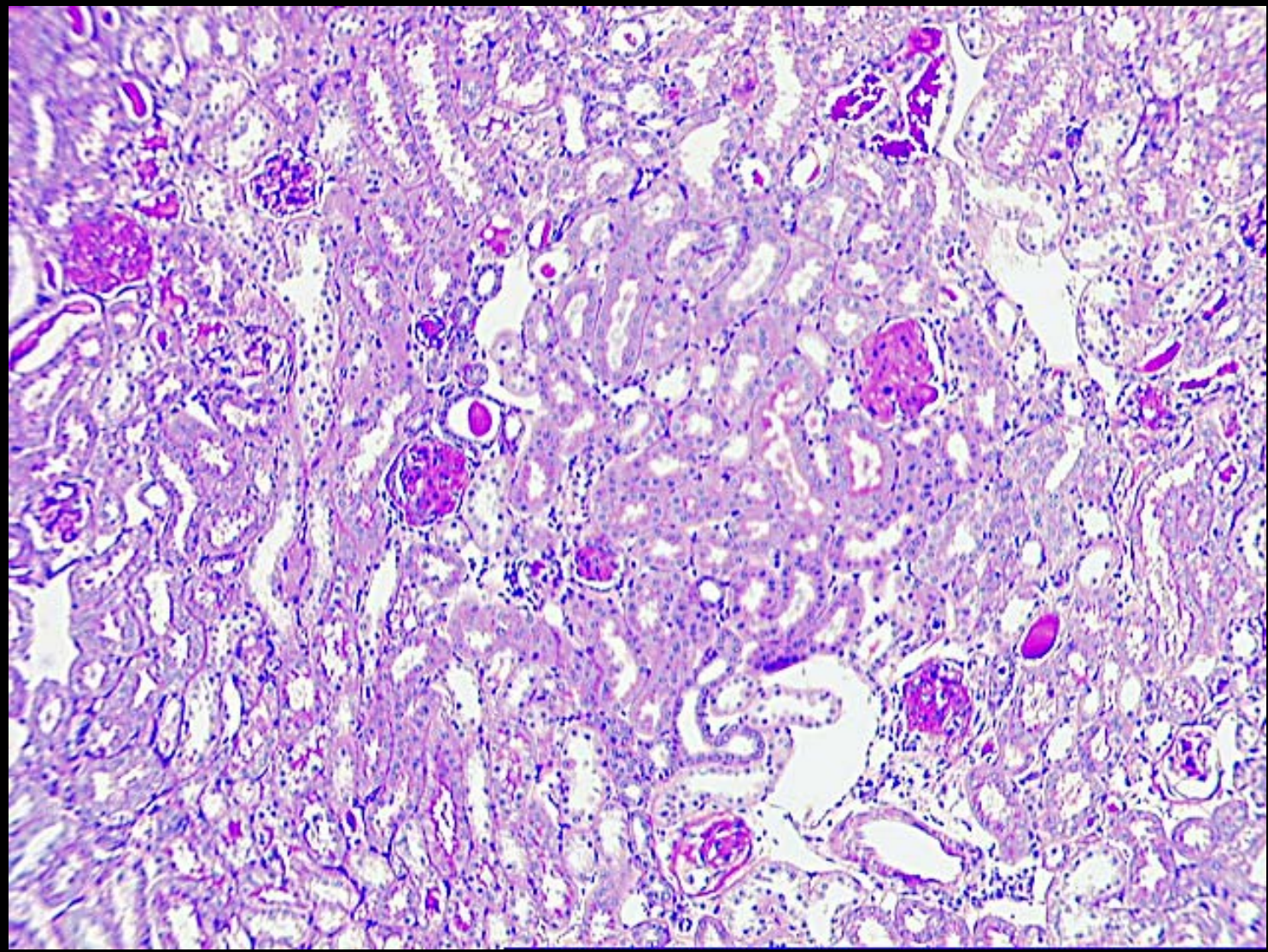


eNOS activity in cells transfected with polymorphic eNOS genotypes

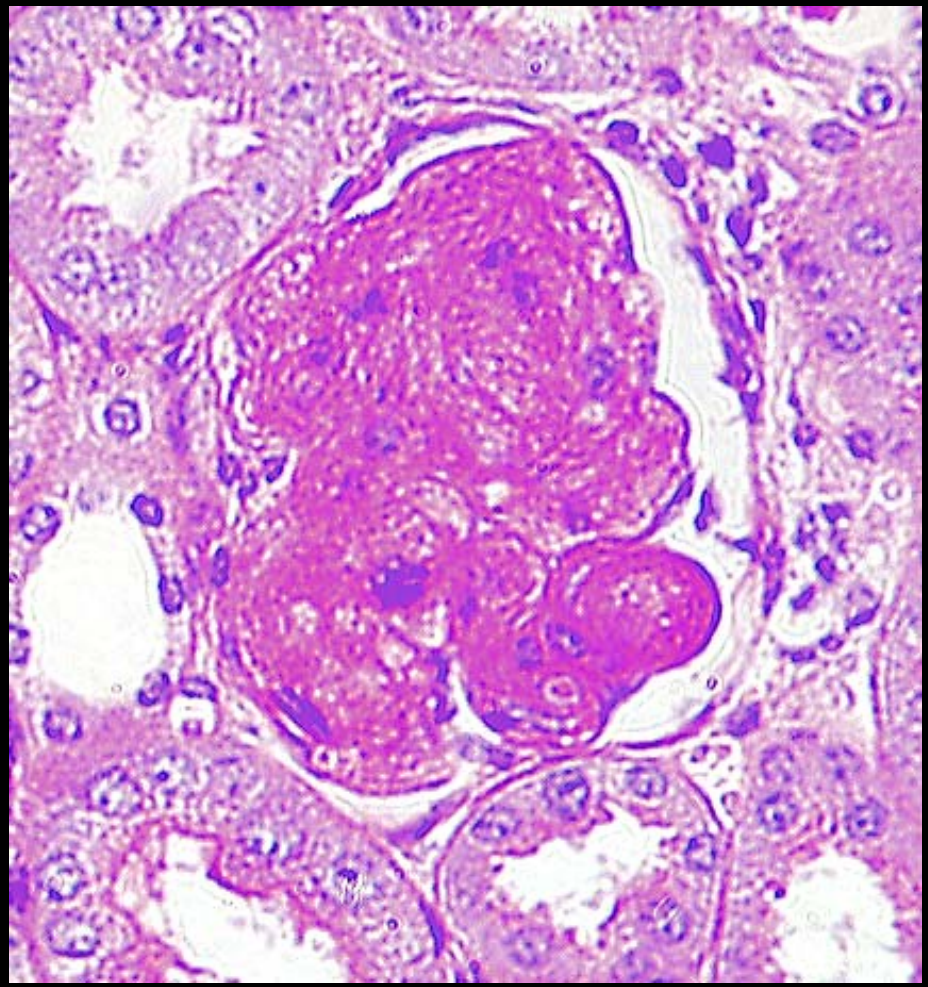


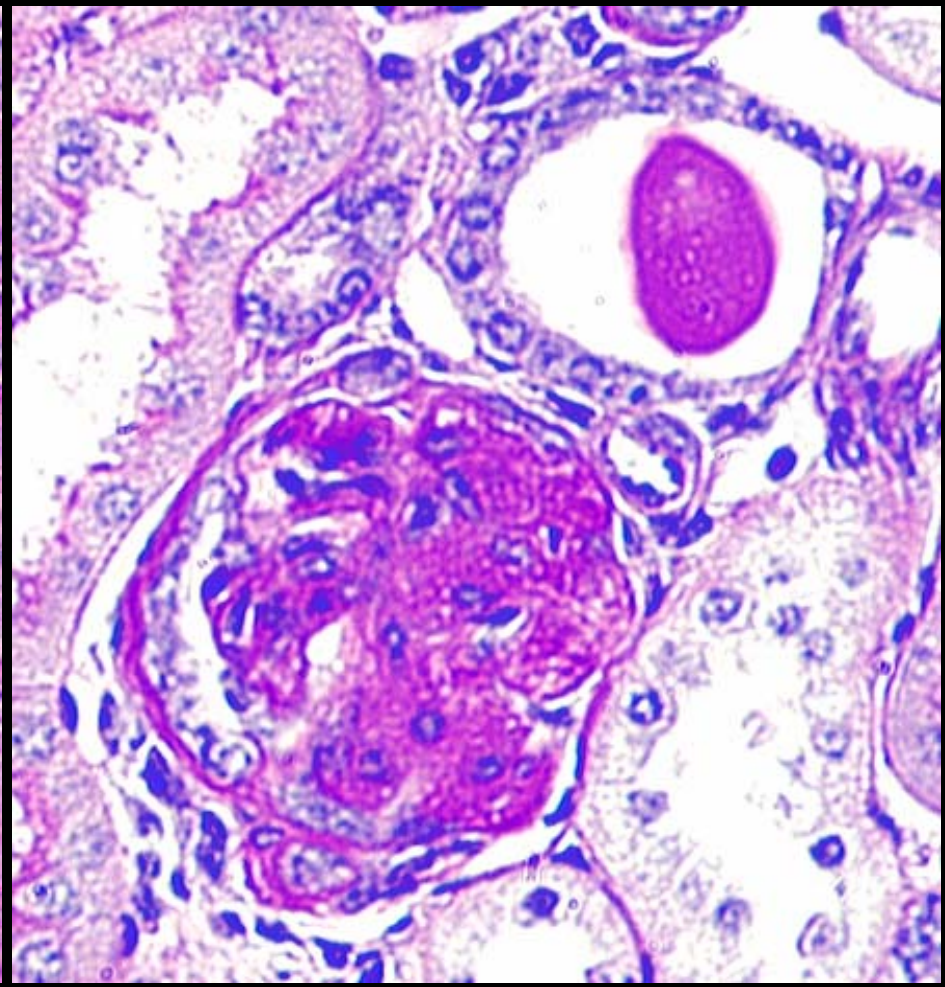
Hypertension. 2002 Oct;40(4):535-40
Diabetes Res Clin Pract. 2004 Sep;65(3):257-65.

