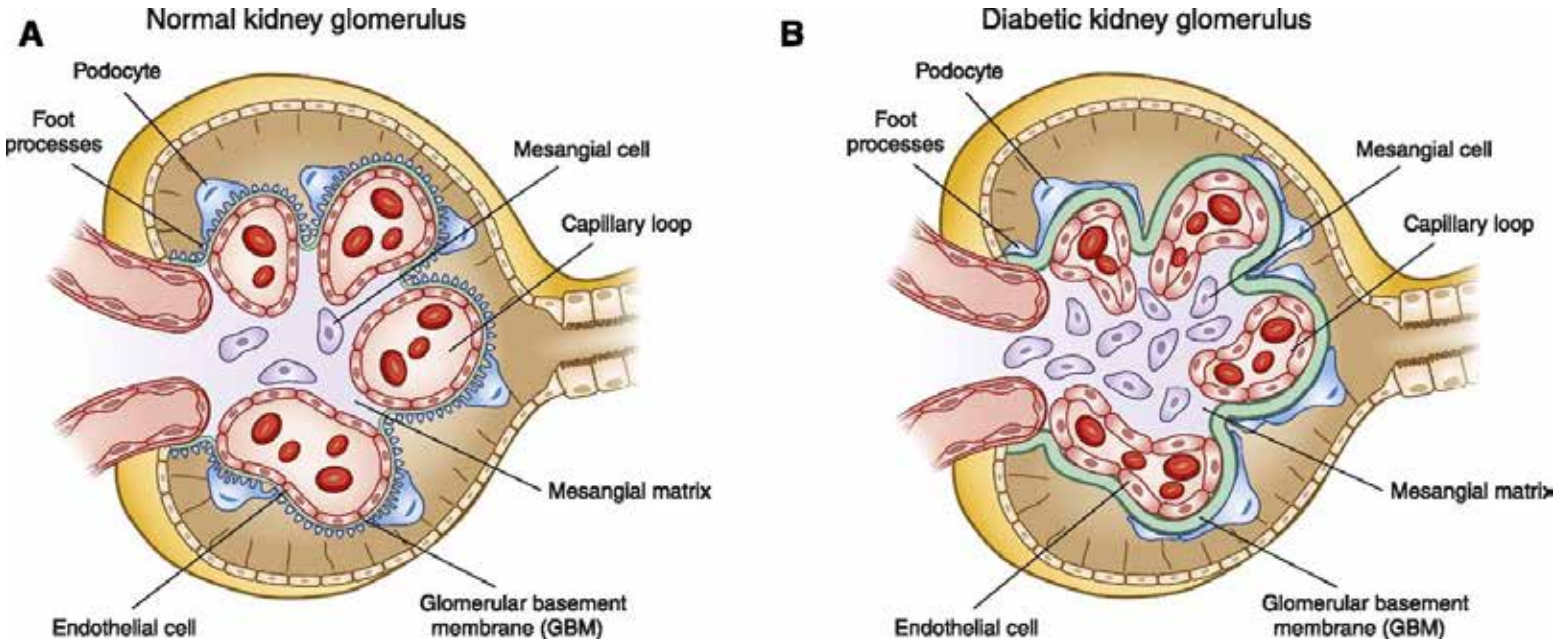
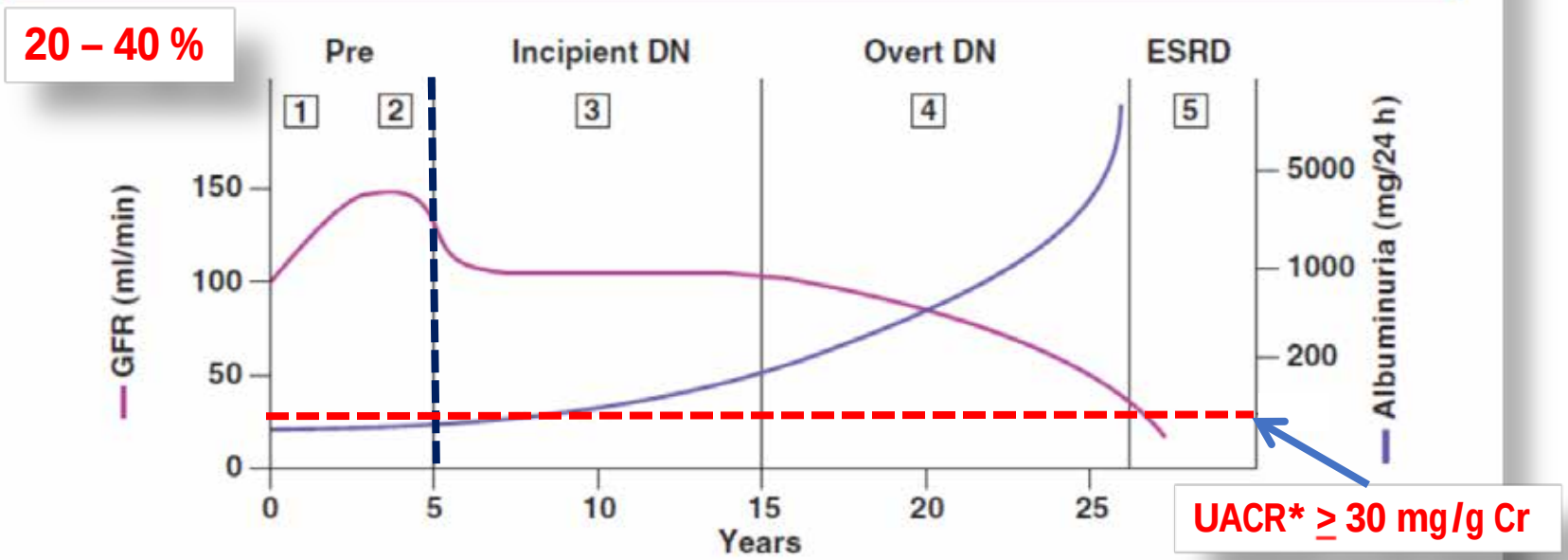


Παθογένεια της Διαβητικής Νεφρικής Νόσου

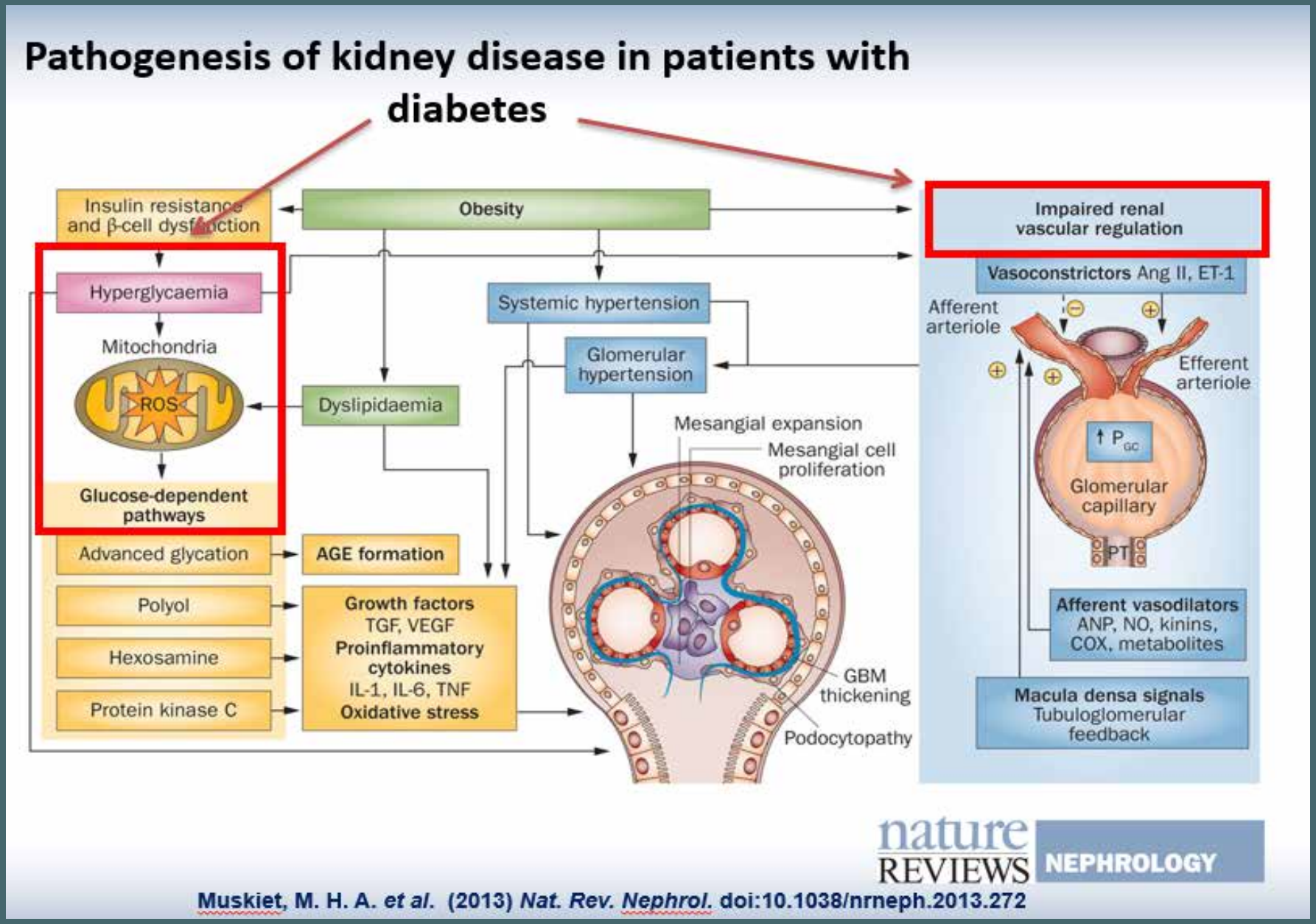
Μάριος Θ. Θεοδωρίδης - Νεφρολόγος, Διευθυντής ΕΣΥ
Παν. Νεφρολογική Κλινική Π.Γ.Ν. Αλεξανδρούπολης

Natural History of Type 1 Diabetic Nephropathy



Stage	Pre	Incipient	Overt
Functional	GFR ↑ (25%–50%)	Microalbuminuria, hypertension	Proteinuria, nephrotic syndrome, GFR ↓
Structural	Renal hypertrophy	Mesangial expansion, GBM thickening, arteriolar hyalinosis	Mesangial nodules (Kimmelstiel-Wilson lesions) Tubulointerstitial fibrosis

Παθογένεια της ΔΝΝ - κλασική προσέγγιση



Muskiet, M. H. A. *et al.* (2013) *Nat. Rev. Nephrol.* doi:10.1038/nrneph.2013.272

