Endothéline et maladies rénales

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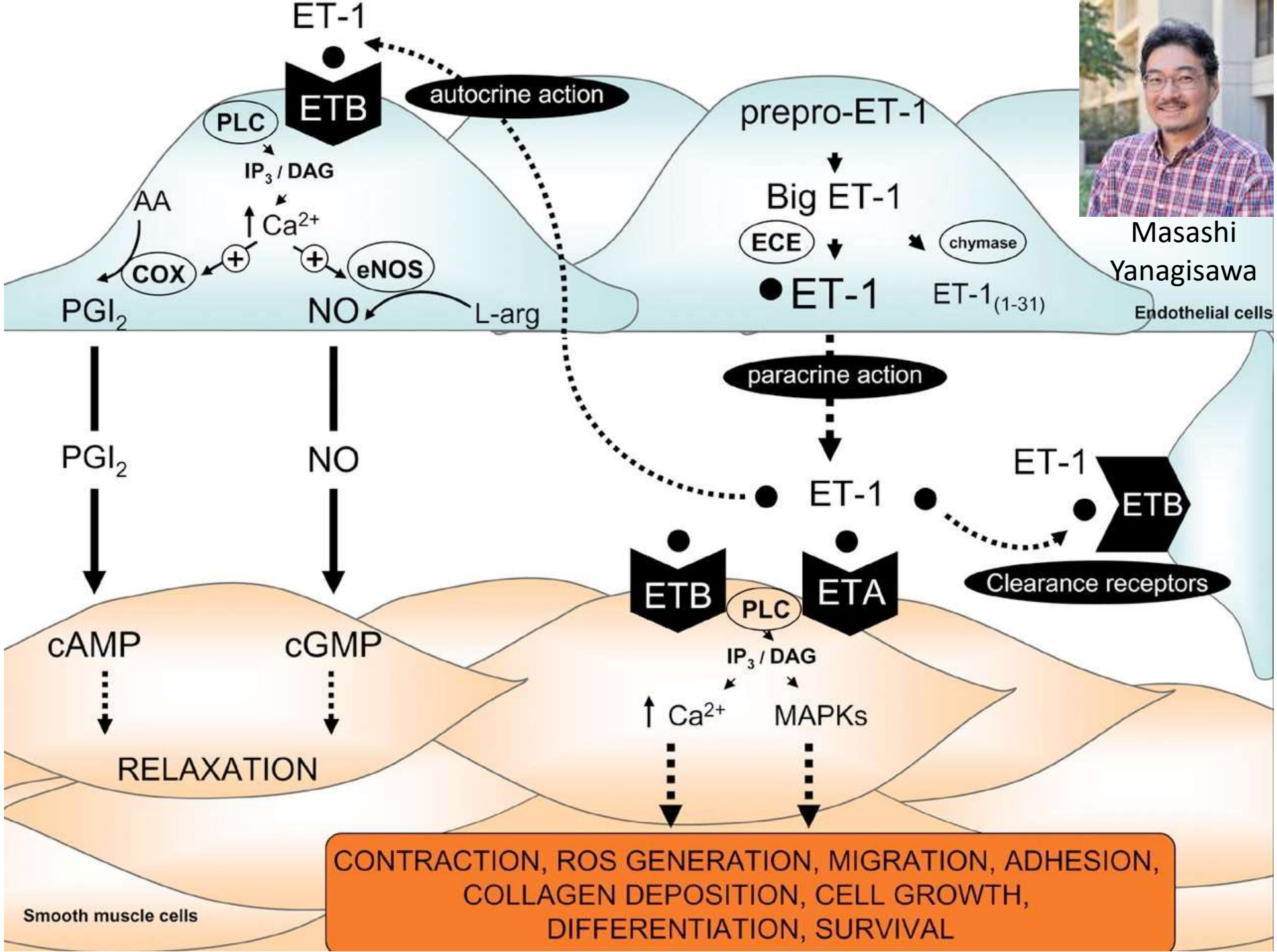
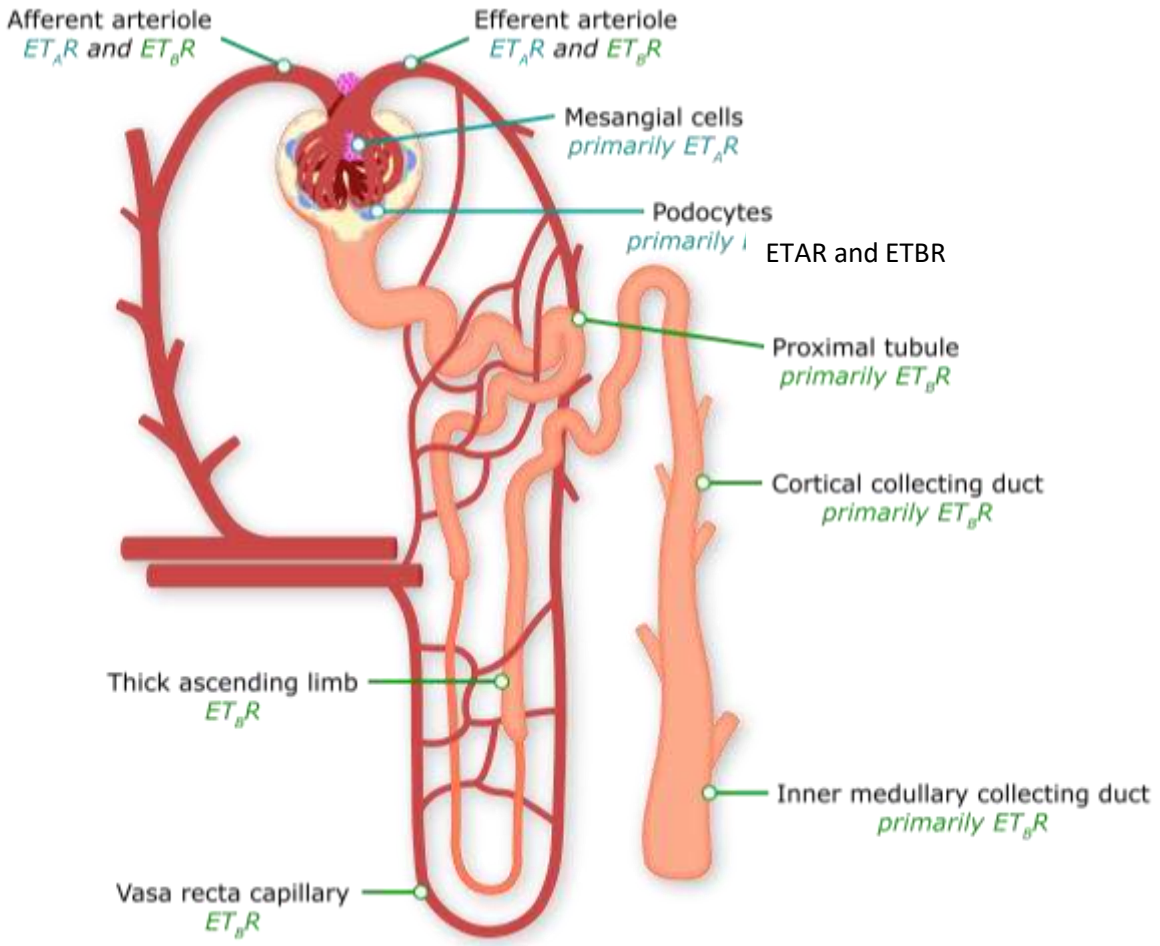


Masashi Yanagisawa

Endothelin-1 is the Most Biologically Relevant Endothelin to Kidney Physiology

- Highly stable molecule[1]
- Extremely potent vasoconstrictor[1]
- Long lasting effects[1]
- Produced most prominently in the kidney[2] and involved in many disease models[4].

Endothelin Receptors Are Ubiquitously Expressed in the Kidney[1,3,4]

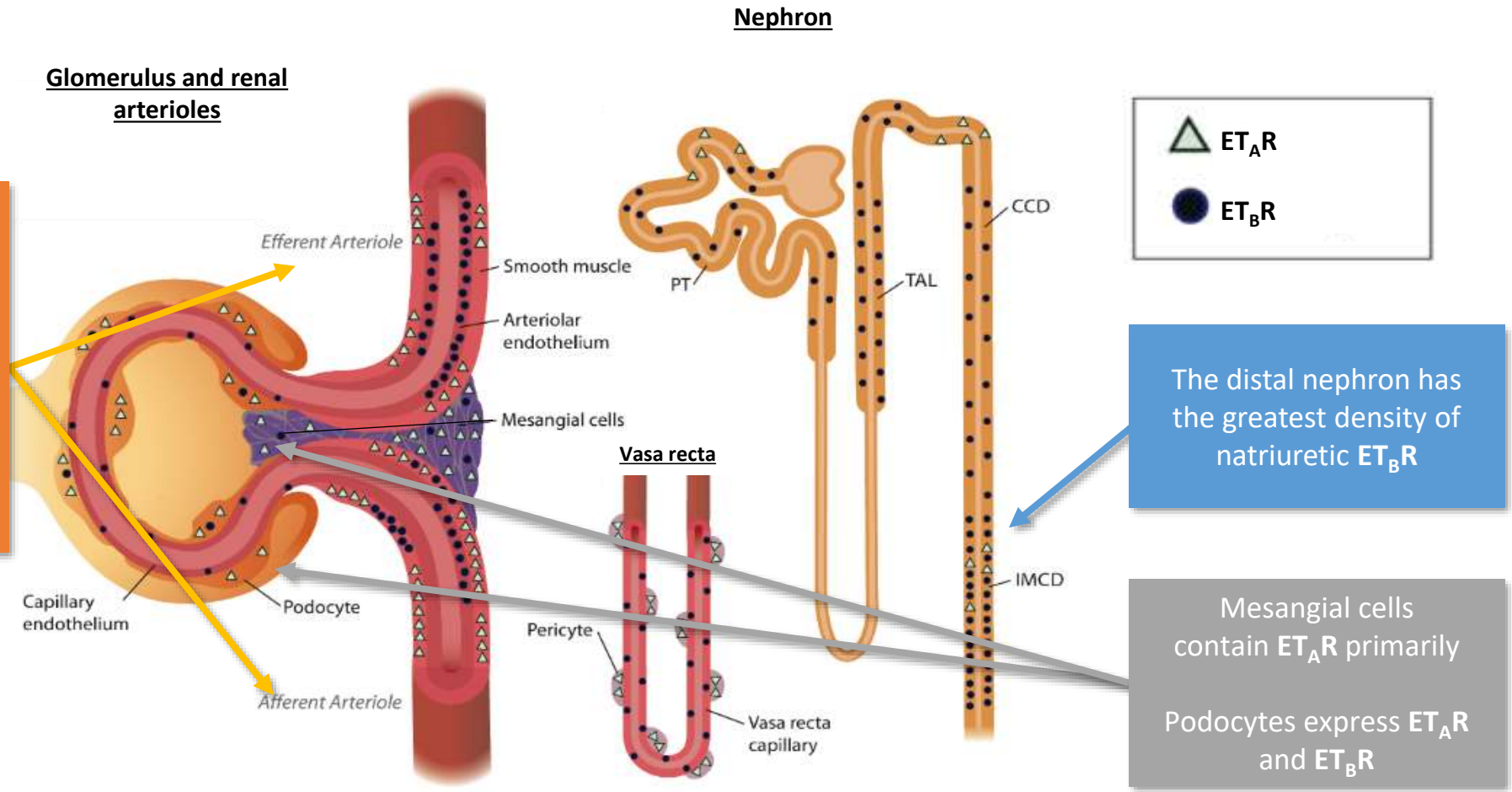


ET_AR, endothelin type A receptor; ET_BR, endothelin type B receptor.

1. Kohan D, *et al. Physiol Rev* 2011; **91:**1–77;
2. Kitamura K, *et al. Biochem Biophys Res Commun* 1989; **162:**38–44;
3. Maguire JJ & Davenport AP. *Semin Nephrol* 2015; **35:**125–136.
4. Fligny C, *et al. Contrib Nephrol*. 2011;172:120-138.

The Density of ET_AR and ET_BR Differs Across Different Renal Compartments

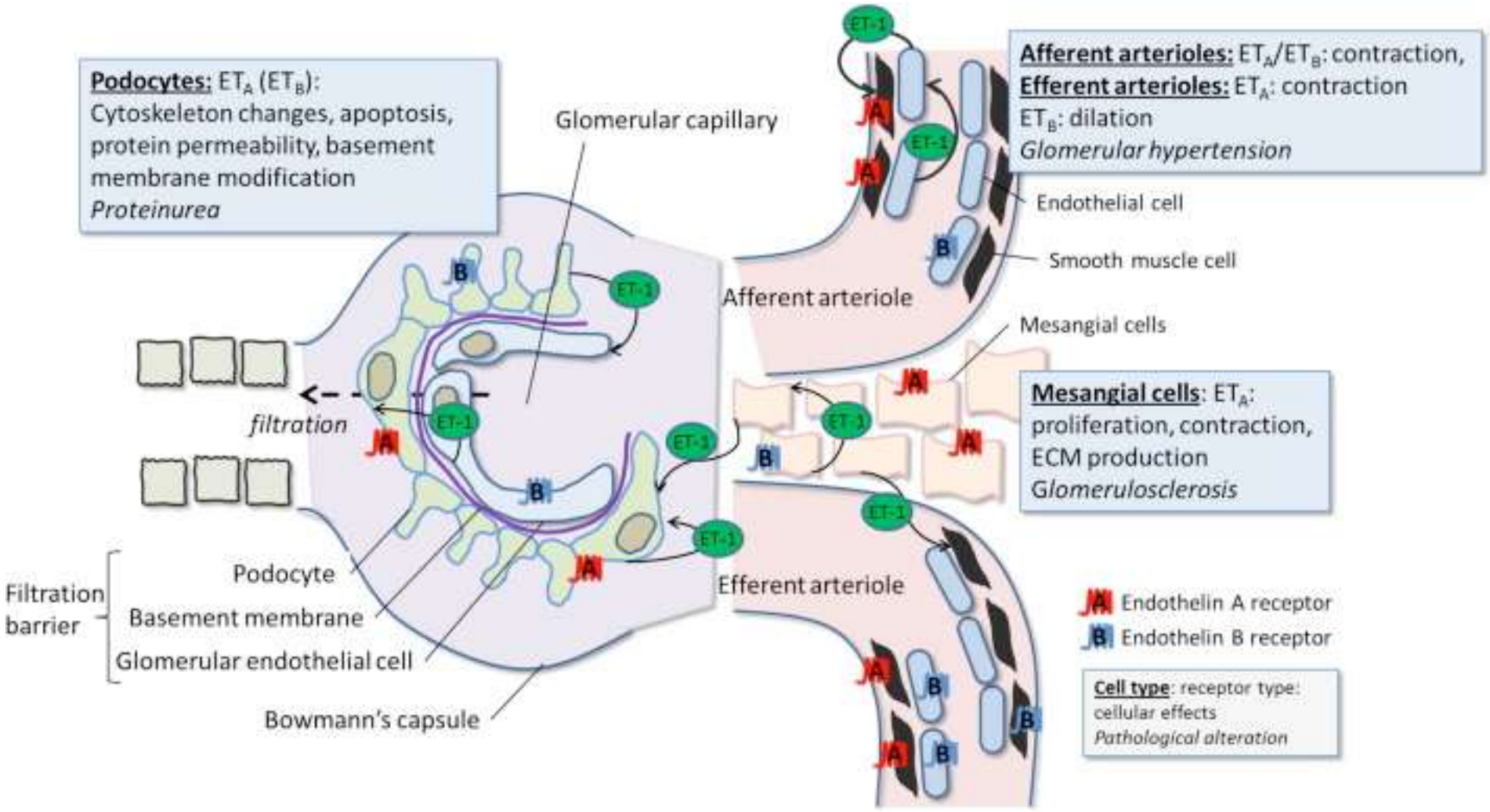
- ***Afferent and Efferent arterioles*** smooth muscle express vasoconstrictive **ET_AR**, mediating many of the physiologic actions of ET-1
- The endothelium express vasodilatory **ET_BR**



The actions of ET-1 are complex. ET-1 effect is exerted through the ET_AR-mediated balance of afferent and efferent arteriole actions; this balance changes in different settings.
Note: The amount of ET receptor shown in a given area is representative of the level of ET receptor activity in that region.
CCD = cortical collecting duct; ET_AR = endothelin receptor type A; ET_BR = endothelin receptor type B;
IMCD = inner medullary collecting duct; PT = proximal tubule; TAL = thick ascending limb.

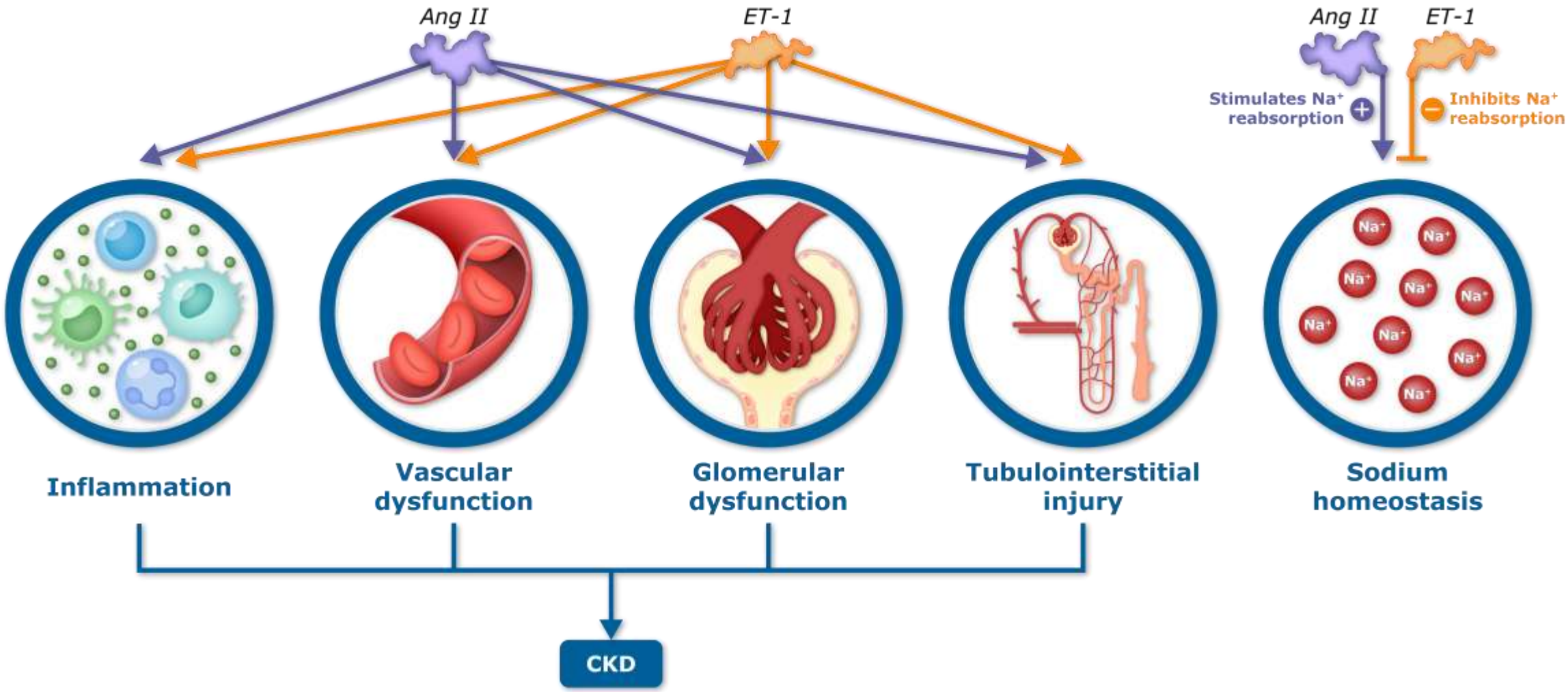
Maguire JJ & Davenport AP. *Semin Nephrol* 2015; **35:**125–136; Figure: Kohan DE, *et al. Compr Physiol* 2011; **1:**883–919.

Preferential vascoconstrictive action of ET-1 on the efferent arteriole



Vignon-Zellweger N et al. Life Sciences 91 (2012) 490–500

ET-1 and Ang II Act in Tandem to Promote CKD Progression via Multiple Mechanisms

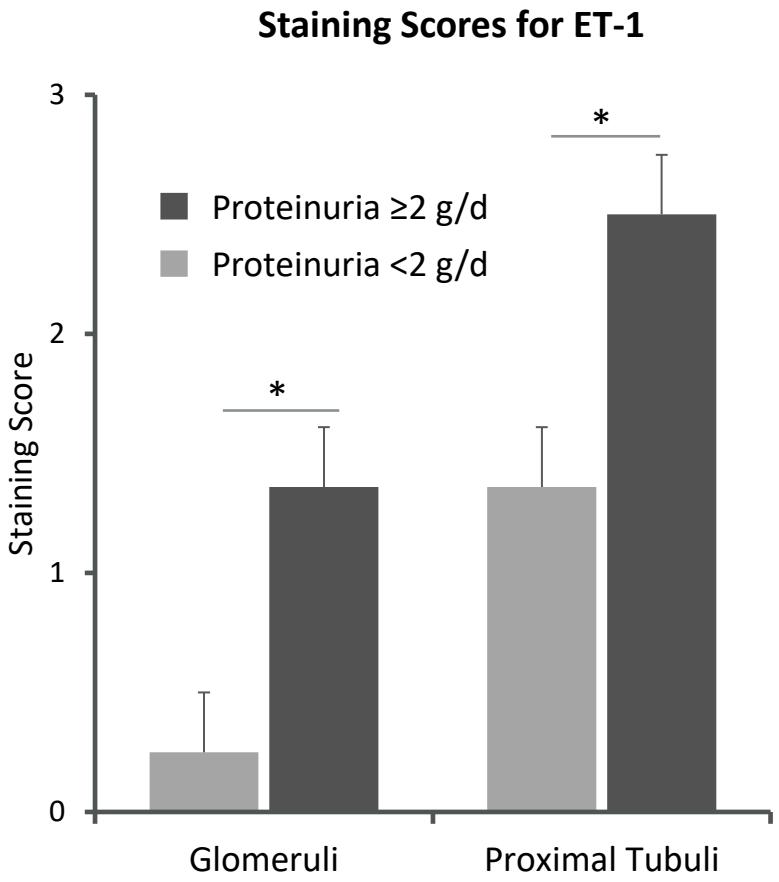


Ang II: angiotensin II
CKD: chronic kidney disease
ET-1: endothelin-1.

Kohan DE & Barton M. *Kidney Int* 2014; **86:**896–904;
Lenoir O, *et al. J Am Soc Nephrol*. 2014;25(5):1050-62;
Komers R & Plotkin H. *Am J Physiol Regul Integr Comp Physiol* 2016; **310:**R877–R884;
Raina R, *et al. Kidney Dis* 2020; **6:**22–34

Increased Levels of ET-1 Are Seen in the Biopsies from Patients with IgA Nephropathy

Immunohistochemical analyses of kidney biopsies of patients with IgA nephropathy (n=16)



Key findings from study

Expression of **ET-1** in **glomeruli and proximal tubular epithelial cells** was significantly greater among patients with **higher-grade proteinuria** (≥2 g/day) than among patients with lower-grade proteinuria (<2 g/day) or controls

*P<0.05 versus IgA nephropathy with lower-grade proteinuria; Staining scores: 0 = no staining; 1 = weak staining; 2 = intermediate staining; 3 = strong staining.
ET-1, endothelin-1; IgA, immunoglobulin A.
Lehrke I, *et al. J Am Soc Nephrol* 2001; **12:**2321–2329.

Several Studies Suggested a Role for ET-1 in IgA Nephropathy

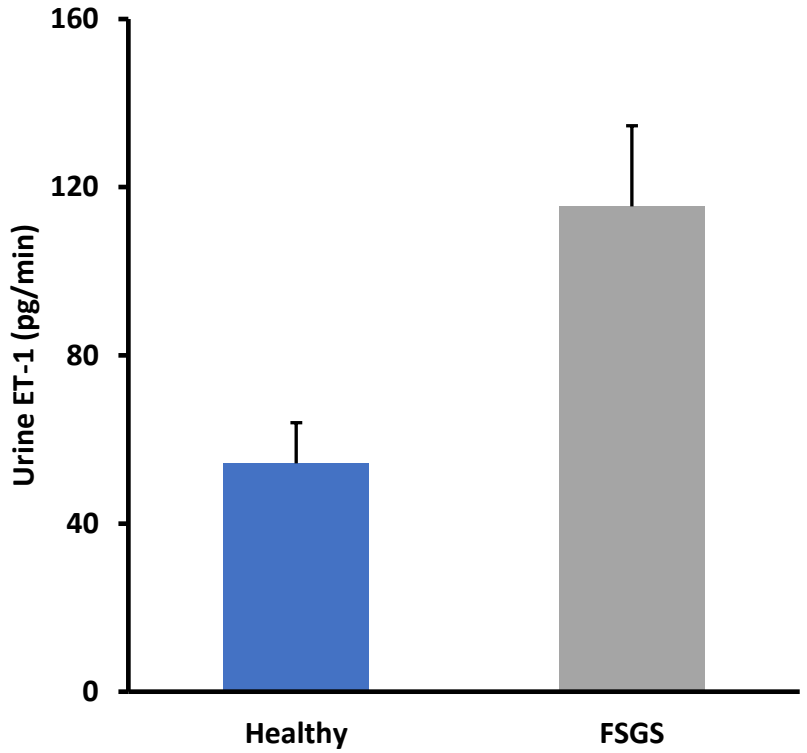
- **Elevated ET-1** in kidney biopsies from patients with IgA nephropathy **correlates with proteinuria** and 1-year progression[1–4]
- Leukocytes from patients with IgA nephropathy **stimulate mesangial cell production of ET-1**[5,6]
- Immune cells, including B-lymphocytes, express endothelin receptors; **monocytes from patients with IgA nephropathy have increased ET-1 expression**[7,8]
- Specific **ET_AR antagonism** in a murine model of IgA nephropathy **reduced proteinuria** and **downregulated pro-inflammatory**, **pro-fibrotic**, and **pro-sclerotic** pathways[9]

ET-1, endothelin-1; ET_AR, endothelin type A receptor; IgA, immunoglobulin A.

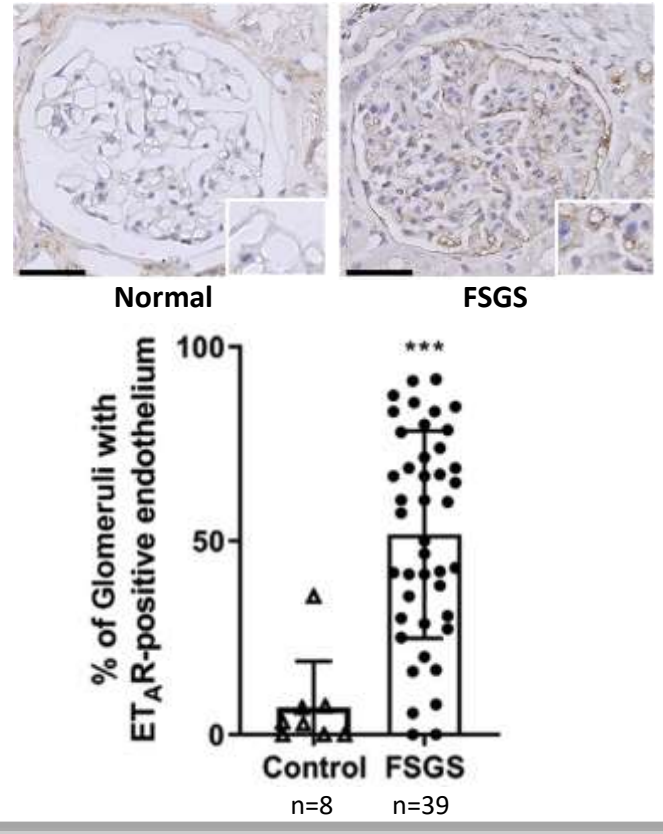
1. Rastaldi M, *et al. Nephrol Dial Transplant* 1998; **13:**1668–74; 2. Zanatta C, *et al. Ren Fail* 2012; **34:**308–15; 3. Lehrke I, *et al. J Am Soc Nephrol 2001;* **12:**2321–2329;
4. Tycová I, *et al. Physiol Res* 2018; **67:**93–105; 5. Chen H, *et al. Nephron* 2001; **89:**274–279; 6. Ebefors K, *et al. BMC Nephrol* 2016; **17**(40);
7. Nakamura T, *et al. Lancet* 1993; **342:**1147–1148;8. Elisa T, *et al. J Immunol Res* 2015; **2015:**147616; 9. King A, *et al. KI Reports* 2021; **6:**S164.

Expression of Both ET-1 and ET_AR Is Elevated in Patients with Primary FSGS

Urinary ET-1 in patients with primary FSGS[1]



ET_AR staining in patients with FSGS*,[2]



* Includes patients with hypertension, atherosclerosis, and/or obesity if it was unclear whether these clinical findings caused FSGS.
ET-1, endothelin-1; ET_AR, endothelin type A receptor; FSGS, focal segmental glomerulosclerosis.

1. Chen HC, *et al. J Clin Lab Anal* 2001; **15:**59–63; 2. van de Lest N, *et al. Kidney Int Reports* 2021; **6:**1939–1948.