eNOS-/- /db/db BKS background PAS

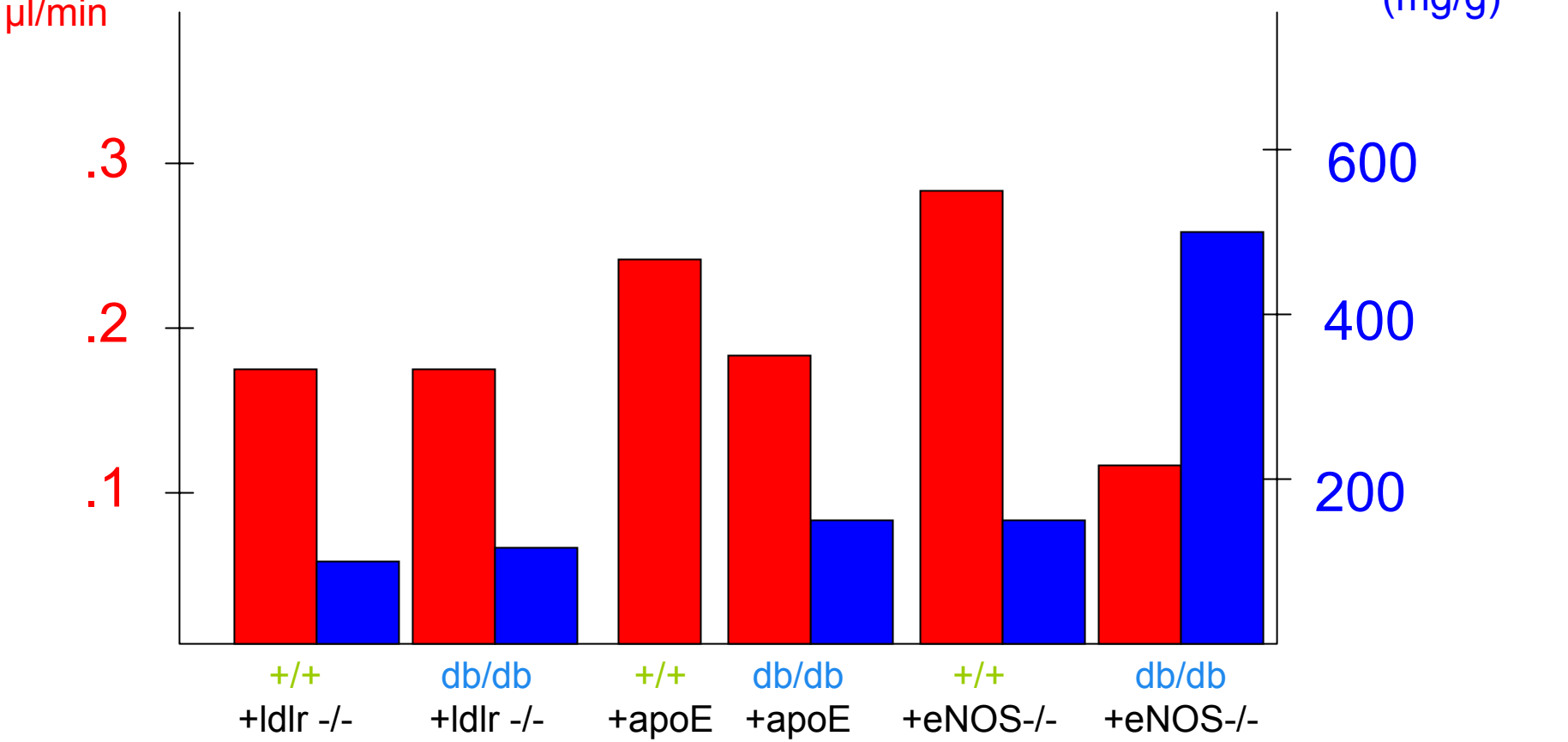


eNOS-/- /db/db BKS background PAS

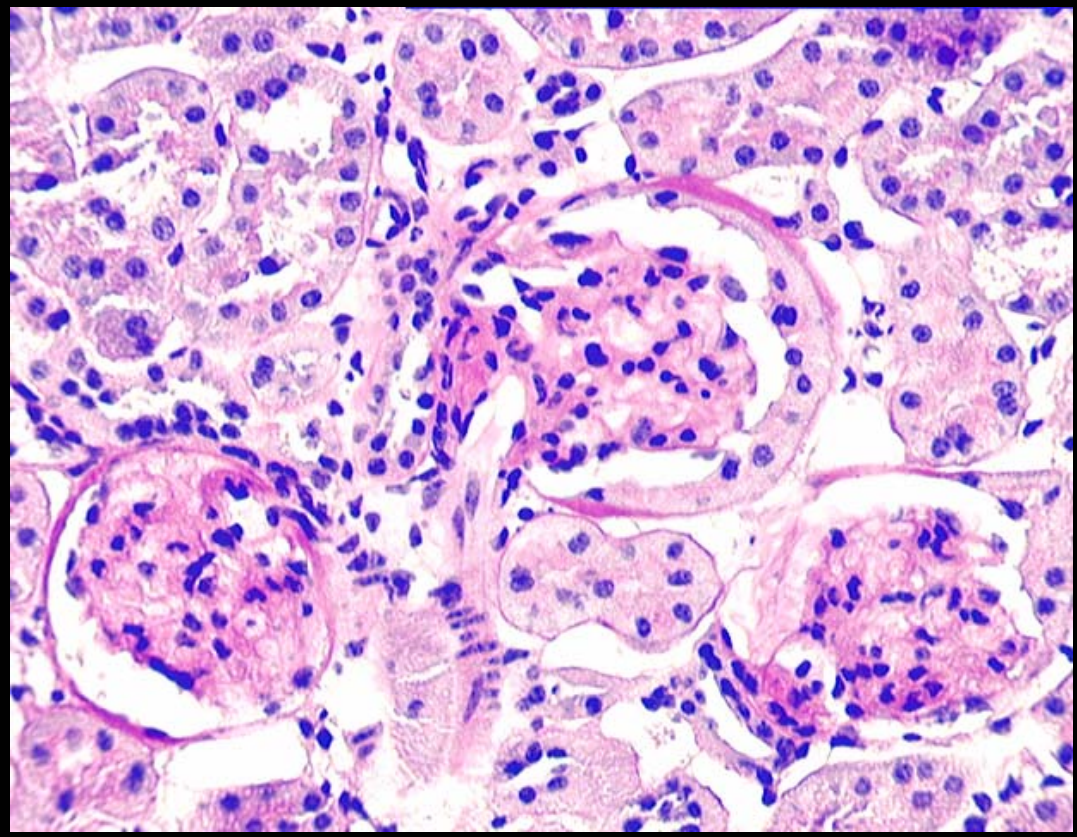




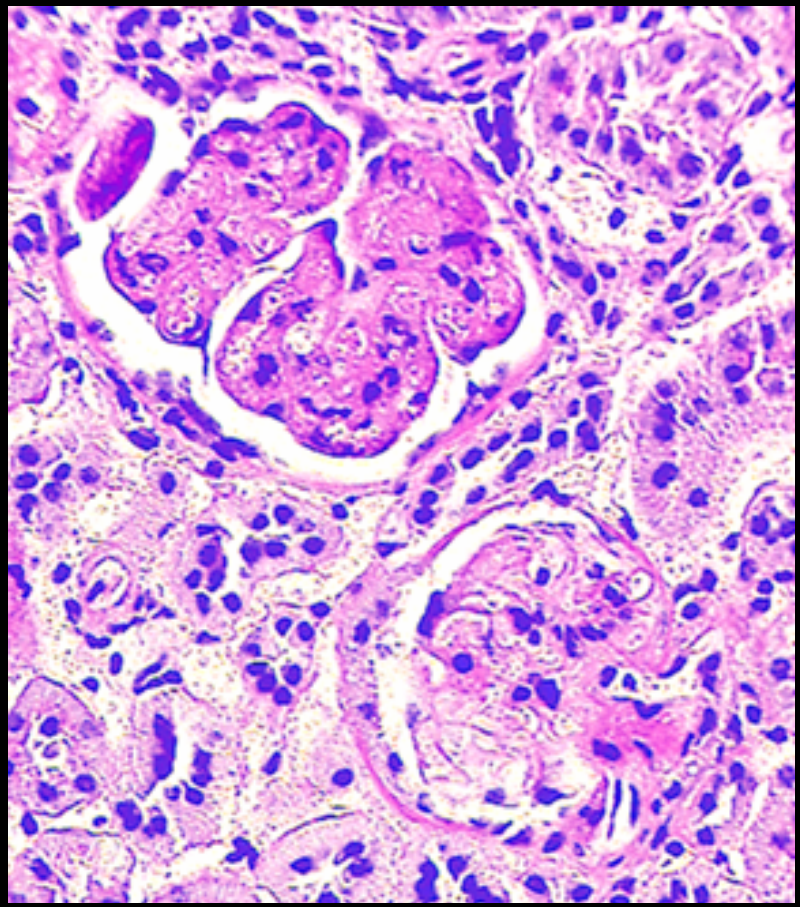
C57/B6 Background (male)
26 wks
n=2-4

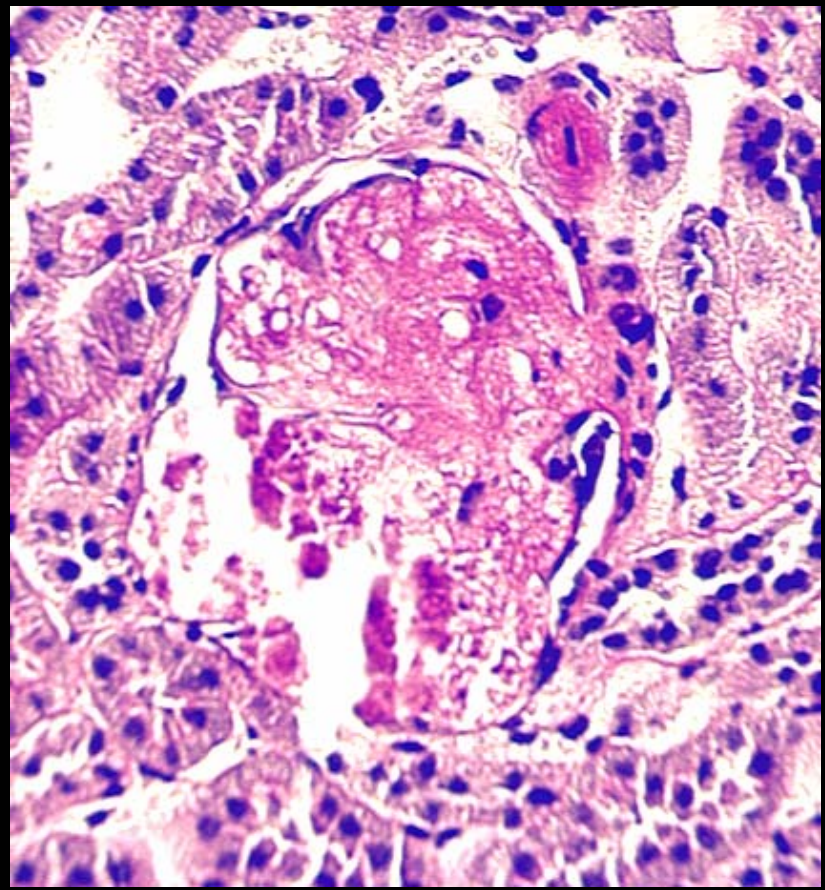


C57BL/6 db/db eNOS-/-



eNOS-/- /db/db C57/B6 background PAS





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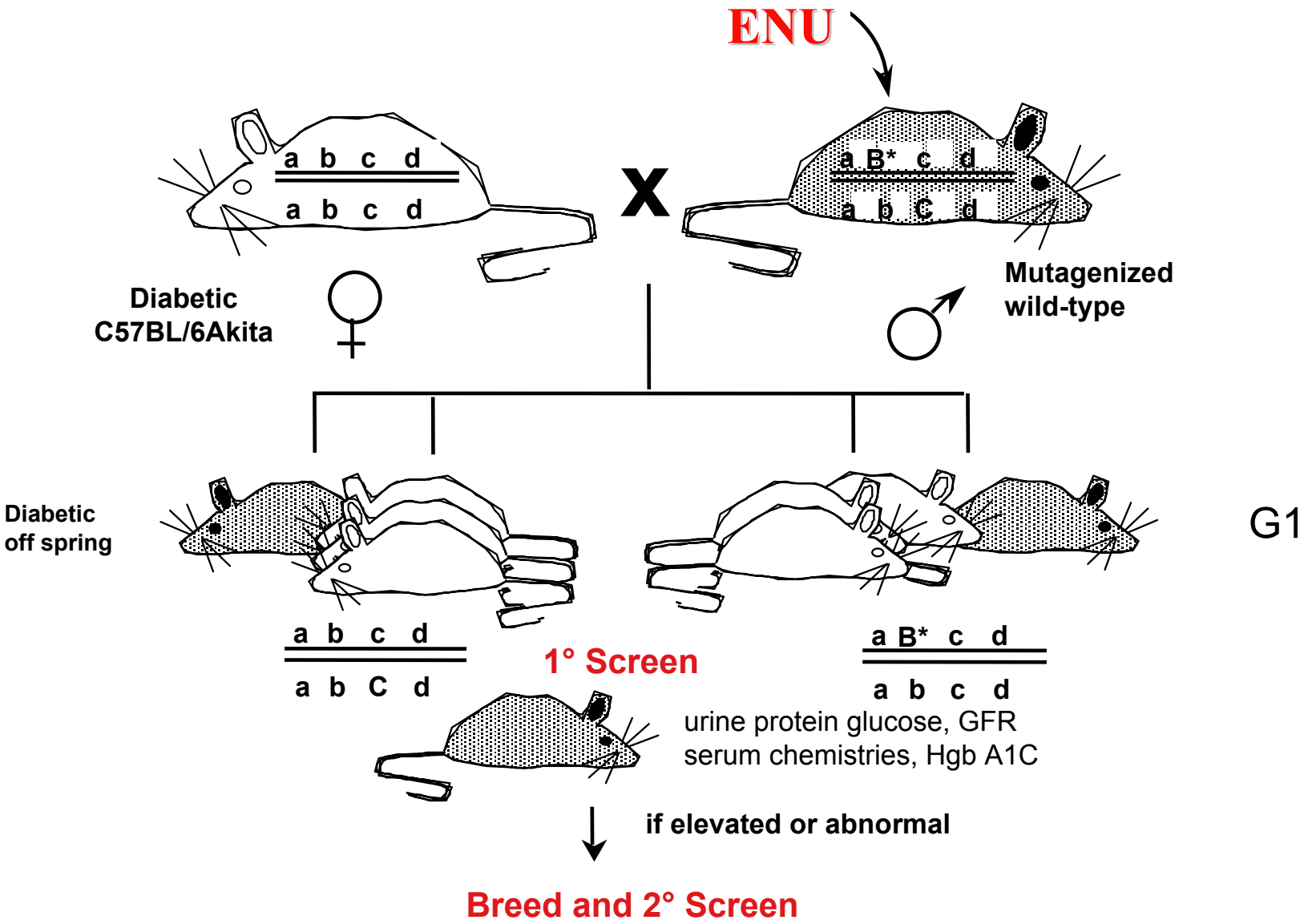
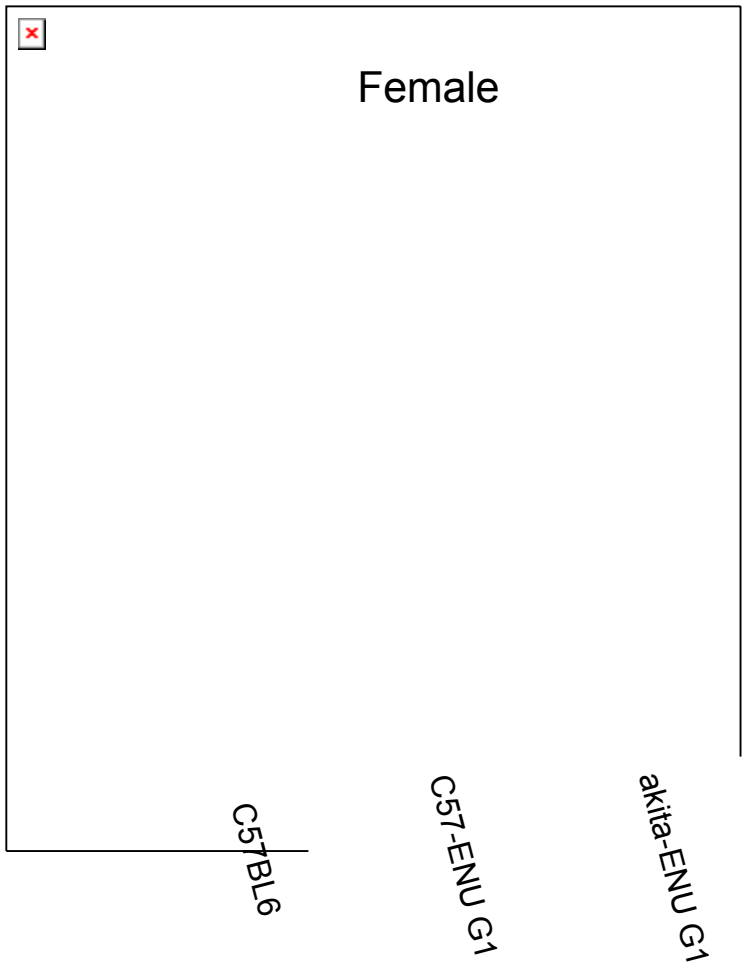
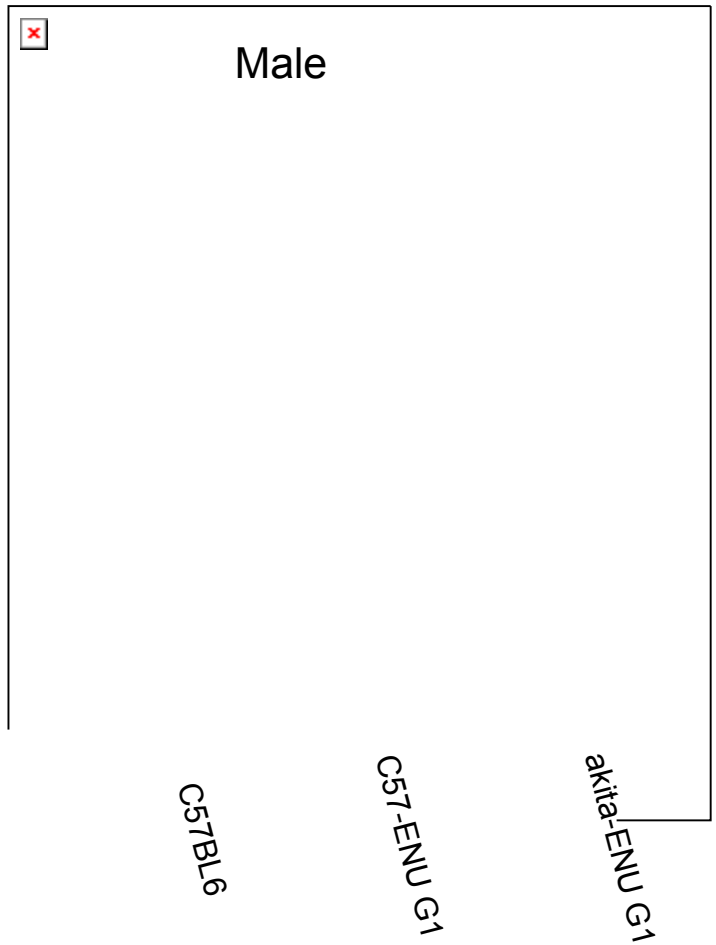
- **Random mutagenesis: ENU Akita**
- Strain dependence: KK and DBA2 STZ