Αιμοδυναμική απορρύθμιση του σπειράματος

IGF-1
Glucagon
Nitric oxide -NO
VEGF
Prostaglandin

Υπεργλυκαιμία

Υπερινσουλιναιμία

AII

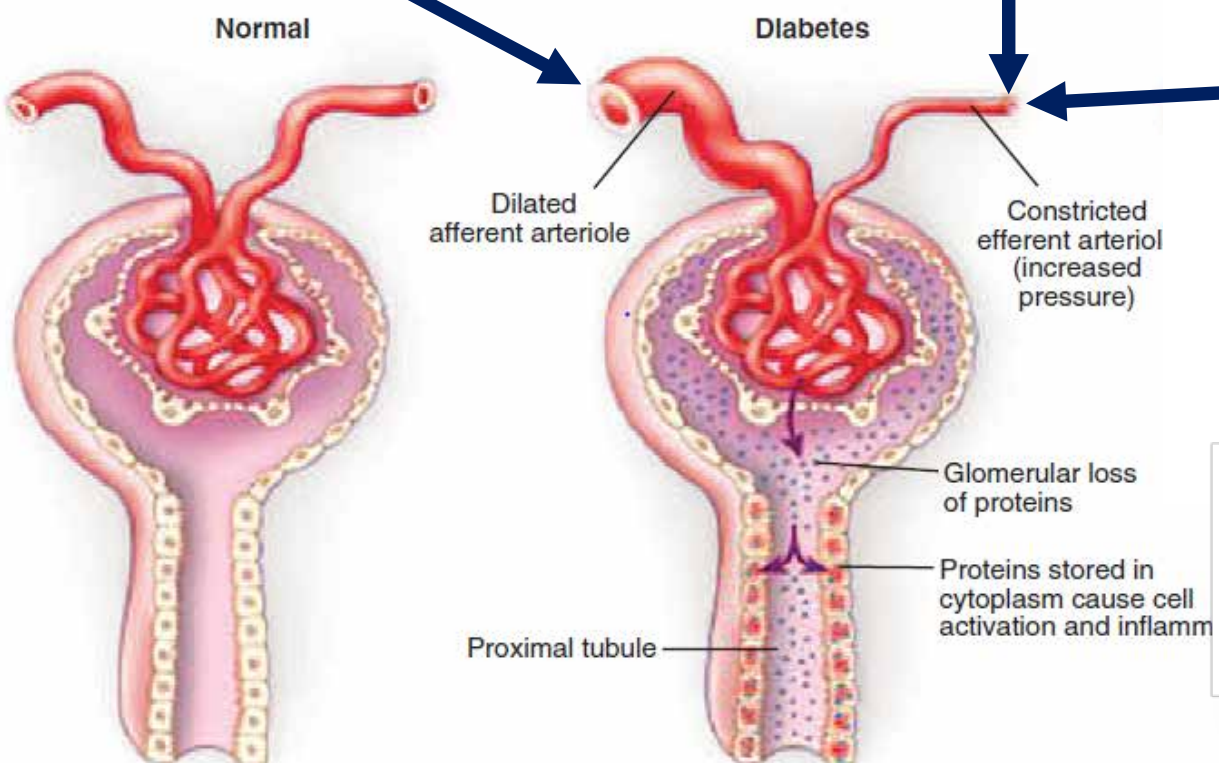
ROS

Ενδοθηλιακή βλάβη

ET1

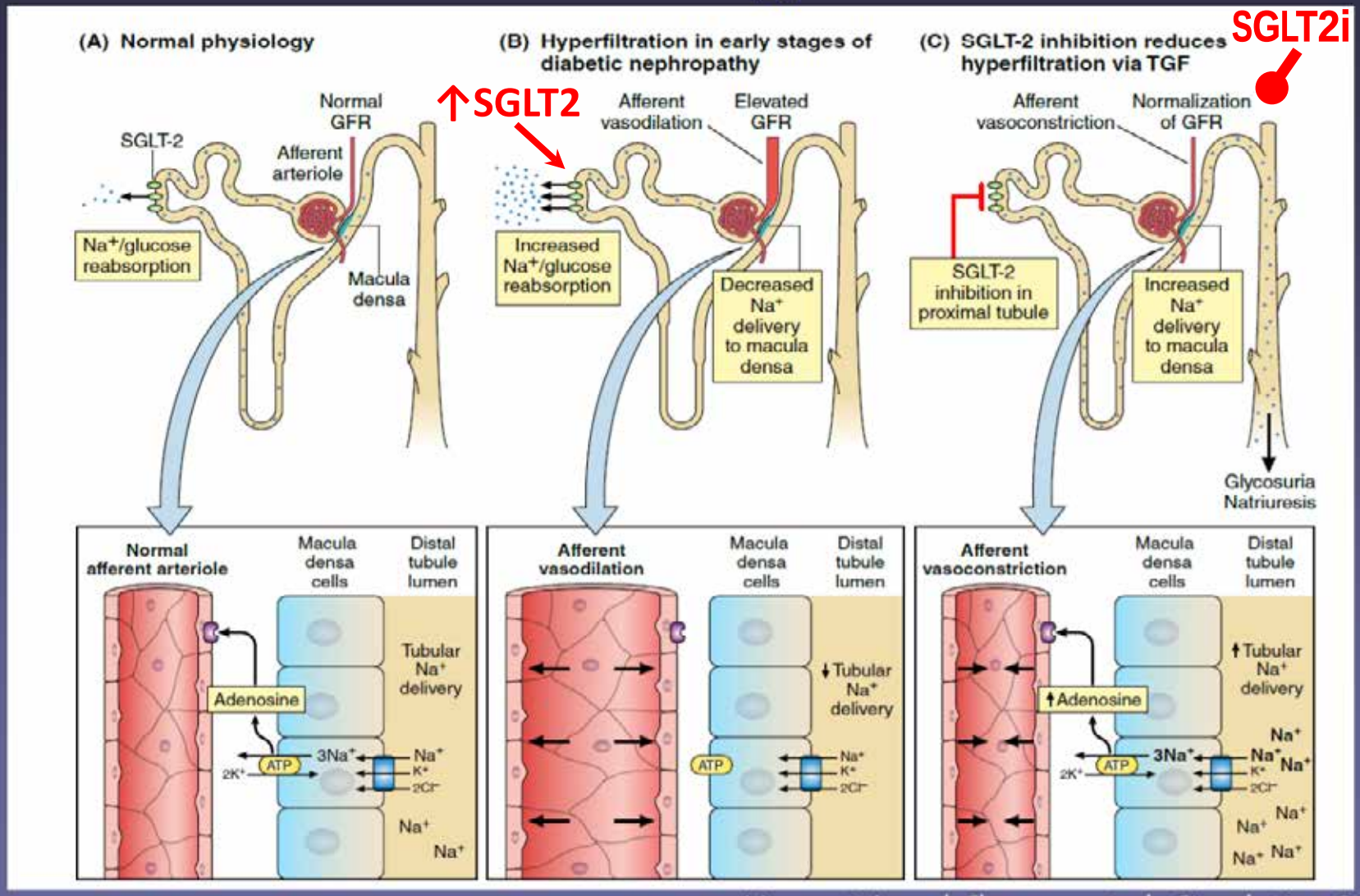
ET-A receptors

Βλάβη ποδοκυττάρων
Οξειδωτικό Στρες
Φλεγμονή
Ίνωση



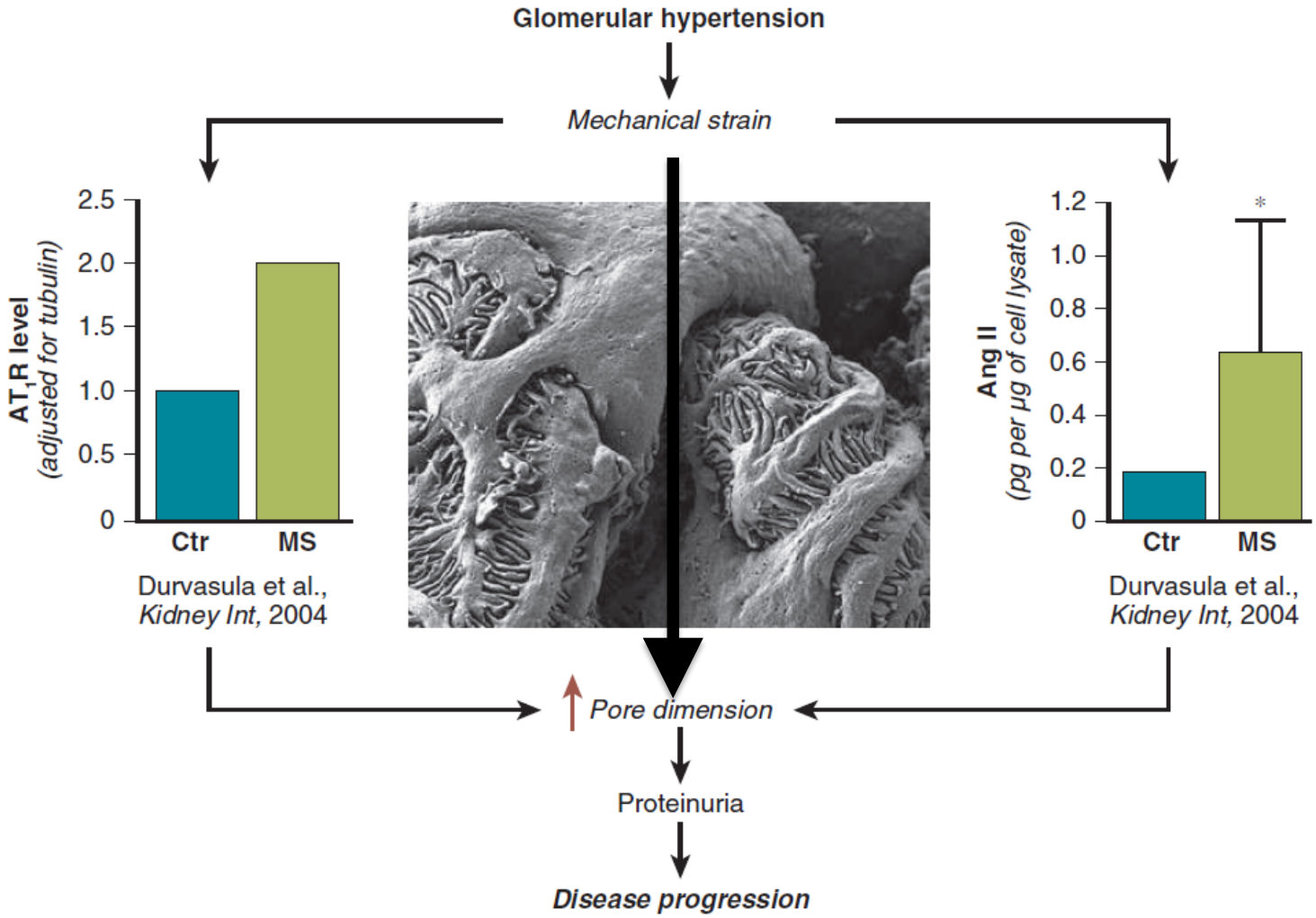
Y.-C. Lin et alJ Formos Med Assoc. 2018 Aug;117(8):662-675

The "Tubular Hypothesis"



Heerspink and Cherney et al. Circulation 2016

Glomerular Hypertension Predisposes to Proteinuria and Renal Disease Progression



Durvasula RV , et al. Kidney Int.2004;65:30–39.