Outline of talk

Which kidney diseases should we target (in addition to IgA nephropathy & FSGS)

Considering :

- **Modes of action <u>not targeted</u> by SGLT2i or MRA**
- **Medical needs in Nephrology *AND beyond***
- **Risk-Benefit balance**
    - Glomerular diseases:

      - FSGS, Alport's syndrome
      - Sickle Cell Nephropathy, ANCA GN

    - AKI, AKI to CKD transition

    - Dialysis & Transplant

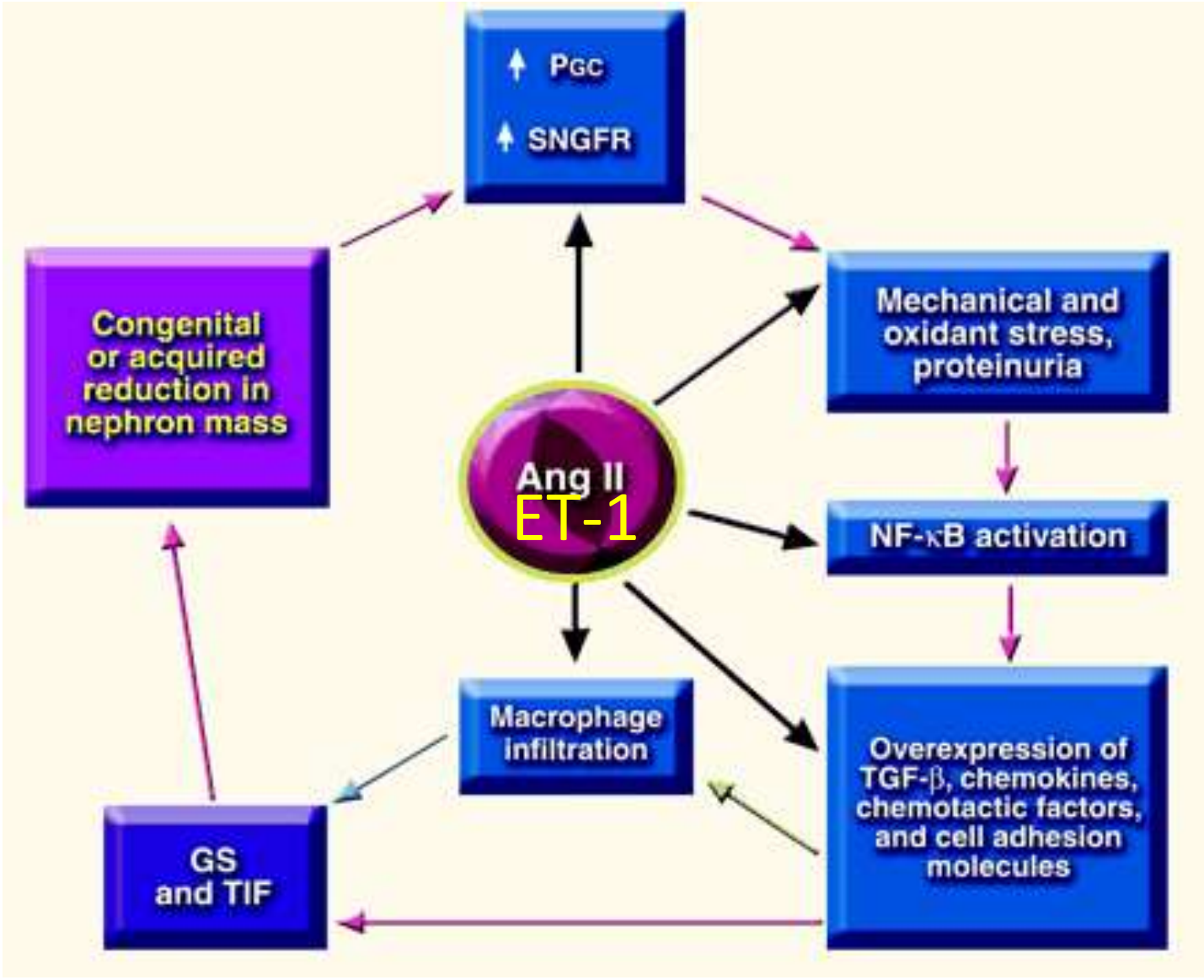
Why blocking ET-1 might be beneficial in kidney diseases

Modes of Action:

- Increases renal blood flow
- Relative efferent to afferent vasodilatation
- Reduction in filtration fraction
- Beneficial effects on top of RAS blockade
- Beneficial effects on top of RAS & SGLT2 blockade
- SGLT2i might offset some of the side effects of ET blockers

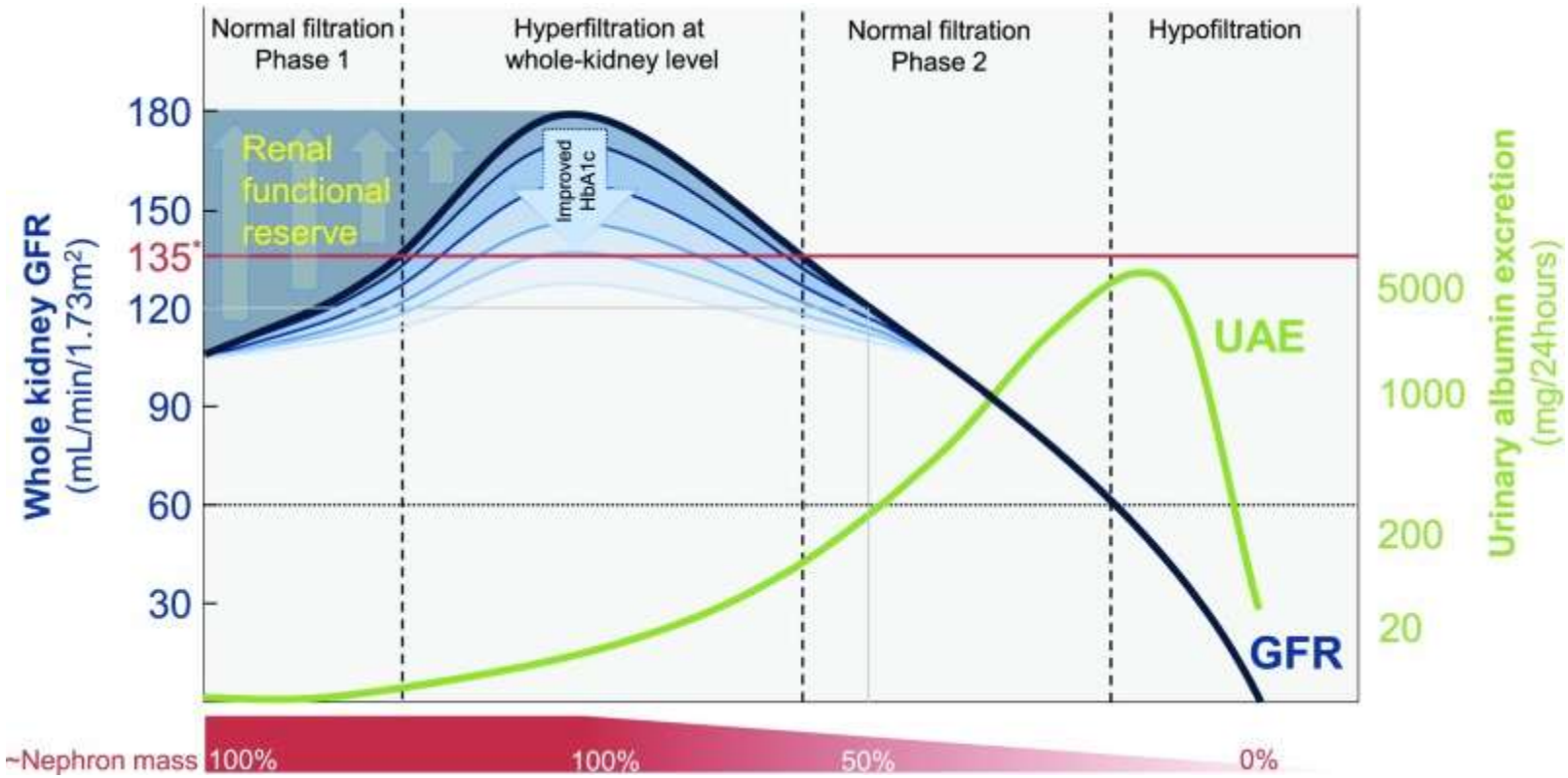
Hemodynamic and non hemodynamic Factors

Glomerular hyperfiltration and hypertension Central role of RAS & ET-1



Brenner, BM et al:
J Clin Invest 110:1753, 2002

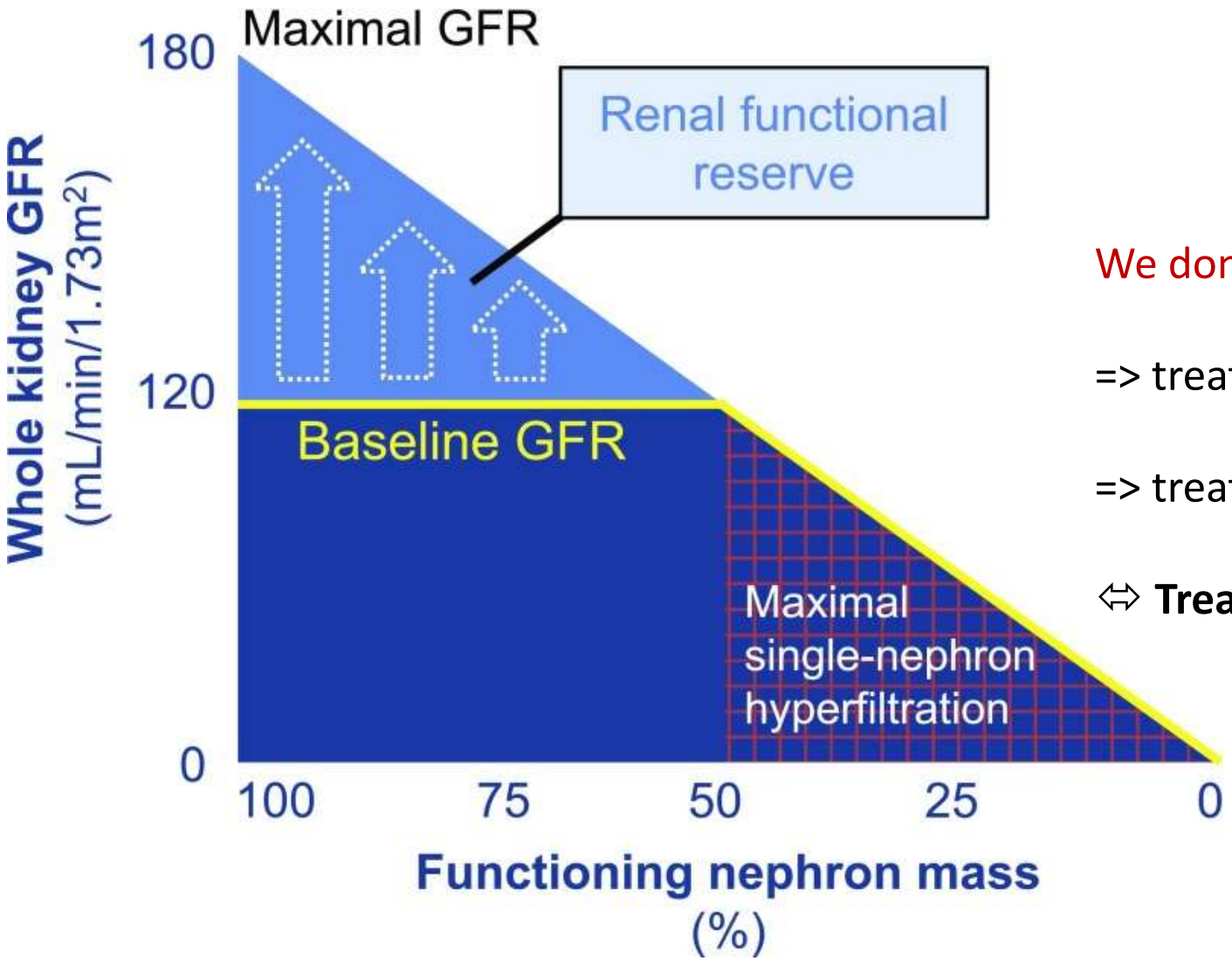
Glomerular hyperfiltration in DKD



- The prevalences of hyperfiltration at the whole-kidney level vary greatly: between 10% and 67% in T1DM and 6%–73% in patients with T2DM.
- GFR increases by about 27% and 16% in recently diagnosed patients with T1DM and T2DM, respectively.
- Hyperfiltration predisposes to progressive nephron damage by increasing glomerular hydraulic pressure (P_{GLO}) and transcapillary convective flux of ultrafiltrate and, although modestly, macromolecules.

Tonneijck L, et al JASN 2017;28(4):1023-1039.

Schematic representation of renal functional reserve



We don't measure this !

=> treat everyone with nephron reduction

=> treat always when history of high GFR

⇔ **Treat everyone** ☺

Tonneijck L, et al JASN 2017 Apr;28(4):1023-1039.

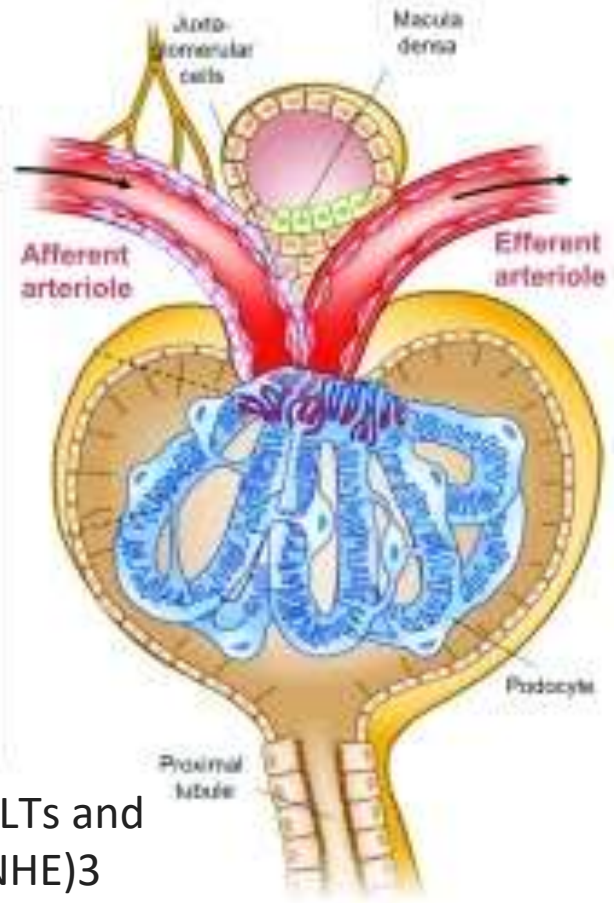
Pathogenesis of glomerular hyperfiltration in diabetes

High glucose

Glomerular hypertrophy

Insulin

Factors causing a net reduction of afferent arteriolar resistance
Vascular factors
Nitric oxide bioavailability
COX-2 prostanoids
Kallikrein-kinins
Atrial natiuretic peptide
Angiotensin(1-7)
Hyperinsulinemia *per se*
Tubular signals
Inhibition of tubuloglomerular feedback (macula densa signals)



Factors causing a net increase of efferent arteriolar resistance
Vascular factors
Angiotensin-II
Thromboxane A2
Endothelin-1 (ETA receptor)
Reactive oxygen species

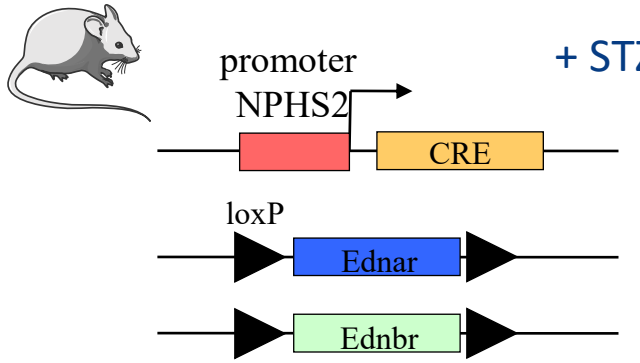
Upregulation of ET-1 and of SGLTs and sodium-hydrogen exchanger (NHE)3

Endothelin 1 in diabetic nephropathy

- Elevated levels of ET1 in patients with type II diabetes (*Takahashi 1990; Verhaar 1998, Mather 2002*)
- A primary disturbance in ET1 production from vascular endothelium exists as an early phenomenon (*Donatelli 1994, Anfossi 2007*)
- Correlation between plasma and urine levels of ET1 and diabetic nephropathy (*Lee 1994, De Mattia 1998, Zanatta 2008, Sasser 2012*)
- Dual ET1 receptor and and ETA selective blockers show encouraging results in diabetic nephropathy (*Chade 2006, Sasser 2007*)
- Atrasentan lowers albuminuria in type II diabetic patients (*Zeeuw 2014*)
- Atrasentan restores the glycocalyx in GFB in diabetic nephropathy (*Boels 2016*)

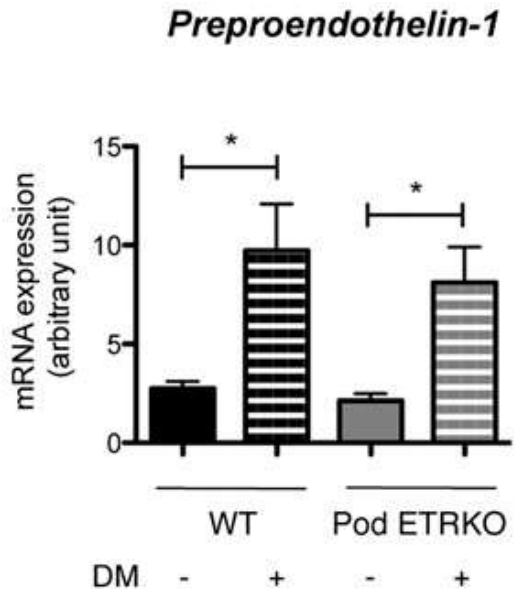
Beyond the hemodynamic effects
A direct effect of ET-1 on glomerular cells?

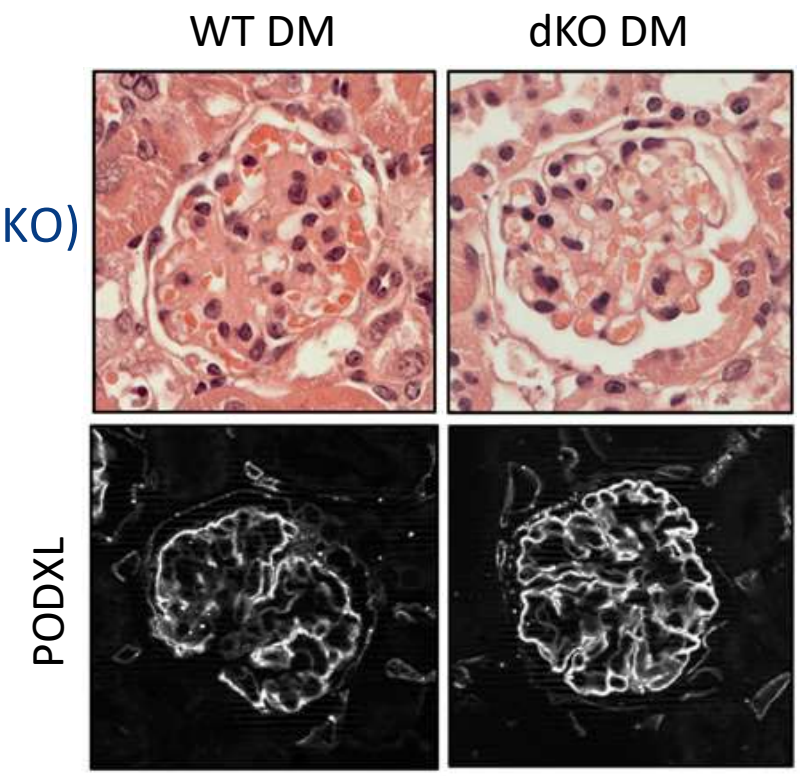
Endothelin 1 mediates direct Podocyte injury in diabetic nephropathy

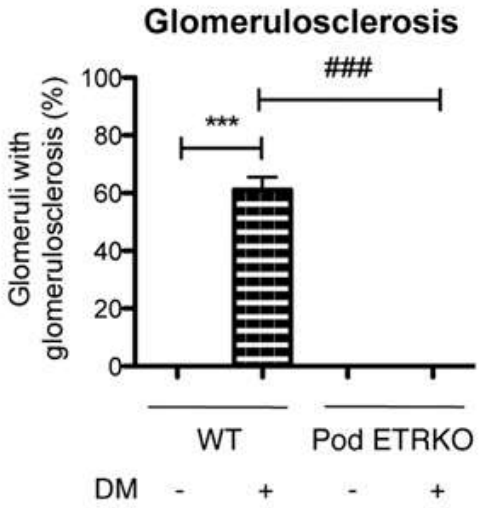


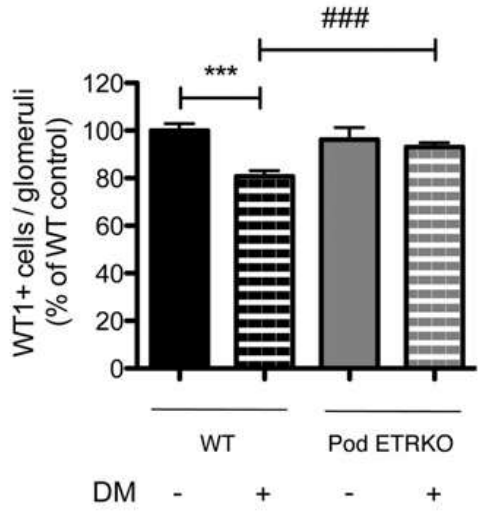
+ STZ ➔ Diabetes Mellitus

Podocyte-selective ETAR/ETBR deletion (dKO)







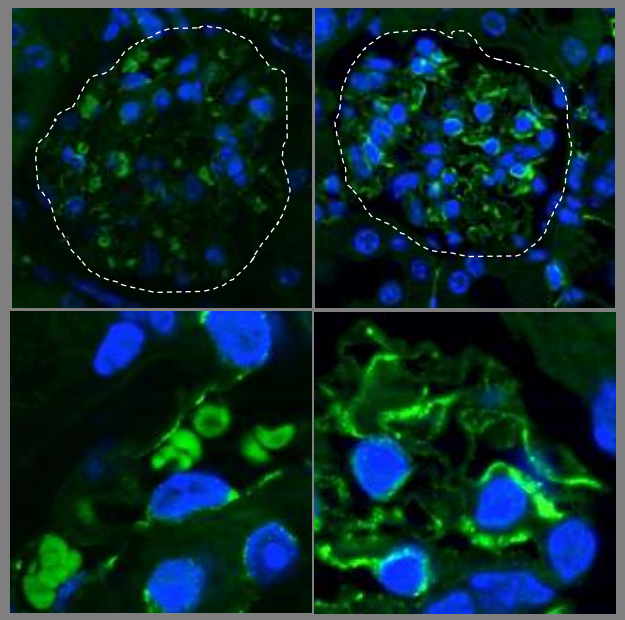


Podocyte ETR deletion prevents glomerulosclerosis and podocyte injury

Lenoir O. et al. JASN 2014

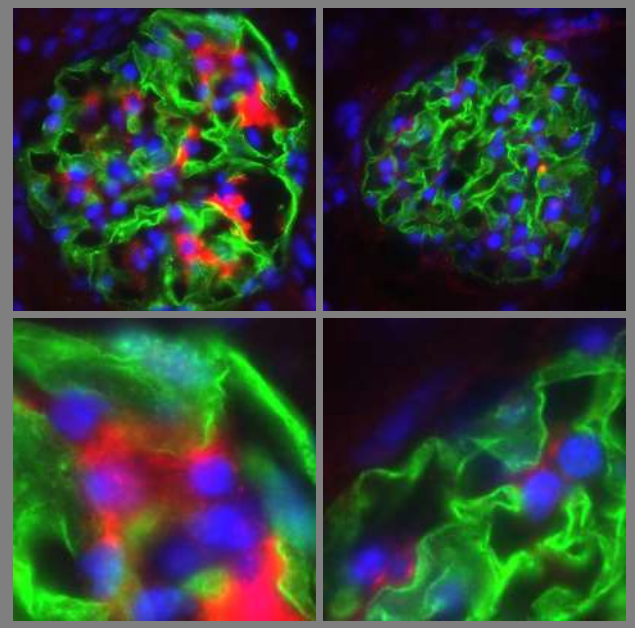
Podocyte ETR activation mediates GEnC injury and mesangial « activation » in diabetic nephropathy

WT DM dKO DM



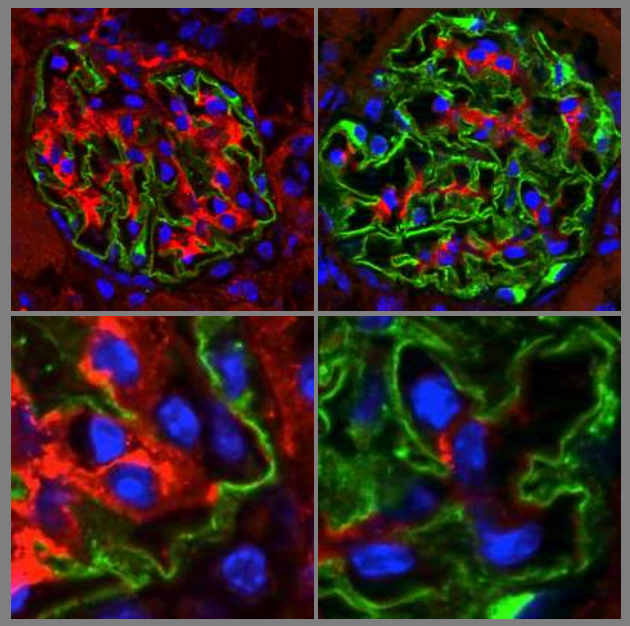
CD31 / DAPI

WT DM dKO DM

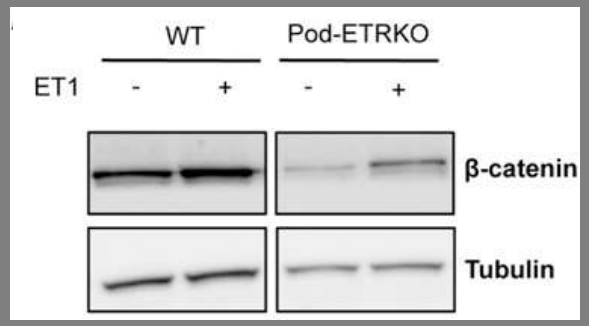


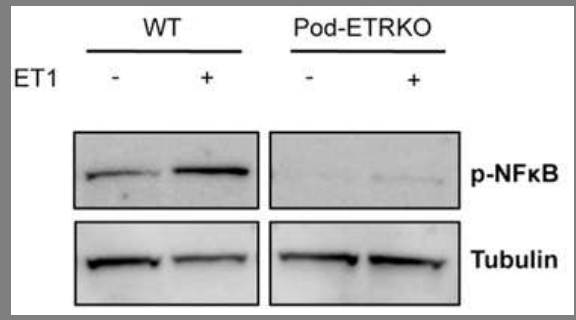
NPHS2/NFKB/DAPI

WT DM dKO DM



NPHS2/βCAT/DAPI

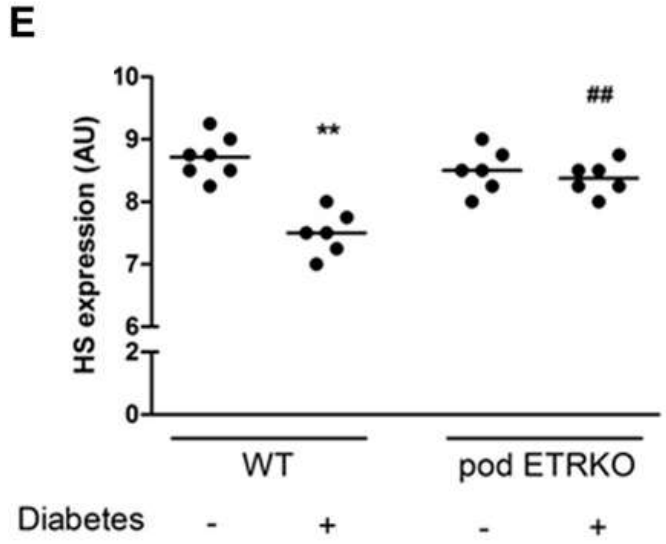


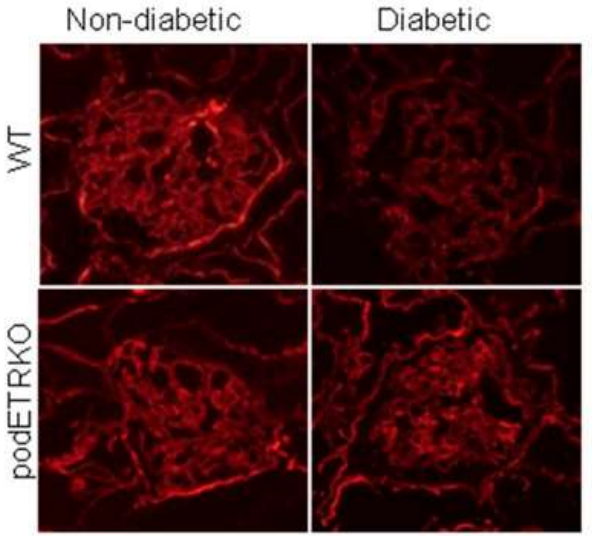


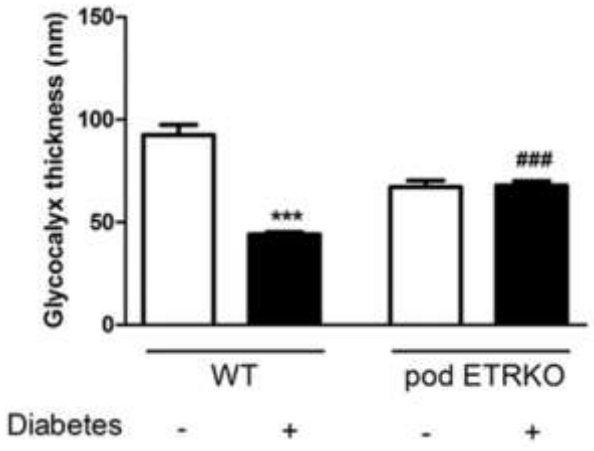
Lenoir O. et al. JASN 2014

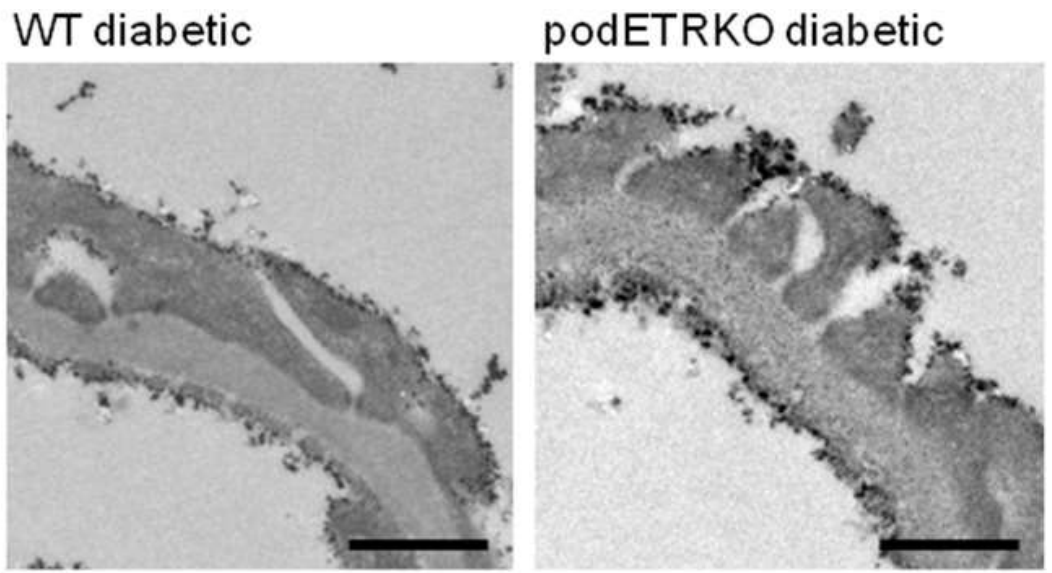
Podocyte ETR signaling promotes β-catenin and NFκB in glomeruli