Mutagenesis: a sensitized screen

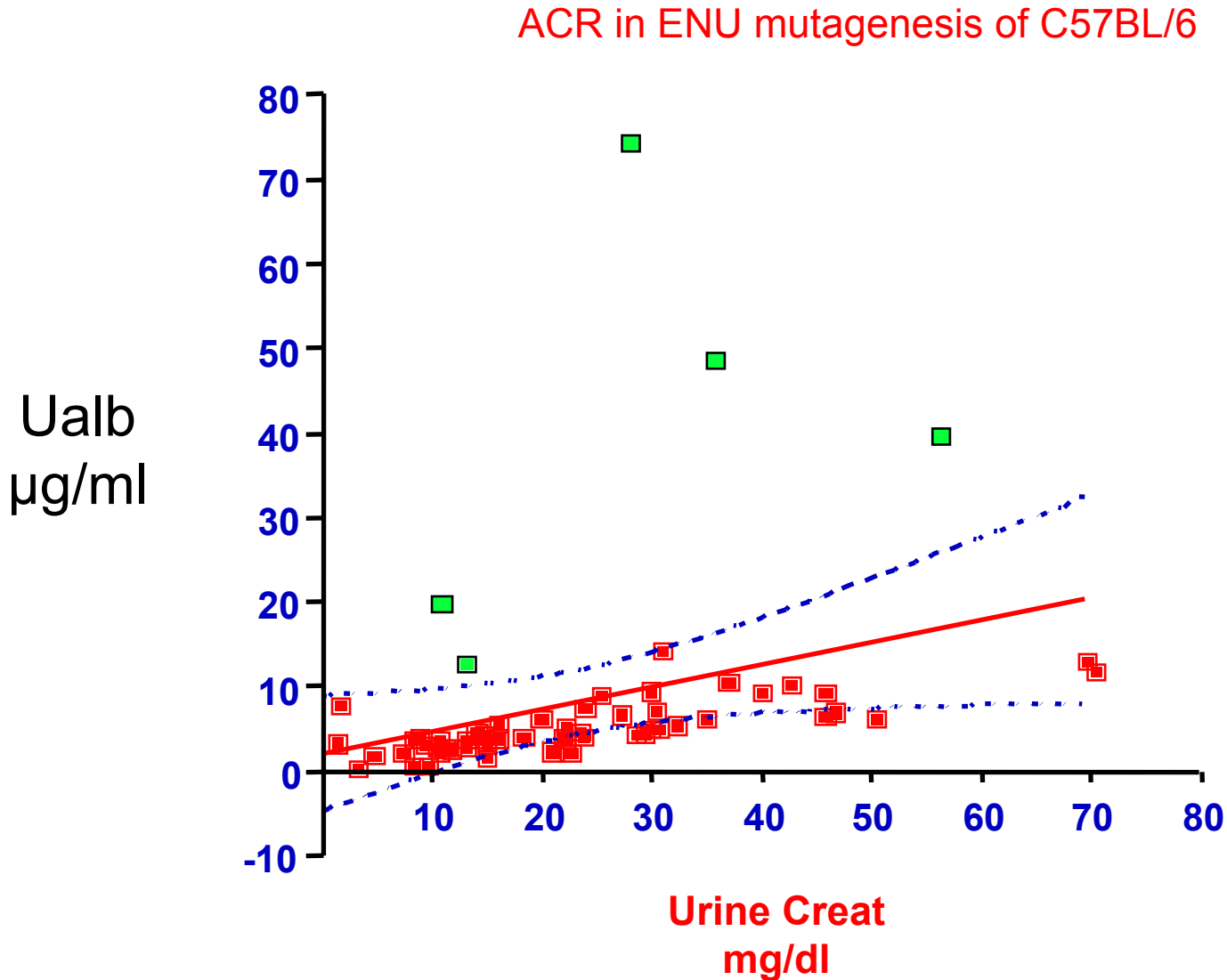


Blood glucose concentrations in ENU G1 mice genotyped for Akita mutation

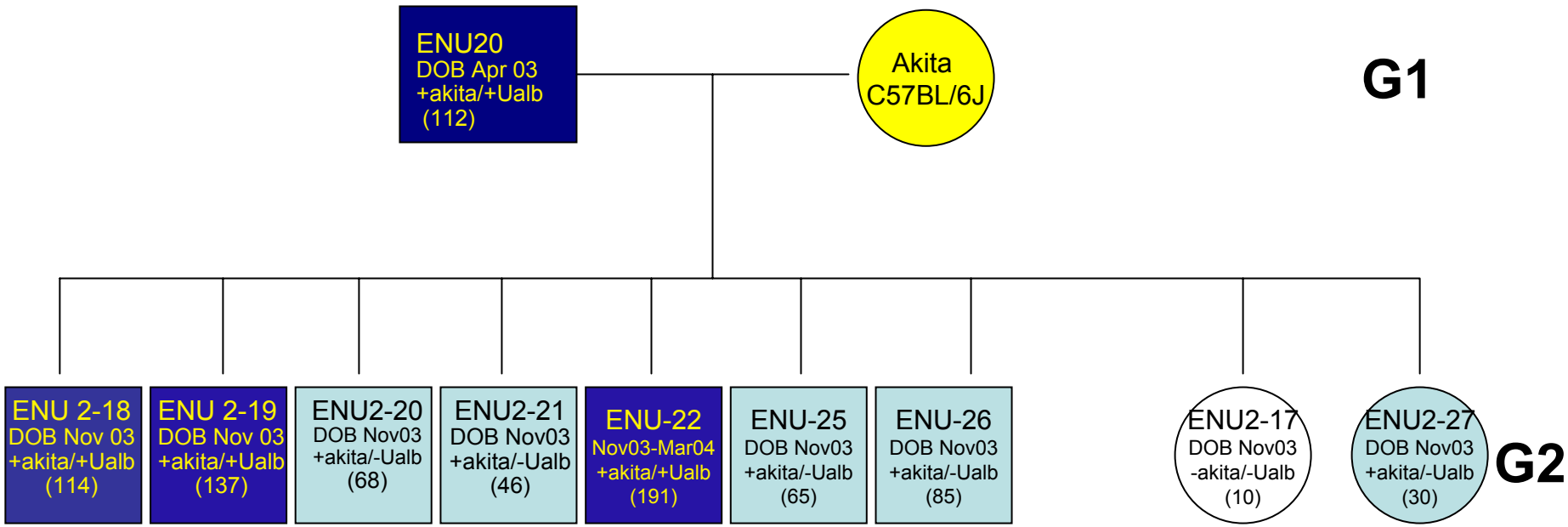




Identification of ACR “out-lyers”



Progeny of ENU20



- The trait is heritable.
- For a dominant trait linked to hyperglycemia we expect 50% of akita mice will be positive for albuminuria and in this line = 3/8 akita were positive for albuminuria

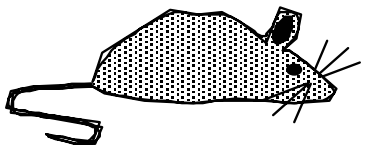
Characterization of Mutant Lines

ENU founders line (G1)	Total # of G2 mice	Total # of G3 mice	# of mice phenotyped	ACR (µg/mg)		
				Akita -	Akita+	albuminuric Akita+
ENU10	6	currently breeding	6	36.0 ±9.3 n=2	43.4±2.9 n=2	**98.0 ±3.6** n=2
ENU76	28	38	50	19.5 ±7.7 n=16	43.5±23.4 n=14	**125.4 ±6.2** n=3
ENU20	20	51	70	11.8 ±7.7 n=11	33.5±20.5 n=13	**108.8 ±44.5** n=9
ENU161	6	currently breeding	6	22.3 ±6.4 n=2	35.9±7.2 n=2	**110.1 ±9.0** n=2

Detailed Phenotyping of G3 to G5 Akita + progeny

1° Screen of Akita+ albuminuric mutants

Blood chemistries for G1 mutants



ID#	Na	K	Cl	Tco2	BUN	Creat	Glu	Hct%
ENU 10	143	4.7	107	19	32	0.6	>700	47
ENU 20	142	4.3	108	21	18	0.7	686	47
ENU 76	145	5.1	116	20	37	0.8	>700	52

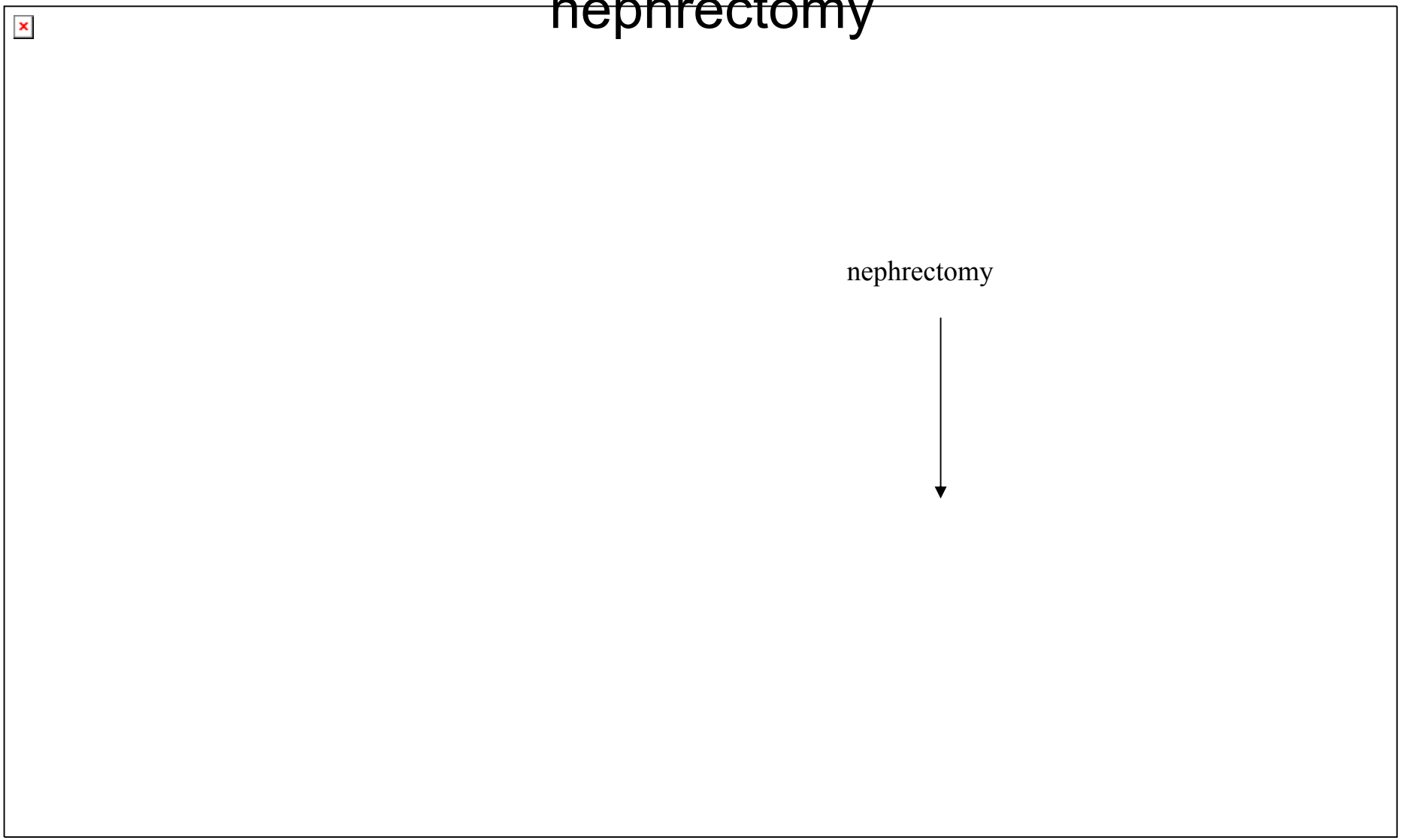
Values obtained with *iSTAT* performed in Vandy MMPC