Mechanisms of Interstitial Damage Induced by Proteins

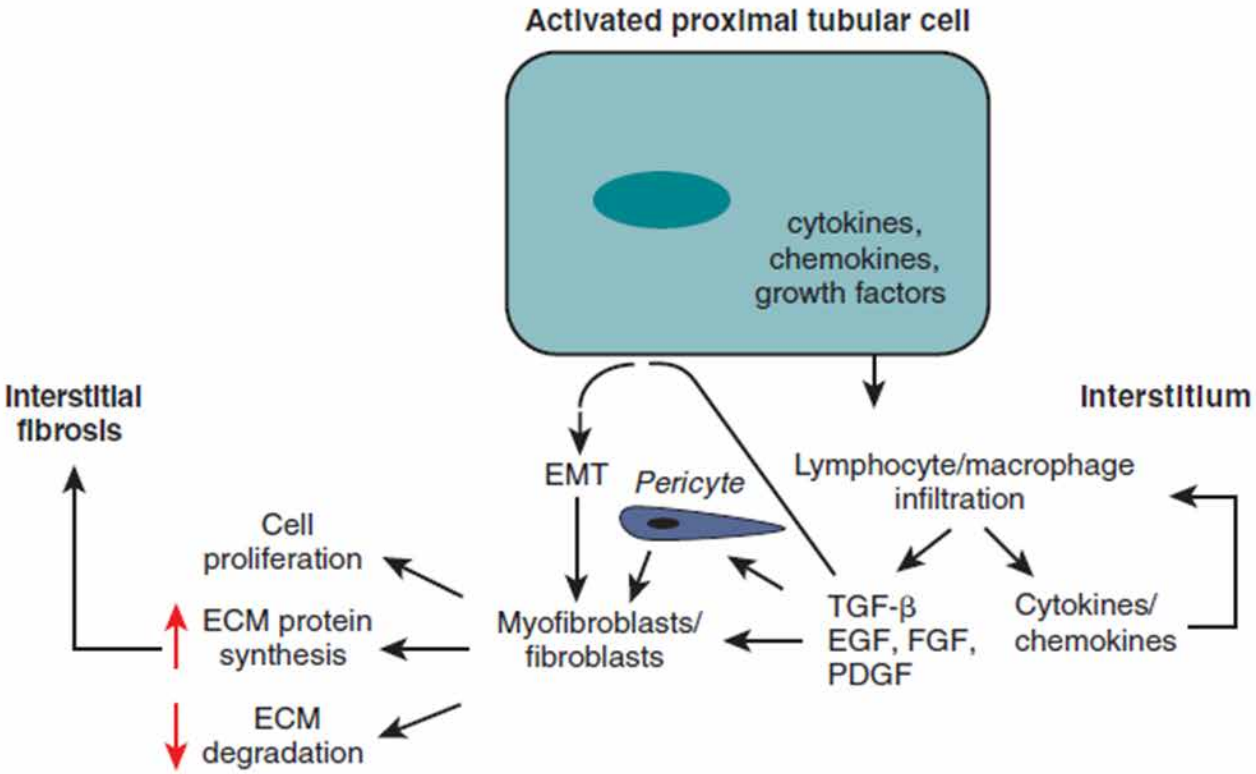
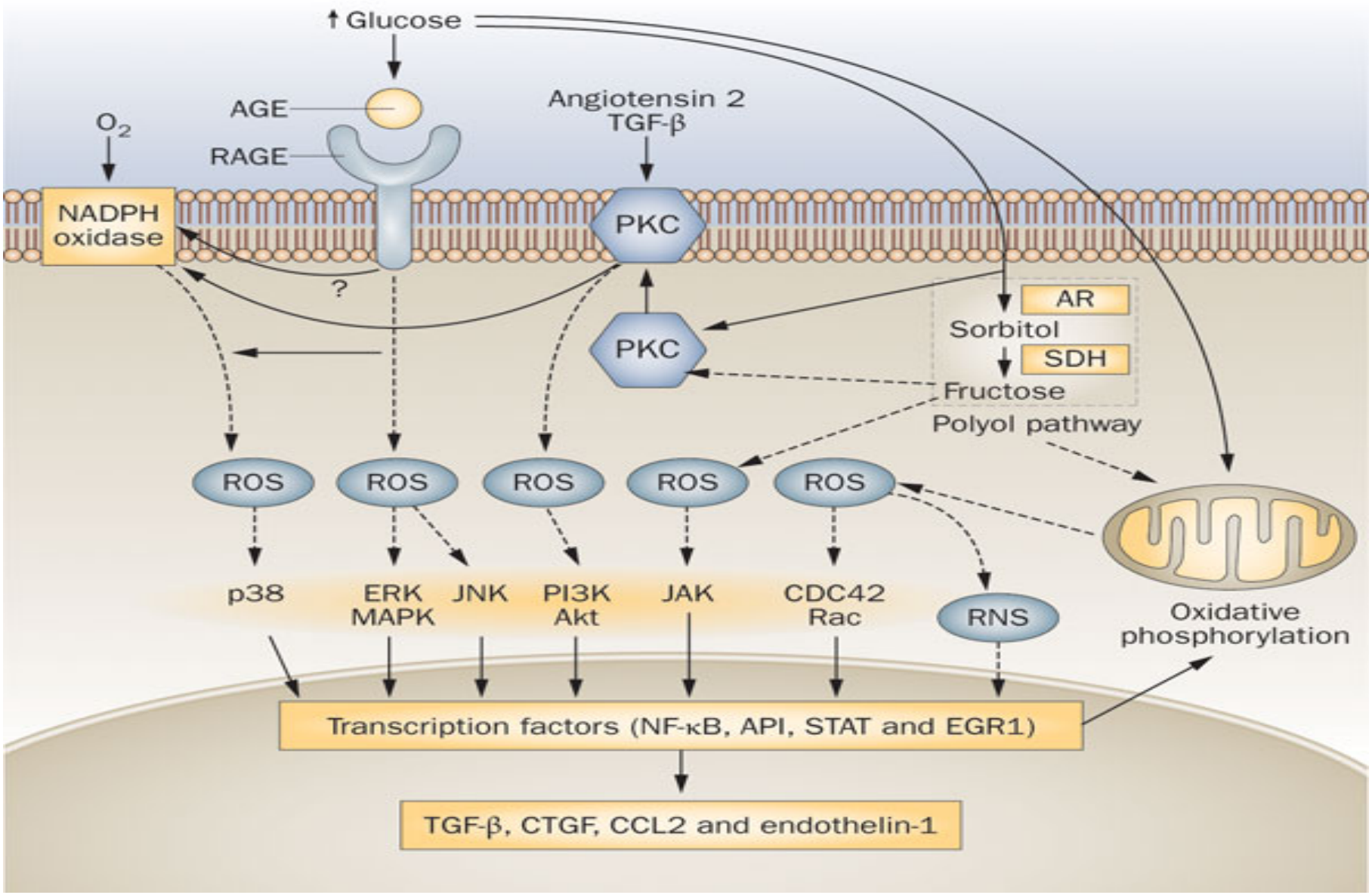


Fig. 78.7 Mechanisms of interstitial damage induced by proteins. Cytokines, chemokines, and growth factors are released from the activated tubule into the interstitium, where they contribute to recruit inflammatory cells and lymphocytes, causing progressive fibrosis. ECM, Extracellular matrix; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transdifferentiation; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β.

Μεταβολική απορρύθμιση του σπειράματος



Different pathways and networks involved in the initiation and progression of diabetic kidney disease.

Radica Z. Alicic et al. CJASN 2017;12:2032-2045

Γλυκόζη, Fructose, Uric Acid & ROS

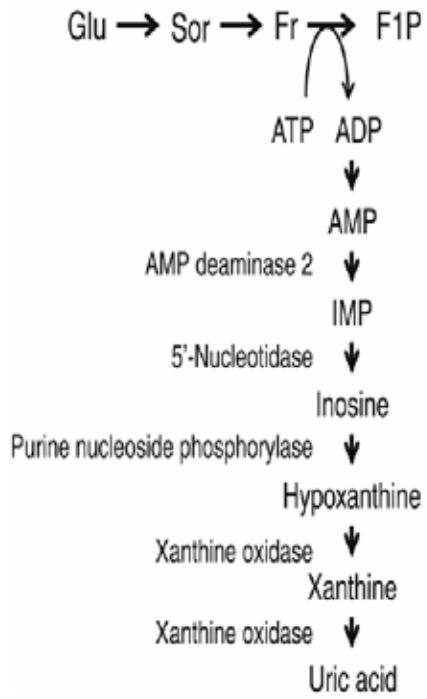


Fig 1.
From polyol pathway to uric acid production. F1P, fructose-1-phosphate; Fr, fructose; Glu,

Sharaf El Din et al. / Journal of Advanced Research 8 (2017) 537

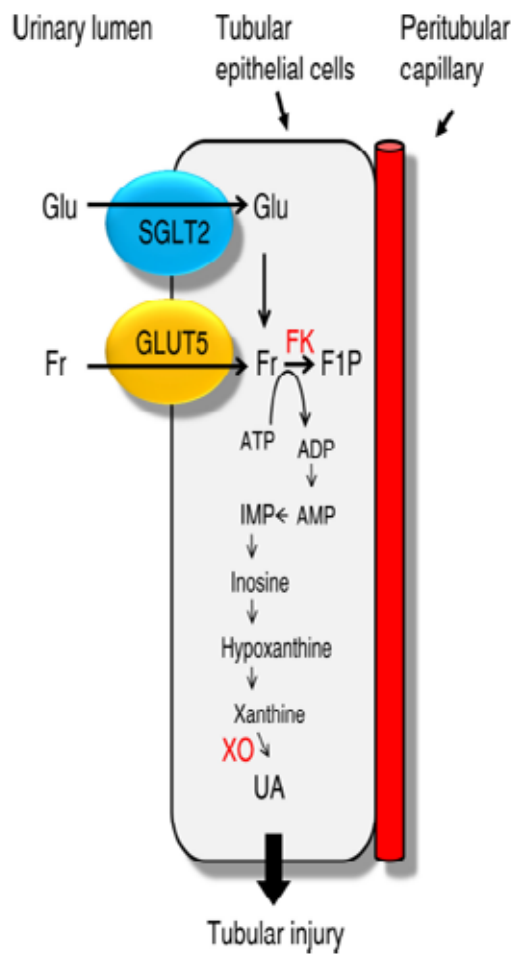
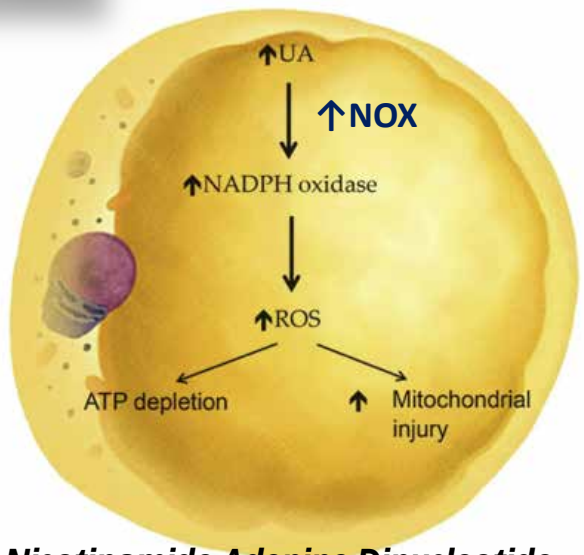
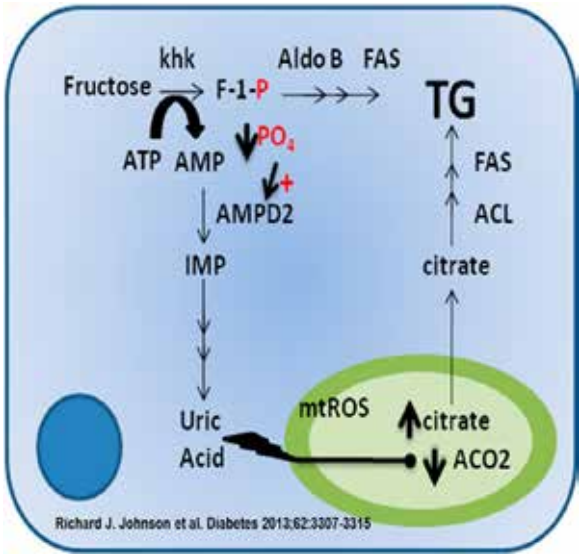


Fig 3.
A proposed mechanism for tubular injury in diabetes. F1P, fructose-1-phosphate; FK