Podocyte ETR activation mediates GEnC injury in diabetic nephropathy



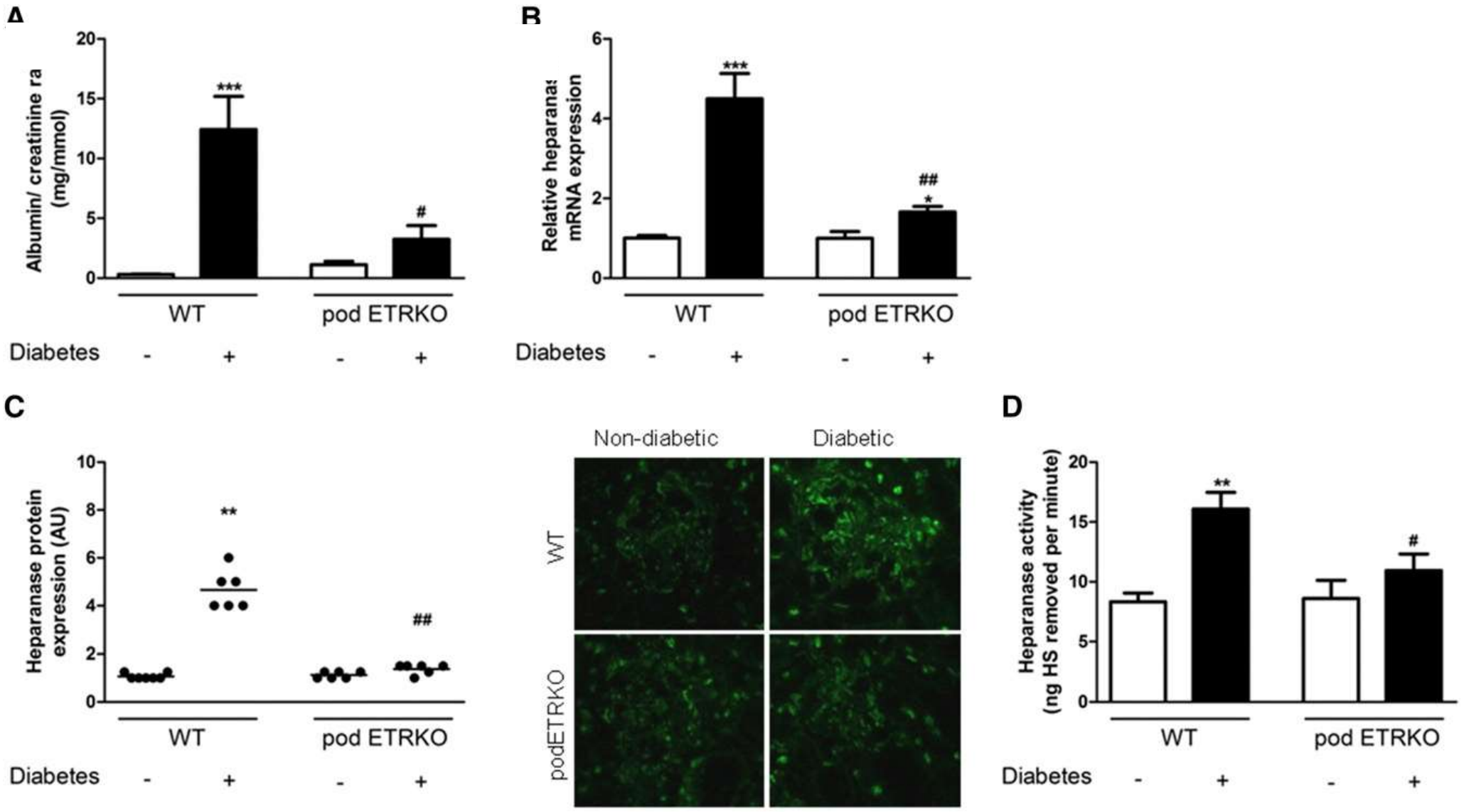






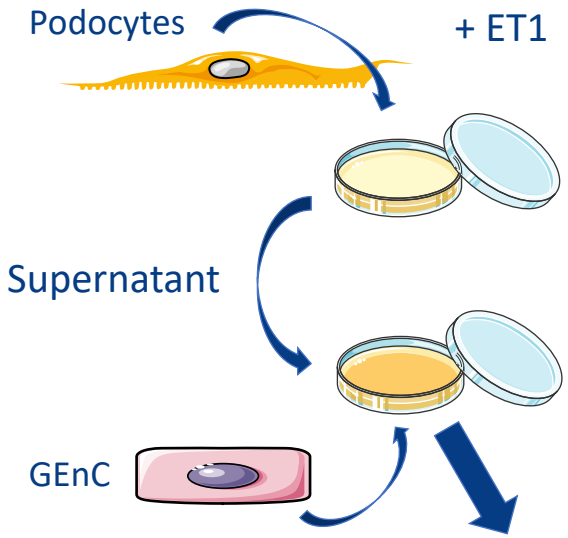
Garsen, Lenoir et al. JASN 2016

Podocyte ETR activation mediates glomerular Heparanase activity

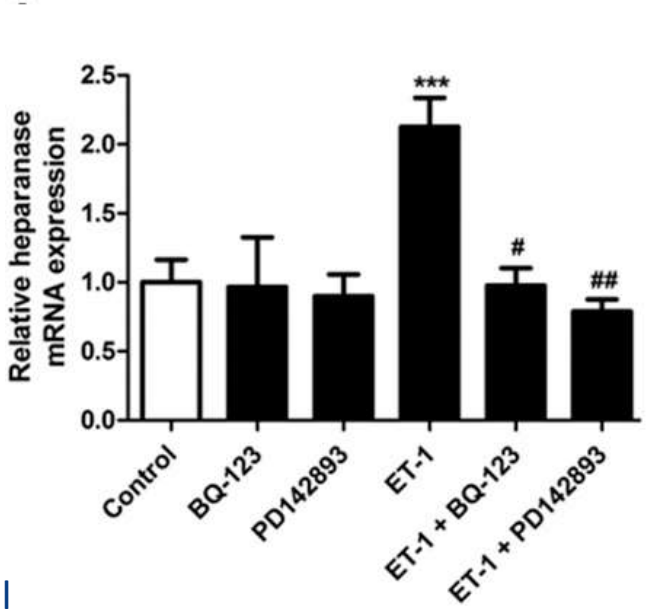


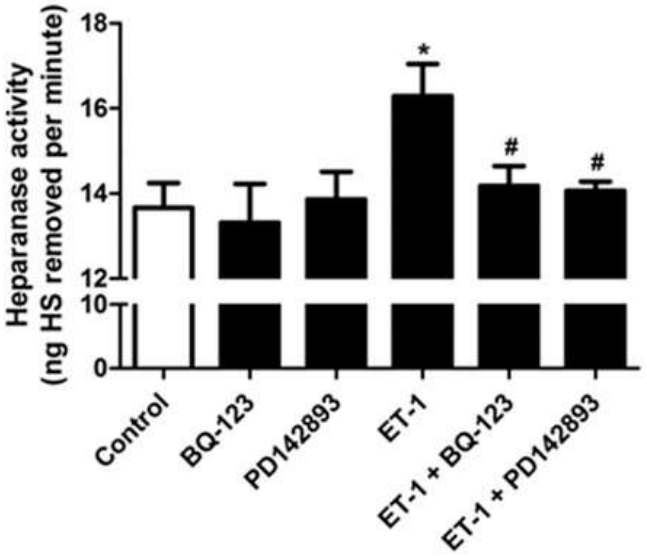
Garsen, Lenoir et al. JASN 2016

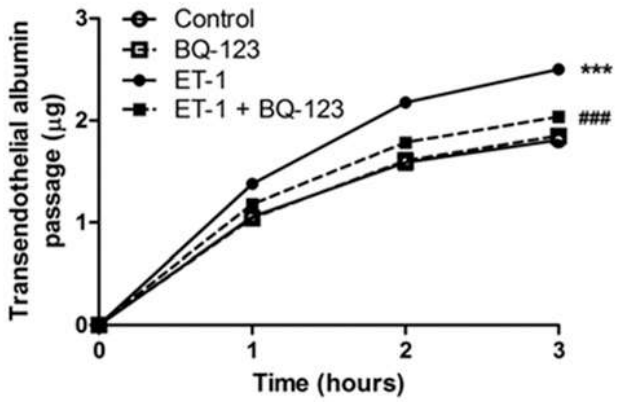
Podocyte ETR activation mediates GEnC injury in diabetic nephropathy

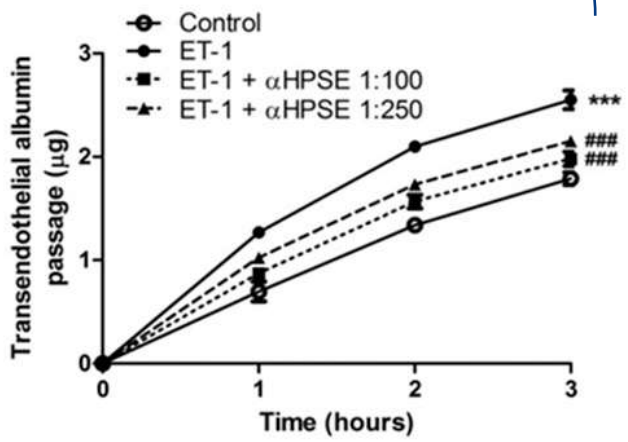


Transendothelial albumin passage

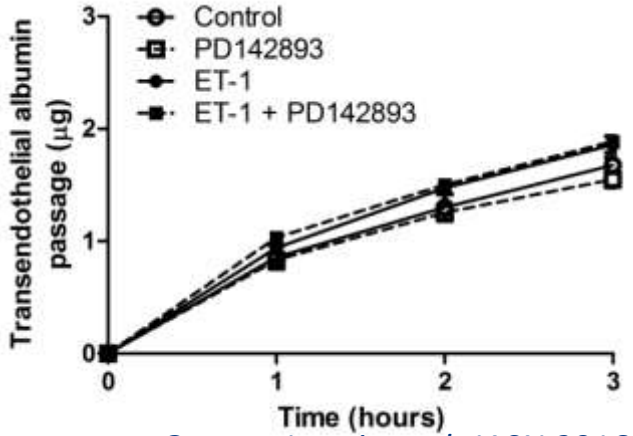






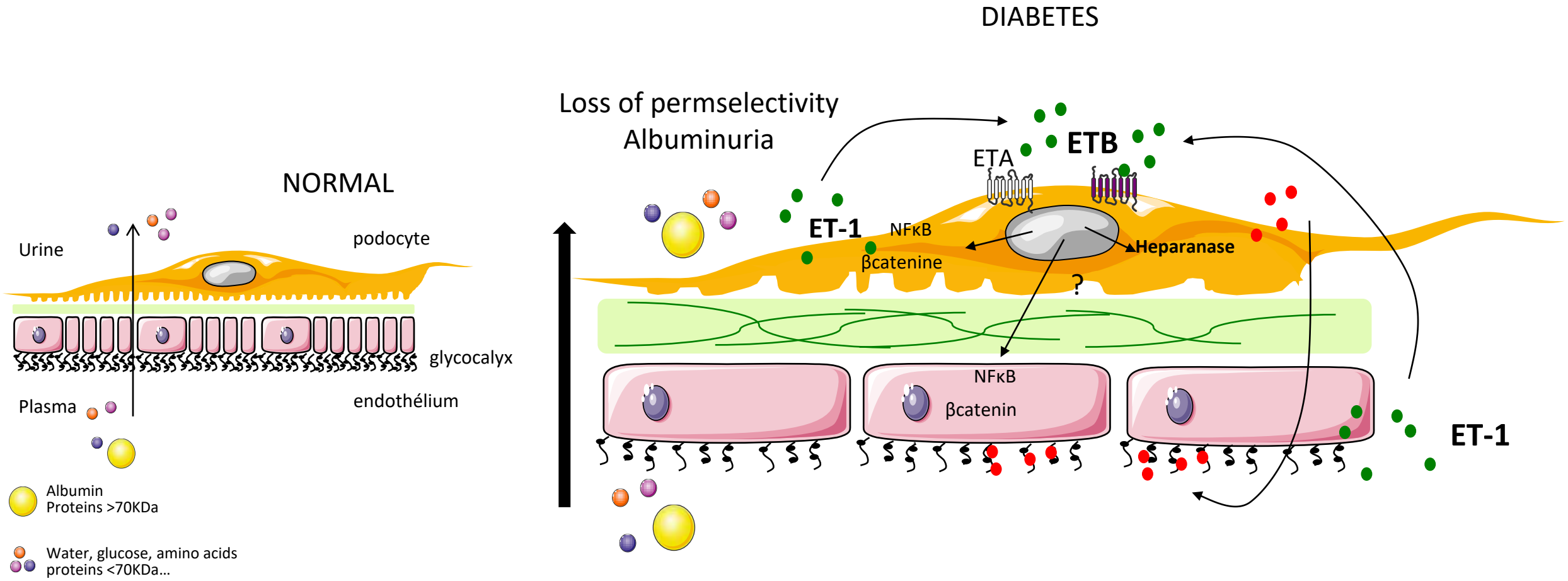


GEnC w/o podocyte SN



Garsen, Lenoir et al. JASN 2016

Podocyte ETR activation mediates GEnC injury in diabetic nephropathy



Garsen, Lenoir et al. J Am Soc Nephrol 2016

Sickle Cell Disease: another Condition with Chronic Hyperfiltration

Table 3. Renal Hemodynamics and Urinary Measures in Male Subjects

Variables	Subjects With SS Disease				Controls, Mann-Whitney Test*				*P* Value
	No.	Med	Min	Max	No.	Med	Min	Max	
GFR, mL/min per 1.73 m^2	32	137	21	210	8	105	89	123	<.005
ERPF, mL/min per 1.73 m^2	30	879.5	175.0	1314.0	8	490.5	269.0	588.0	<.001
ERBF, mL/min per 1.73 m^2	30	1132	198	1663	8	839	516	996	<.001
FF, %	30	16	11	24	8	22	19	33	<.001
Specific gravity	33	1.010	1.002	1.014	8	1.022	1.018	1.030	<.001
UFR during creatinine excretion, mL/min	33	1.6	0.7	2.7	8	0.8	0.5	1.7	<.005
UFR during cimetidine CrCl, mL/min	33	1.8	0.8	4.1	8	1.8	0.9	4.3	.92
Creatinine excretion, mmol/kg per day	33	0.17	0.13	0.34	8	0.17	0.16	0.19	.77
Cimetidine creatinine excretion, mmol/kg per day	33	0.15	0.10	0.19	8	0.14	0.09	0.18	.58
CrCl, mL/min per 1.73 m^2	33	144.0	32.5	419.0	8	91.0	79.0	99.0	<.001
Cimetidine CrCl, mL/min per 1.73 m	33	114	20	216	8	69	46	113	<.002
Albumin excretion rate, µg/min	33	8.3	2.4	666.8	8	5.4	3.5	7.64	<.03
Albumin creatinine ratio, mg/mmol	33	1.5	0.4	193.5	8	0.5	0.4	1.0	<.002

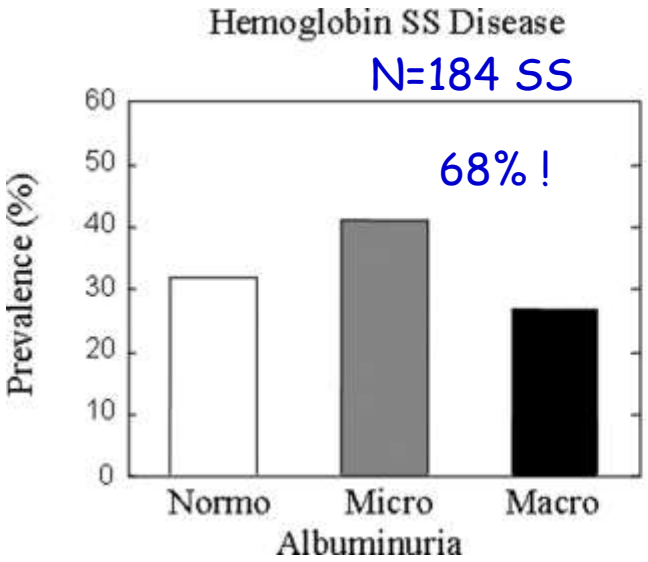
GFR+30%
RPF+79%
RBF+35%*

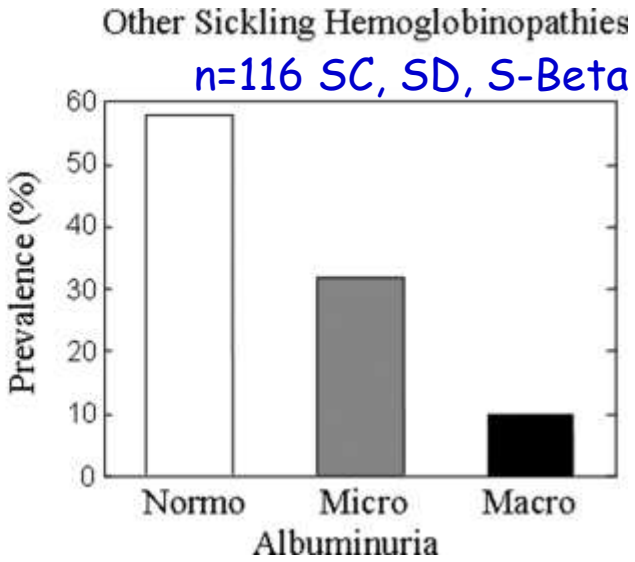
Abbreviations: CrCl, creatinine clearance; ERBF, effective renal blood flow; ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration rate; max, maximum value; med, median value; min, minimum value; SS, homozygous sickle cell; UFR, urine flow rate.
SI conversion factor: To convert creatinine clearance to microliters per second, multiply by 0.01667.
*Those with normal (AA) genotype.

* Hb: -74%

Thompson, J. et al. Arch Intern Med 2007;167:701-708.

High Prevalence of Albuminuria and CKD in Adults with Sickle Cell Hemoglobinopathies



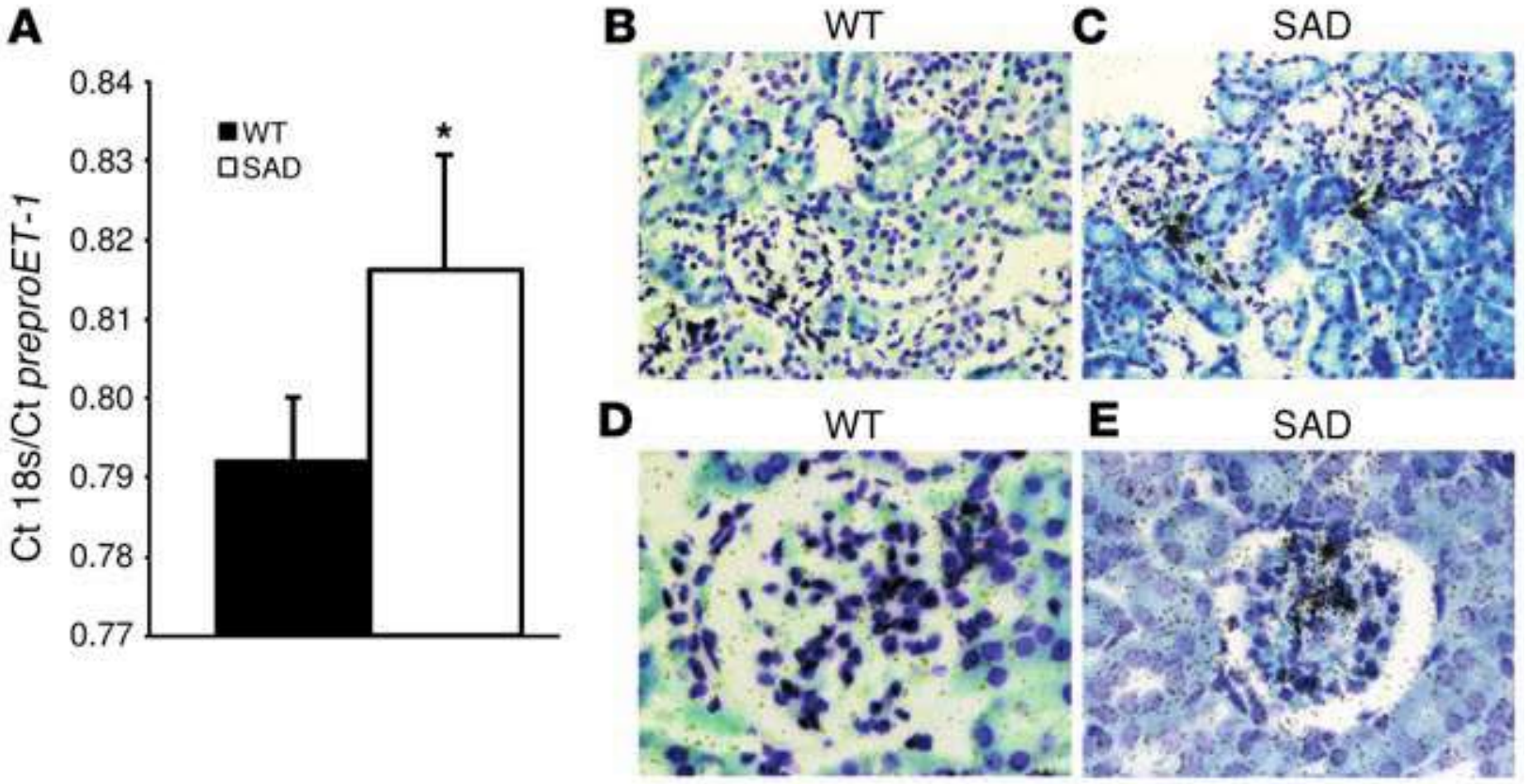


CRF: 21%

Macroalbumin excretion rate: 300 mg/g creatinine

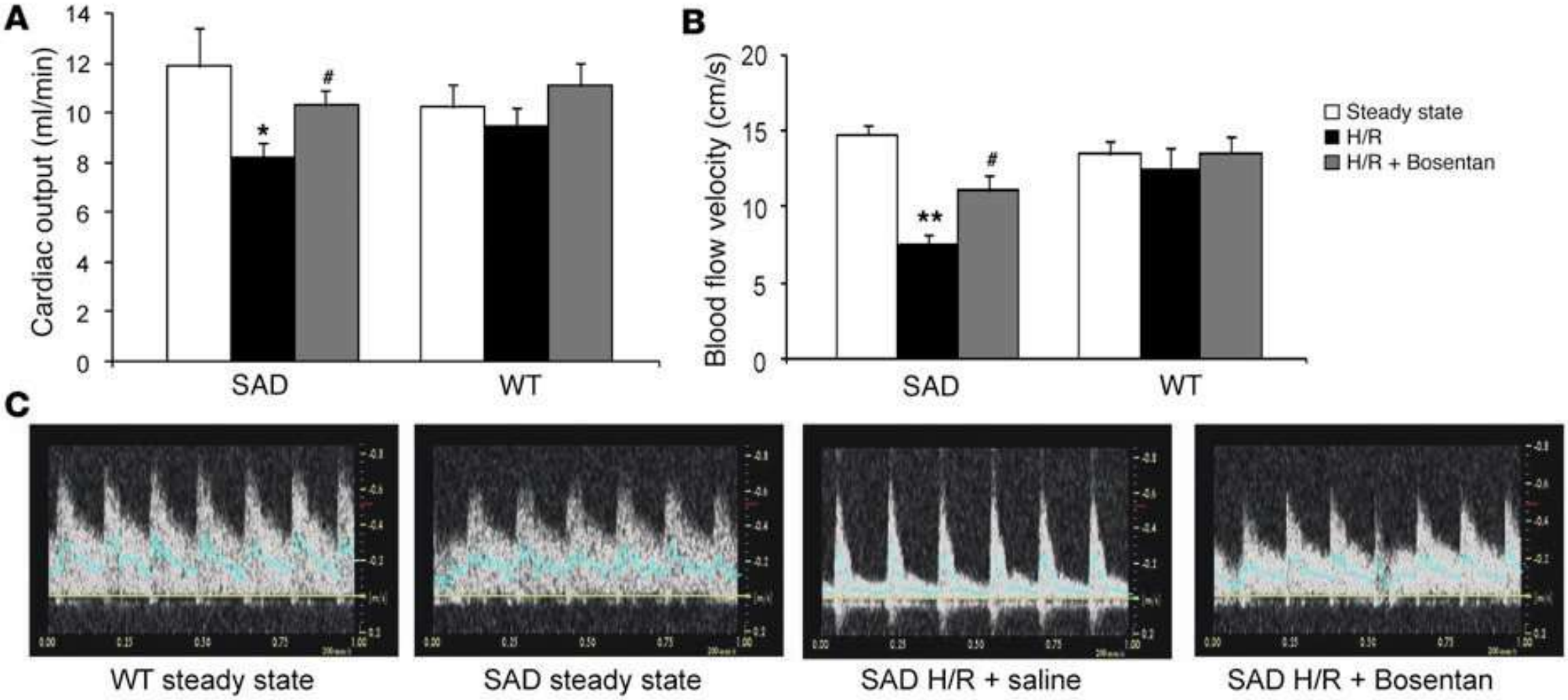
Guasch, A. et al. J Am Soc Nephrol 2006;17:2228-2235

Vascular and Glomerular production of ET-1 in Sickle Cell Nephropathy



Sabaa N et al. J Clin Invest. 2008 May;118(5):1924-33.

Sickle cell disease: strong ET-1-dependent control of the renal vascular resistances during vaso-occlusive crisis



Sabaa N et al. J Clin Invest. 2008 May;118(5):1924-33.

Sickle cell nephropathy

BASIC RESEARCH www.jasn.org

Long-Term Endothelin-A Receptor Antagonism Provides Robust Renal Protection in Humanized Sickle Cell Disease Mice

Malgorzata Kasztan,* Brandon M. Fox,* Joshua S. Speed,* Carmen De Miguel,* Eman Y. Gohar,* Tim M. Townes,† Abdullah Kutlar,‡ Jennifer S. Pollock,*§ and David M. Pollock*§

*Cardio-Renal Physiology and Medicine, Department of Medicine, and †Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, Alabama; and ‡Division of Hematology and Oncology, and §Department of Medicine, Medical College of Georgia, Augusta University, Augusta, Georgia

Kasztan, *JASN* 2018

Outline of talk

Which kidney diseases should we target (in addition to IgA nephropathy & FSGS)

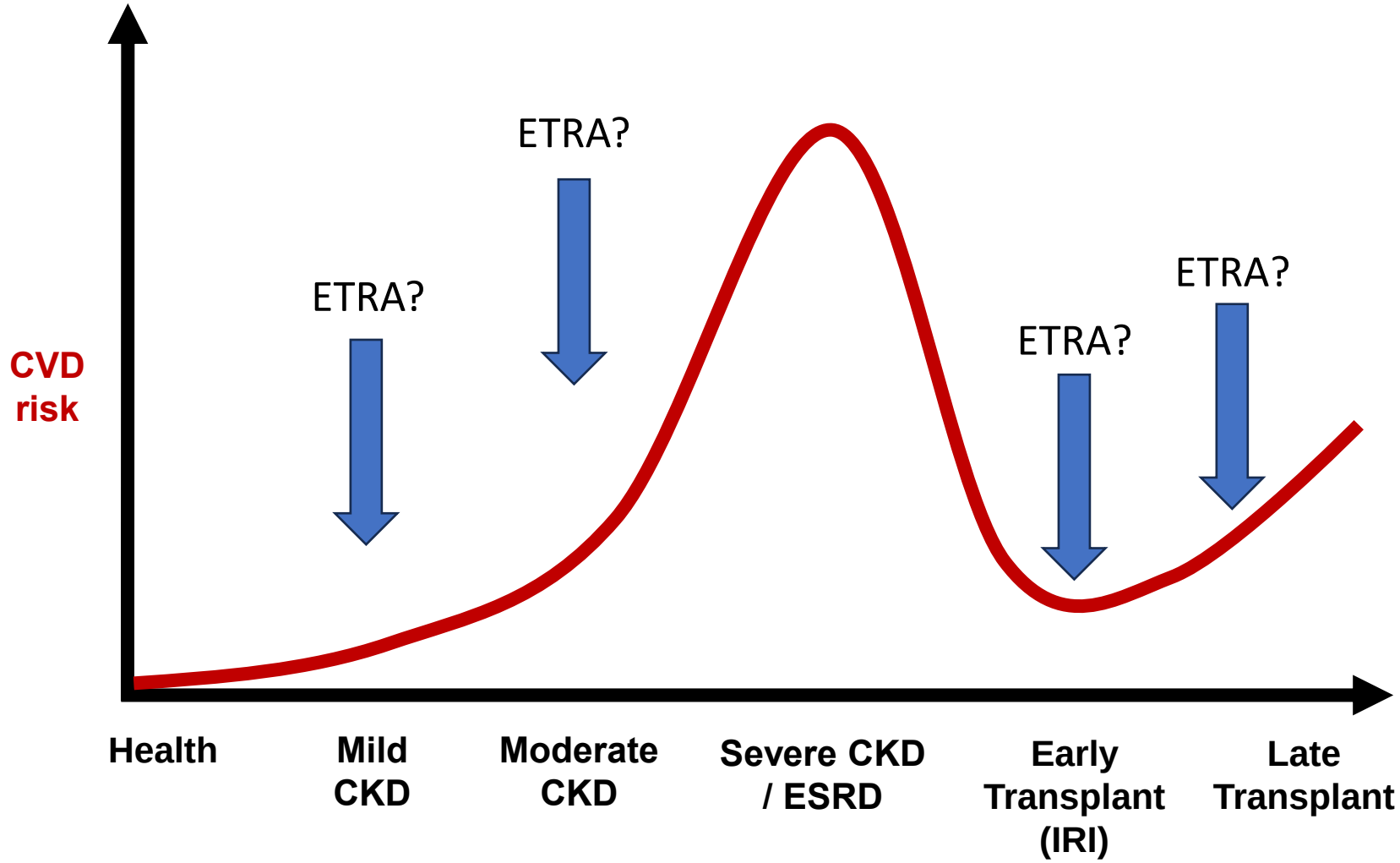
Considering :

- **Modes of action not targeted by SGLT2i or MRA**
- **Medical needs in Nephrology *AND beyond***
- **Risk-Benefit balance**