24 hour urine albumin microscopy
GFR
serum chemistries, TG, cholesterol Hgb A1C
renal anatomy (ultrasound +micro CT scan)

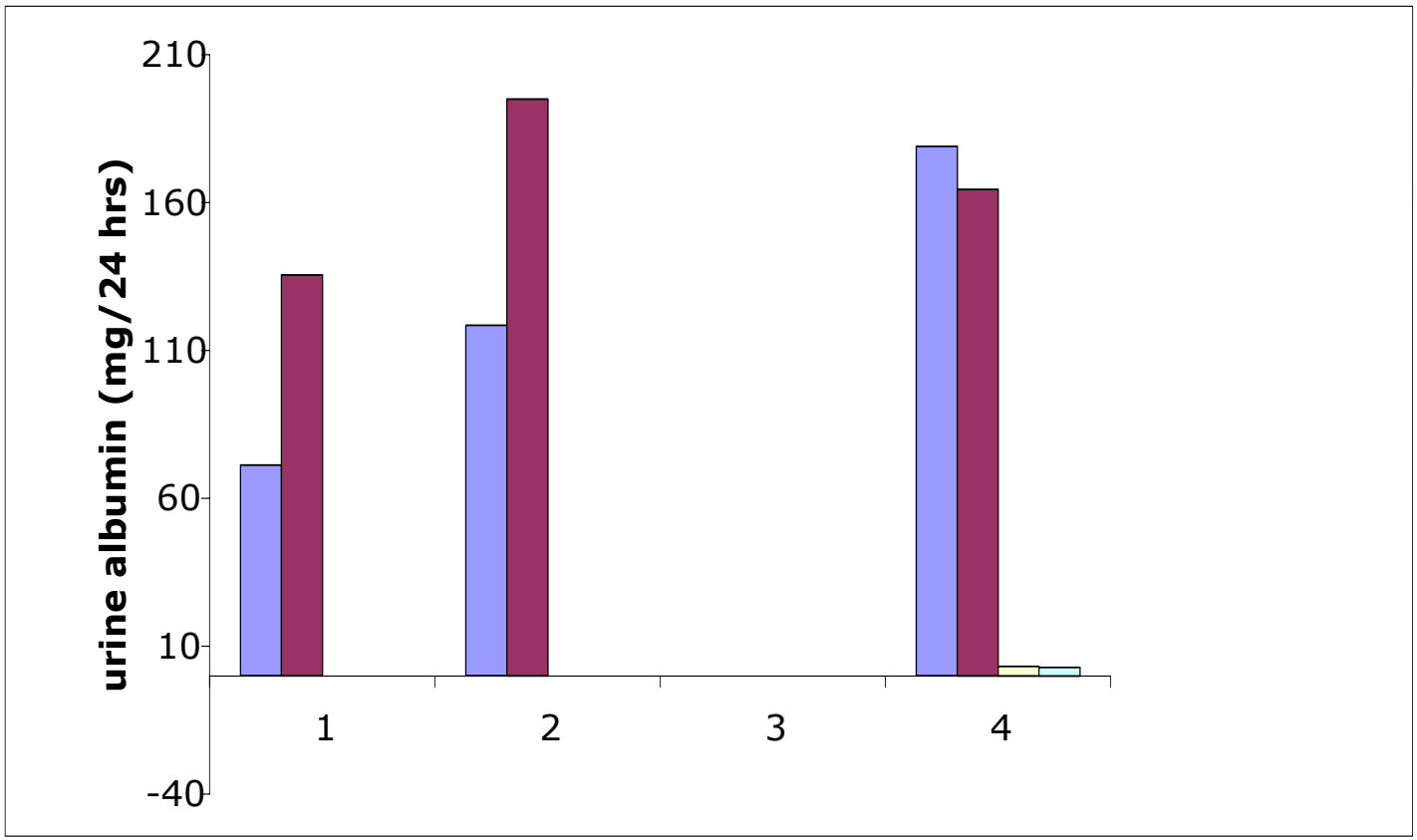
2° Screen

Renal histopathology
Blood pressure/telemetry
+
Uropathy
Retinopathy
Neuropathy
Cardiovascular
Wound healing?

Time course of ACR in mouse urine in ENU founders before and after unilateral nephrectomy

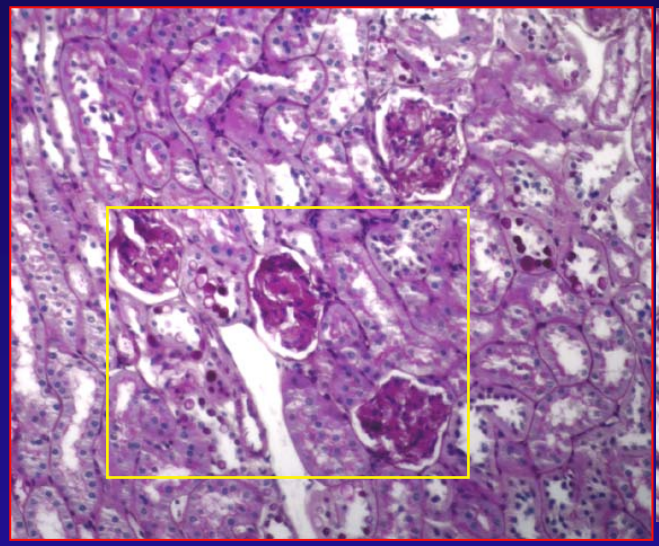


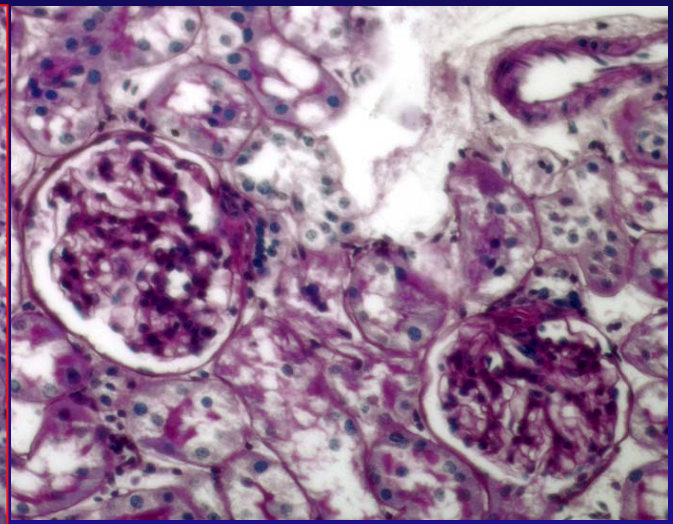
24 hour albumin excretion in ENU founders before and after unilateral nephrectomy



Renal Histopathology in ENU mutants

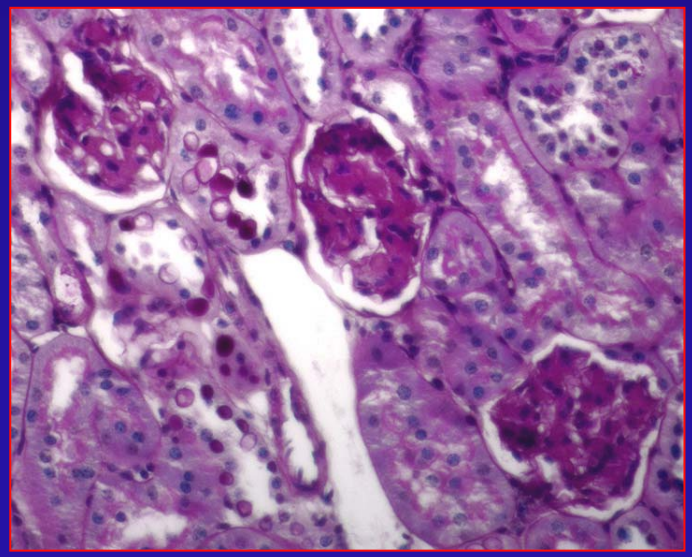
ENU 76

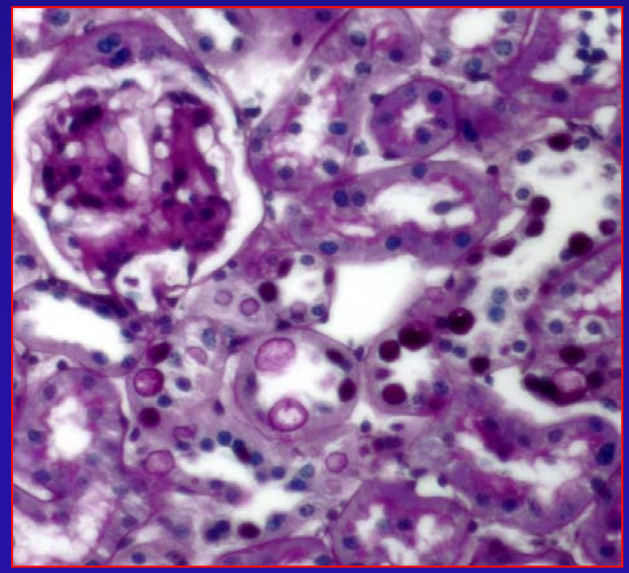




ENU 129 PAS
Non-albuminuric

ENU 76





ENU 10

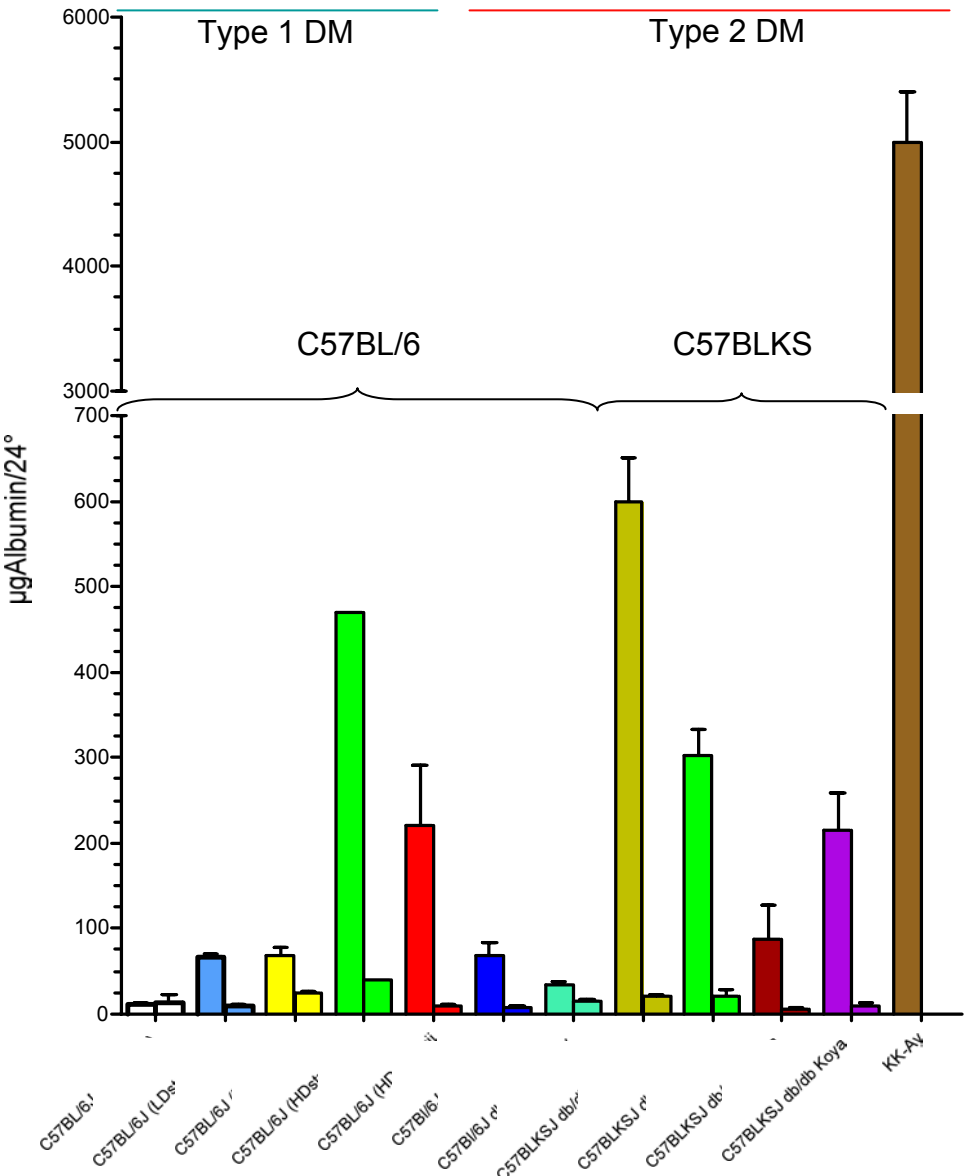
Generating mouse mutants with Diabetic Nephropathy