Nicotinamide Adenine Dinucleotide



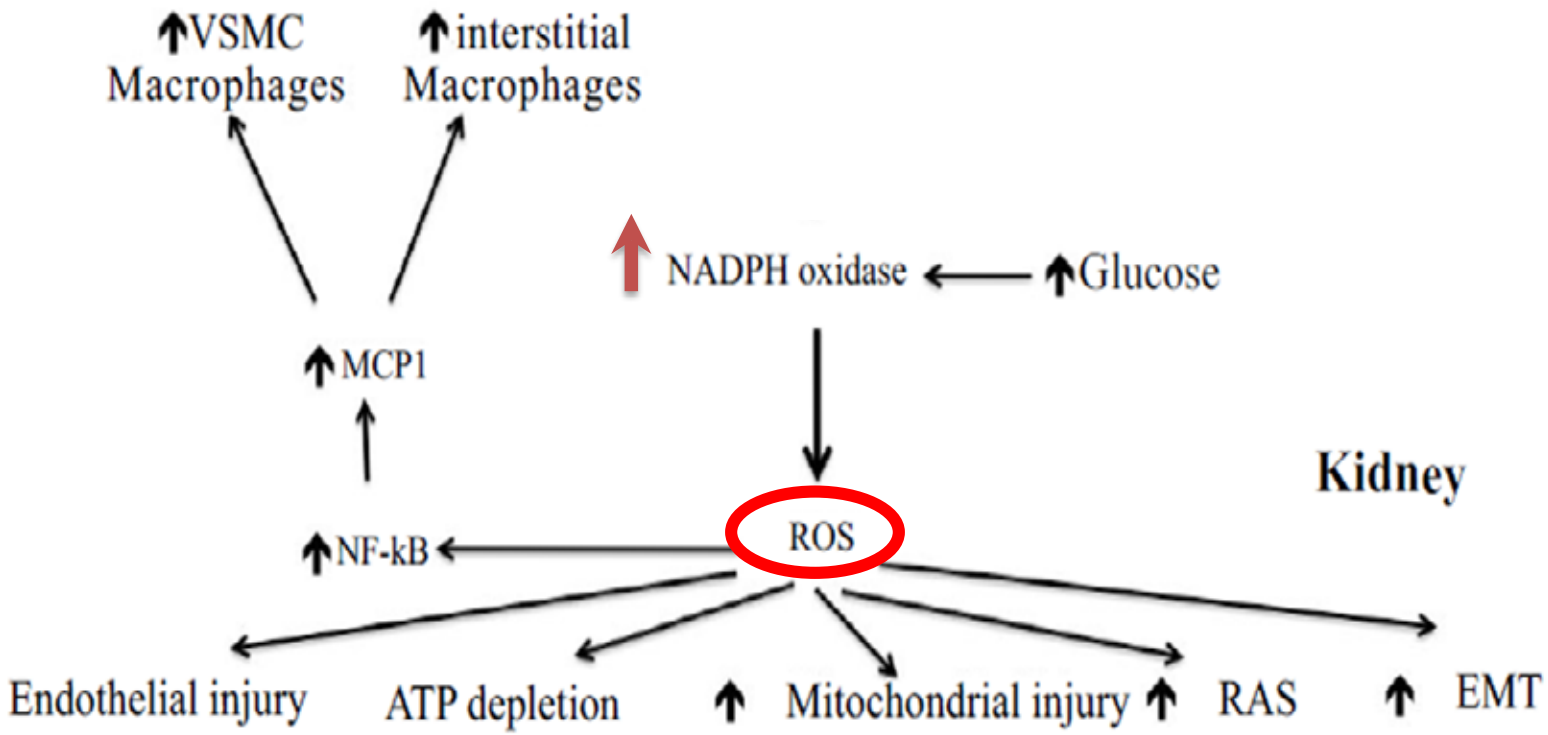


Figure 3 Different pathogenic mechanisms of kidney injury possibly induced by uric acid. UA= uric acid; ROS= reactive oxygen species; NF-κB= Nuclear Factor kappa B; MCP1= Macrophage Chemoattractant protein-1; RAS= Renin angiotensin system; EMT= Epithelium mesenchyme transition; VSMC= Vascular smooth muscle cells. NADPH: Nicotinamide Adenine Dinucleotide

Abdulazim DO (2017) Clin Exp Nephrol Vol 2 No 2: 35.

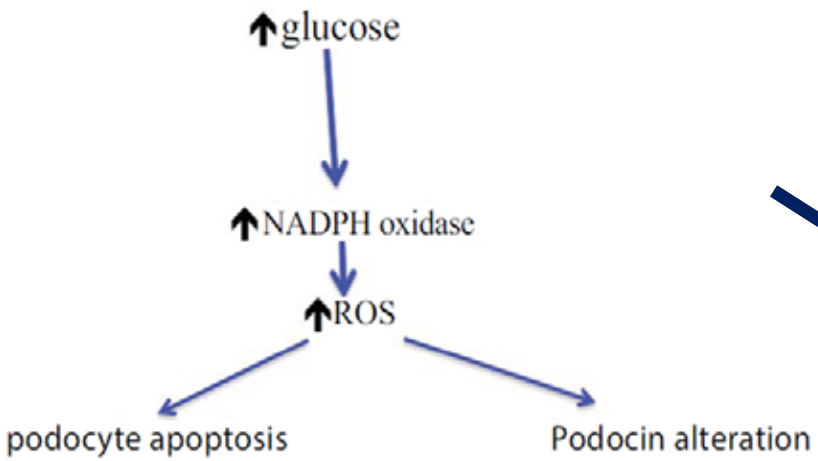
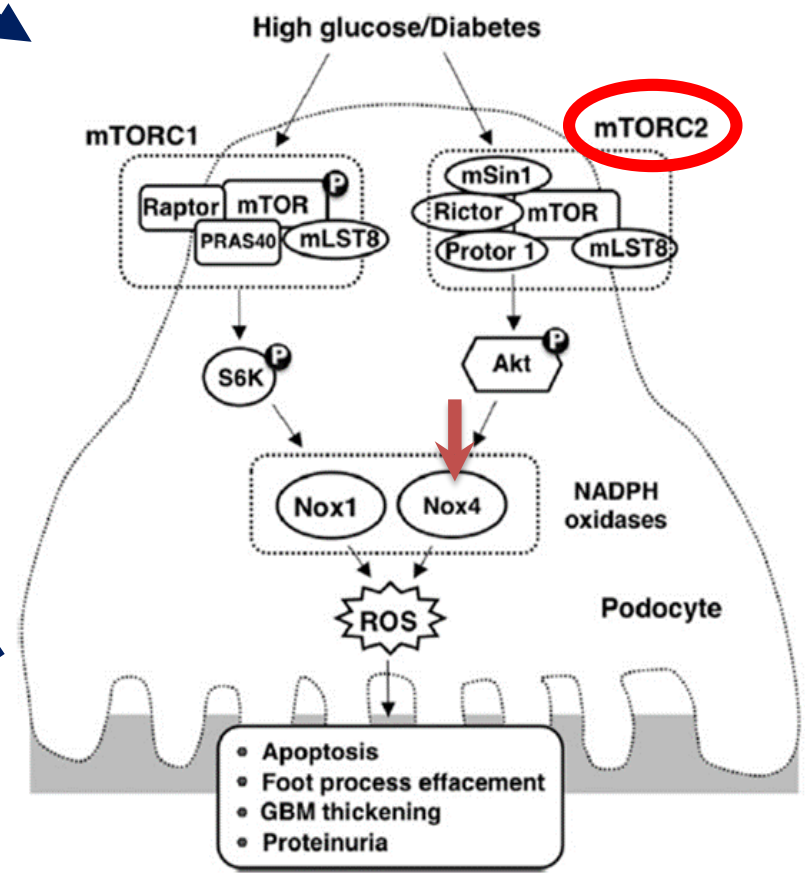
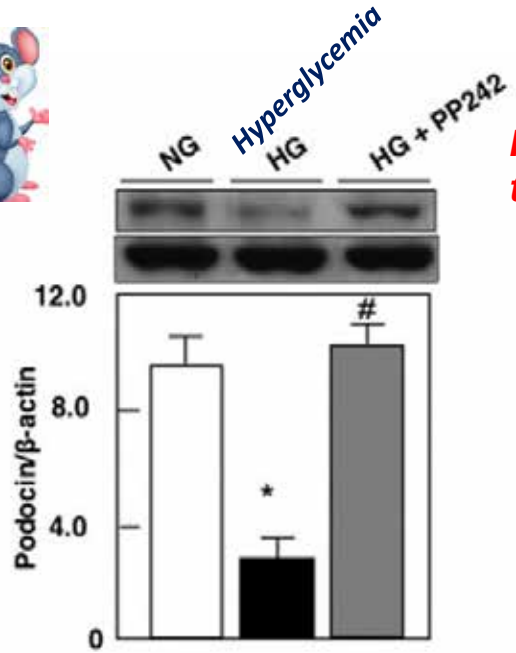


Figure 2 Reactive oxygen species mediated podocyte injury and podocin protein alteration. NADP= Nicotinamide adenine dinucleotide phosphate; ROS= Reactive oxygen species.

Proposed mechanism of HG/diabetes-induced podocyte depletion/apoptosis





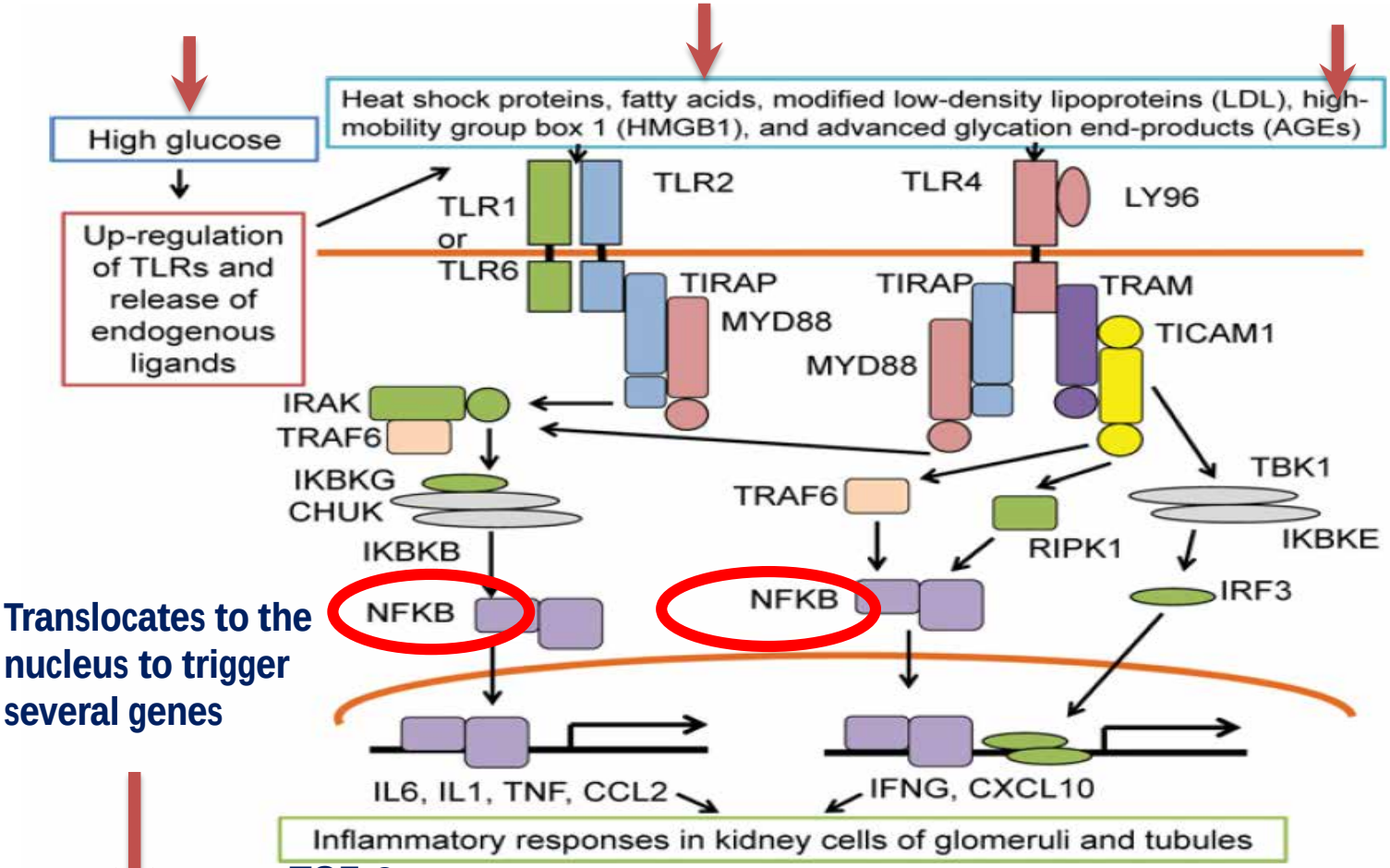
Inhibition of the mTORC

NADPH oxidase is encoded by the NOX1 gene

Eid S et al Antioxid Redox Signal 2016; 25: 703-719.