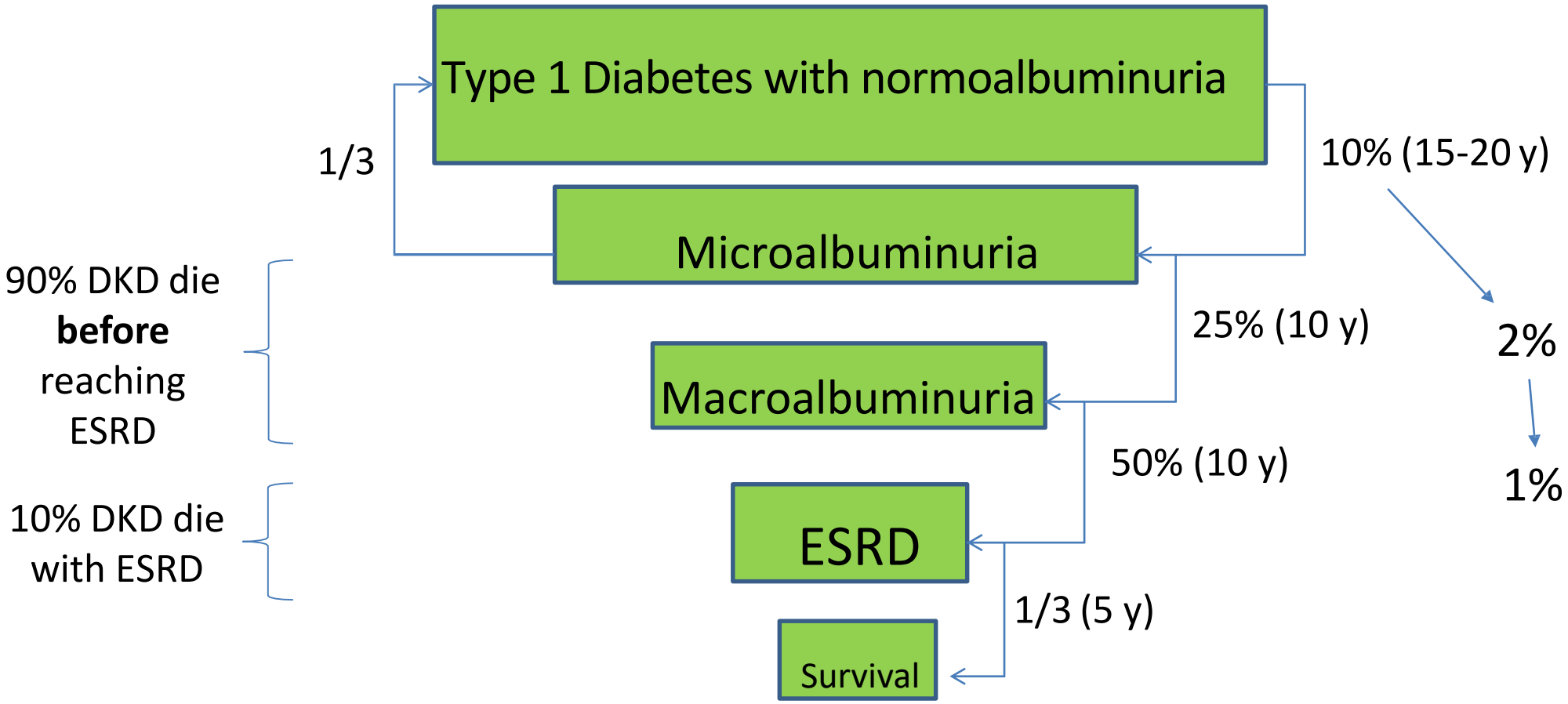
=>

- Glomerular diseases
- Sickle Cell Nephropathy, ANCA GN, Alport's syndrome
- AKI, AKI to CKD transition
- Dialysis & Transplant

Cardiovascular risk in kidney disease



Progression of DKD: treat the kidney AND the whole CV system



- ***How do we know who should be treated more aggressively?***
- ***Do we treat DKD at different stages similarly?***

Alicic RZ, Rooney MT, Tuttle KR. *CJASN* 2017;12:2032-2045

Why blocking ET-1 might be beneficial in cardiovascular disease in the context of kidney disease

- Reduces blood pressure
- Reduces arterial stiffness
- Improves endothelial function
- Improves fibrinolytic capacity

- Beneficial effects on top of RAS blockade
- Beneficial effects on top of RAS & SGLT2 blockade
- SGLT2i might offset some of the side effects of ET blockers

ET-1 & hypertension

CLINICAL EPIDEMIOLOGY www.jasn.org

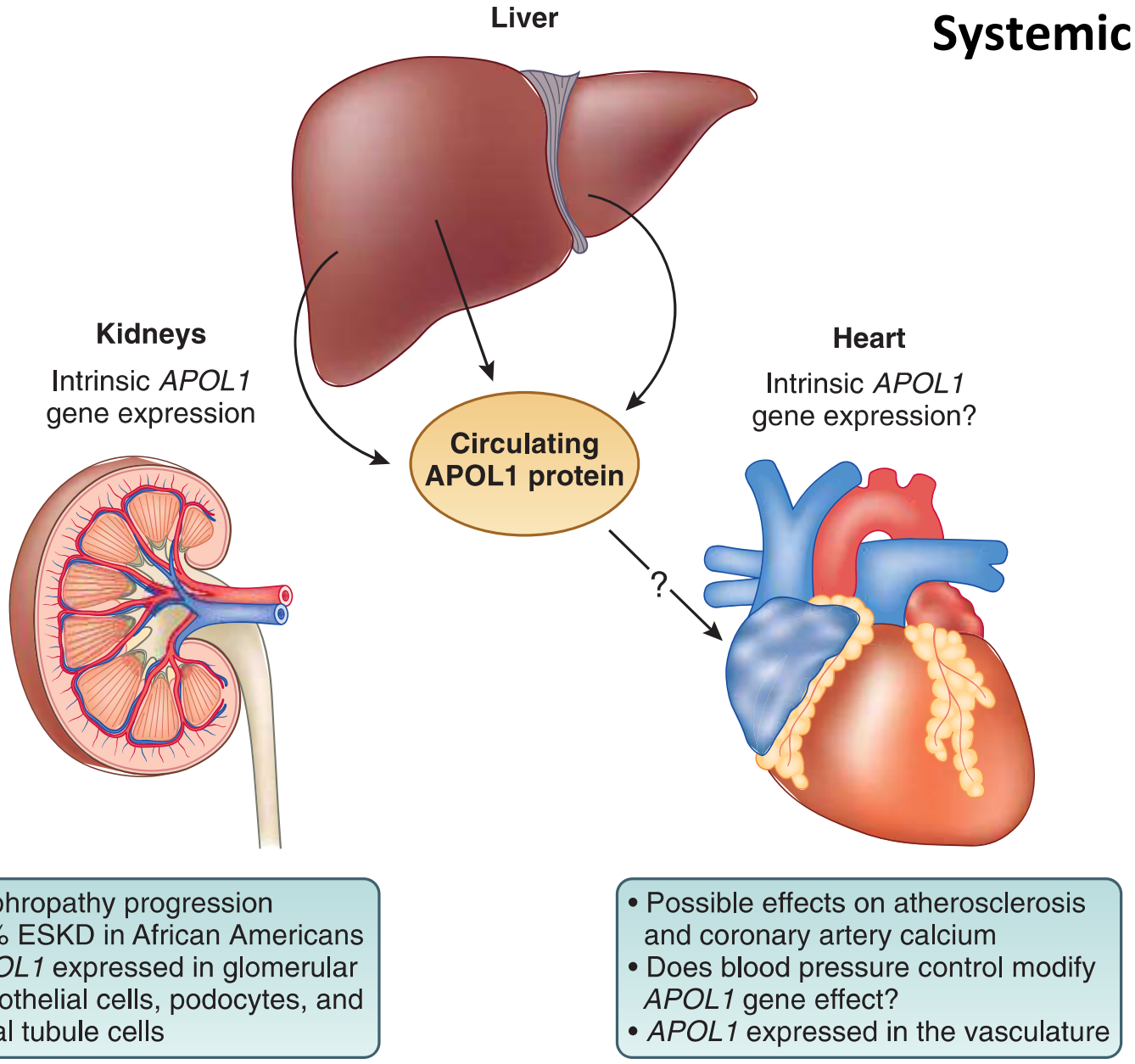
Association between Endothelin-1 Levels and Kidney Disease among Blacks

Casey M. Rebholz,*† Jane L. Harman,‡ Morgan E. Grams,†§ Adolfo Correa,‖¶ Daichi Shimbo,** Josef Coresh,*† and Bessie A. Young††

*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; †Welch Center for Prevention, Epidemiology, and Clinical Research, Baltimore, Maryland; ‡Program in Prevention and Population Sciences, Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; §Division of Nephrology, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland; Departments of ‖Pediatrics and ¶Medicine, University of Mississippi Medical Center, Jackson, Mississippi; **Department of Medicine, Columbia University Medical Center, New York, New York; and ††Division of Nephrology, Department of Medicine, Veterans Affairs Puget Sound Health Care System and the University of Washington, Seattle, Washington

Rebholz, *JASN* 2017

Systemic endothelial injury in APOL1 disease?



APOL1 genotype is associated with

- albuminuria,
- subclinical atherosclerosis,
- incident myocardial infarction,
-and mortality in older African Americans.

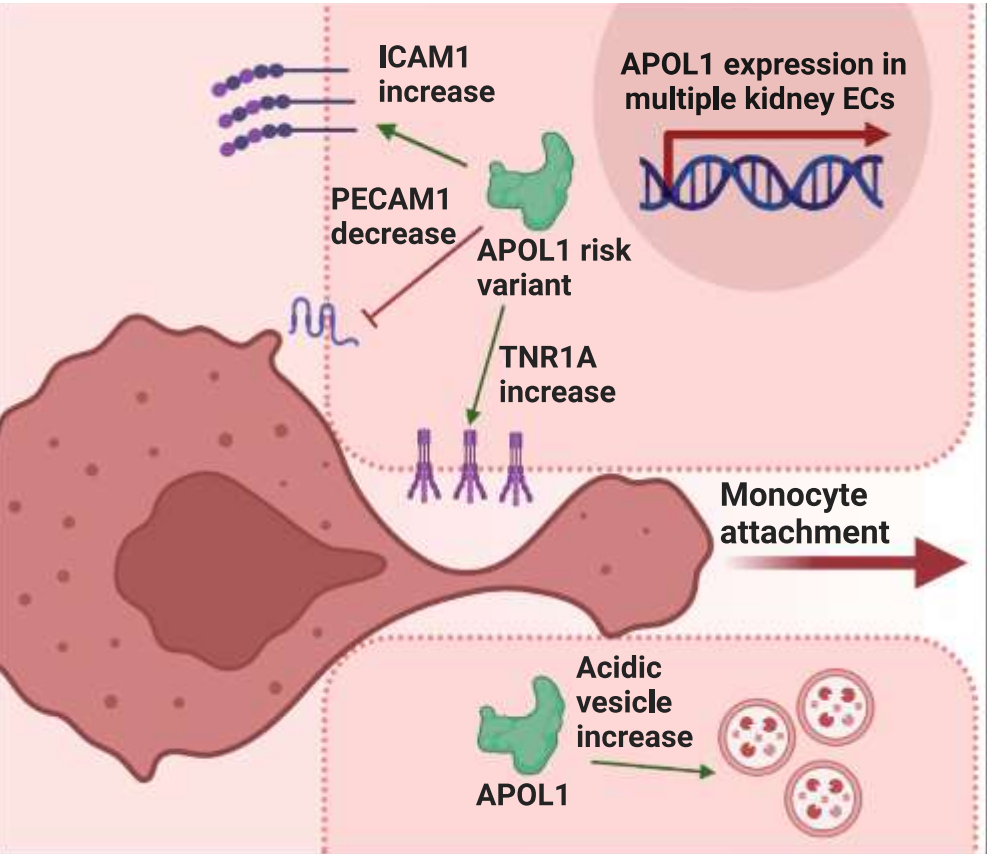
Relative Risk for FSGS >10

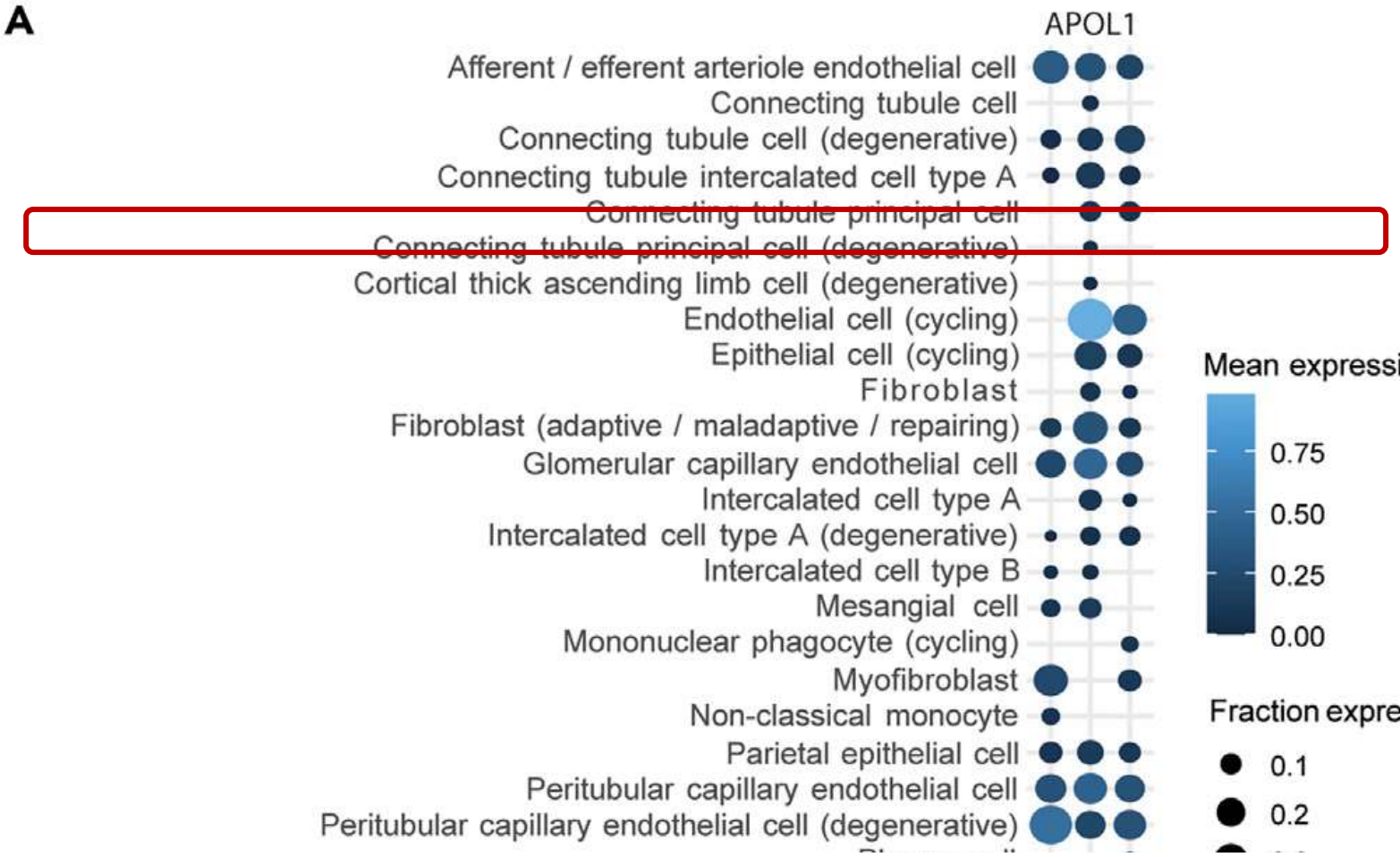
More intensive BP control in individuals with CKD and 2 APOL1 renal-risk variants may be associated with longer survival.

Ku E et al. *Strict blood pressure control associates with decreased mortality risk by APOL1 genotype*. Kidney Int. 2017; 91: 443-450

Robinson TW and Freedman BI, Kidney International (2017) 91, 276–278

APOL1 promotes endothelial cell activation

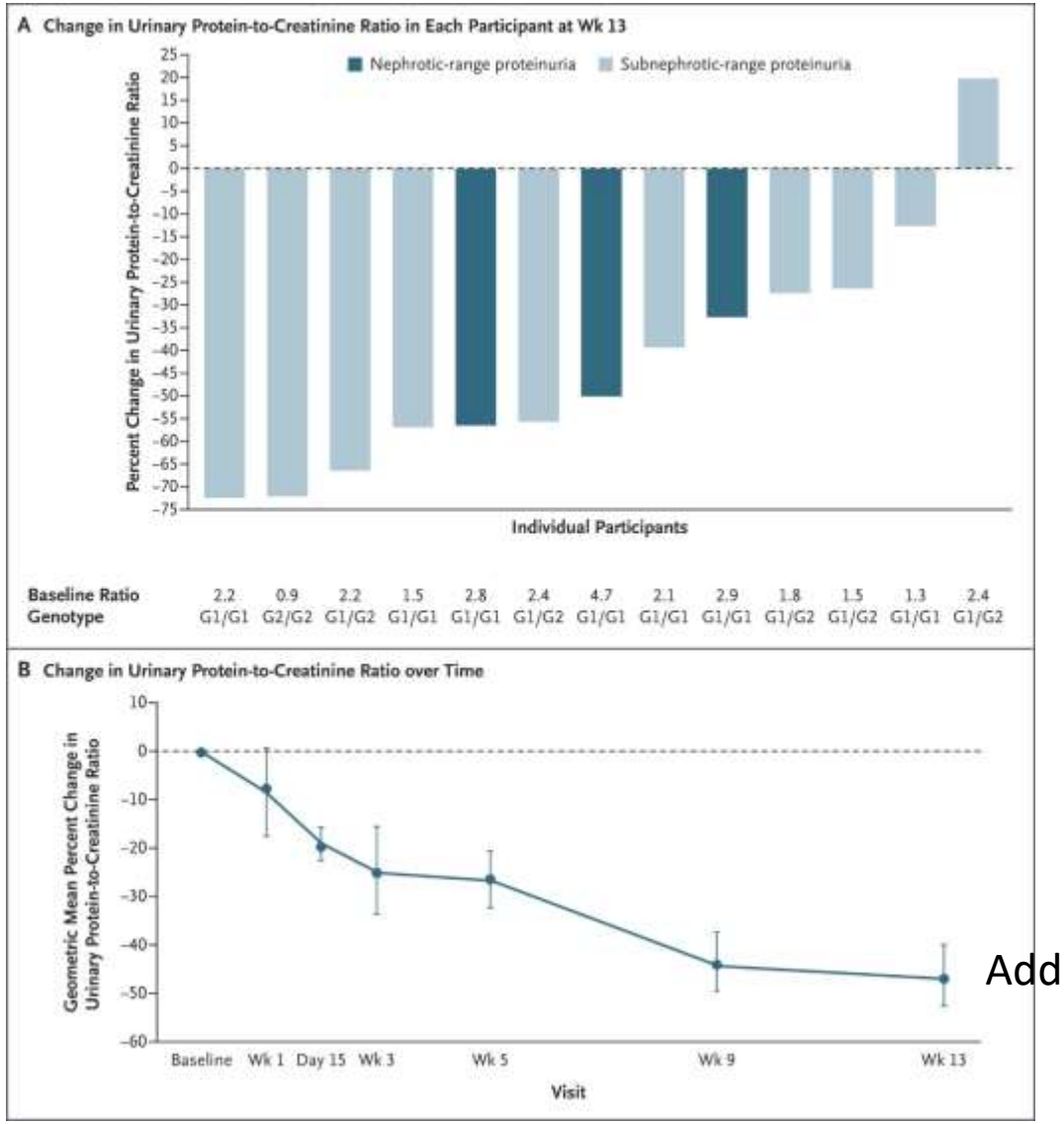




Heatmaps showing differential gene expression (log2 fold change) of EC markers from glomerulus kidney biopsies. Each heatmap shows expression profiles from individuals with nephrotic syndrome and either low-risk or high-risk APOL1 genotypes

Carracedo et al., iScience 26, 106830, June 16, 2023

Identification of APOL1 as a risk gene for CKD in African ancestor and use APOL1 inhibitor as a new therapy for CKD



Map Apol1 gene

↓

Mechanisms?

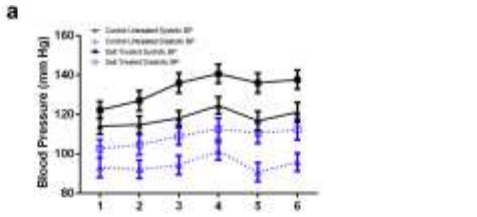
↓

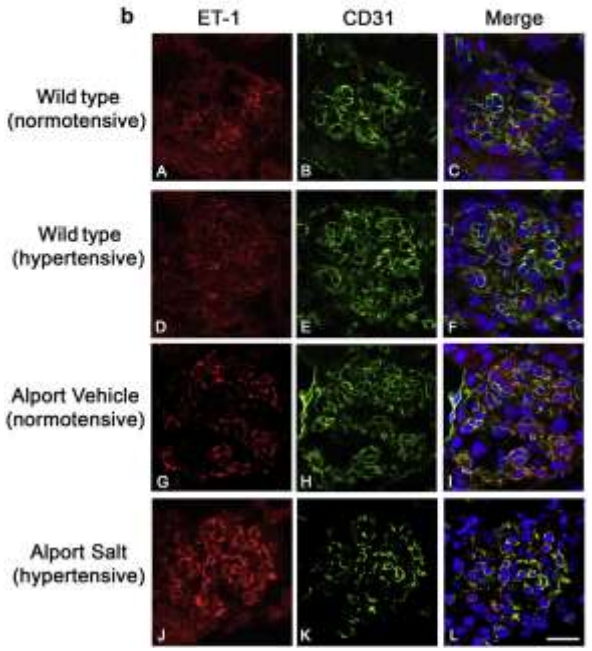
Medicine Apol1 inhibitors

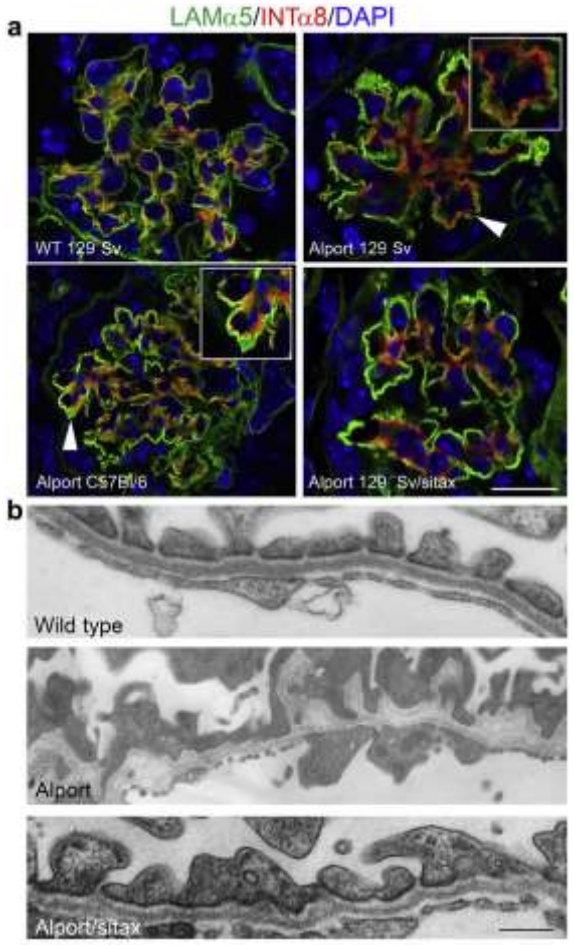
Adding ETRA?

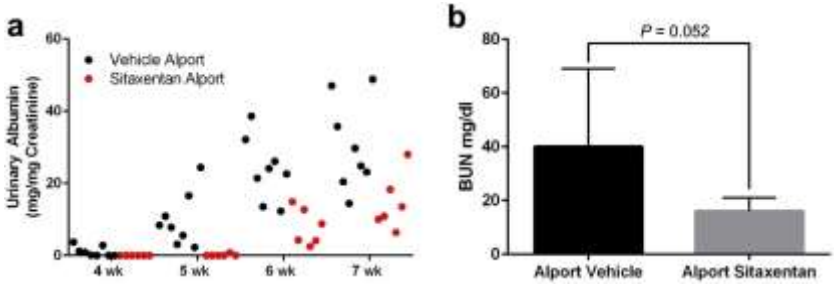
N Engl J Med 2023; 388:969-979
DOI: 10.1056/NEJMoa2202396

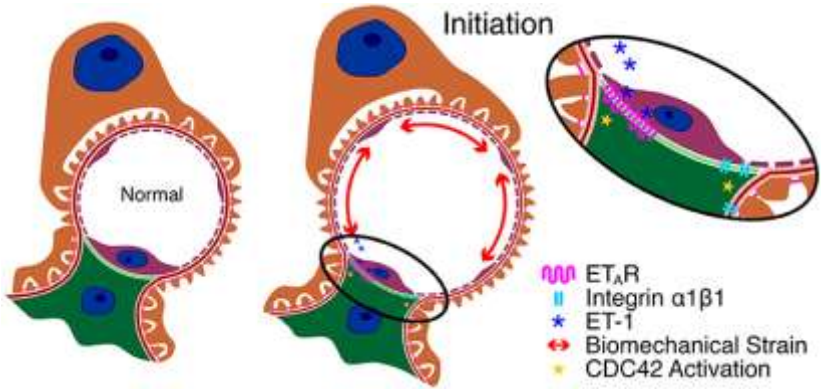
Alport's syndrome

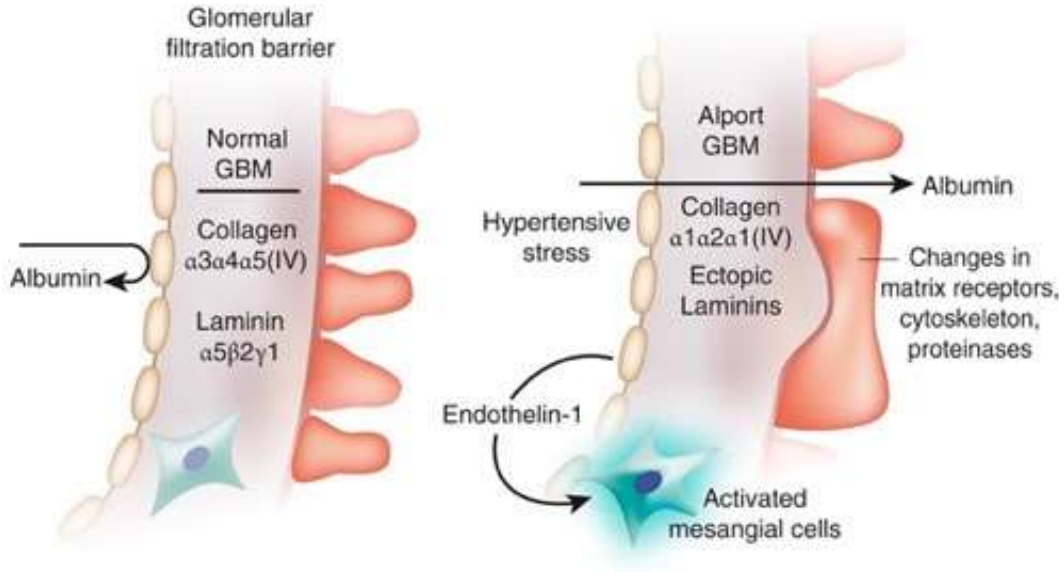








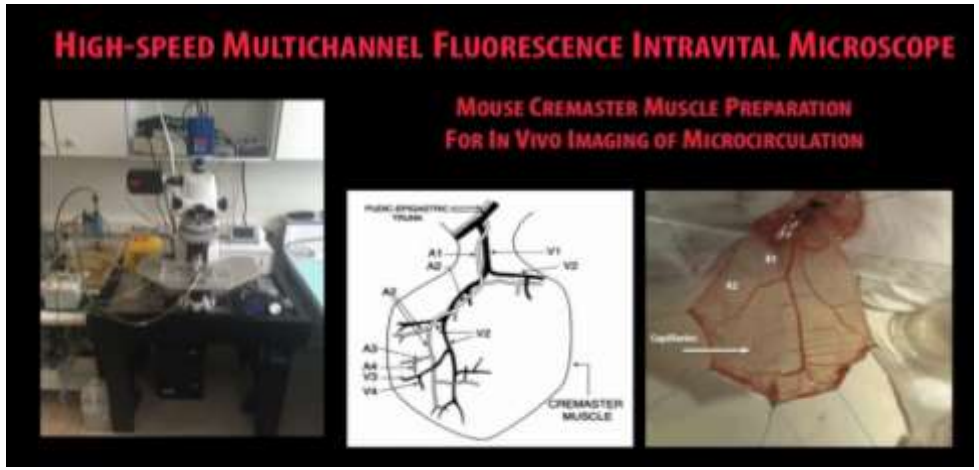




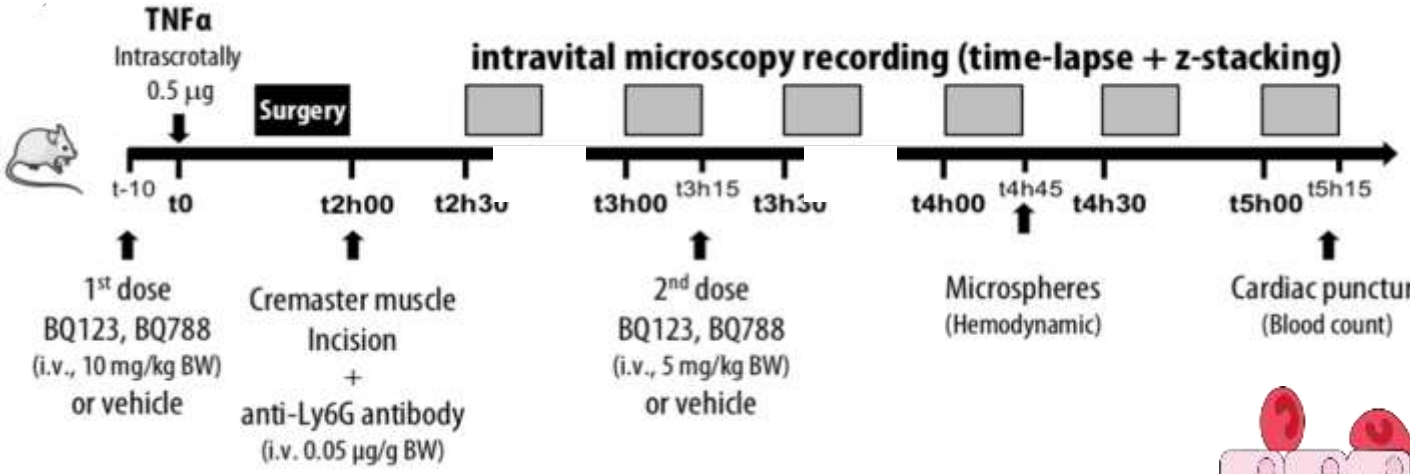
Dufek B et al. *Kidney Int* 2016
Cosgrove D et al. *Front Med* (Lausanne). 2022

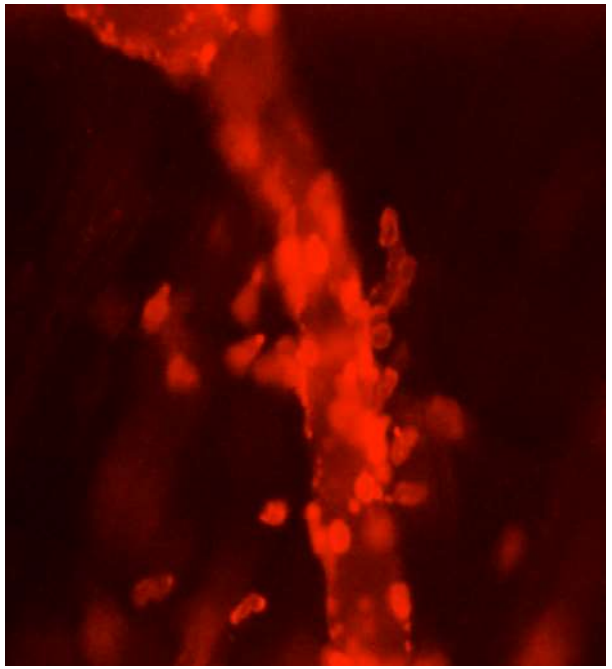
Dale R. Abrahamson, *Kidney Int* 2016

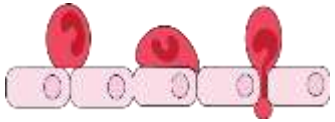
Neutrophils: targets for ET-1?