- Candidate Genes: eNOS db/db KLS
- Random mutagenesis: ENU Akita
- **Strain dependence:** KK and DBA2 STZ

MOUSE MODELS OF DIABETIC NEPHROPATHY
(In Press J. Am. Soc. Nephrol. For Publication Jan 2005)

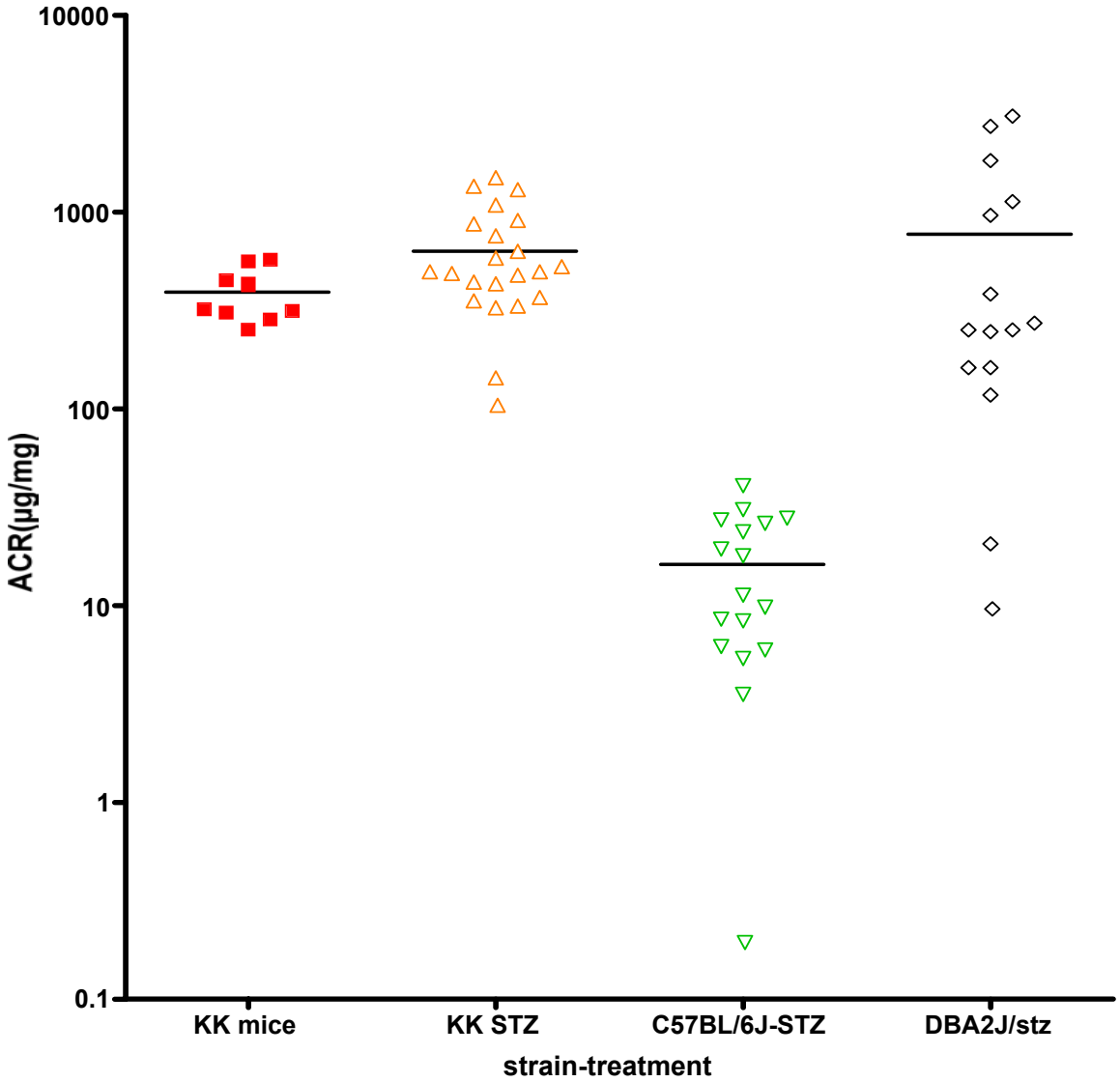
Matthew D. Breyer[1], Erwin Böttinger[2], Frank C. Brosius, III[3], Thomas M. Coffman[4], Raymond C. Harris[1], Charles W. Heilig[5], and Kumar Sharma[6] (For the AMDCC)

1

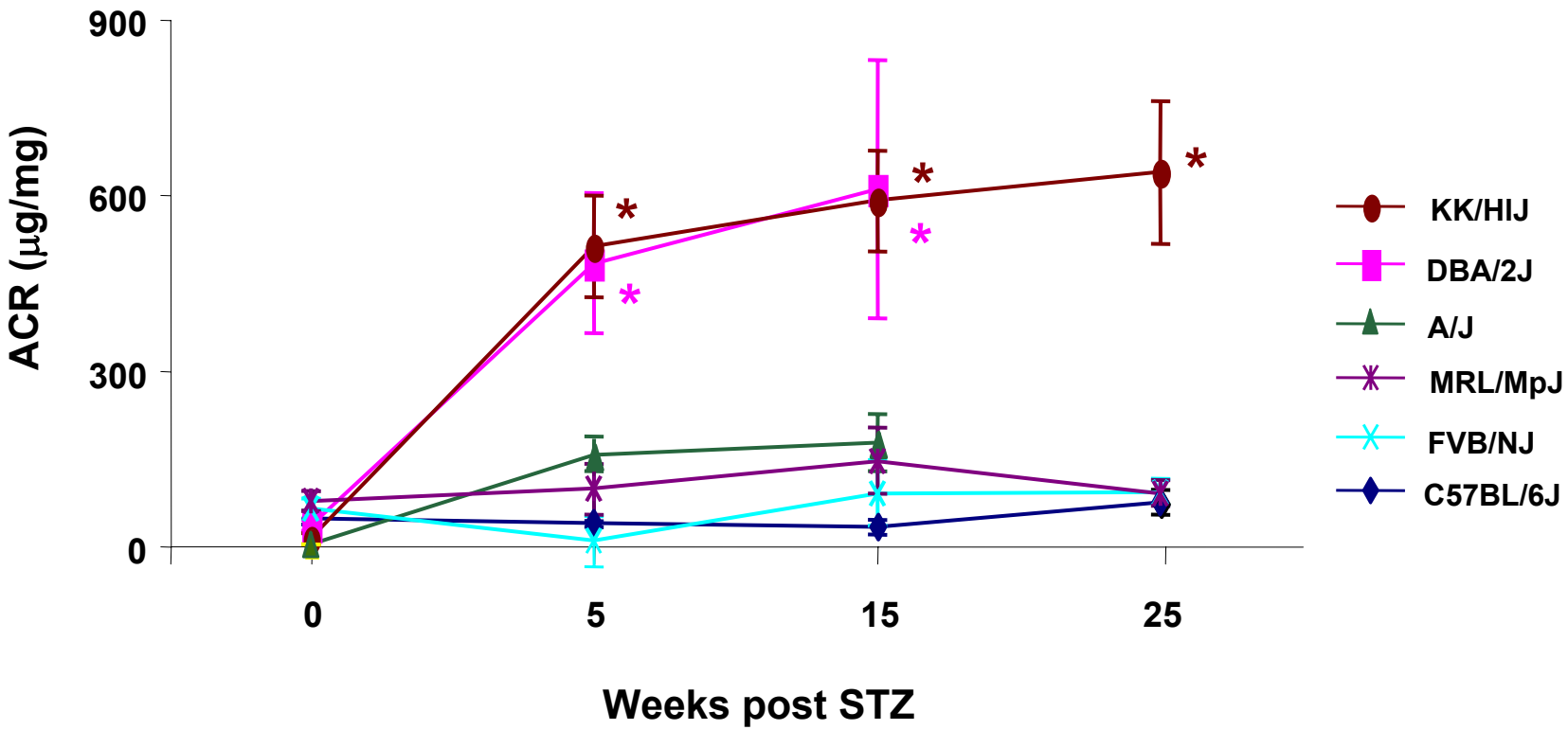


24hour Urine Albumin excretion reported in the literature for mouse models of Diabetes