Toll-like receptors (TLRs) & NFkB

TLRs are membrane receptors which activate immune cell responses



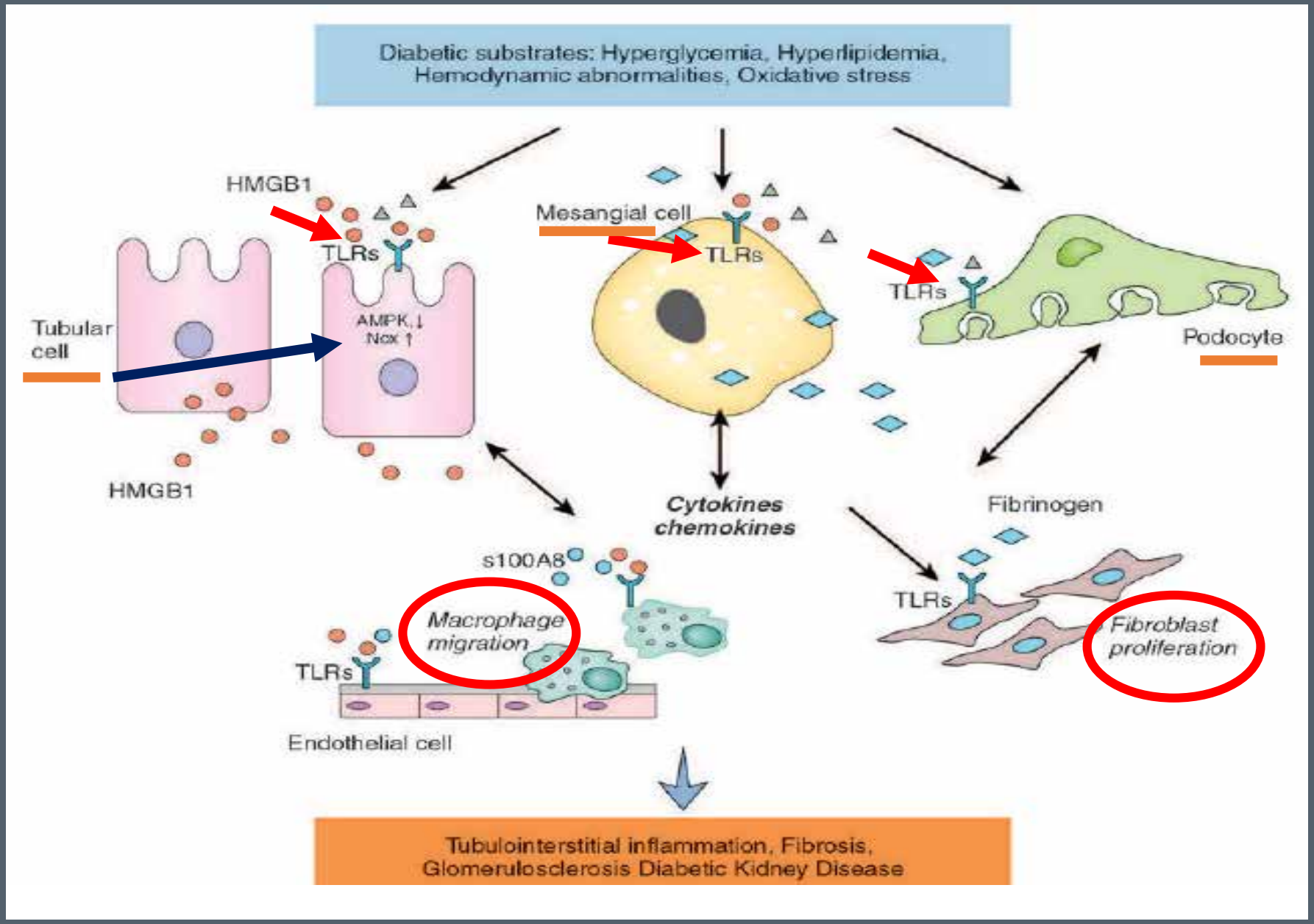
Translocates to the nucleus to trigger several genes

TGF-β

Chemokine ligand 2 (CCL2) ή (MCP-1)
Μόρια προσκόλλησης (ICAM1) → **Διήθηση με Μακροφάγα** → **↑ εναπόθεση κολλαγόνου**

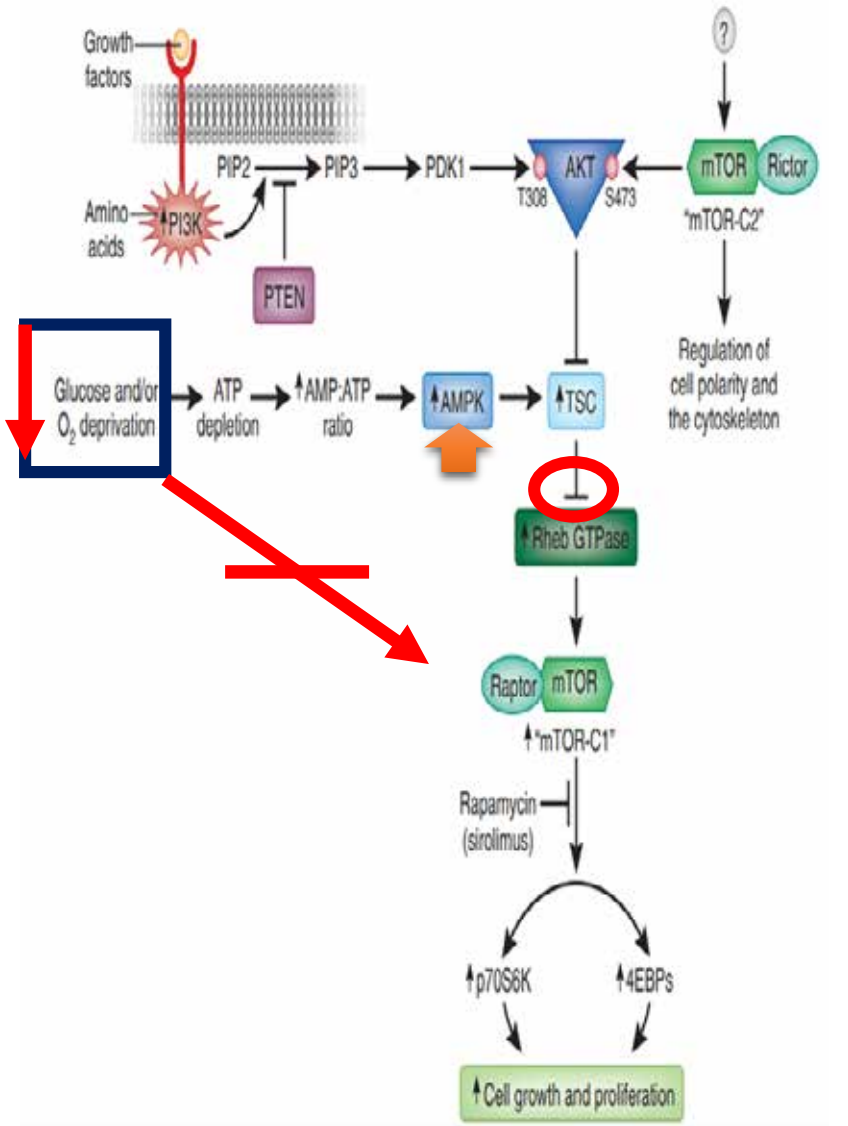
J. Wada and H. MakinoClinical Science (2013) 124, 139–152

Ο ρόλος των Toll-like receptors (TLRs) στη ΔΝΝ

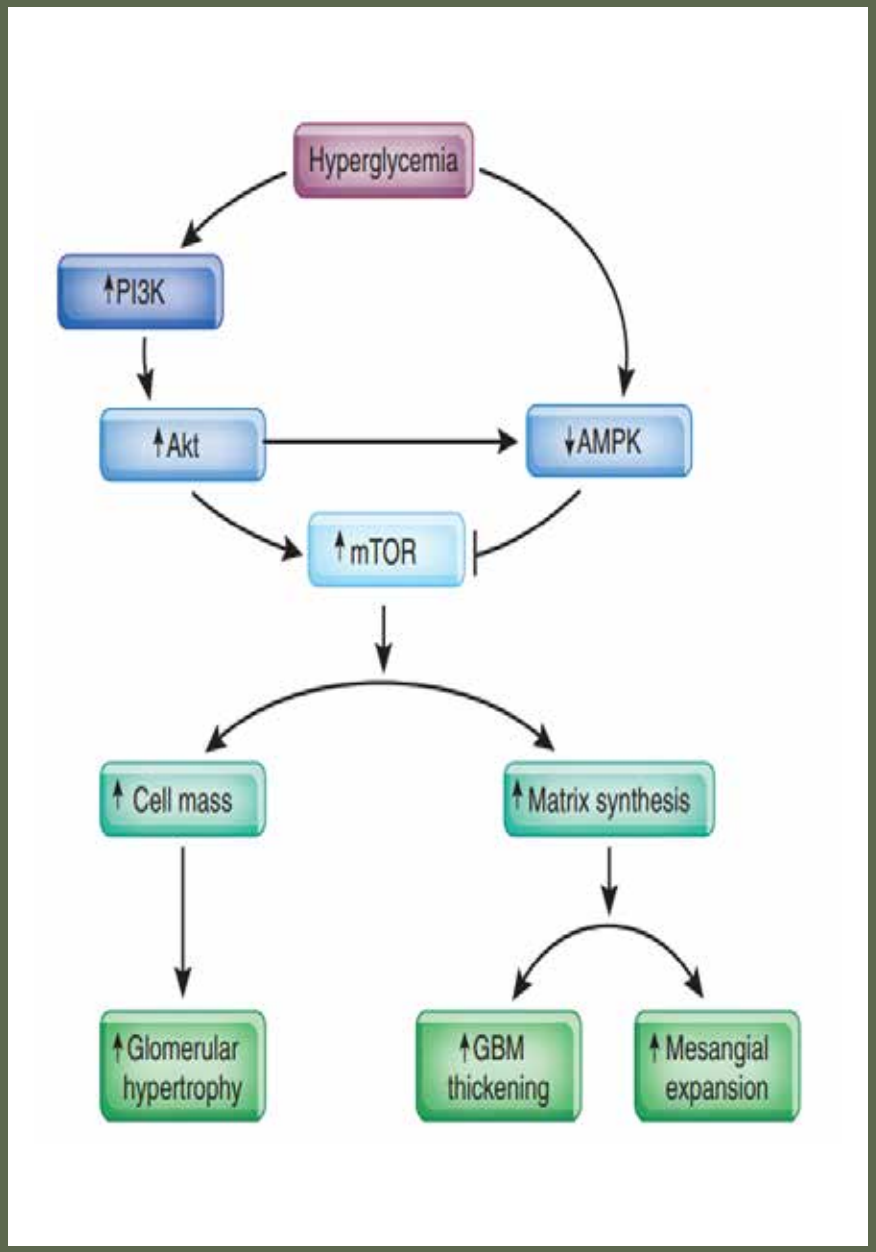


Lin Met al. J Am Soc Nephrol. 2012;23(1):86–102.