BQ123: specific ETA antagonist
BQ788: specific ETB antagonist

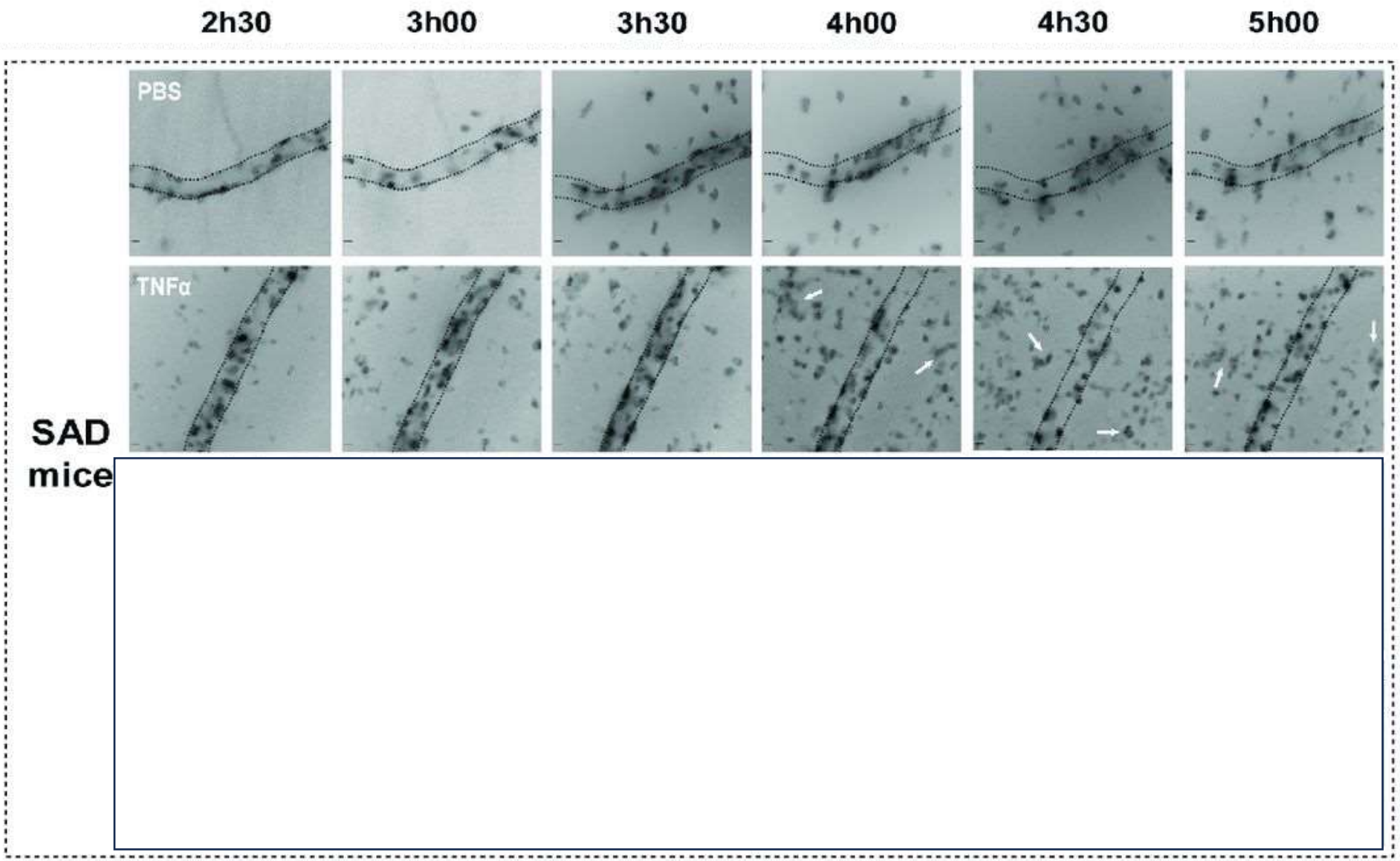






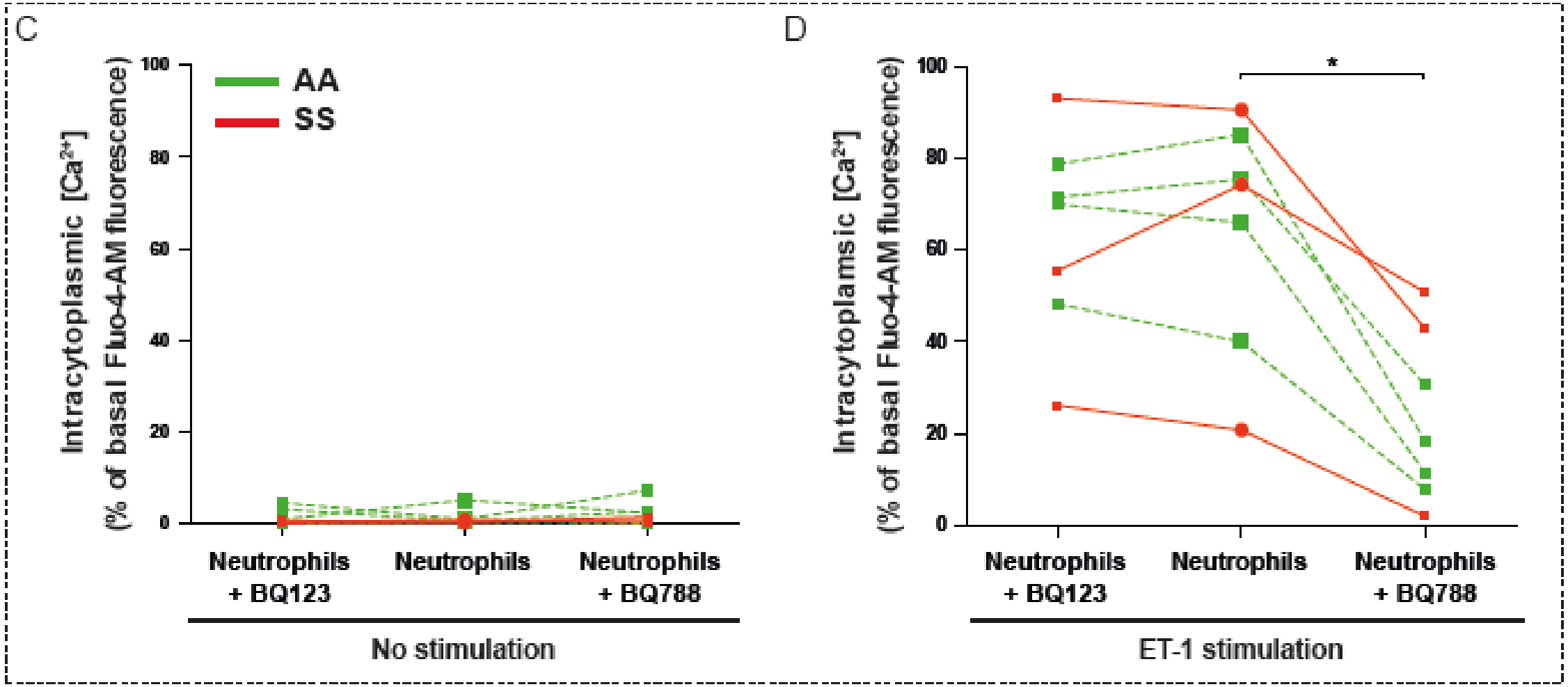
- Rolling
- Adhesion
- Transmigration

Kinetics of neutrophil recruitment and transmigration in the inflamed microcirculation



Koehl B, Nivoit P, El Nemer W, Lenoir O, et al. **Haematologica 2017**;102:1161-1172

ETB Activation Elicits Intracellular Calcium Mobilization in Human Neutrophils



Koehl B, Nivoit P, El Nemer W, Lenoir O, et al. **Haematologica 2017**;102:1161-1172

USE OF ETRA FOR IMMUNE KIDNEY DISEASES INVOLVING NEUTROPHILS?

=> ANCA vasculitis?

=> AKI?

=> ?

ANCA-associated vasculitis

www.kidney-international.org clinical investigation

Arterial stiffness, endothelial dysfunction and impaired fibrinolysis are pathogenic mechanisms contributing to cardiovascular risk in ANCA-associated vasculitis

see commentary on page 963

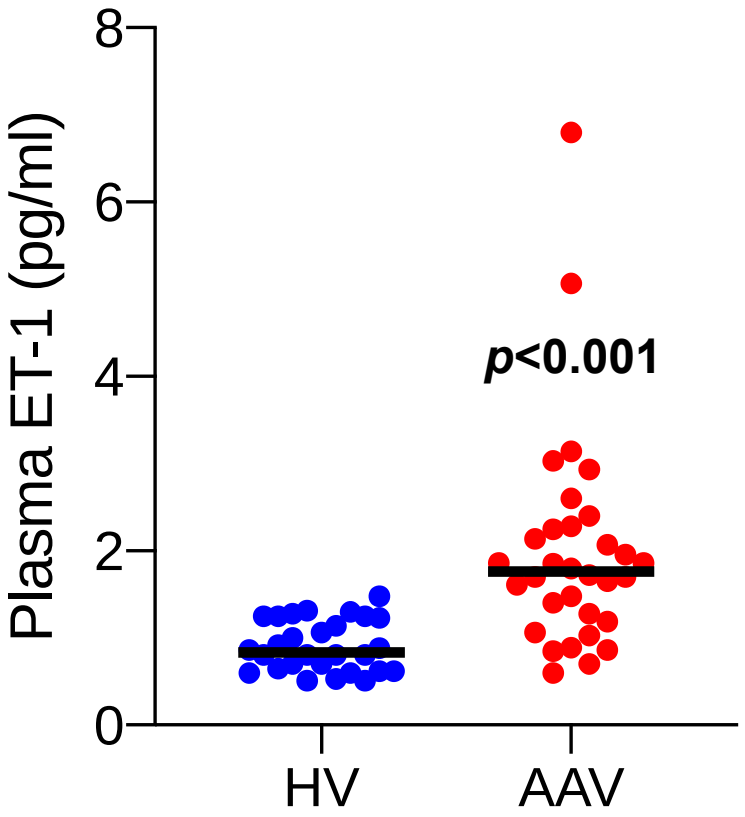
OPEN

Tariq E. Farrah[1,2,3], Vanessa Melville[2], Alicja Czopek[1], Henry Fok[4], Lorraine Bruce[1], Nicholas L. Mills[1,5], Matthew A. Bailey[1], David J. Webb[1,2], James W. Dear[1] and Neeraj Dhaun[1,2,3]

[1]British Heart Foundation Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK; [2]Clinical Research Centre, University of Edinburgh, Western General Hospital, Edinburgh, UK; [3]Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK; [4]Department of Clinical Pharmacology, Kings College London, St Thomas' Hospital, London, UK; and [5]Usher Institute, University of Edinburgh, Edinburgh, UK

Farrah, *Kidney Int* 2022

ANCA-associated vasculitis



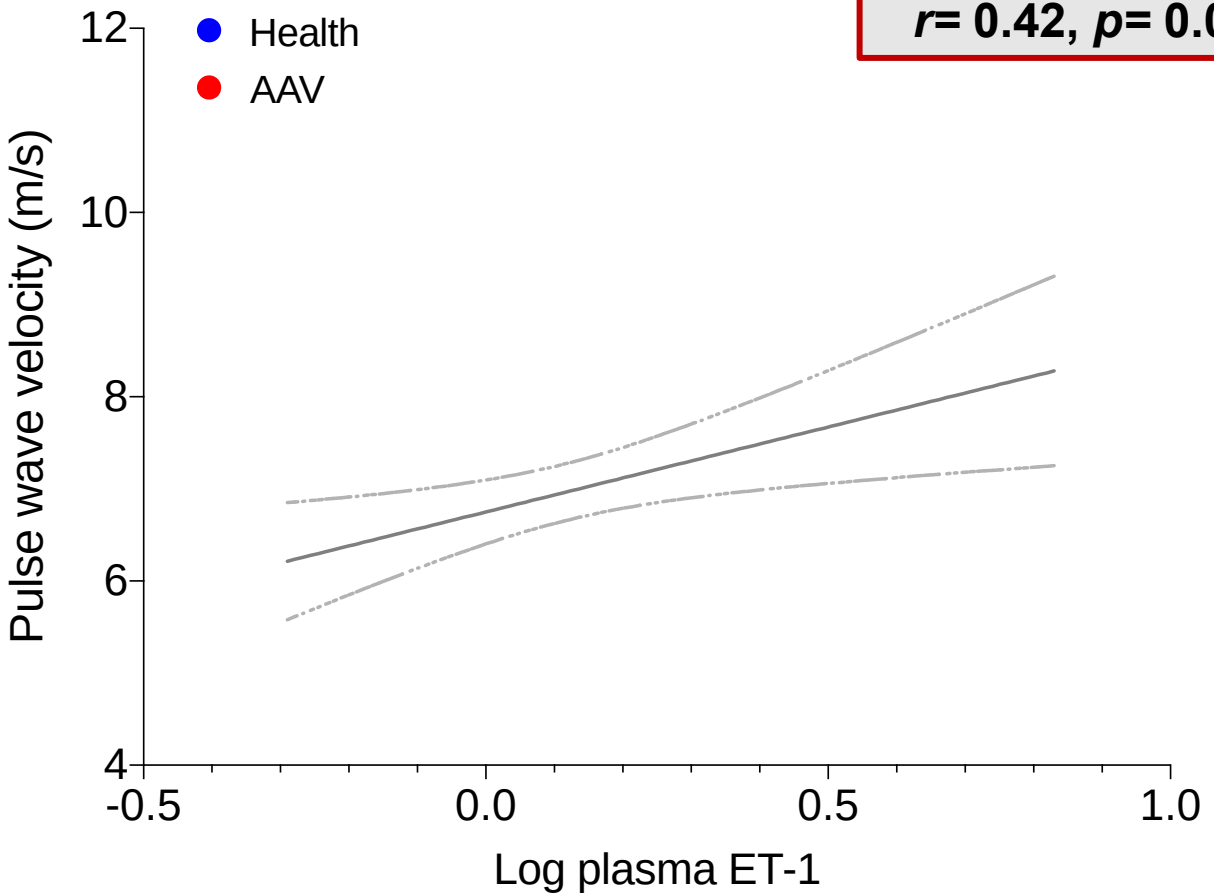
Median plasma ET-1

AAV = 1.8 pg/mL

HV = 0.9 pg/mL

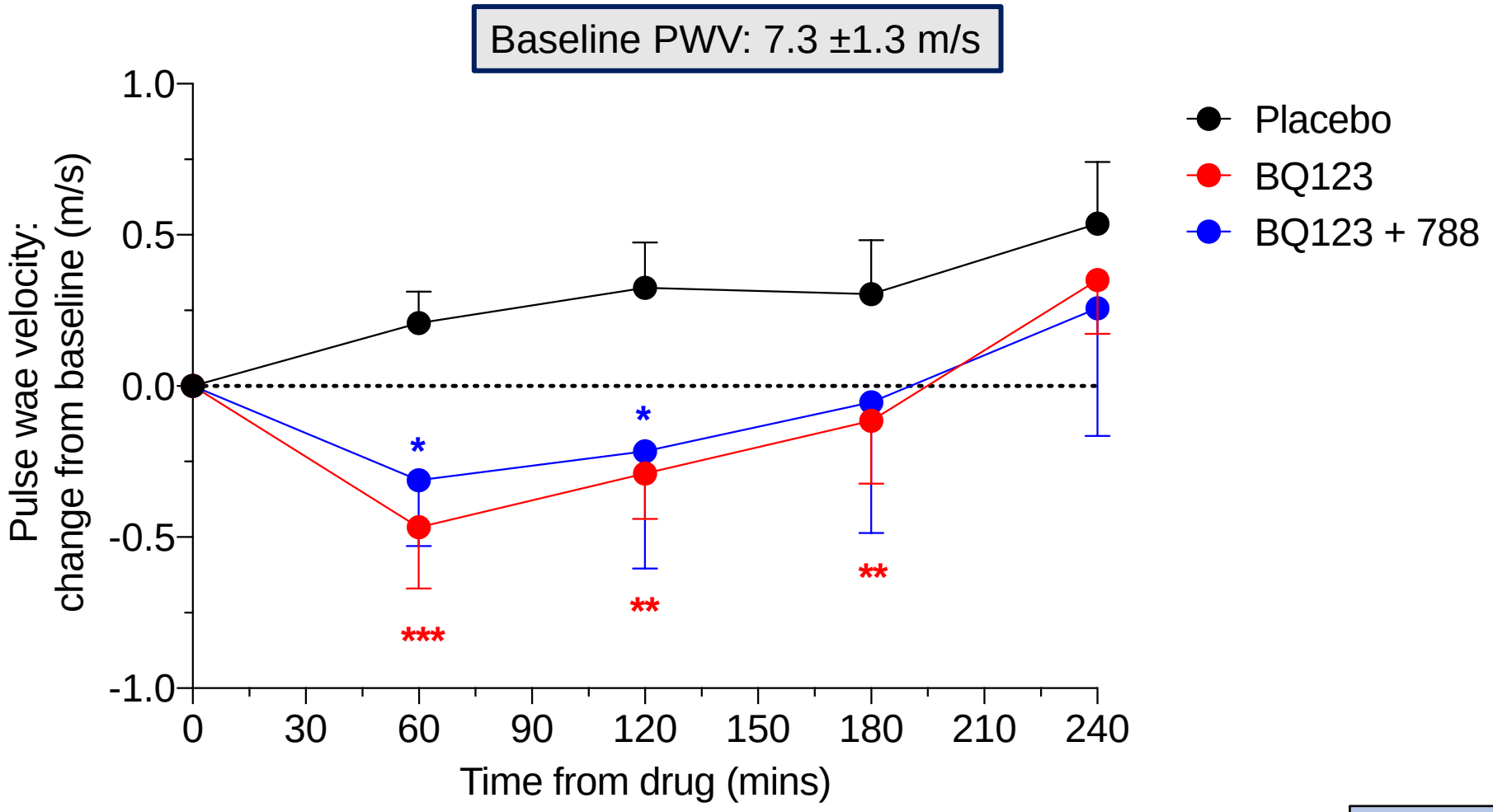
CKD = 2.1 pg/mL

Plasma ET-1 & arterial stiffness



Farrah, *Kidney Int* 2022

Pulse wave velocity



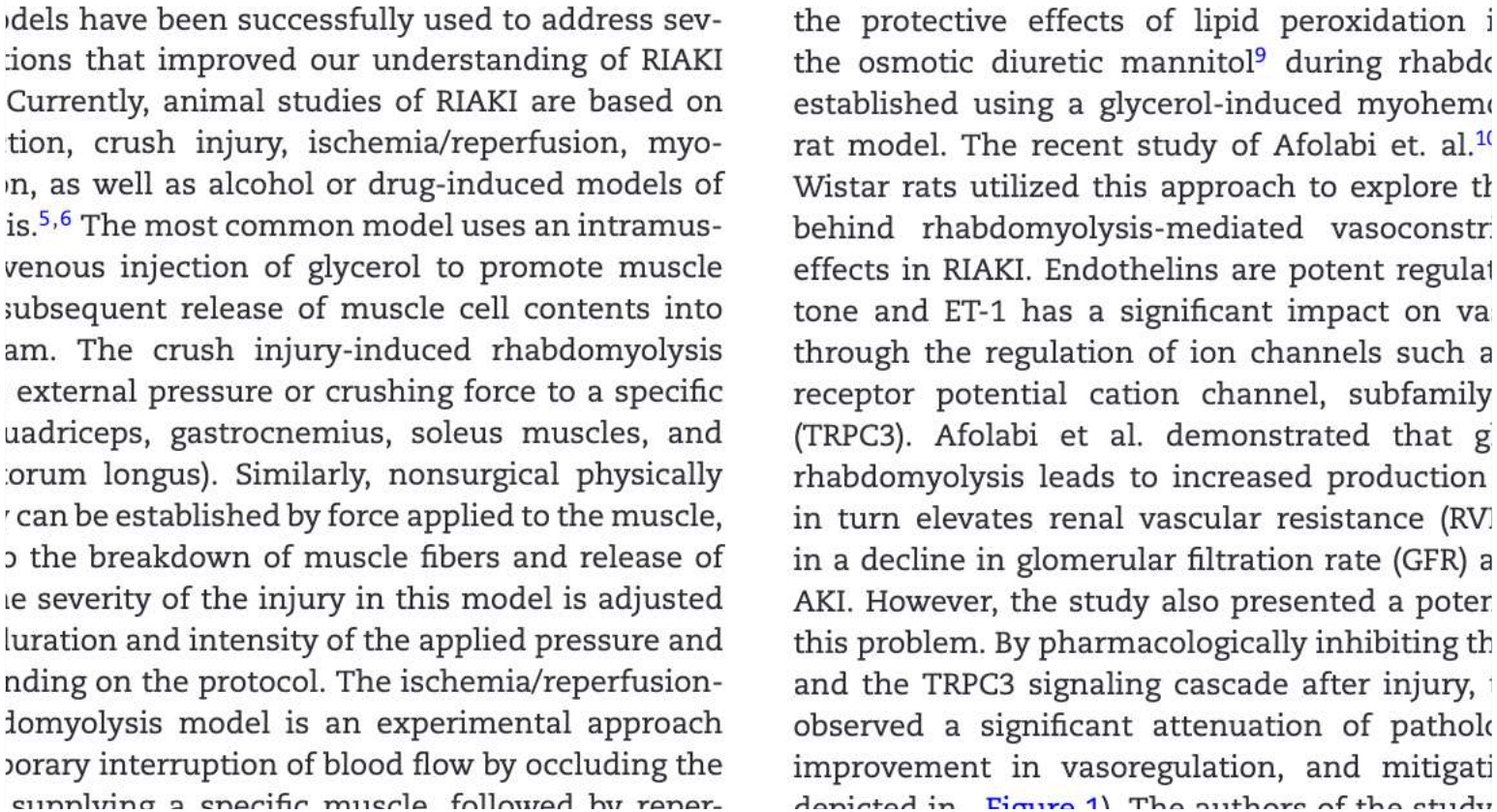
Farrah, *Kidney Int* 2022

AKI & chronic kidney/cardiovascular disease

- AKI mortality is high: 2 million deaths worldwide per year
- AKI survivors: ~30% left with CKD
- Remaining 70%: 28-fold increased risk of developing CKD & cardiovascular disease
- Currently, no treatments that prevent progression of AKI to CKD

Role of endothelin in acute renal failure due to rhabdomyolysis in rats.

Karam H, Bruneval P, Clozel JP, Löffler BM, Bariéty J, Clozel M. J Pharmacol Exp Ther. 1995;274(1):481-6.

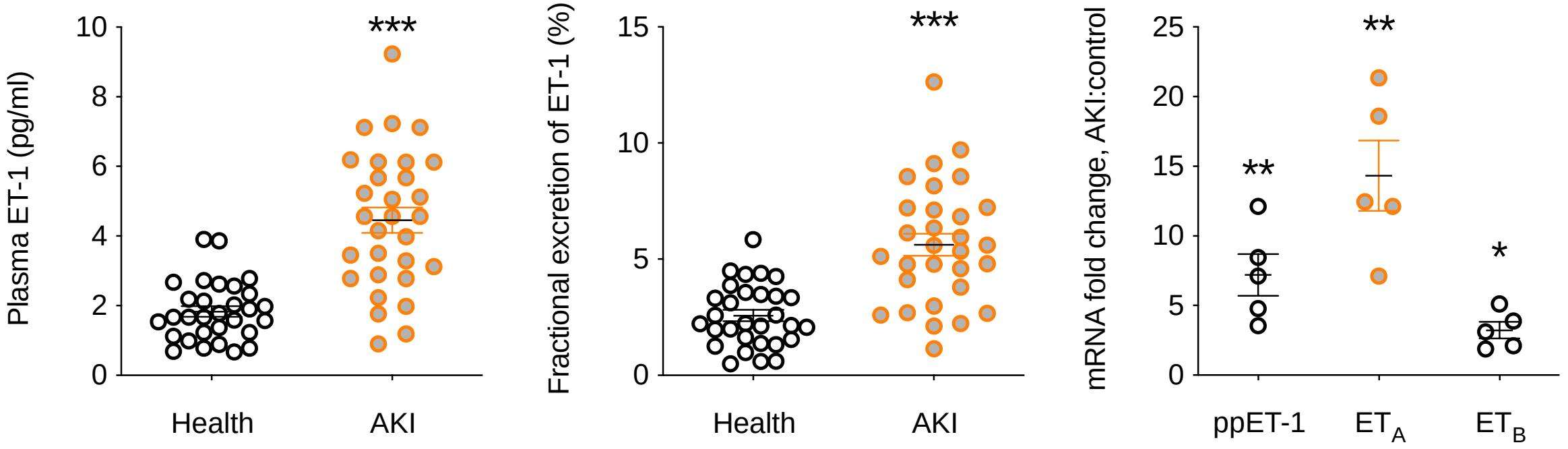


ɔdels have been successfully used to address sev-
ːions that improved our understanding of RIAKI
Currently, animal studies of RIAKI are based on
tion, crush injury, ischemia/reperfusion, myo-
ɔn, as well as alcohol or drug-induced models of
is.[5,6] The most common model uses an intramus-
venous injection of glycerol to promote muscle
subsequent release of muscle cell contents into
am. The crush injury-induced rhabdomyolysis
external pressure or crushing force to a specific
uadriceps, gastrocnemius, soleus muscles, and
ːorum longus). Similarly, nonsurgical physically
ˈ can be established by force applied to the muscle,
ɔ the breakdown of muscle fibers and release of
ıe severity of the injury in this model is adjusted
luration and intensity of the applied pressure and
nding on the protocol. The ischemia/reperfusion-
ɗomyolysis model is an experimental approach
ɔorary interruption of blood flow by occluding the
supplying a specific muscle, followed by reper-

the protective effects of lipid peroxidation i
the osmotic diuretic mannitol[9] during rhabdo
established using a glycerol-induced myohem
rat model. The recent study of Afolabi et. al.[10]
Wistar rats utilized this approach to explore th
behind rhabdomyolysis-mediated vasoconstri
effects in RIAKI. Endothelins are potent regulat
tone and ET-1 has a significant impact on va
through the regulation of ion channels such a
receptor potential cation channel, subfamily
(TRPC3). Afolabi et al. demonstrated that g
rhabdomyolysis leads to increased production
in turn elevates renal vascular resistance (RVI
in a decline in glomerular filtration rate (GFR) a
AKI. However, the study also presented a poter
this problem. By pharmacologically inhibiting th
and the TRPC3 signaling cascade after injury,
observed a significant attenuation of patholo
improvement in vasoregulation, and mitigati
depicted in Figure 1). The authors of the study

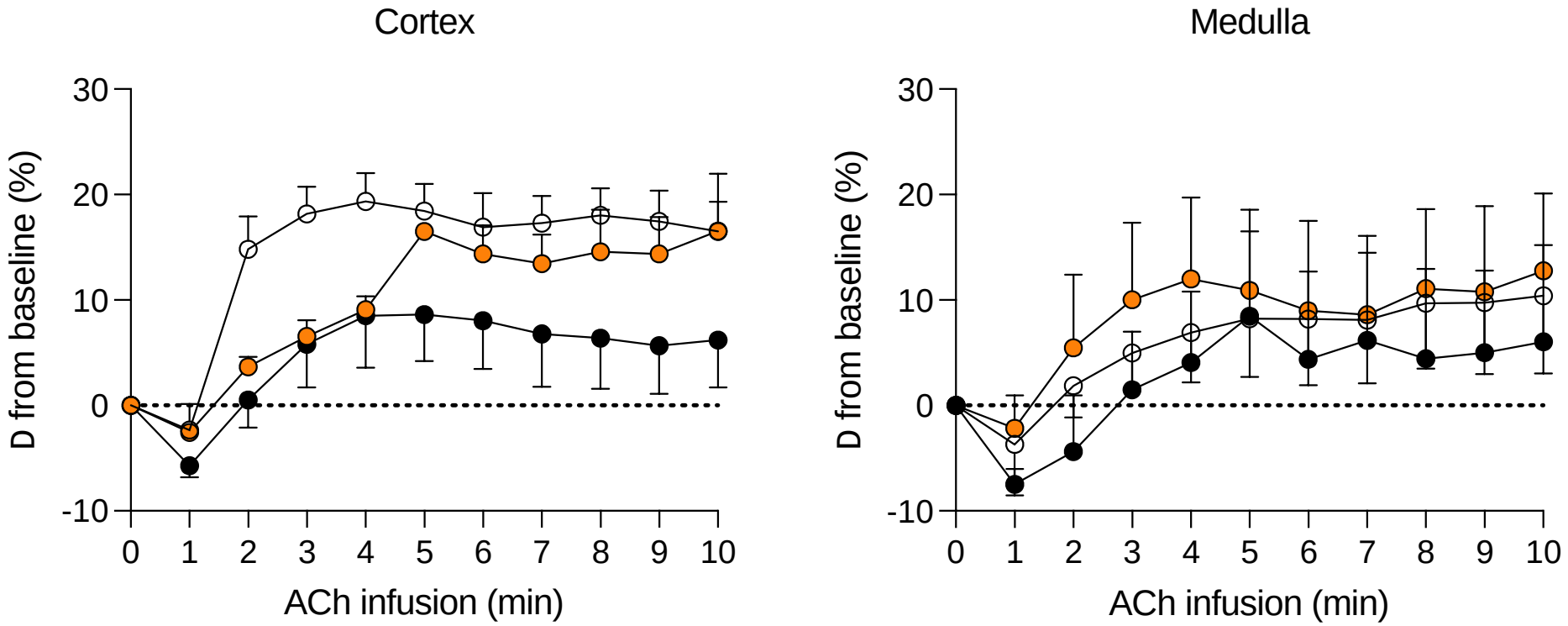
Afolabi JM et al. Post-injury inhibition of Endothelin-1 dependent renal vasoregulation mitigates rhabdomyolysis- induced acute kidney injury. Function (Oxf). 2023;4(4): zqad022

Patients with AKI have an upregulated ET system



Czopek *et al, Sci Transl Med,* 2022

Endothelin-A receptor antagonism improves renal hemodynamics

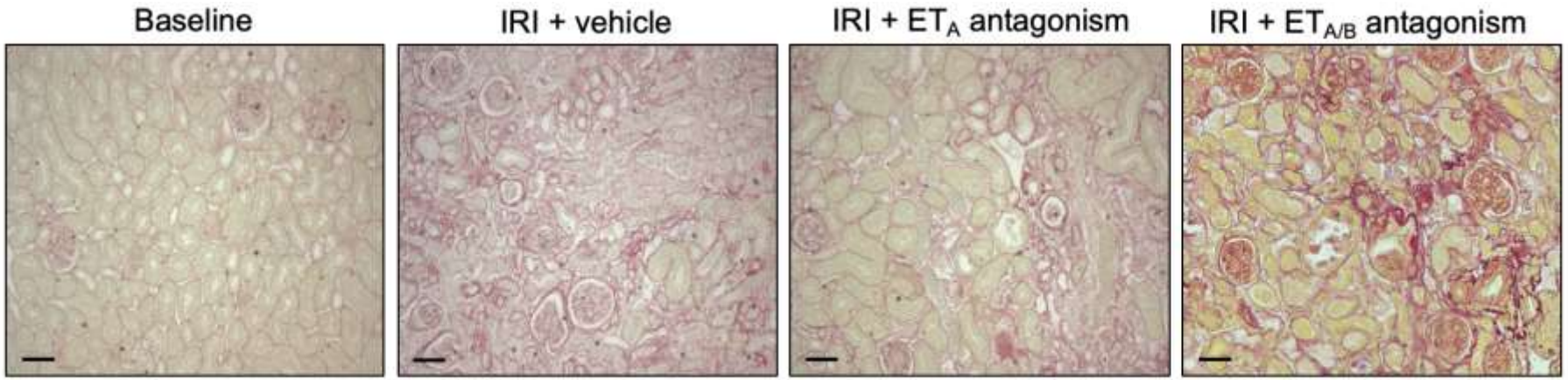


p <0.001 for baseline *vs.* IRI + vehicle
p <0.05 for IRI + vehicle *vs.* IRI + ET_A antagonism