Individual data for Ualb:Cre excretion in diabetic mice

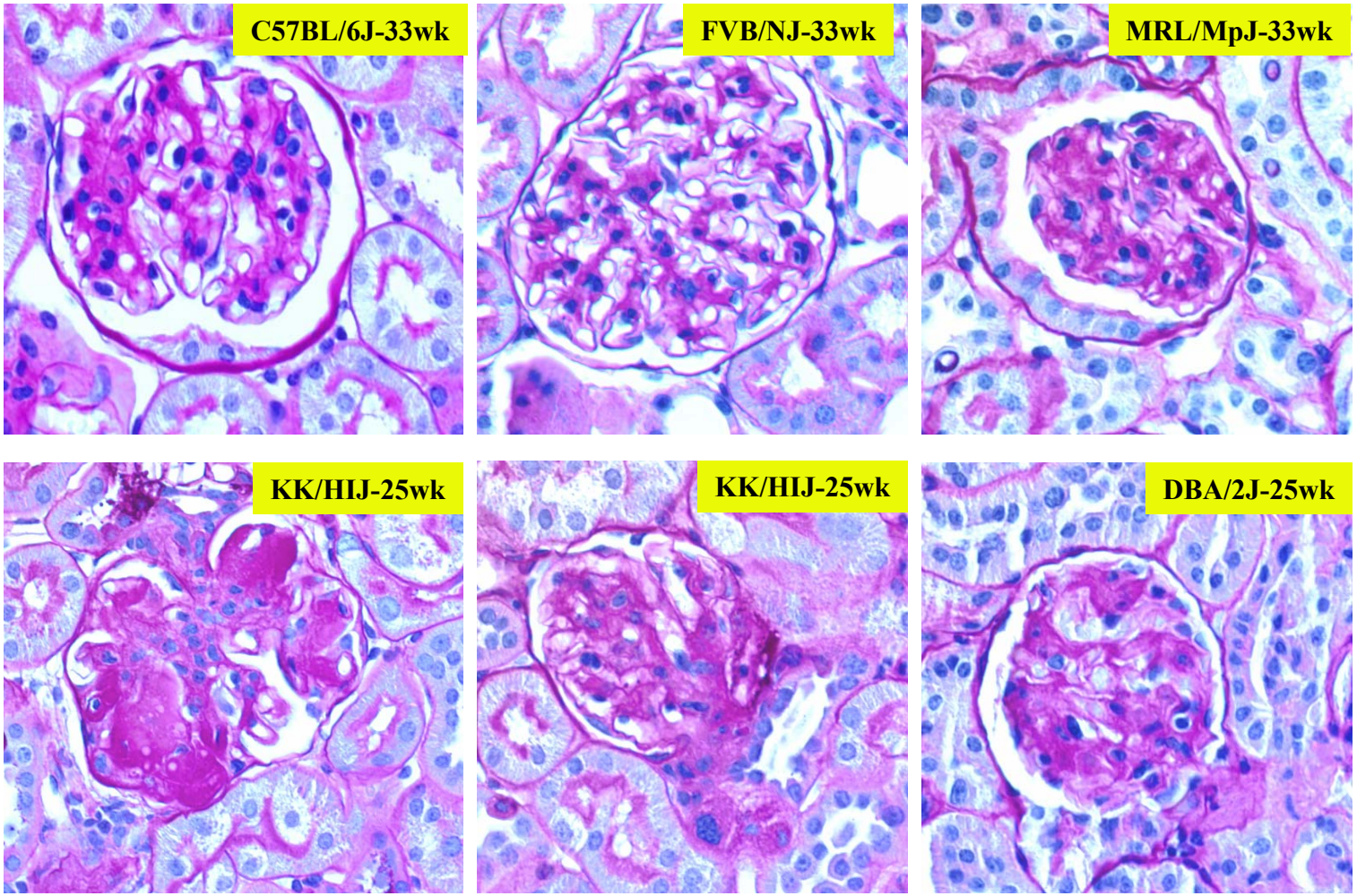


Urinary albumin excretion in type 1 diabetic inbred mice

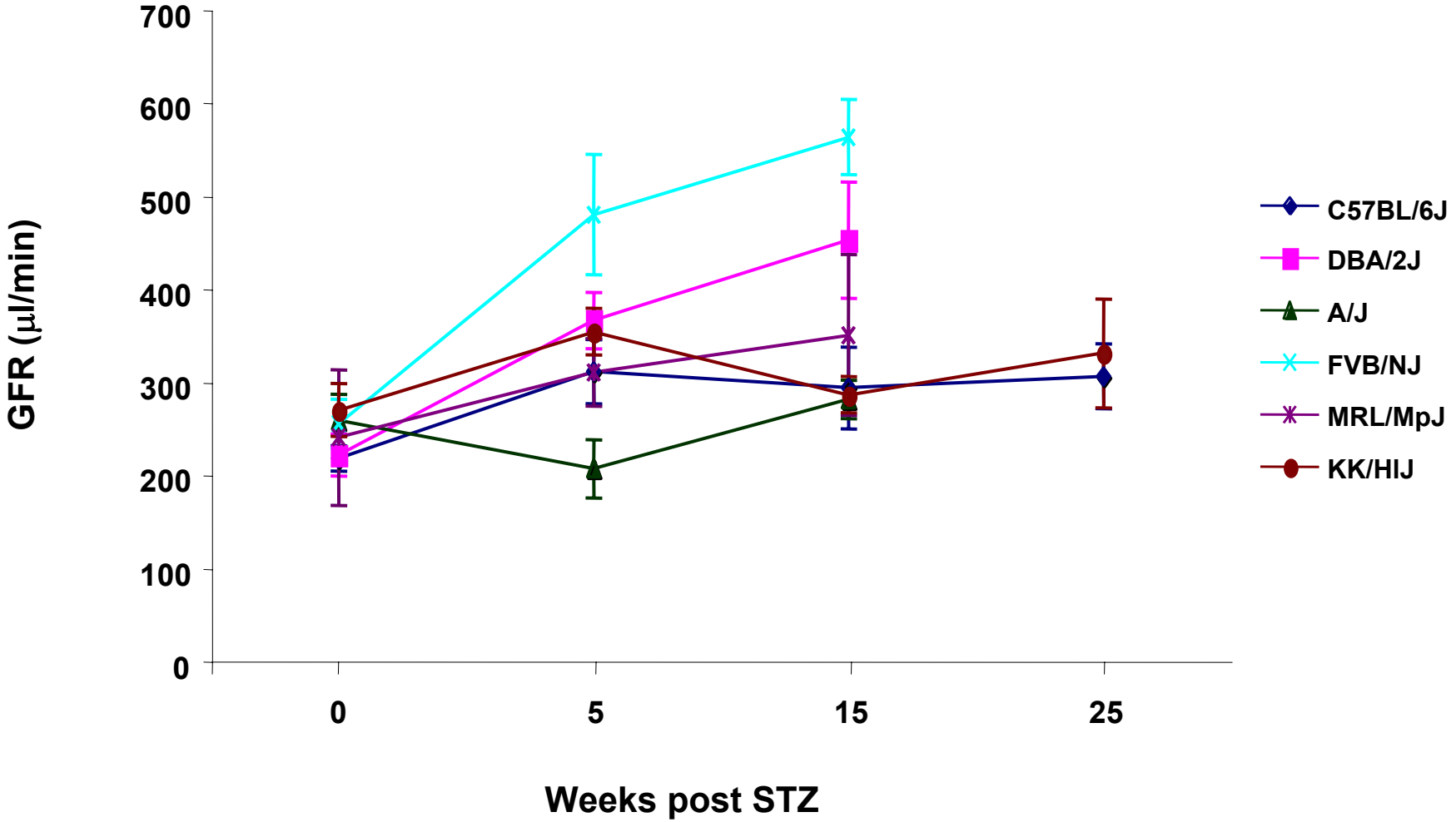


ANOVA: * vs ACR at same time point in A/J, FVB/NJ, MRL/MpJ, and C57BL/6J, $P<0.05$

Glomerular morphology in diabetic inbred strains

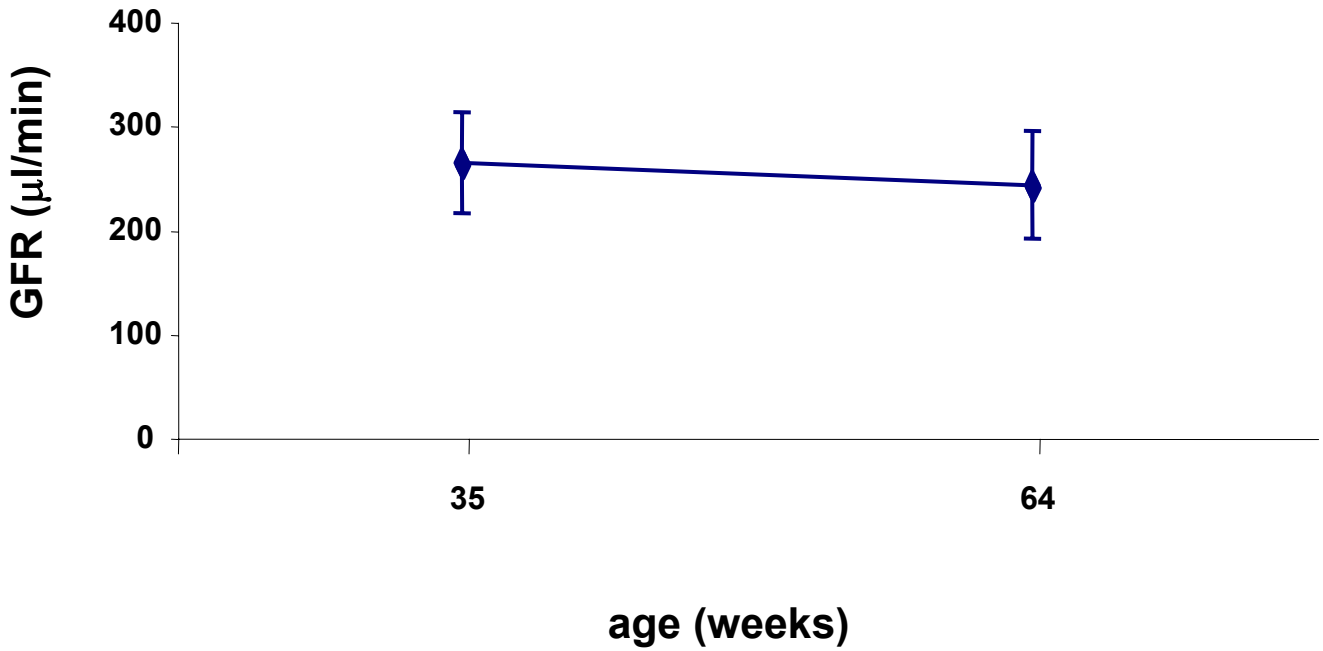


GFR in diabetic inbred mice



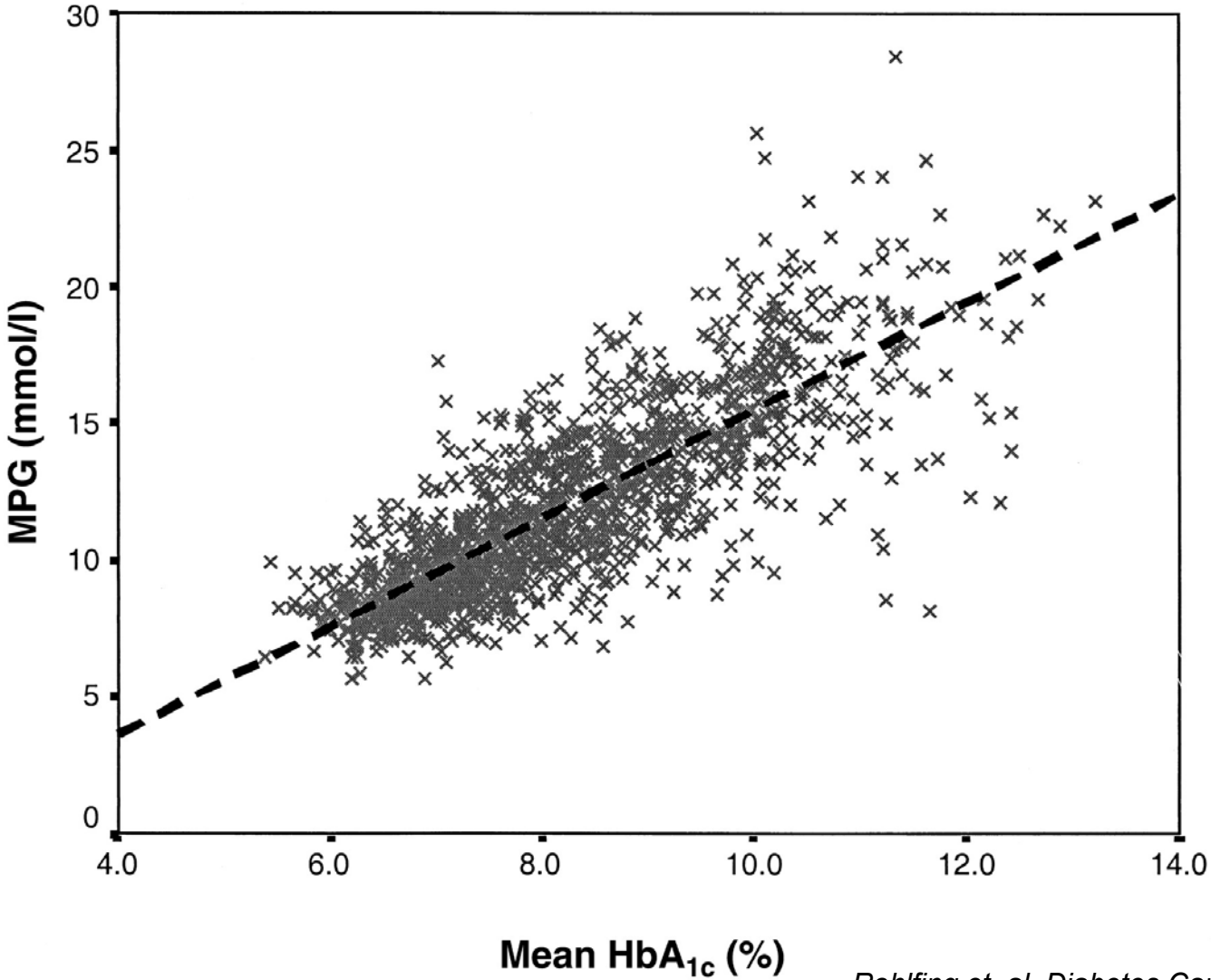
ANOVA: 5th week vs basal, P<0.05 in
C57BL/6J, DBA/2J, FVB/NJ, and KK/HIJ strains

GFR in Non-STZ injected KK/HIJ mice



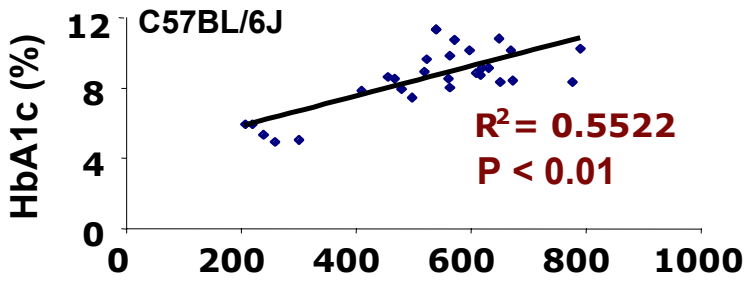
Glycated Hemoglobins and blood glucose in mouse and man

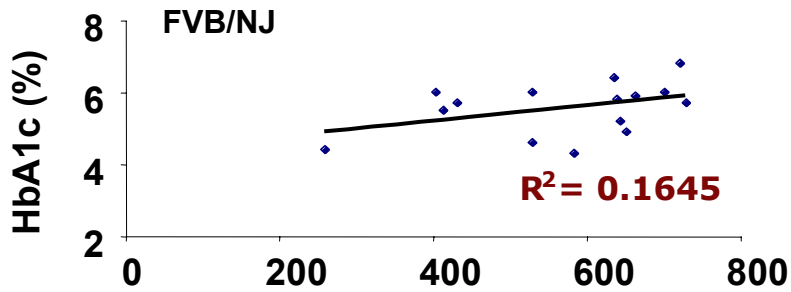
Relationship between HbA1c and Mean Plasma Glucose

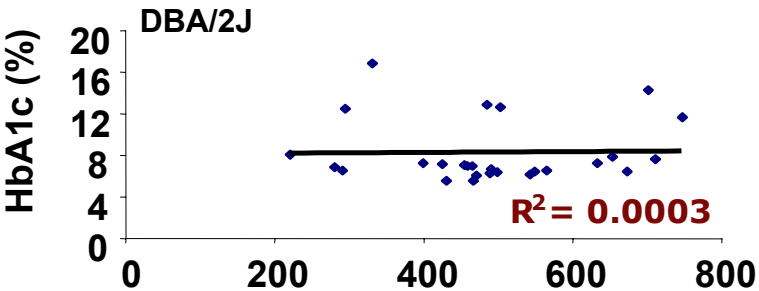


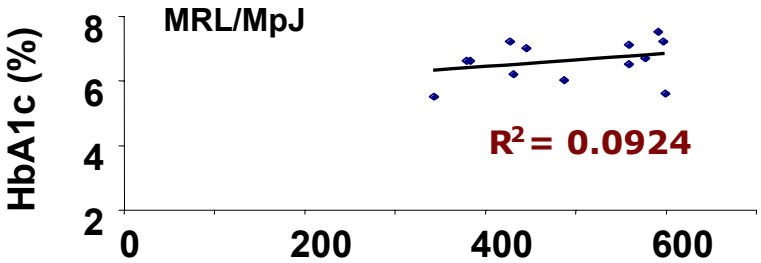
Rohlfing et. al. Diabetes Care 25:275-278, 2002