Ο ρόλος των Mammalian Target Of Rapamycin (mTOR) στη ΔΝΝ



adenosine monophosphate kinase (AMPK)



W. Lieberthal et al J Am Soc Nephrol 20: 2493–2502, 2009

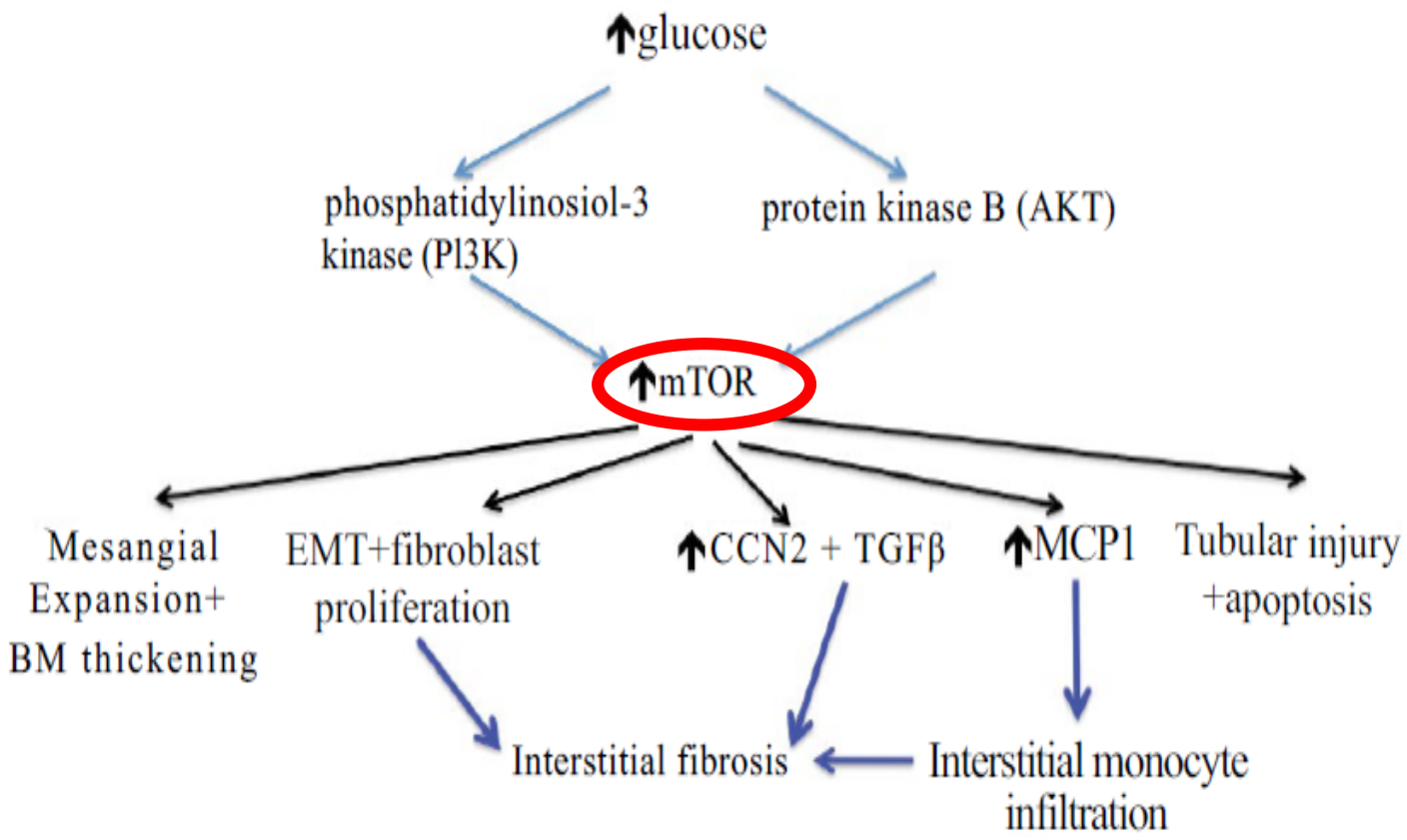
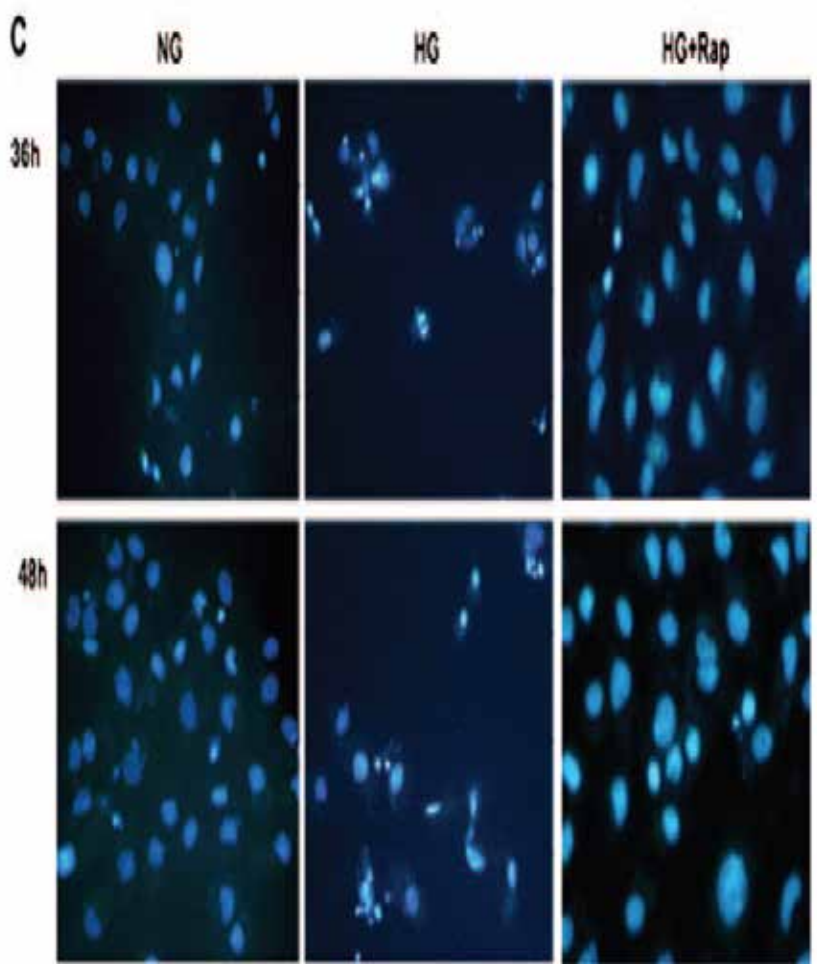
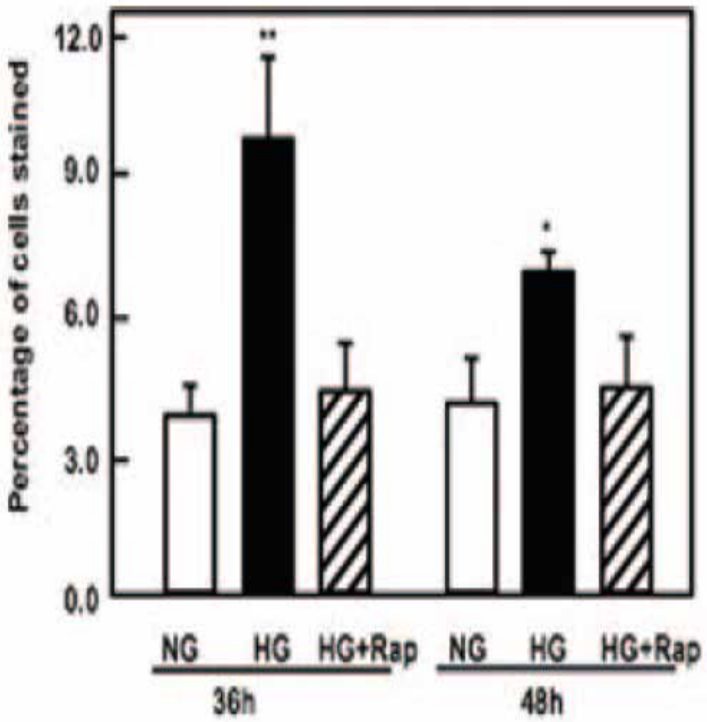


Figure 5 Consequences of mTOR activation induced by hyperglycemia. mTOR= mammalian target of rapamycin; BM= basement membrane; EMT= Epithelium mesenchyme transition; CCN2= Connective tissue growth factor; TGFβ= Transforming Growth Factor β; MCP1= Macrophage chemoattractant protein.

Abdulazim DO (2017) Clin Exp Nephrol Vol 2 No 2: 35.

The mTOR Pathway Promotes Apoptosis of Tubular Epithelial Cells in Diabetes





C. Velagapudi et al Am Soc Nephrol 22: 262–273, 2011.

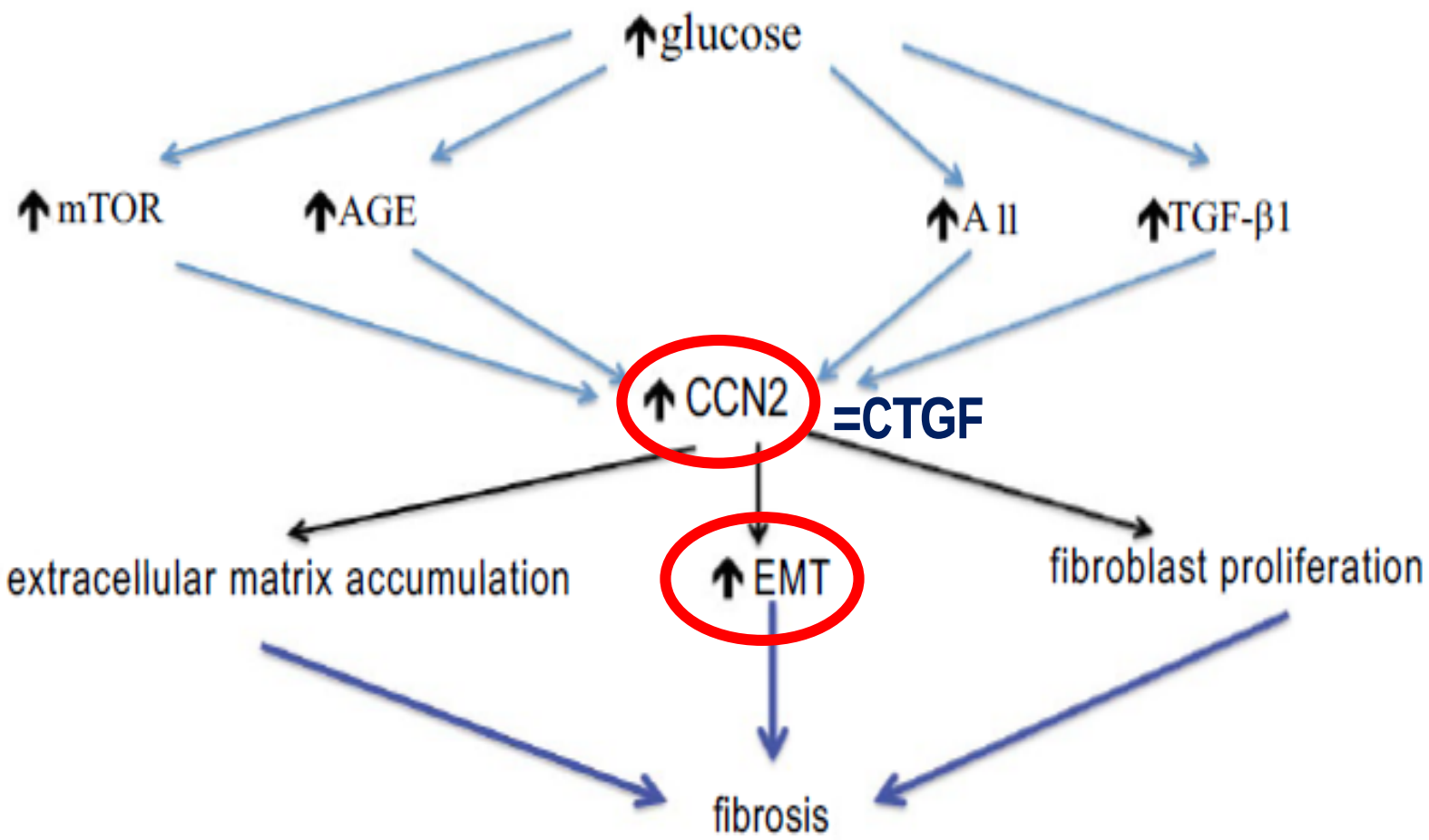


Figure 6 CCN2 mediated glomerular and interstitial fibrosis. mTOR= Mammalian target of rapamycin; AGE= Advanced glycation endproducts; A II= Angiutensin II; TGFβ= Transforming Growth Factor β; CCN2= Connective tissue growth factor; EMT= Epithelium mesenchyme transition.