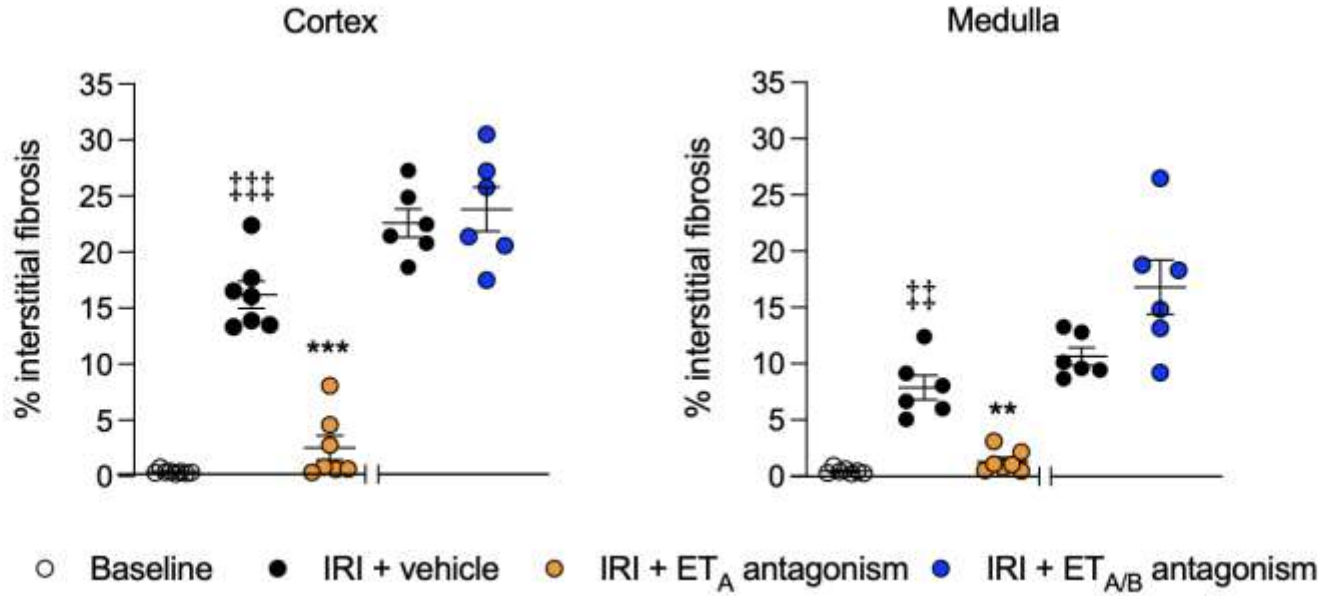
p =ns for baseline *vs.* IRI + vehicle
p =ns for IRI + vehicle *vs.* IRI + ET_A antagonism

Czopek *et al, Sci Transl Med,* 2022

Selective, but not dual, endothelin receptor antagonism prevents the transition from AKI to CKD



4 Wk



Czopek *et al, Sci Transl Med,* 2022

Dialysis

- CKD increasingly common and number of patients requiring dialysis (& unsuitable for transplantation) projected to increase globally

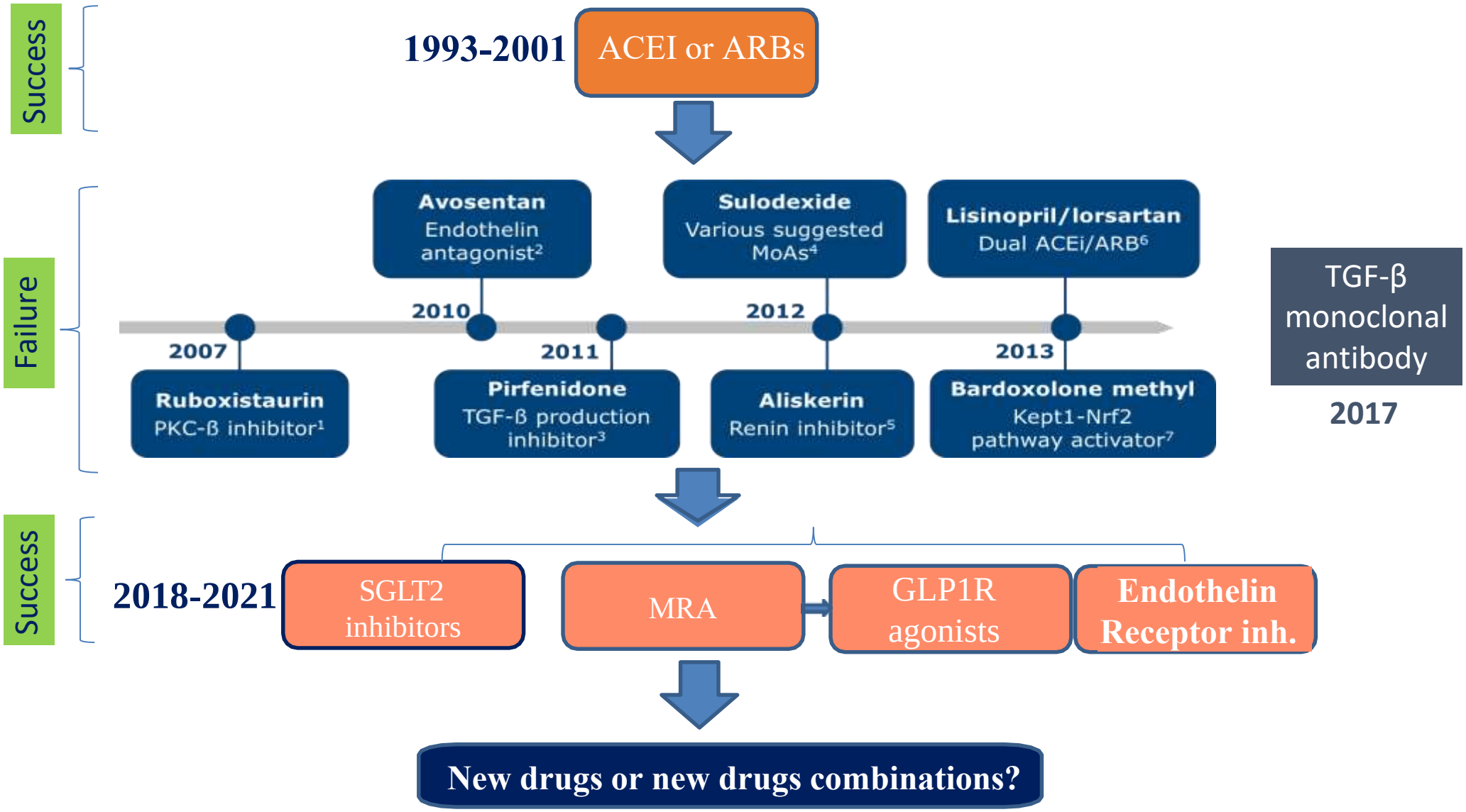
- Number of dialysis complications in which ET-1 may play a role
  - Hypertension
  - Increased CVD risk (arterial stiffness, LVH)
  - Pain

- **Teratogenicity and fluid retention not an issue here**

- Captive and co-morbid patient group (they come to hospital x3/week!)

- **Renal AND Cardiovascular benefit is expected**

Time line of drug discovery for DKD



Acting on multiple levels to slow kidney diseases

ETRA, MR?

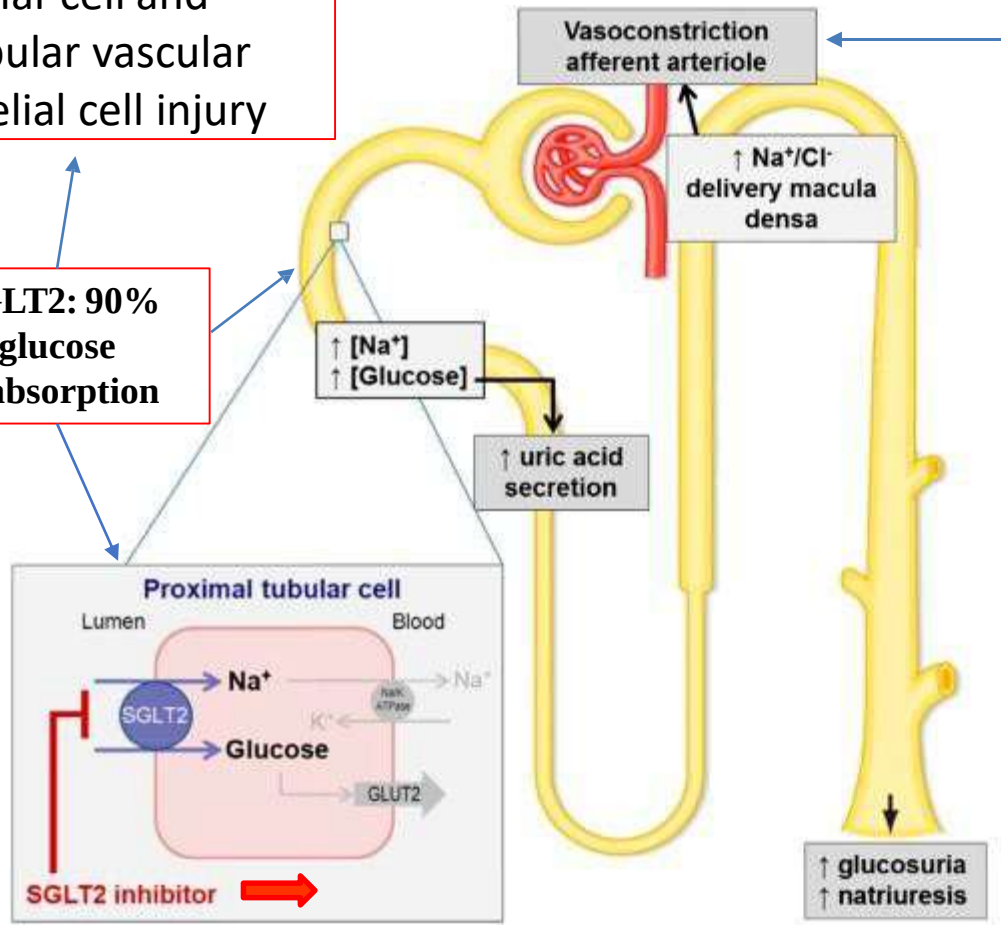
Tubular cell and peritubular vascular endothelial cell injury

ETRA, ACEI, ARB, SGLT2i MRA

- hypoxia
- oxidative stress
- inflammation

promoting vasodilation of efferent arteriole

ETRA, ACEI, ARB, SGLT2i



Clinical findings (SGLT2i)

- ↓ Plasma glucose
- ↓ Body weight
- ↓ Blood pressure
- ↓ Plasma uric acid
- ↓ Glomerular hyperfiltration

ETRA

- PMN
- Mast Cells

- Interstitial fibrosis, fibroblasts
- Systemic endothelium

ETRA, MRA

Combination of ET & SGLT2 inhibition

brief report www.kidney-international.org

New insights from SONAR indicate adding sodium glucose co-transporter 2 inhibitors to an endothelin receptor antagonist mitigates fluid retention and enhances albuminuria reduction

see commentary on page 301

OPEN

Hiddo J.L. Heerspink[1], Donald E. Kohan[2] and Dick de Zeeuw[1]

[1]Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; and [2]Division of Nephrology, University of Utah, Salt Lake City, Utah, USA

Hiddo J.L. Heerspink et al. The Lancet 2019

Heerspink, *Kidney Int* 2021

Major studies on endothelin receptor antagonists in diabetic and non-diabetic CKD currently in the pipeline

The safety and efficacy of combining ETRA with SGLT2 inhibitors are being evaluated

in the PROTECT open-label extension (NCT03762850) and the SPARTACUS (NCT05856760) trials (IgAN) : **Sparsentan**

In the ASSIST (IgAN) and AFFINITY (DKD) trials: **Atrasentan**

In the ZODIAC (DKD) and ZENITH (proteinuric CKD) trials: **Zibotentan**

Study name (acronym) ID	Phase	Study population	Drugs	Planned enrolment	Outcomes (primary; secondary and others)
Atrasentan in Patients With IgA Nephropathy (ALIGN); NCT04573478	Phase 3	Biopsy-proven IgA nephropathy	Atrasentan versus placebo (all receiving RAS inhibitors, some receiving background SGLT2i)	320 subjects	Change in proteinuria (UPCR) Change in GFR Composite endpoints (reduction in eGFR, dialysis, transplantation, mortality) Change in proteinuria (24-h collection)
Randomized, Double-blind, Placebo-controlled, Crossover Study of Atrasentan in Subjects With IgA Nephropathy (ASSIST); NCT05834738	Phase 2	Biopsy-proven IgAN	Atrasentan versus placebo (both on a background SGLT2i and RAS inhibitors)	52 subjects	Change in proteinuria (UPCR) Change in proteinuria at 24 weeks of treatment (UPCR)
Atrasentan in Patients With Proteinuric Glomerular Diseases (AFFINITY); NCT04573920	Phase 2	Proteinuric glomerular diseases (IgAN, FSGS, Alport syndrome, diabetic kidney disease)	Atrasentan (all receiving RAS inhibitor; diabetic kidney disease patients receiving SGLT2i)	100 subjects	Change in proteinuria (IgAN, FSGS and Alport patients) Change in albuminuria (diabetic kidney disease patients)
Zibotentan and Dapagliflozin in Patients With Type 2 Diabetes and Elevated Albuminuria (ZODIAC); NCT05570305	Phase 2	Type 2 diabetes mellitus with albuminuria	Zibotentan and dapagliflozin versus placebo (all receiving RAS inhibitor)	38 subjects	Change from baseline in albuminuria after 4 weeks combined zibotentan and dapagliflozin treatment versus 4 weeks treatment with zibotentan alone Changes in extracellular fluid, body weight, N-terminal prohormone of brain natriuretic peptide, GFR, haematocrit, systolic blood pressure, diastolic blood pressure, renin–angiotensin–aldosterone system markers, copeptin
Study to Investigate Efficacy, Safety, and Tolerability of Zibotentan/Dapagliflozin Compared to Dapagliflozin in Participants With Chronic Kidney Disease and High Proteinuria (ZENITH High Proteinuria); NCT06087835	Phase 3	CKD with high proteinuria (UACR >700 mg/g or UPCR >1000 mg/g)	Zibotentan and dapagliflozin versus dapagliflozin (all receiving RAS inhibitor)	1500 subjects	Change in GFR from baseline Change in UPCR Change in UACR Time to any component of the composite endpoint (eGFR decline, ESRD, death) Change in systolic blood pressure Proportion of participants achieving UPCR <1000 mg/g and >30% reduction
A Study to Investigate Safety and Effect of Sparsentan in Combination With SGLT2 Inhibition in Participants With IgAN (SPARTACUS); NCT05856760	Phase 2	Biopsy-proven IgAN	Sparsentan (all receiving background SGLT2i)	60 subjects	Change in UACR UACR <0.2 g/g Reduction in UACR (30% and 50%) UACR and UPCR at each visit eGFR at each visit Blood pressure at each visit
A Study of the Safety and Activity of Sparsentan for the Treatment of Incident Patients With Immunoglobulin A Nephropathy (SPARTAN); NCT04663204	Phase 2	Incident biopsy-proven IgAN	Sparsentan	12 subjects	UPCR eGFR Change in proteinuria (24-h) Abnormalities in clinical laboratory assessments and vital signs Incidence of AEs, serious AEs, AEs leading to discontinuation, AEs leading to death
ETA and AT1 Antagonism in ANCA-vasculitis (SPARVASC); NCT05630612	Phase 2	ANCA-associated vasculitis in remission	Sparsentan versus irbesartan	40 subjects	Forearm blood flow Fibrinolytic capacity Blood pressure Arterial stiffness Systemic haemodynamics Proteinuria

GLOMERULAR DISEASES

Given the effects of endothelin antagonism are analogous to those of RAS blockade – increase in renal blood flow; reduction in intraglomerular pressure; reduction in filtration fraction – but are additive to those of RAS blockade, it is likely that any glomerular disease might benefit from this approach.

BEYOND GLOMERULI

Endothelin antagonism may provide additional therapeutic effects via specific cellular actions on:

Podocytes *(with impact of surrounding other glomerular cell types) =>* ***all podocytopathies***

Neutrophils *(**Sickle Cell Disease, ANCA vasculitis ?, AKI?, Dialysis? Transplant?**)*
Monocytes *(**HD, Transplant, ANCA vasculitis, IgAN**)*
Mast cells *(AKI, SCD, IgAN, all CKD?)*

Endothelial cells *(**AKI, IgAN, IgAV, AAV, Scleroderma, Preeclampsia, SCD, DKD, HUS, CKD**)*

VSMCs, ***Pericytes?, Fibroblasts?*** *(AKI, all CKD?)*

The Paris GlomGang @PARCC-HEGP

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

EDINBURGH KIDNEY RESEARCH GROUP

KIDNEY DISEASE

Endothelin blockade prevents the long-term cardiovascular and renal sequelae of acute kidney injury in mice

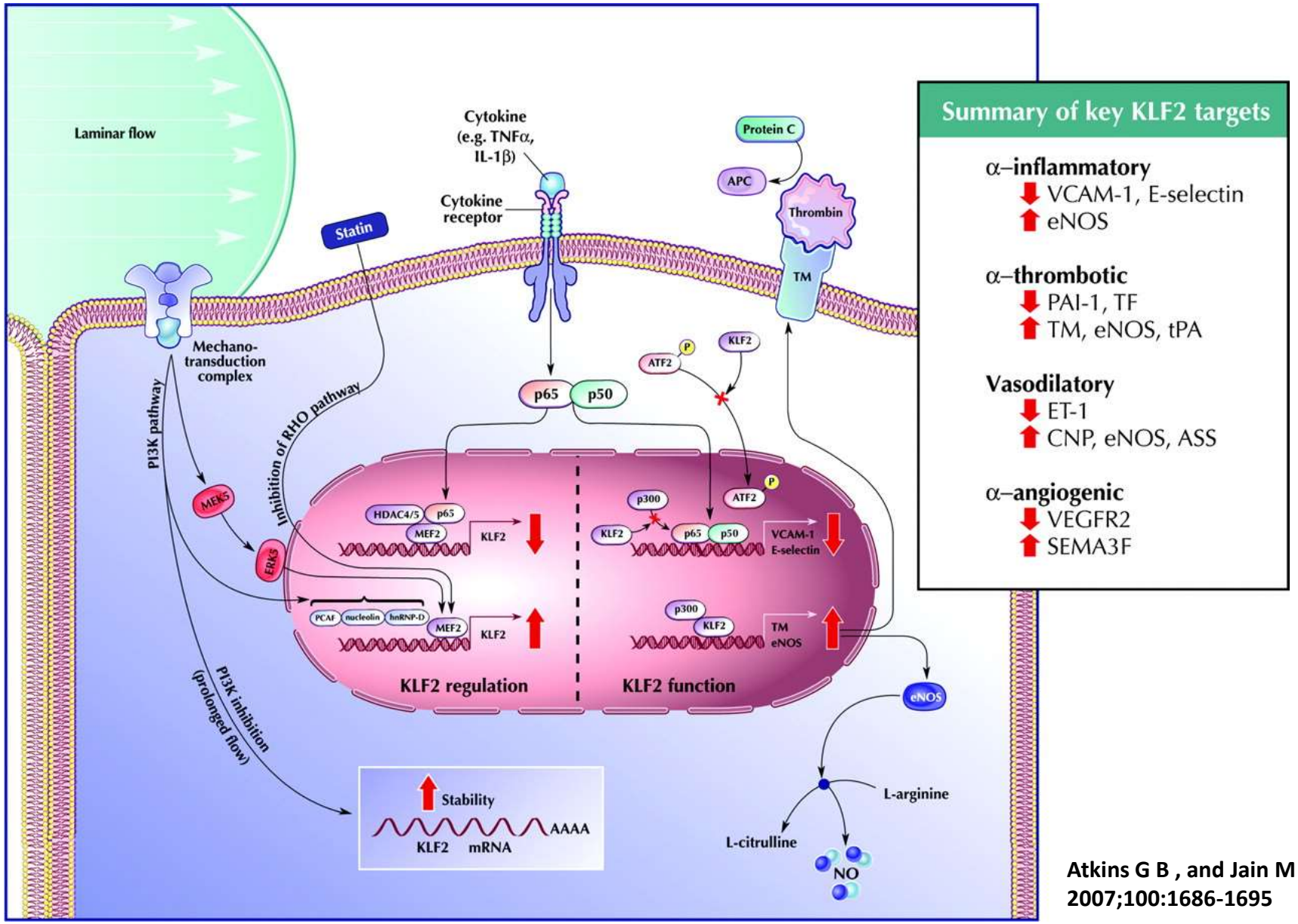
Alicja Czopek[1†], Rebecca Moorhouse[1†], Peter J. Gallacher[1], Dan Pugh[1,2], Jessica R. Ivy[1], Tariq E. Farrah[1,2], Emily Godden[1], Robert W. Hunter[1,2], David J. Webb[1], Pierre-Louis Tharaux[3], David C. Kluth[1,2], James W. Dear[1], Matthew A. Bailey[1‡], Neeraj Dhaun[1,2,3‡*]

Acute kidney injury (AKI) is common and associated with increased risks of cardiovascular and chronic kidney disease. Causative molecular/physiological pathways are poorly defined. There are no therapies to improve long-term outcomes. An activated endothelin system promotes cardiovascular and kidney disease progression. We hypothesized a causal role for this in the transition of AKI to chronic disease. Plasma endothelin-1 was threefold higher; urine endothelin-1 was twofold higher; and kidney preproendothelin-1, endothelin-A, and endothelin-B receptor message up-regulated in patients with AKI. To show causality, AKI was induced in mice by prolonged ischemia with a 4-week follow-up. Ischemic injury resulted in hypertension, endothelium-dependent and endothelium-independent macrovascular and microvascular dysfunction, and an increase in circulating inflammatory Ly6C^{high} monocytes. In the kidney, we observed fibrosis, microvascular rarefaction, and inflammation. Administration of endothelin-A antagonist, but not dual endothelin-A/B antagonist, normalized blood pressure, improved macrovascular and microvascular function, and prevented the transition of AKI to CKD. Endothelin-A blockade reduced circulating and renal proinflammatory Ly6C^{high} monocytes and B cells, and promoted recruitment of anti-inflammatory Ly6C^{low} monocytes to the kidney. Blood pressure reduction alone provided no benefits; blood pressure reduction alongside blockade of the endothelin system was as effective as endothelin-A antagonism in mitigating the long-term sequelae of AKI in mice. Our studies suggest up-regulation of the endothelin system in patients with AKI and show in mice that existing drugs that block the endothelin system, particularly those coupling vascular support and anti-inflammatory action, can prevent the transition of AKI to chronic kidney and cardiovascular disease.

Czopek *et al, Sci Transl Med,* 2022

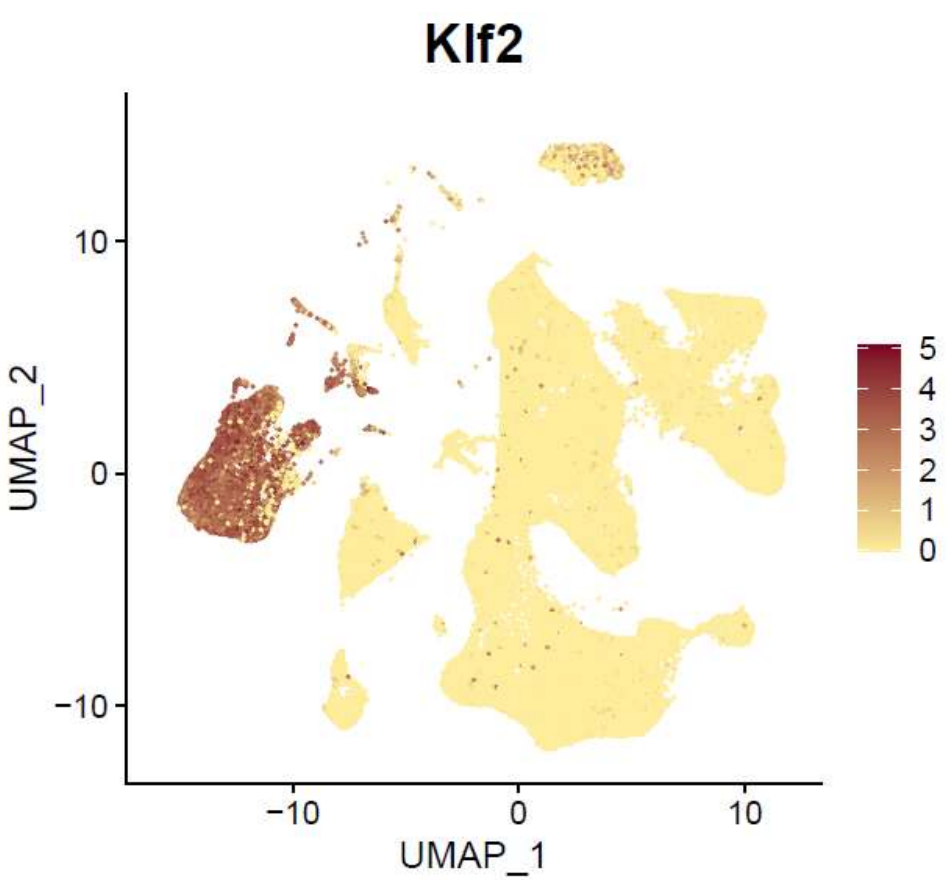
Merci

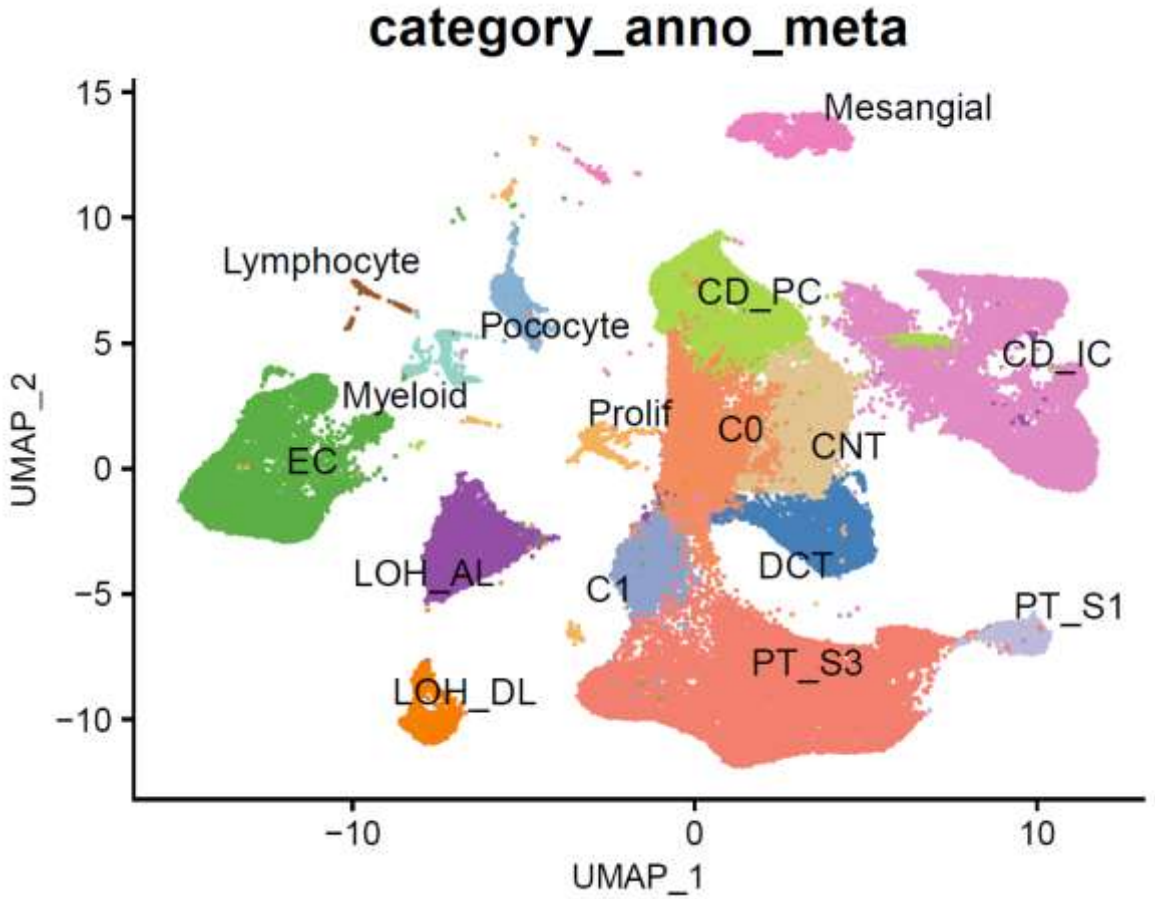
KLF2 is regulated by shear stress in endothelial cells and has endothelium-protective effects, *including via repression of*