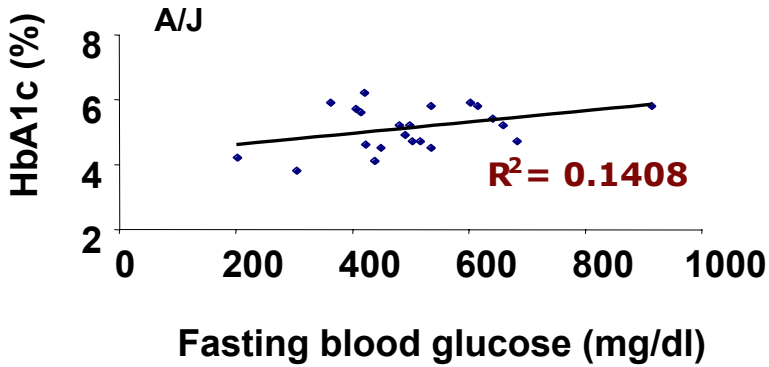
Correlation Between Blood Glucose and HbA1c in diabetic inbred mice

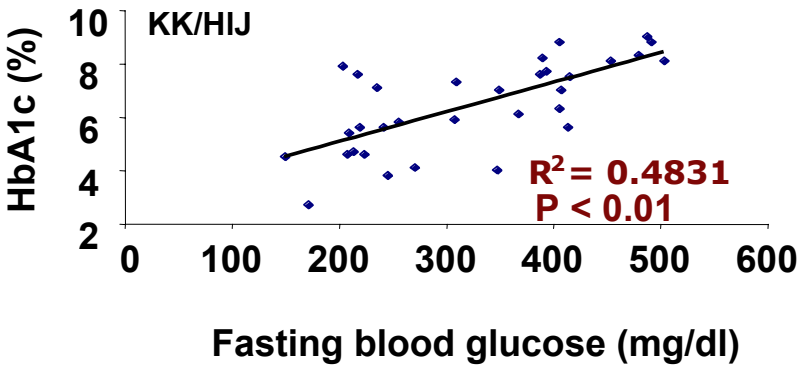






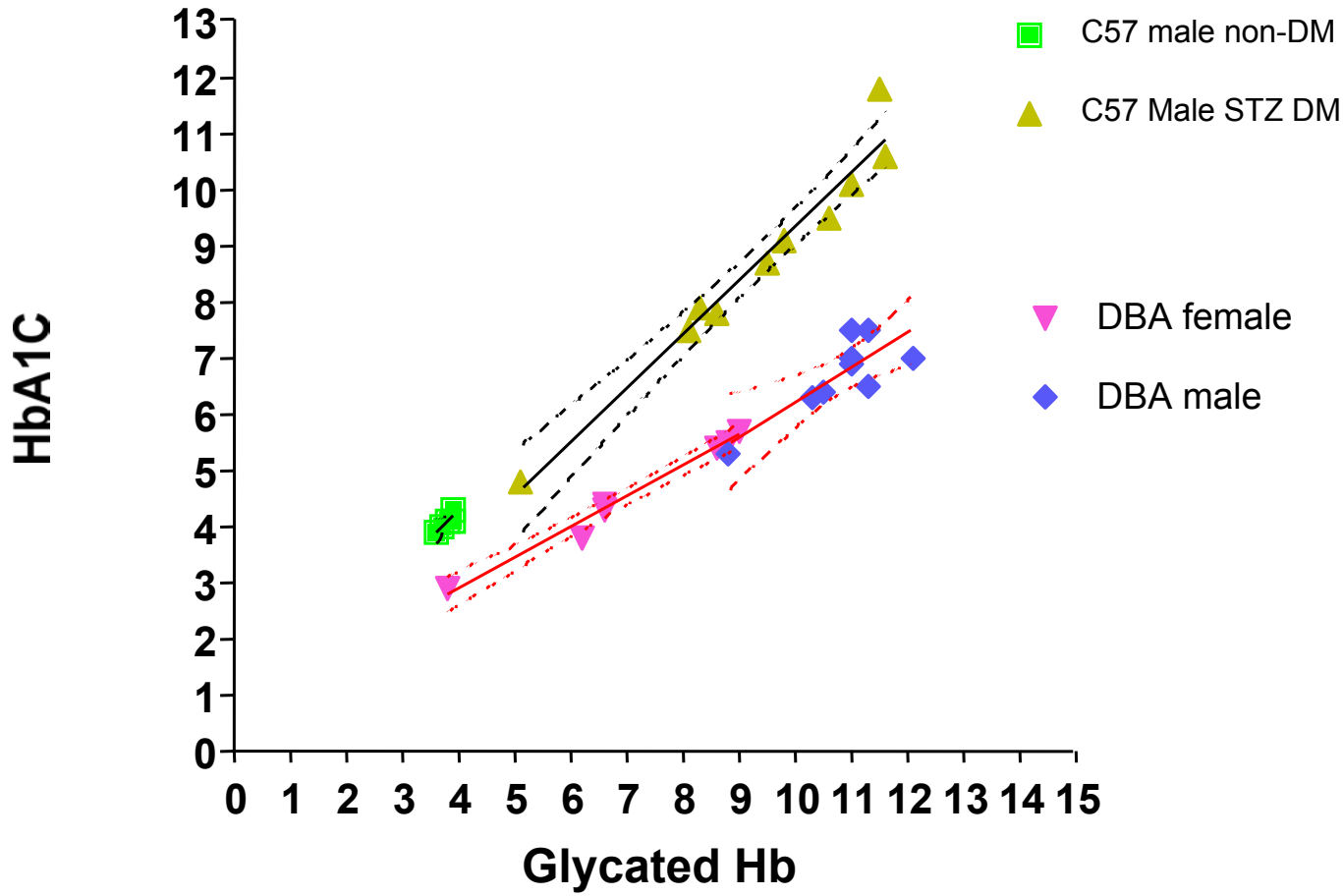




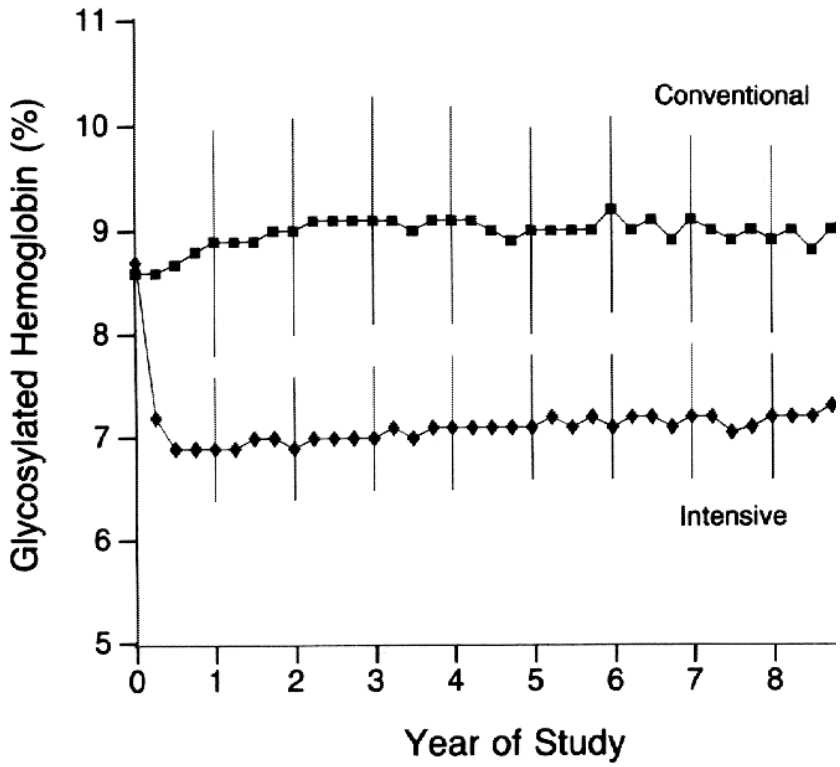


Biorad Variant HPLC Glycated Hb vs DCA2000

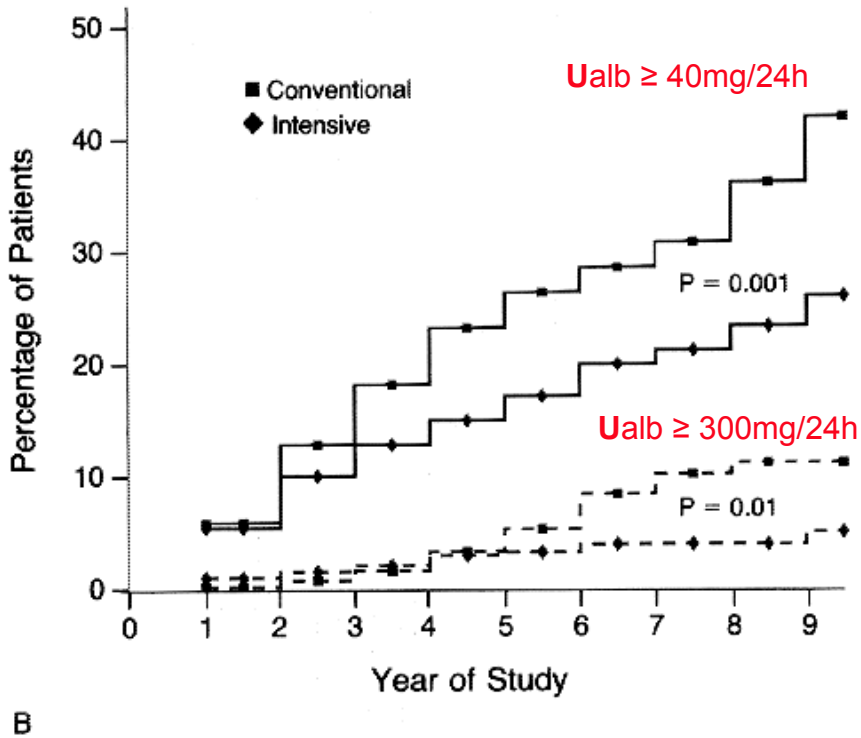
Correlation between Glycated Hb and HbA1C by strain



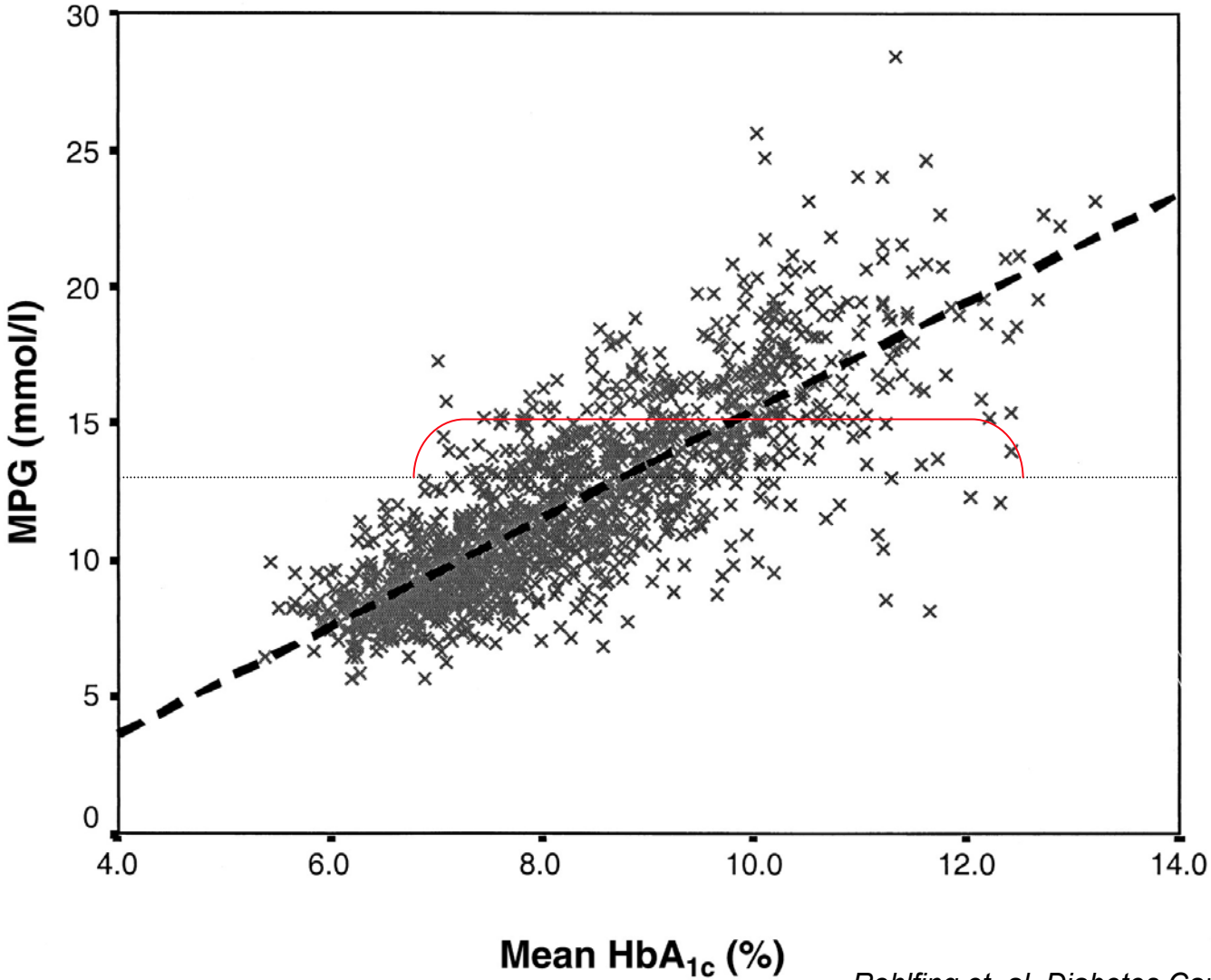
Reducing HgA1c is associated with reduced complications: results from DCCT