Abdulazim DO (2017) Clin Exp Nephrol Vol 2 No 2: 35.

Αναστολείς του CNN2 και μείωση της UAER

Table 2: Agents for nonspecific inhibition of CCN2 expression in diabetic nephropathy.

Agents	Subjects	Treatment plan	Pathway	Outcome
Losartan [43]	T1 DN patients	50, 100, and 150 mg/day for 2 months, then 100 mg for 36 months		Losartan persistently decreased urinary CCN2 excretion, which correlated with a slower rate of decline in GFR
Spironolactone [46]	MCs, PTCs T2DM rats	100 nM for 24 h; 20 mg/kg/day, p.o. for 8 months	TGF-beta1-independent pathway	Spironolactone suppressed the production of CCN2 in MCs, PTCs, and T2DM rat model. Spironolactone reduced urinary protein and albumin excretion.
Fasudil [52]	T1DM rats	10 mg/kg/day IG for 30 days	Rho/Rho-kinase pathway	Fasudil inhibited CCN2 expression in the renal cortex of diabetic rats, with no affection of plasma glucose, blood pressure, and creatinine clearance in the diabetic rats. Fasudil suppressed urinary excretion of albumin.
Fasudil [54]	HMCs	HG 30 mmol/L fasudil 25, 50, and 100 μmol/L for 12, 24, 36, 48, and 72 h	Rho/Rho-kinase pathway	Fasudil reduced CCN2 mRNA expression and protein secretion.
Fluorofenidone [65] (AKF-PD)	MMC	TGF-β1 (1 ng/mL) fluorofenidone (2 mM) for 24 hours	ERK and p38 pathways	Fluorofenidone reduced TGF-β1-induced CCN2 expression.
Fluorofenidone [66] (AKF-PD)	HK2	TGF-β1 (5 ng/mL) AKF-PD 1 mM, 2 mM For 48 h	Downregulation of p-Smad2 and p-Smad3 proteins.	AKF-PD downregulated TGF-β1-induced CCN2 expression and attenuated EMT.
Exendin-4 [73]	HMC	HG 30 mmol/L Ex-4 0.03, 0.3, and 3 nmol/L for 24 hours	cAMP/PKA pathway	Exendin-4 decreased HG-induced the expression of TGF-β1 and CCN2.

Songyan Wang et al Journal of Diabetes Research Volume 2015

Activation of a local tissue angiotensin system in podocytes by mechanical strain[1]

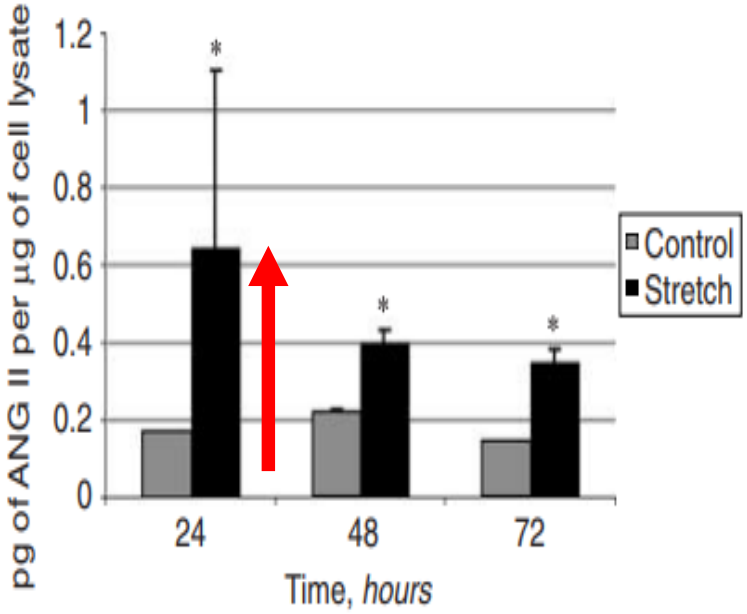


Fig. 1. Mechanical strain increases angiotensin II (ANG II) production by cultured podocytes. Angiotensin II levels were measured in total cell lysates by competitive enzyme-linked immunosorbent assay (ELISA). Compared to nonstretched controls, stretching primary culture mouse podocytes increased angiotensin II levels at all time points. $^*P < 0.05$.

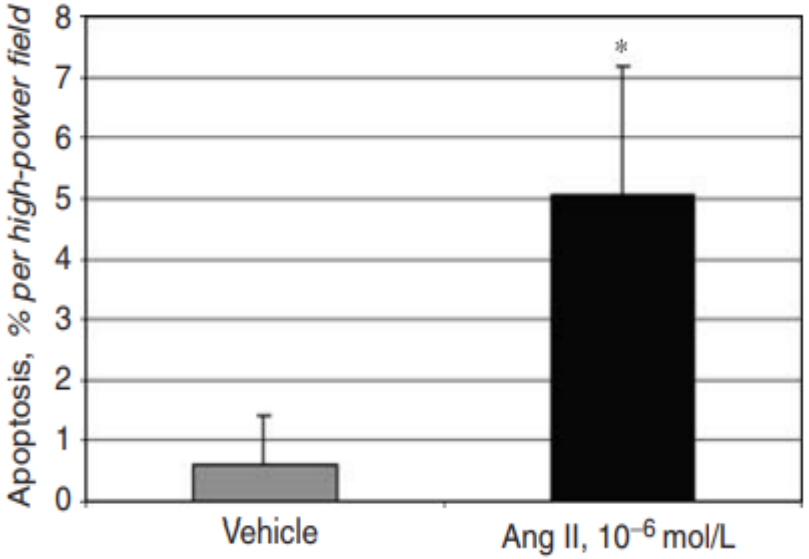


Fig. 7. Exogenous angiotensin II increases apoptosis of cultured podocytes. Conditionally immortalized mouse podocytes grown in 1% fetal bovine serum (FBS) were exposed to exogenous angiotensin II (10^{-6} mol/L) or vehicle. Apoptosis was measured at 24 hours by Hoechst staining. Angiotensin II increased podocyte apoptosis 8.5-fold compared to podocytes treated with vehicle alone (5.1% vs. 0.6%, respectively, $P < 0.001$).

Durvasula RV, et al. (2004) Kidney Int 65: 30-39

Tubulo-glomerular feedback → Hypeperfusion, Shear stress → ↑Angiotensin II

Notch1 receptor ← ***transmembrane receptor***

↑active Notch1 intracellular domain

↑snail protein ← ***transcription factor***

Podocytes

Nucleus → Apoptosis

↓Nephrin

↑proteinuria

Figure 7 Mechanism of podocyte injury and proteinuria induced by angiotensin II.

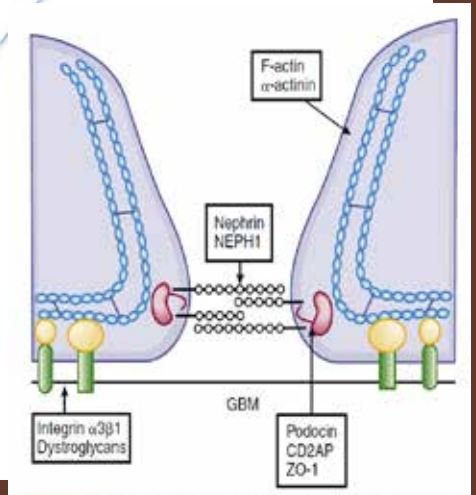
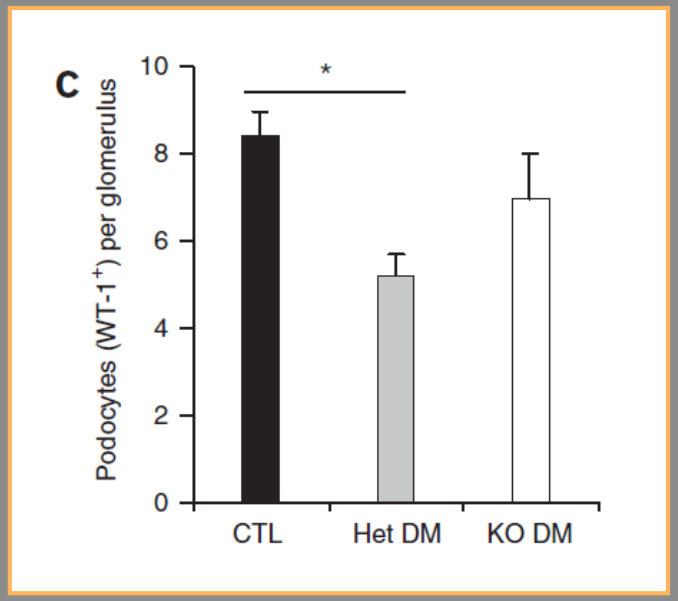
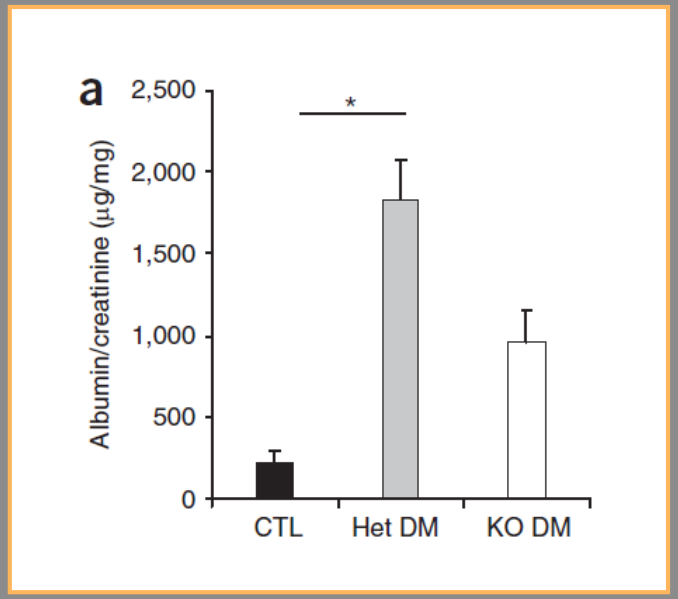