Atkins G B , and Jain M K *Circulation Research*. 2007;100:1686-1695

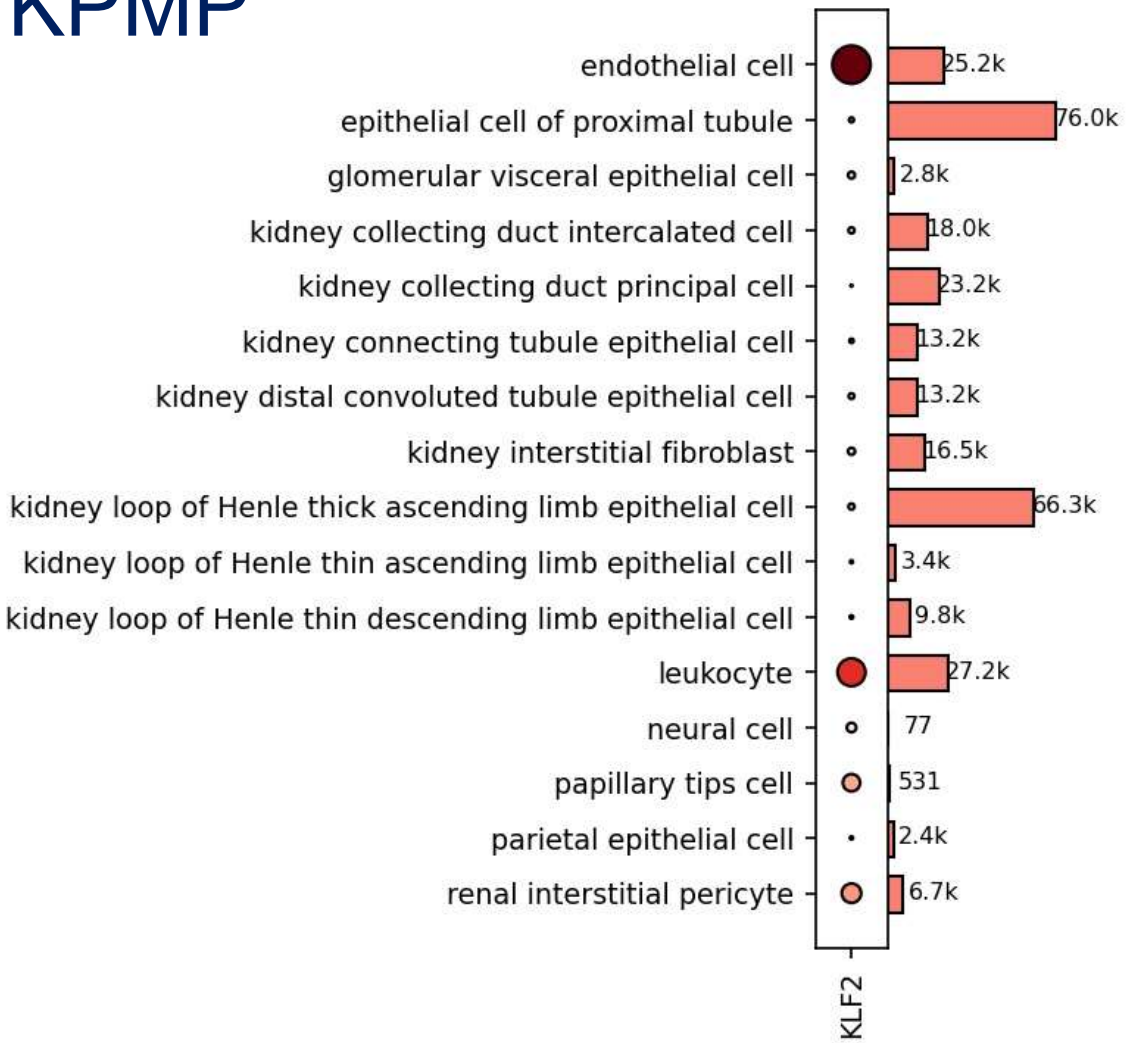
Single cell data suggest that KLF2 expresses mostly in endothelial cells

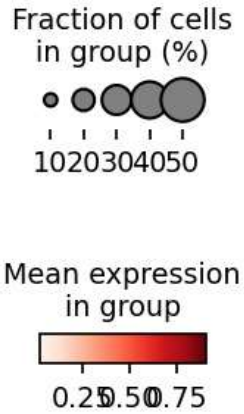




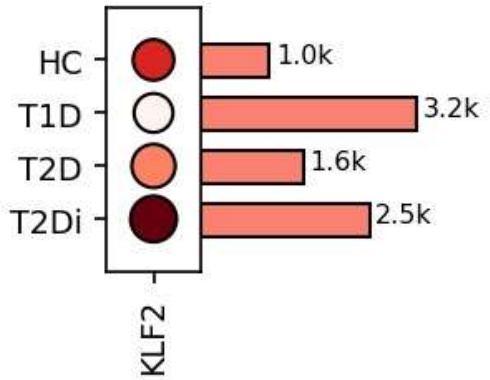
ALTERATION OF KLF2 in DKD

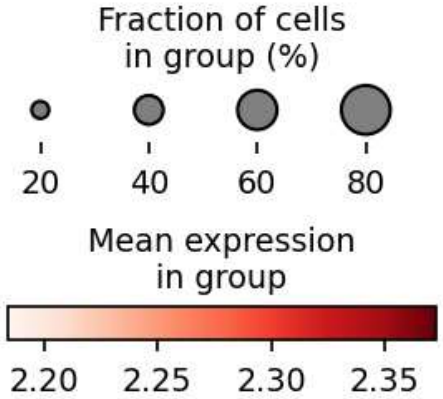
KPMP





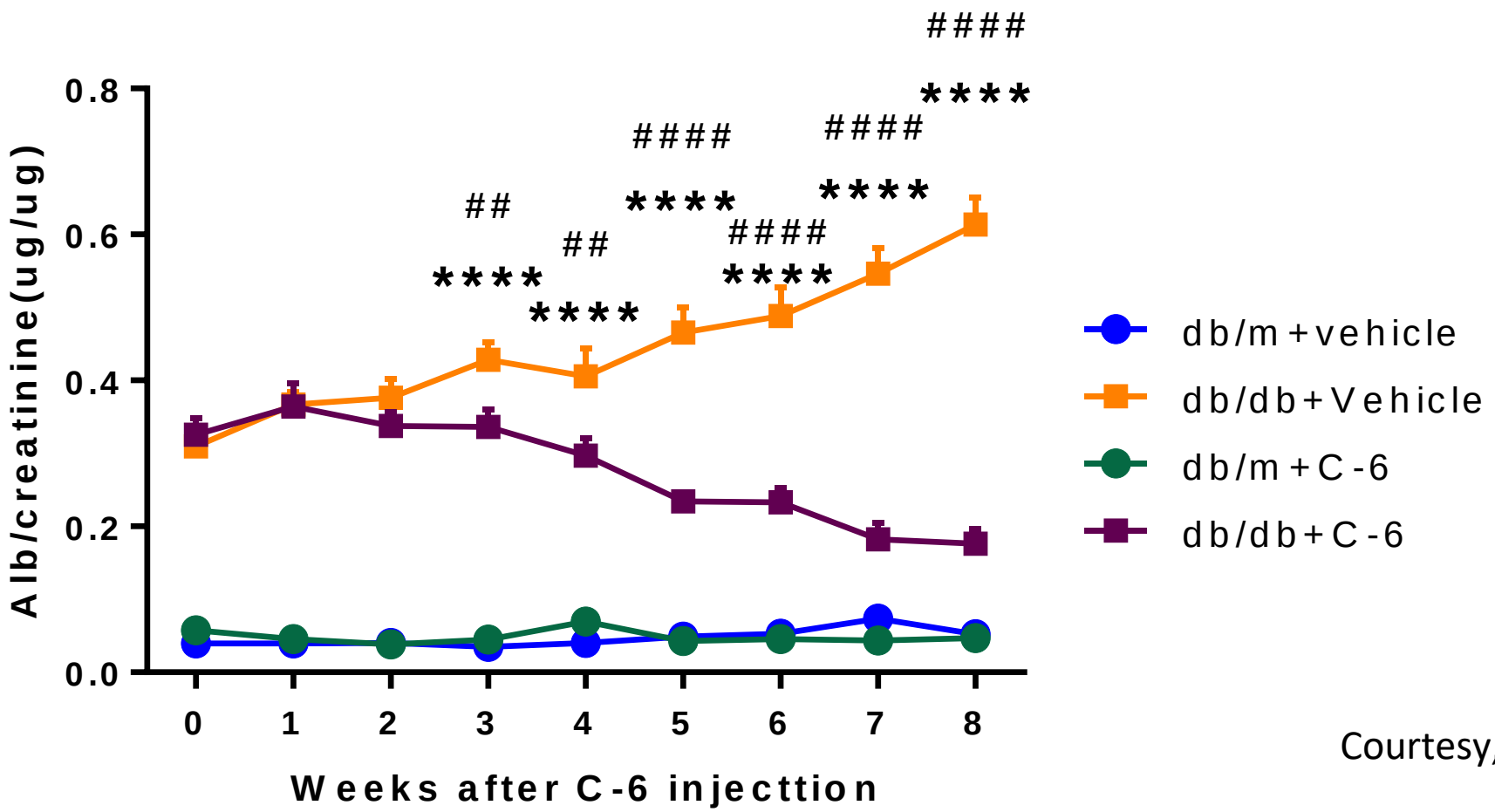
T2DM





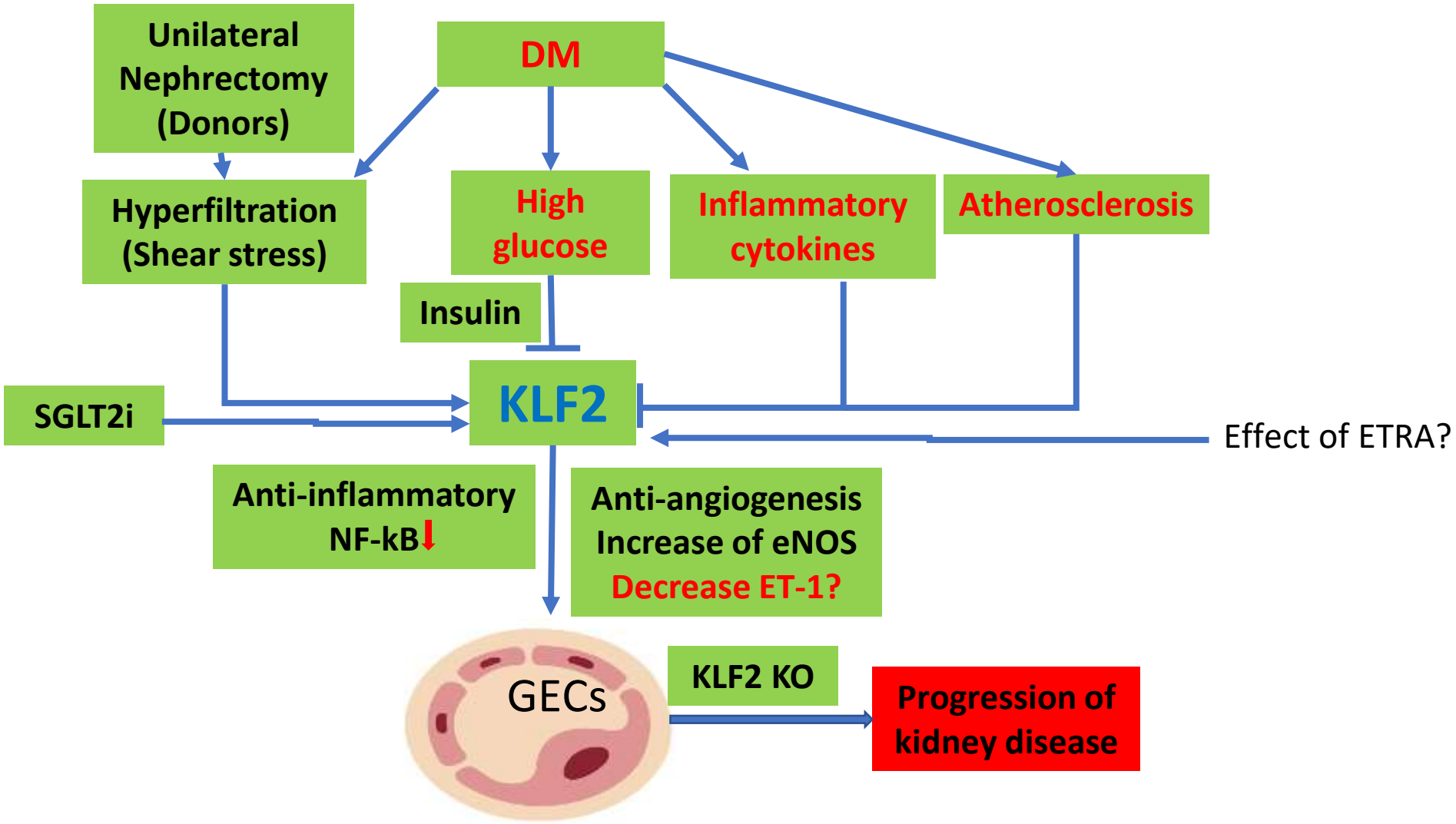
Treatment of KLF2 agonist ameliorates albuminuria in db/db mice

=> suggests a contribution of Endothelial injury to DKD



Courtesy, Cijiang John He

Summary of KLF2 data in kidney disease



Endothelin-1 induces mucosal mast cell degranulation and tissue injury via ETA receptors. Boros M et al. Clin Sci (Lond). 2002 Aug:103 Suppl 48:31S-34S

Mast Cells Mediate Acute Kidney Injury through the Production of TNF in cisplatin-induced renal injury.
Summers SA et al. J Am Soc Nephrol. 2011; 22(12): 2226–2236.

Selective ET(A) receptor blockade protects against cisplatin-induced acute renal failure in male rats
Mai M Helmy et al. Eur J Pharmacol. 2014:730:133-9

Endothelin system expression in the kidney following cisplatin-induced acute kidney injury in male and female mice
Gales A et al. Can J Physiol Pharmacol. 2022; 100(9): 868–879

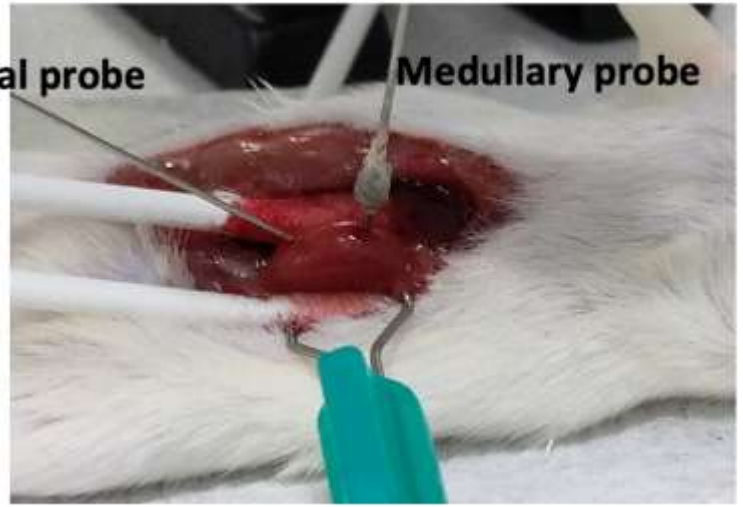
MC infiltration was correlated with an increase in serum creatinine between tissue collection and follow up in IgA nephropathy (Ehara T et al. Contribution of mast cells to the tubulointerstitial lesions in IgA nephritis. Kidney Int 1998;54:1675-83; Silva GE et al. Mast cells, TGF-beta1 and alpha-SMA expression in IgA nephropathy. Dis Markers 2008;24:181-90. Hiromura K et al. Tubulointerstitial mast cell infiltration in glomerulonephritis. Am J Kidney Dis 1998;32:593-9; Kurusu A, et al. Relationship between mast cells in the tubulointerstitium and prognosis of patients with IgA nephropathy. Nephron 2001;89:391-7).

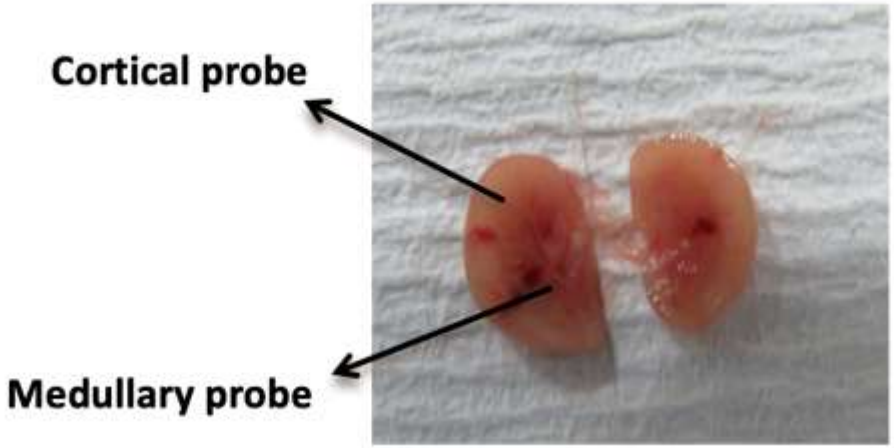
Mast cells in rapidly progressive glomerulonephritis. Tóth T et al. J Am Soc Nephrol 1999;10:1498-505

Interstitial fibrosis, a common manifestation of kidney disease, **was positively correlated with the degree of MC infiltration** (Pardo J, Diaz L, Errasti P, et al. Mast cells in chronic rejection of human renal allografts. Virchows Arch 2000;437:167-72 17,20-22; Kondo S et al. Role of mast cell tryptase in renal interstitial fibrosis. J Am Soc Nephrol 2001;12:1668-76; Yamada M et al. Mast cell chymase expression and mast cell phenotypes in human rejected kidneys. Kidney Int 2001;59:1374-81).

Heightened levels of MCs were also associated with worse clinical outcomes in patients with kidney disease, while those with stable or improving renal function had reduced MC infiltration (Silva GE et al. Mast cells, TGF-beta1 and alpha-SMA expression in IgA nephropathy.

Endothelin-A receptor antagonism improves renal hemodynamics





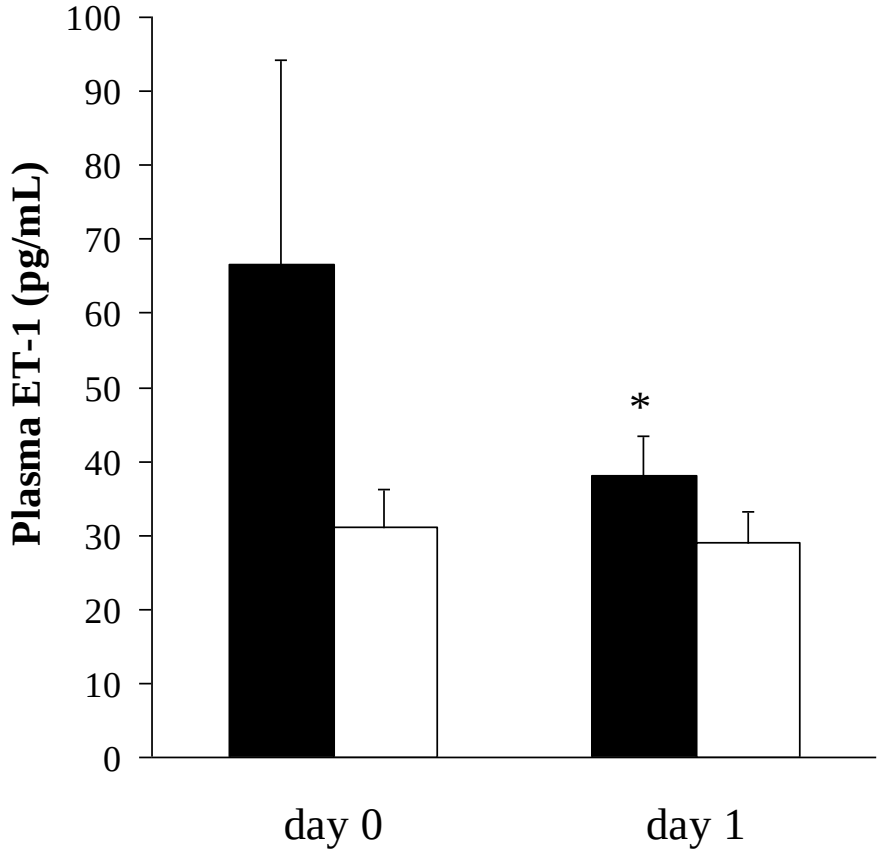
Acute kidney injury

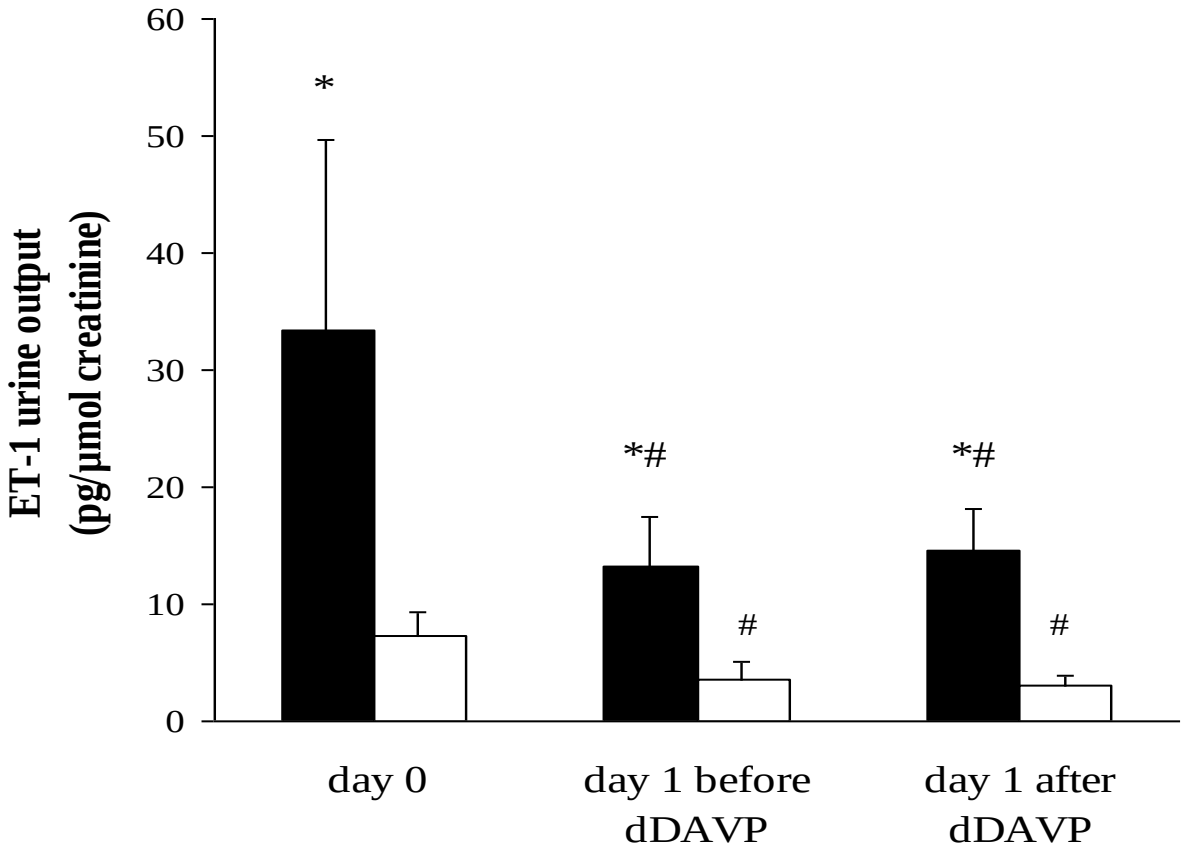
- AKI is a global health problem; affects up to 20% of hospital inpatients
- Costs $24 billion per year in the US; 1% of the annual NHS budget in the UK
- Treatment is supportive

Summary

- The endothelin system is important in renal physiology and disease
- Accepting the advent of SGLT2i, there remain potential (renal) patient groups who might benefit from ET system blockade
  - Areas discussed
  - Kidney disease in the context of anti-angiogenic therapies and preeclampsia
  - Dialysis, transplant
- Industry engagement to take this forward is key

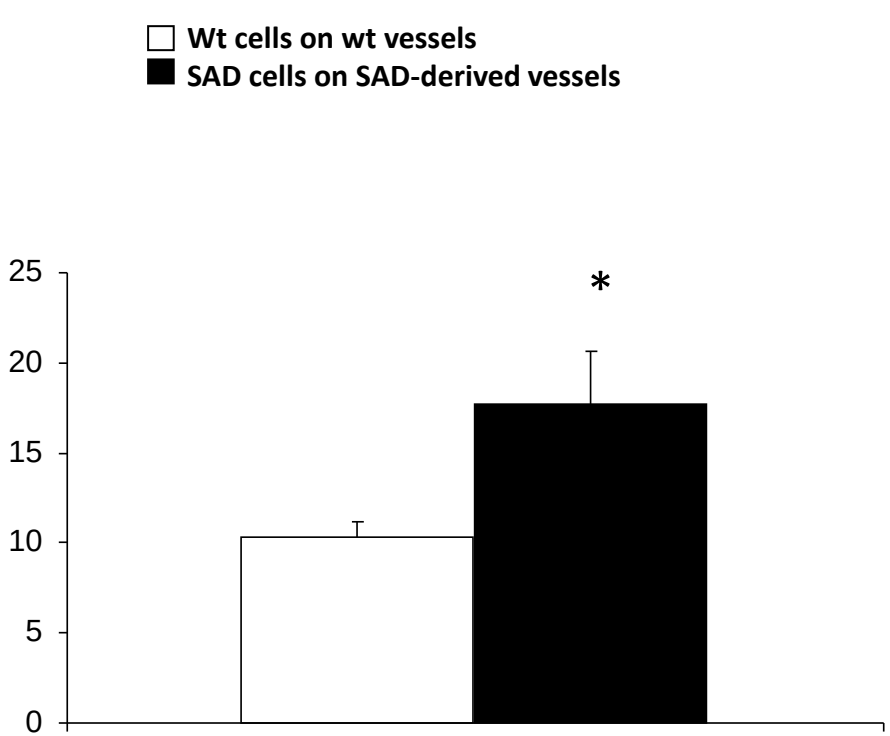
SCD kidney as a potent source of ET-1

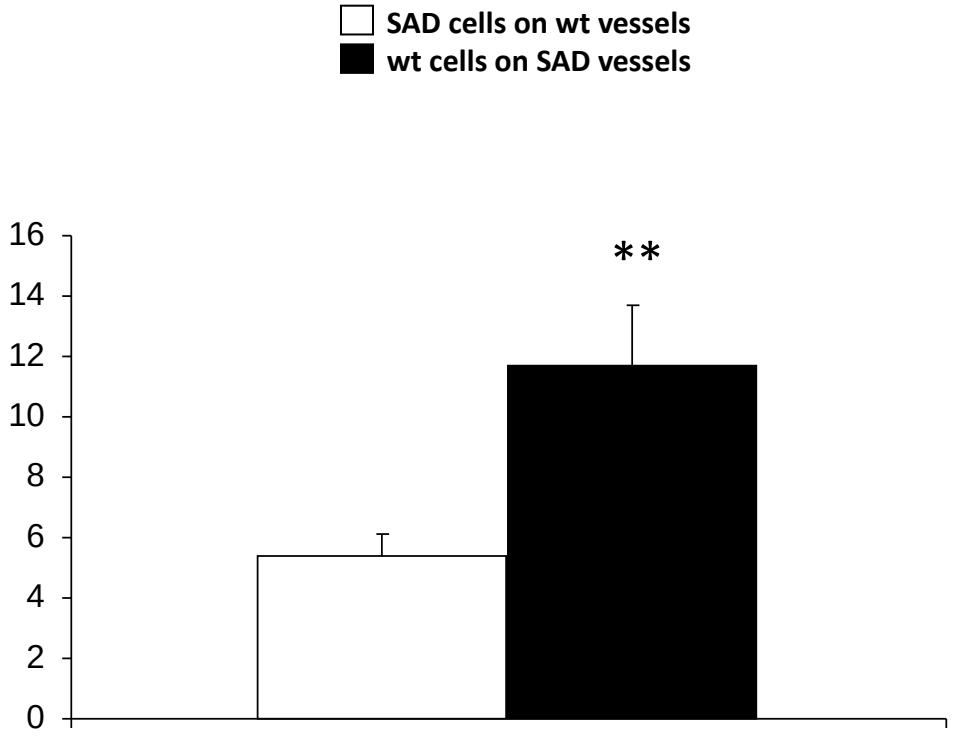




Tharaux P-L et al. Nephrol Dial Transplant, 2005

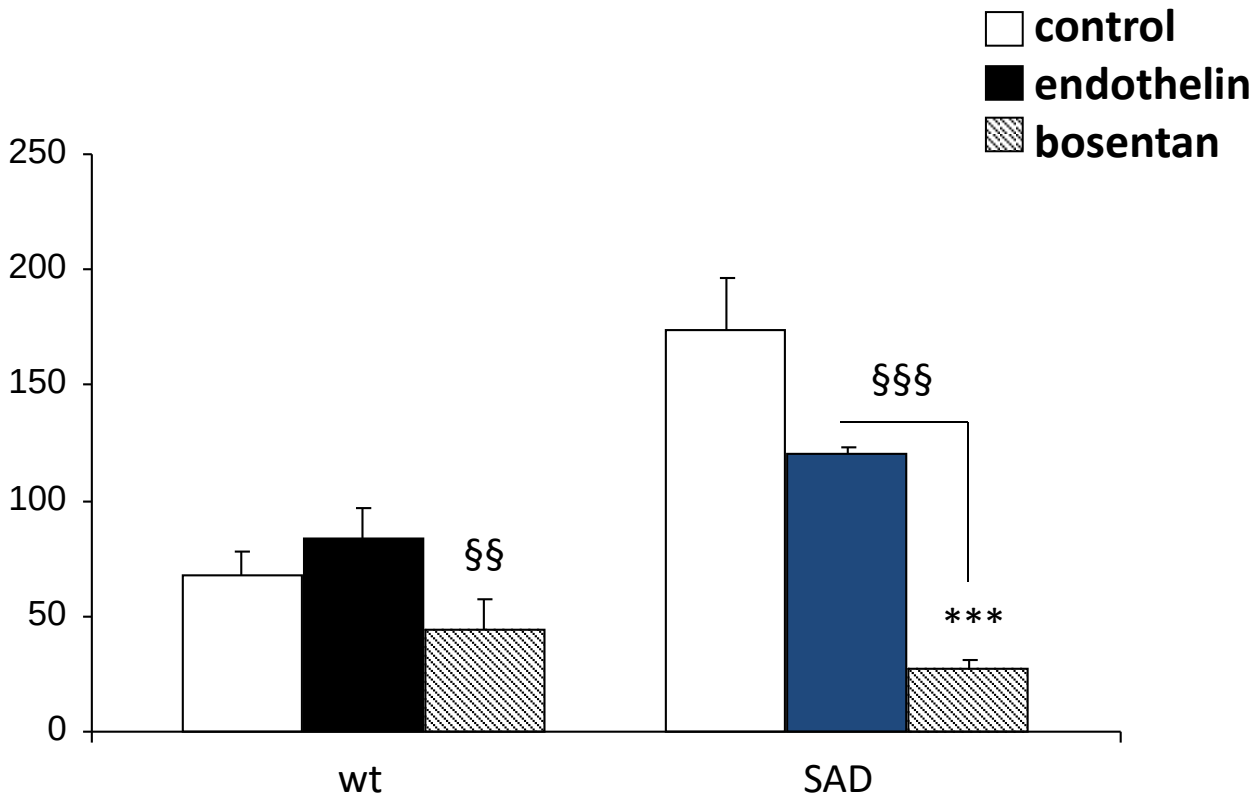
Ex vivo adhesion





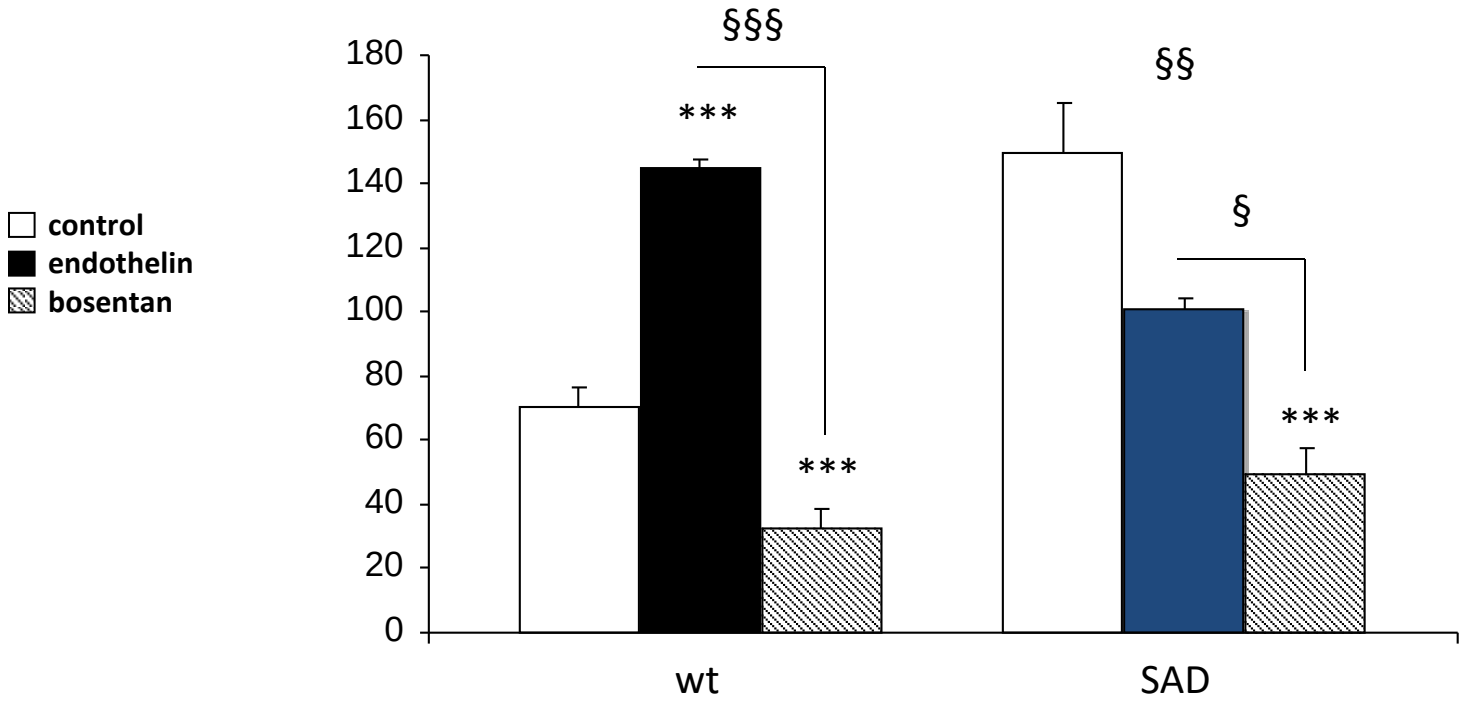
Unpublished

Adhesion of Circulating Mononuclear Cells



Unpublished

Adhesion Of Bone-marrow-derived Cells



Unpublished

ET-1 & hypertension

Am J Physiol Regul Integr Comp Physiol 314: R544–R551, 2018.
First published December 13, 2017; doi:10.1152/ajpregu.00312.2017.