Secondary intervention (mild retinopathy) cohort

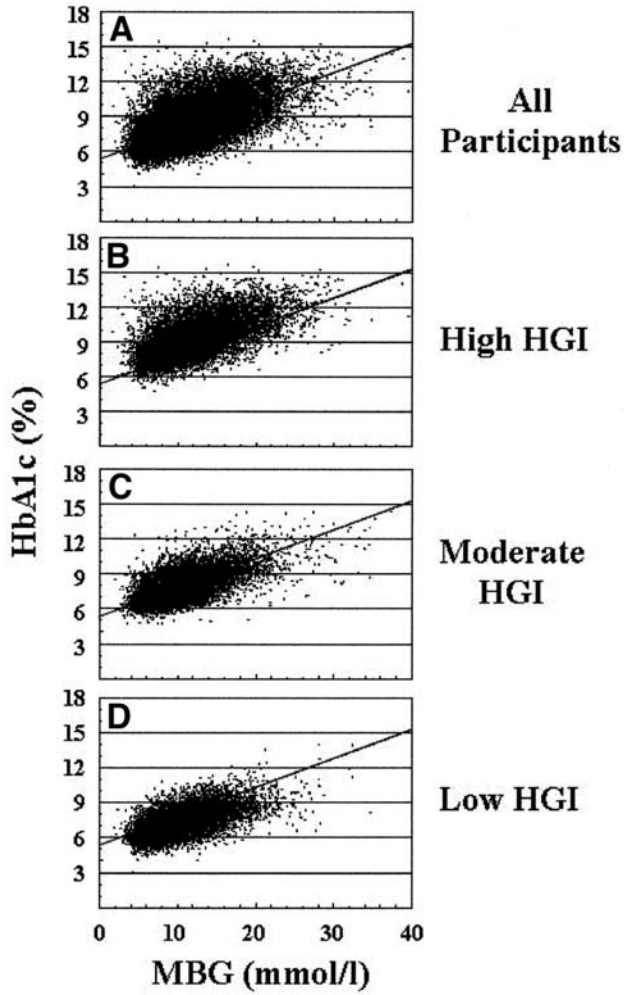


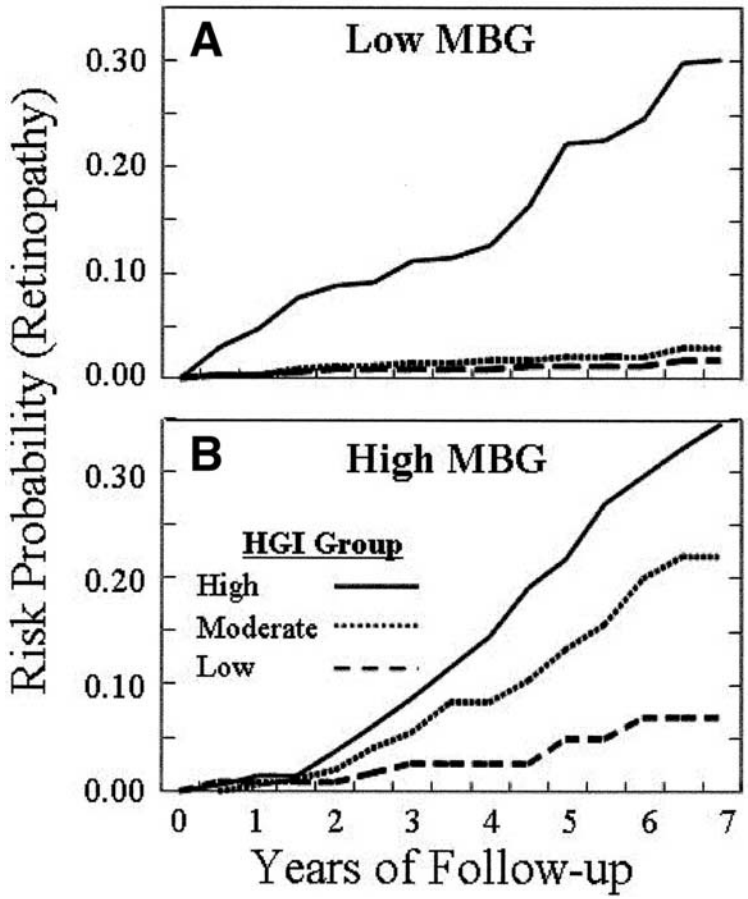
Relationship between HbA1c and Mean Plasma Glucose



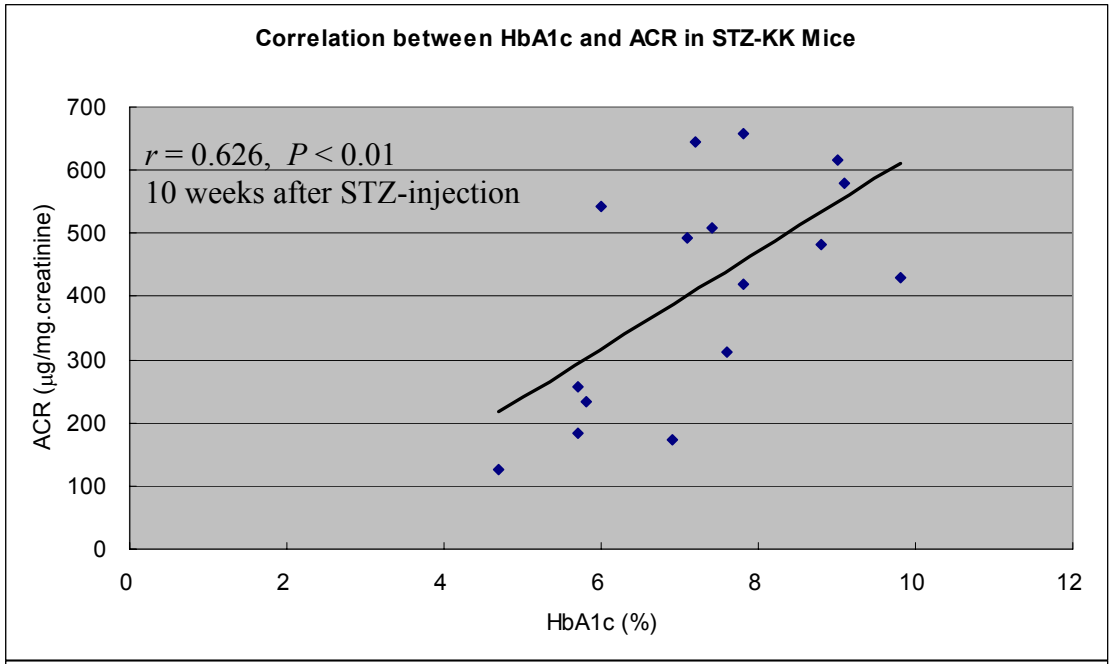
Rohlfing et. al. Diabetes Care 25:275-278, 2002

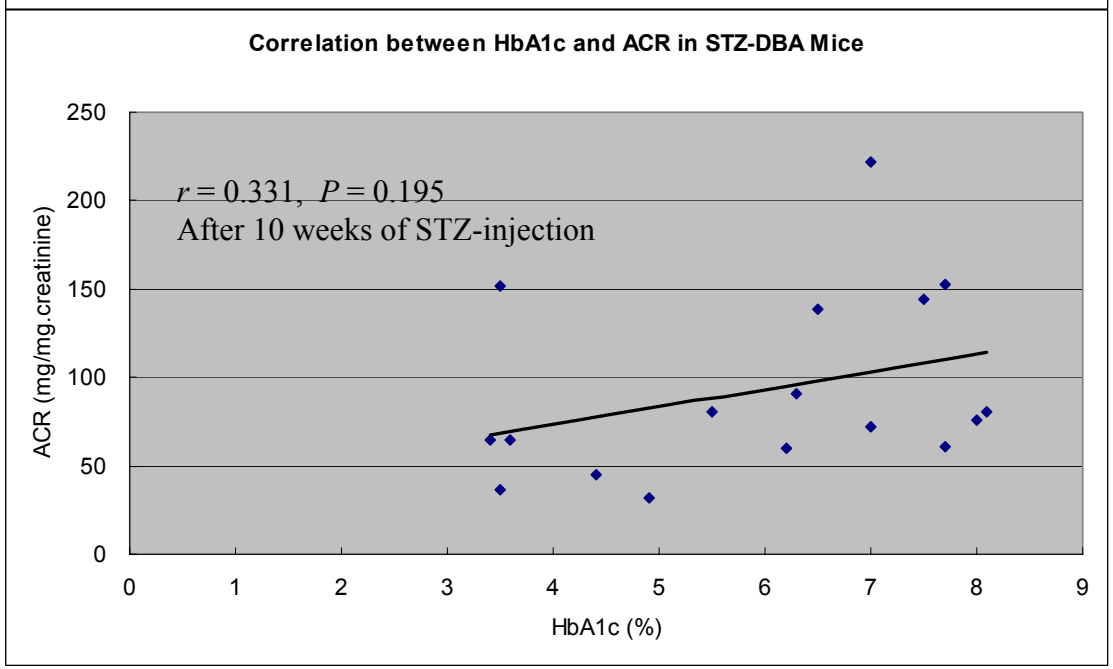
High glycators vs. Low Glycators: Risk of complications





McCarter et. al. Diabetes Care 27:1259-1264, 2004 © 2004


Correlation between HbA1c and ACR in STZ-KK Mice
r = 0.626, P < 0.01
10 weeks after STZ-injection
ACR (μg/mg.creatinine)
HbA1c (%)
0
100
200
300
400
500
600
700
0
2
4
6
8
10
12


Correlation between HbA1c and ACR in STZ-DBA Mice
r = 0.331, P = 0.195
After 10 weeks of STZ-injection
ACR (mg/mg.creatinine)
HbA1c (%)
0
50
100
150
200
250
0
1
2
3
4
5
6
7
8
9

Current Models

*planned, not yet started

Animal model	Background strain	Diabetic Model	current status	Phenotyping (begin-end)	Consortium collaborators
db/db	C57BLKS	Type 2	Final phenotyping		
ApoE-/- db.db	C57BLKS	Type 2	Phenotyping ongoing	11-03 to 12-04	Rockefeller*
LDLr-/- db/db	C57BLKS	Type 2	phenotyping ongoing	11-03 to 12-04	Rockefeller*
eNOS db/db	c57Bl/6J	Type 2	phenotyping ongoing	11-03 to 12-04	Rockefeller*
eNOS-/-	C57BLKS	--	Phenotyping ongoing	11-03 to 12-04	UCLA*
eNOS-/- STZ	C57BLKS	Type 1	phenotyping beginning	10-04 to 9/06	UCLA *
eNOS-/- Akita	c57Bl/6J	Type 1	breeding beginning	10-03 to 6-04	UCLA *
ENU 10-Akita	c57Bl/6J	Type 1	Final phenotyping	6-03 to 3-05	UofM/Rock/CWRUUtah*
ENU 20-Akita	c57Bl/6J	Type 1	Final phenotyping	6-03 to 3-05	UofM/Rock/CWRUUtah*
ENU 76-Akita	c57Bl/6J	Type 1	Final phenotyping	6-03 to 3-05	UofM/Rock/CWRUUtah*
ENU 161-Akita	c57Bl/6J	Type 1	Final phenotyping	6-03 to 3-05	UofM/Rockefeller/Utah*
DBA2 Akita	DBA2	Type 1	Backcross at N5	9/04-9/06	CWRU (Retinopathy) UTAH(Cardiac)*
Akita	C57BL6	TYPE 1	FINAL PHENOTPYING	6-03 to 12-04	CWRU (Retinopathy)
KK-STZ	KK	TYPE 1	25 wk of hyperglycemia	starting c Insulin Rx 10/04-9/06	CWRU (Retinopathy))
KK AKITA	KK	TYPE 1	BACKCROSS AT N3	9/05- 9/06	
KK-Ay (agouti)	KK	TYPE 1I	breeding	10/04 - 9/06	
A/J STZ	A/J	Type 1	Week 16	10/05	CWRU (Retinopathy)
DBA-STZ	DBA	Type 1	Week 16	starting c Insulin Rx 10/04-9/06	CWRU (Retinopathy)
nestin cre (podocyte-neuron) x flox SOD2-STZ	c57Bl/6J	Type 1	Tissue specific kos established	Neuro-renal 11-03 to 12-04	MICHIGAN

Ongoing studies

- eNOS-/-
  - Type 1 models: STZ, Tg pdx1-HNF-6, Akita
  - Later measures of blood pressure, 24° urine albumin excretion, HbA1c, GFR,
- ENU mutagenesis
  - Expand line numbers
  - HPLC Serum Creatinine, Inulin clearance, histopathology, chol/triglycerides in 3-4 lines
  - Backcross Akita mutation to A/J for mapping of mutations
- Strain phenotyping
  - Phenotype KK GFR and DBA GFR at later ages-requires insulin replacement.
  - Eyes Sent to Tim Kern for retinopathy evaluation
  - Backcross Akita mutation to DBA/KK/A-J
  - Validate HbA1C and define correlation between:
    - FBS and 24° glucose monitoring vs. HbA1C
    - ACR and glycated Hb