RESEARCH ARTICLE | *Fluid and Electrolyte Homeostasis*

Diurnal pattern in skin Na^+ and water content is associated with salt-sensitive hypertension in ET_B receptor-deficient rats

Joshua S. Speed,[3] Kelly A. Hyndman,[1] Malgorzata Kasztan,[1] Jermaine G. Johnston,[1] Kaehler J. Roth,[1] Jens M. Titze,[2] and David M. Pollock[1]

[1]*Cardio-Renal Physiology and Medicine, Department of Medicine, Division of Nephrology, University of Alabama at Birmingham, Birmingham, Alabama;* [2]*Cardiovasular and Metabolic Disorders, National University of Singapore Medical School, Singapore; and* [3]*Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi*

Submitted 14 August 2017; accepted in final form 12 December 2017

Speed, *Am J Physiol* 2017

ET-1 & hypertension

Endothelin-1 and End Organ Damage

Three-Month Endothelial Human Endothelin-1 Overexpression Causes Blood Pressure Elevation and Vascular and Kidney Injury

Suellen C. Coelho, Olga Berillo, Antoine Caillon, Sofiane Ouerd, Júlio C. Fraulob-Aquino, Tlili Barhoumi, Stefan Offermanns, Pierre Paradis, Ernesto L. Schiffrin

Abstract—Endothelium-derived endothelin (ET)-1 has been implicated in the development of hypertension and end-organ damage, but its exact role remains unclear. We have shown that tamoxifen-inducible endothelium-restricted human ET-1 overexpressing (ieET-1) mice exhibited blood pressure rise after a 3-week induction in an ET type A (ET_A) receptor-dependent manner, in absence of vascular and renal injury. It is unknown whether long-term ET-1 overexpression results in sustained blood pressure elevation and vascular and renal injury. Adult male ieET-1 and control tamoxifen-inducible endothelium-restricted Cre recombinase (ieCre) mice were induced with tamoxifen and 2.5 months later, were treated with or without the ET_A receptor blocker atrasentan for 2 weeks. Three-month induction of endothelial human ET-1 overexpression increased blood pressure ($P<0.01$), reduced renal artery flow ($P<0.001$), and caused mesenteric small artery stiffening ($P<0.05$) and endothelial dysfunction ($P<0.01$). These changes were accompanied by enhanced mesenteric small artery *Col1A1* and *Col3A1* expression, and perivascular adipose tissue oxidative stress ($P<0.05$) and monocyte/macrophage infiltration ($P<0.05$). Early renal injury was demonstrated by increased kidney injury molecule-1 expression in renal cortex tubules ($P<0.05$), with, however, undetectable lesions using histochemistry staining and unchanged urinary albumin. There was associated increased myeloid ($CD11b^+$) and myeloid-derived suppressive cell ($CD11b^+Gr\text{-}1^+$) renal infiltration ($P<0.01$) and greater frequency of myeloid and renal cells expressing the proinflammatory marker CD36 ($P<0.05$). Atrasentan reversed or reduced all of the above changes ($P<0.05$) except the endothelial dysfunction and collagen expression and reduced renal artery flow. These results demonstrate that long-term exposure to endothelial human ET-1 overexpression causes sustained blood pressure elevation and vascular and renal injury via ET_A receptors. (***Hypertension*. 2018;71:208-216. DOI: 10.1161/HYPERTENSIONAHA.117.09925.) • Online Data Supplement

Key Words: endothelial cells ■ endothelin-1 ■ hypertension ■ inflammation ■ vascular system injuries

Coelho, *Hypertension* 2018

AKI to CKD

Nephrol Dial Transplant (2019) 34: 794–801
doi: 10.1093/ndt/gfy246
Advance Access publication 9 August 2018

Delayed spironolactone administration prevents the transition from acute kidney injury to chronic kidney disease through improving renal inflammation

Jonatan Barrera-Chimal[1,2], Leslie Rocha[1,3], Isabel Amador-Martínez[1,2], Rosalba Pérez-Villalva[1,3], Rafael González[1,3], Cesar Cortés-González[4], Norma Uribe[5], Victoria Ramírez[3], Nathan Berman[1,3], Gerardo Gamba[1,3] and Norma A. Bobadilla[1,3]

[1]Molecular Physiology Unit, Department of Genomic Medicine, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]Unidad de Medicina Traslacional, Department of Genomic Medicine, Instituto de Investigaciones Biomédicas and Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico, [3]Department of Nephrology Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [4]Unidad de Investigación Biomédica en Cáncer, Instituto Nacional de Cancerología, Mexico City, Mexico and [5]Department of Pathology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

IRI: AKI to CKD

- Common & occurs in a variety of settings
  - AKI
  - Renovascular disease
  - Transplant
  - Other surgery (aortic aneuyrsm repair)

- Risk of CKD development & even worse if AKI occurs in pre-existing CKD

- Number of pre-clinical studies support a role for ET-1 but as yet no clinical data

Chade, *JASN* 2015; Chade, *Kidney Int* 2014; Franzen, *Diabetologia* 2015; Stobdan, *PNAS* 2015

AKI to CKD

Life Sciences 228 (2019) 295–304

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Downregulation of endothelin A receptor (ETaR) ameliorates renal ischemia reperfusion injury by increasing nitric oxide production

Long Li[a,b,c,1], Xia Wang[d,1], Long Zheng[a,b,1], Jiawei Li[a], Ming Xu[a,b], Ruiming Rong[a,b], Tongyu Zhu[a,b], Yichen Jia[a,b,*]

[a] Department of Urology, Zhongshan Hospital, Fudan University, Shanghai 200032, China
[b] Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China
[c] Department of Urology, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200011, China
[d] Department of Cardiology, Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai 200030, China

Neutrophils : target for ET-1?

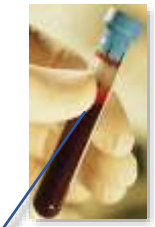
In vivo effects of bosentan on reticulocyte and neutrophil blood counts in WT and SAD mice exposed to hypoxia

	WT ($n = 6$)	WT H/R ($n = 6$)	WT H/R + bosentan ($n = 6$)	SAD ($n = 6$)	SAD H/R ($n = 6$)	SAD H/R + bosentan ($n = 6$)
Hematocrit (%)	44.5 ± 1.0	45.1 ± 0.8	44.8 ± 0.4	43.9 ± 0.2	42.7 ± 1.3	43.2 ± 0.6
Hemoglobin (g/dk)	13.9 ± 0.8	14.2 ± 0.6	14.6 ± 0.3	13.1 ± 0.5	12.5 ± 0.9	13.3 ± 1.1
Reticulocytes (%)	4.5 ± 1.2	6.1 ± 0.8	5.9 ± 0.7	5.3 ± 0.4	6.2 ± 1.2	6.8 ± 1.5
Neutrophils (cells/μl)	862.7 ± 54	1821 ± 266[A]	827 ± 133[B]	2527 ± 229	5681 ± 811[C]	1321 ± 446[D]

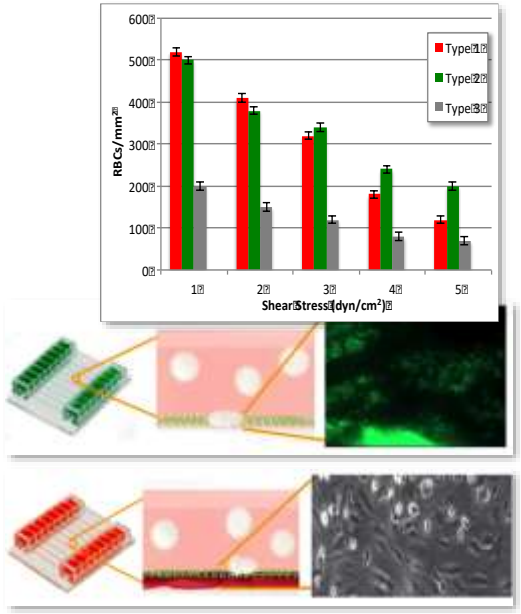
The mice were exposed to hypoxic conditions (8% oxygen) for 46 hours. [A]$P < 0.05$ versus steady state. [B]$P < 0.05$ versus vehicle-treated mice. [C]$P < 0.01$ versus steady state. [D]$P < 0.01$ versus vehicle-treated mice.

Sabaa N et al. J Clin Invest. 2008 May;118(5):1924-33.

Fonctional characterization of human neutrophils



Dynamic adhesion monitoring plateform



ECM proteins :

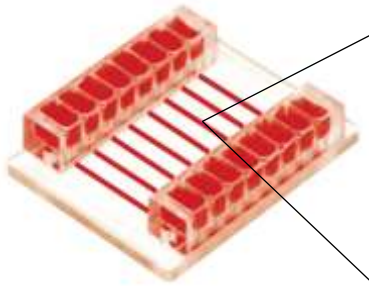
- *Laminin*
- *Fibronectin*
- *Collagens*
- *Thrombospondin*
- *Etc.*

Endothelial cells

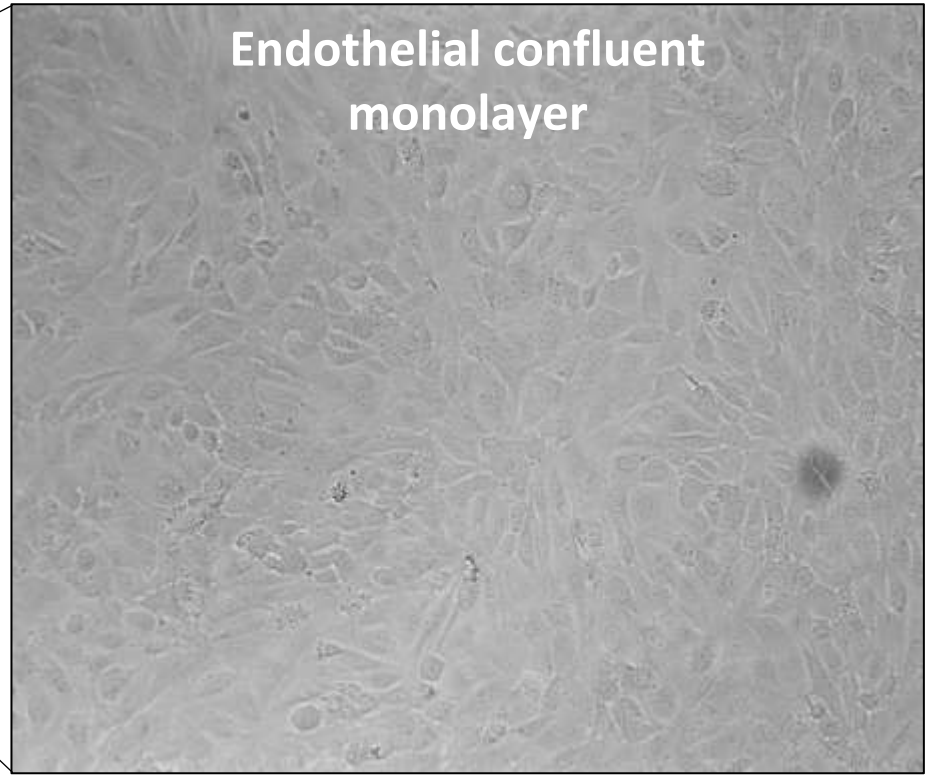
- *Non-activated*
- *Activated (cytokines)*

Fonctional characterization of human neutrophils to endothelial cells

HUVECs and HDMEC are cultured in microfluidic chanels



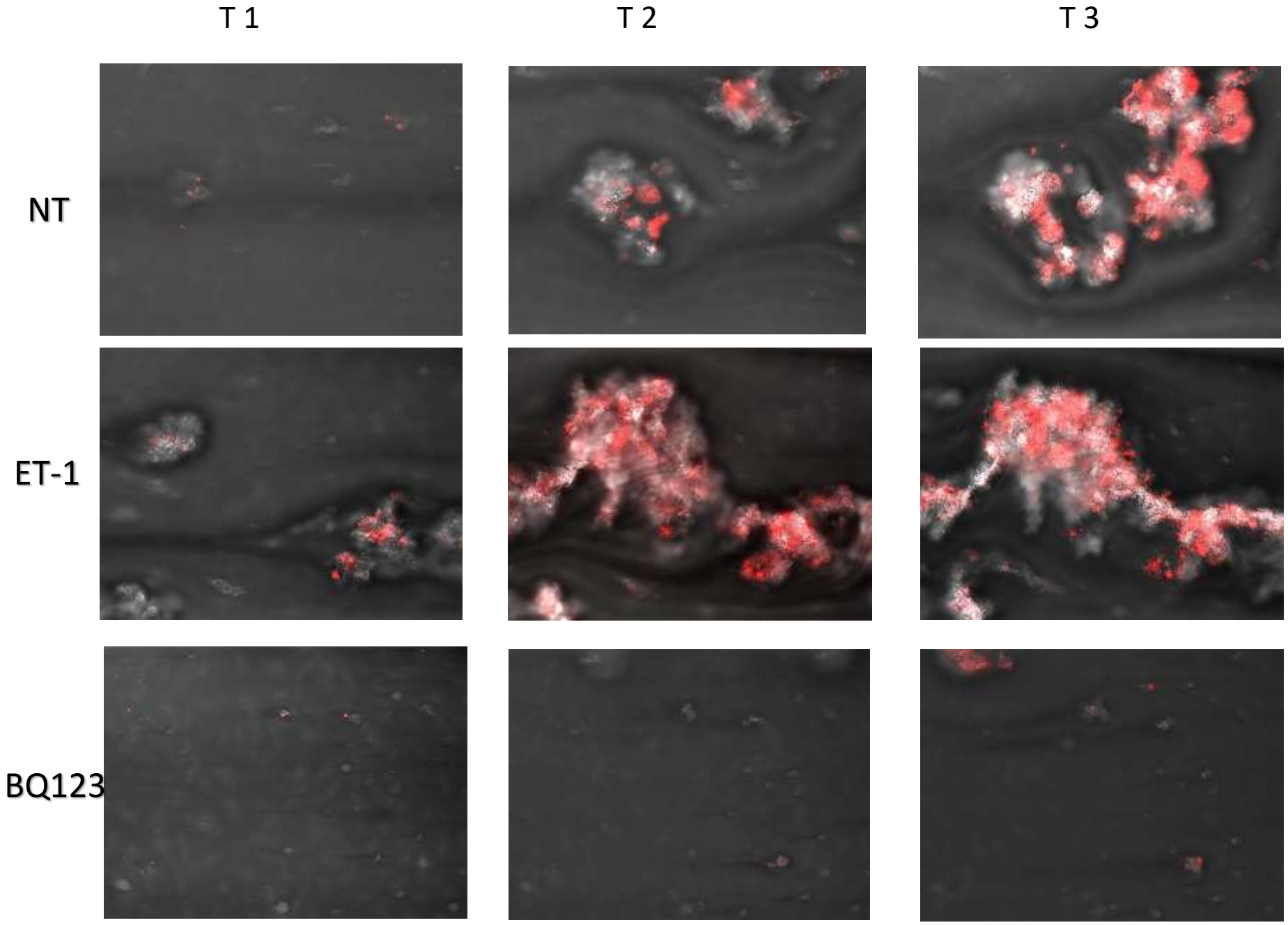
Vena8



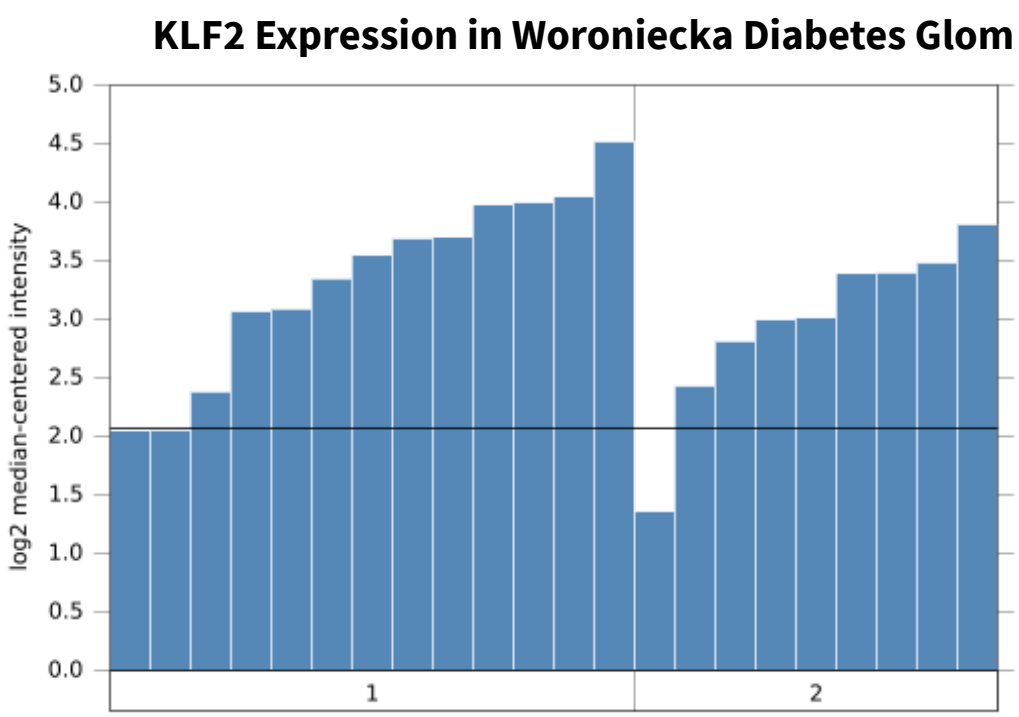
Kima pump (*Cellix*)

4 Nanopumps (*Cellix*)

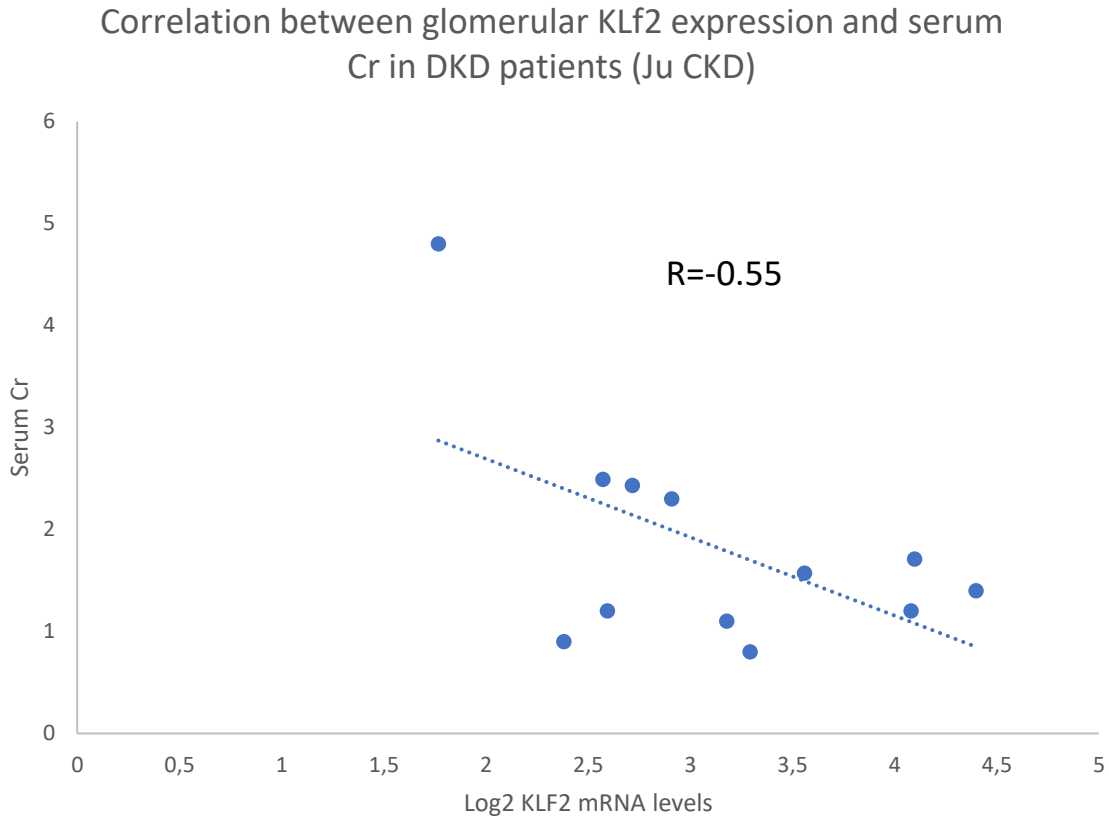
4 conditions in parallel


T 1
T 2
T 3
NT
ET-1
BQ123

KLF2 expression in human DKD by Nephroseq



1. Healthy Living Donor (13)
2. Diabetic Nephropathy (9)



Pre-eclampsia

Clinical Science (2019) **133** 1341–1352
https://doi.org/10.1042/CS20190464

PORTLAND PRESS

Review Article

Endothelin receptor antagonism during preeclampsia: a matter of timing?

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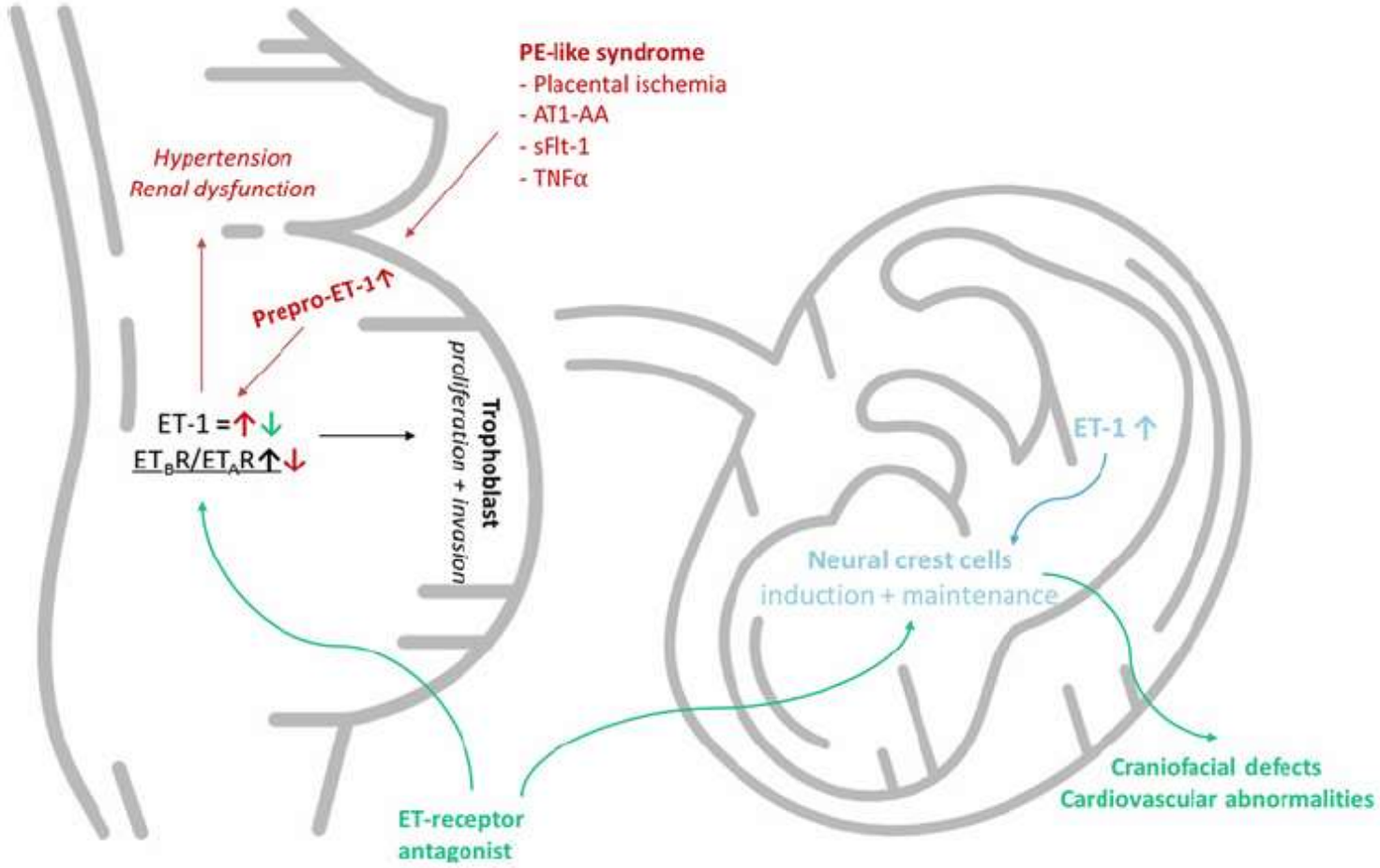
Pre-eclampsia



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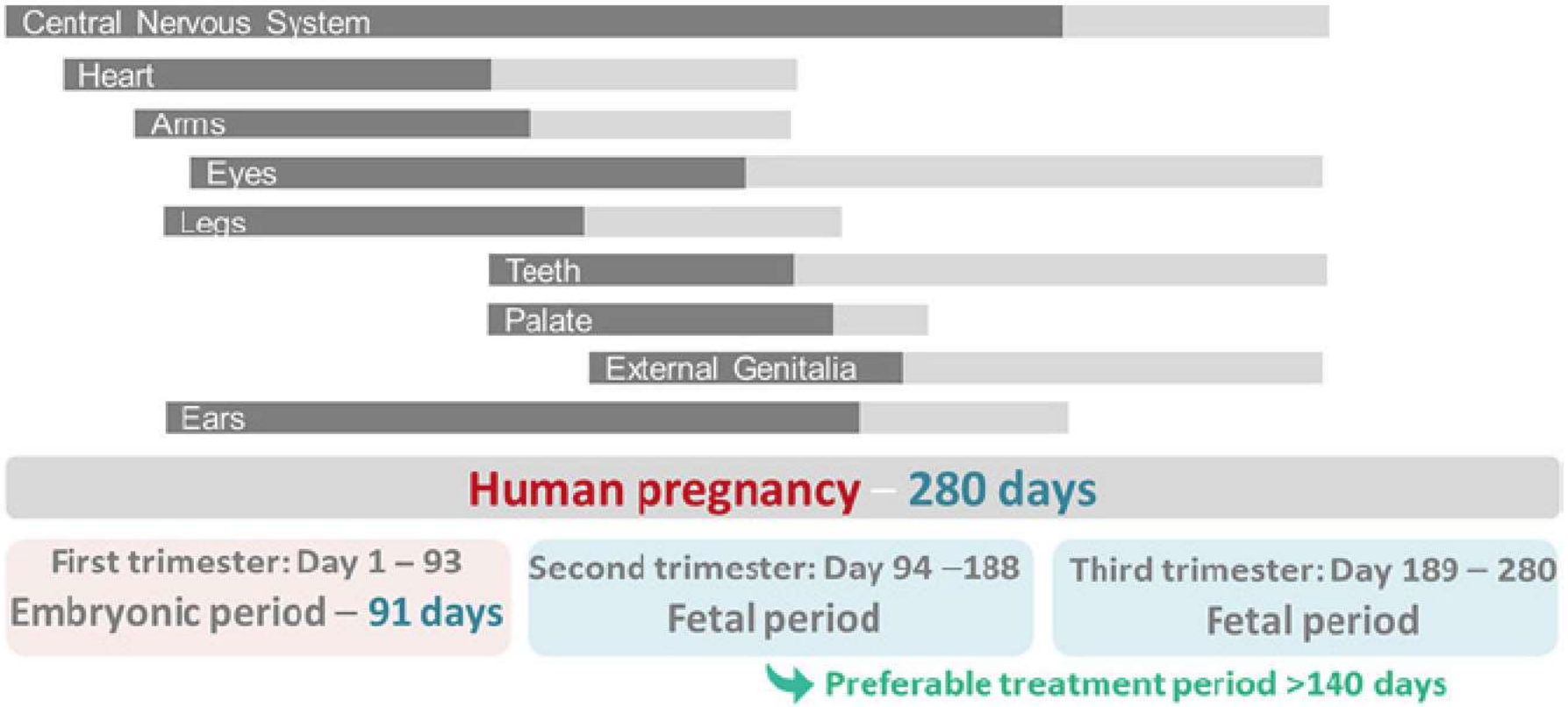
Pre-eclampsia



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