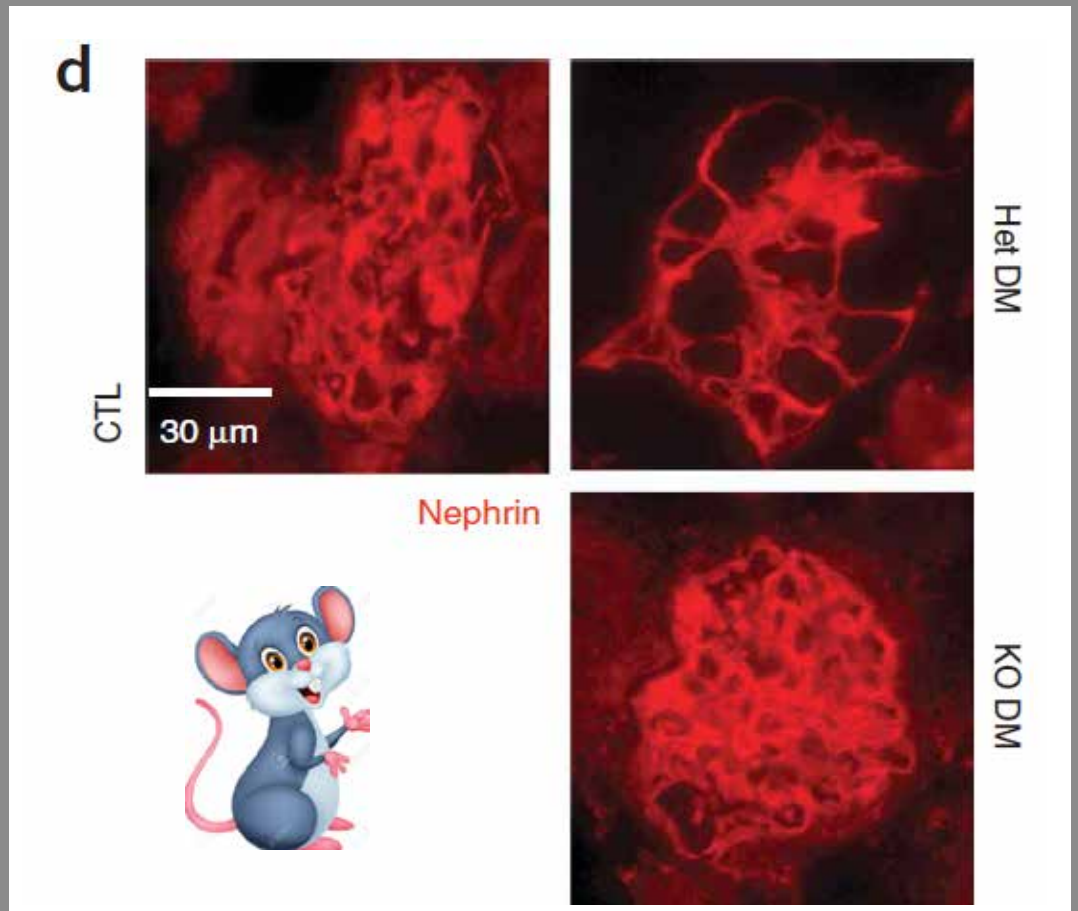
FIGURE 52-1 Hypothetical model of the podocyte slit diaphragm. See text for discussion. (From Jalanko H: Pathogenesis of proteinuria: lessons learned from nephrin and podocin, *Pediatr Nephrol* 18:487-491, 2003.)

Abdulazim DO (2017) Clin Exp Nephrol Vol 2 No 2: 35.

The Notch pathway in podocytes plays a role in the development of glomerular dis.







Recombining **b**inding **p**rotein **:** demonstrate Notch1 signaling

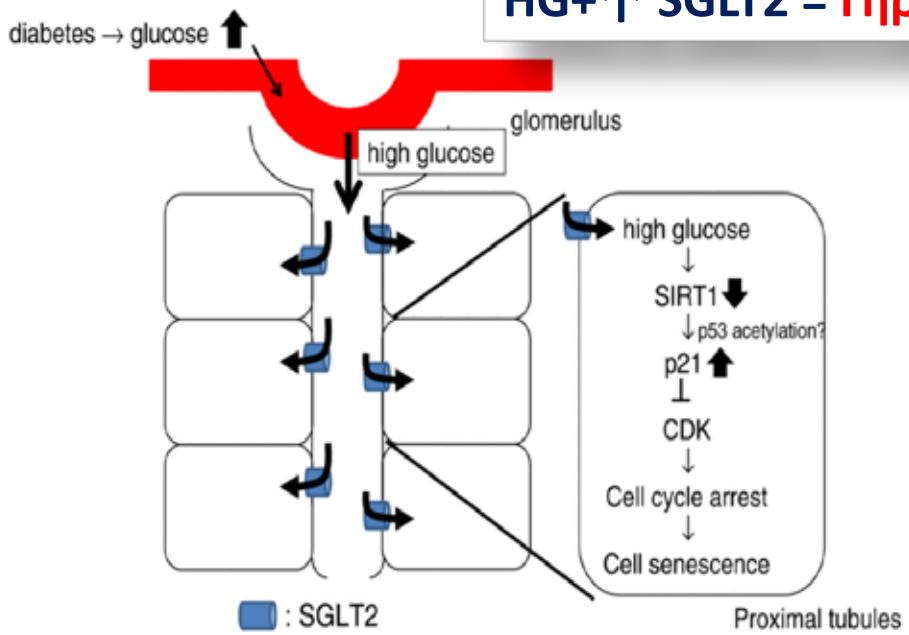
CTL : Control

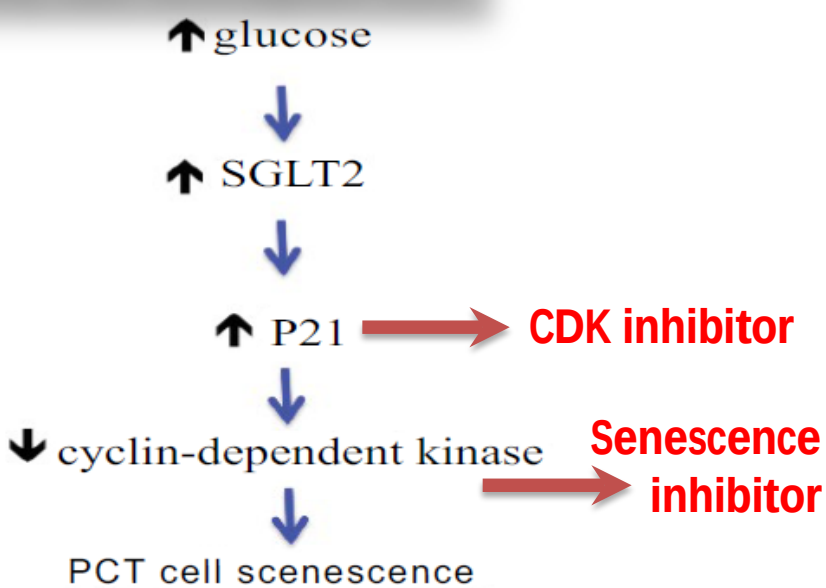
Het DM : DM WITHOUT deletion of Rbpj

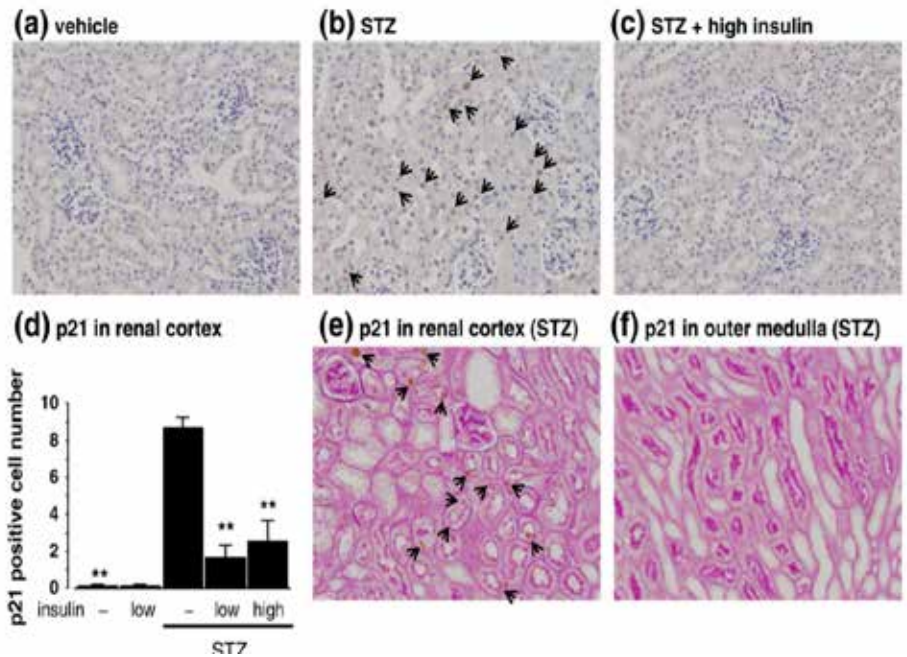
KO DM : DM WITH deletion of Rbpj

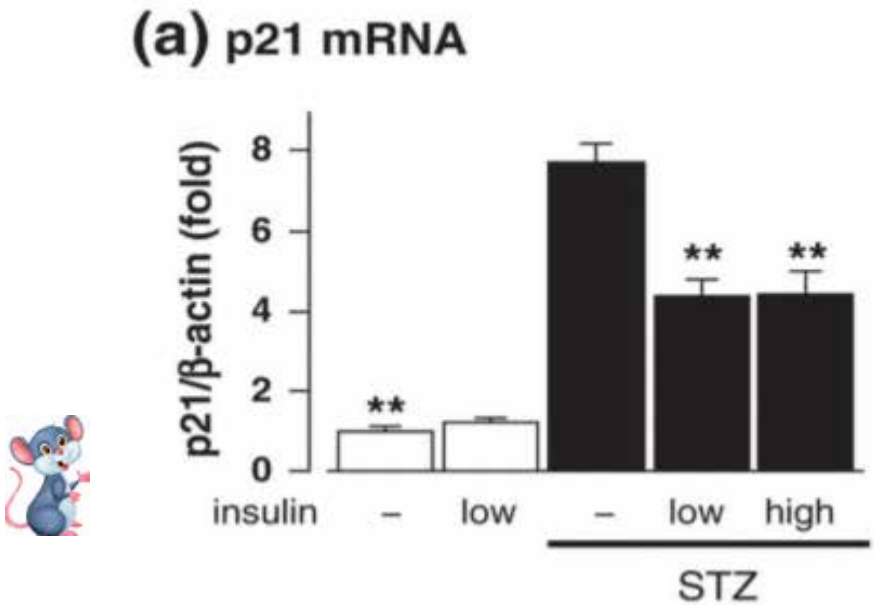
Niranjan Tet al . (2008) Nat Med 14: 290-298.

HG+↑ SGLT2 = Γήρανση των κυττάρων ΕΣΑ







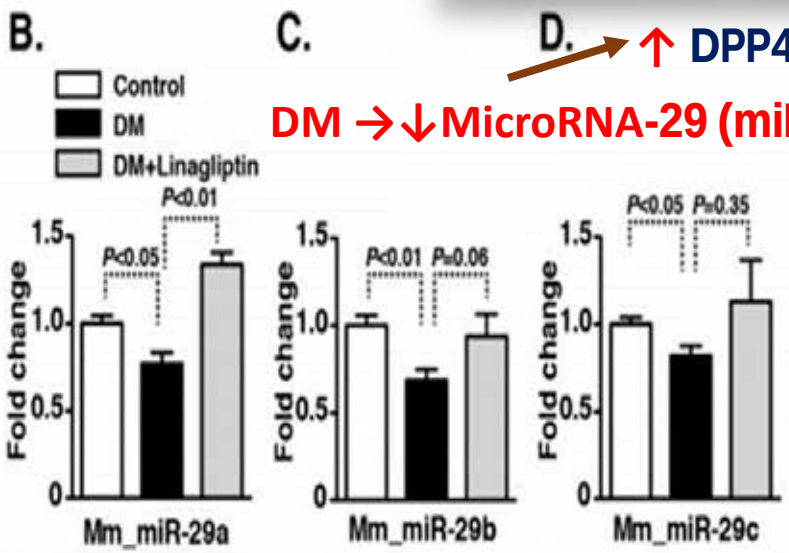


Kitada K, et al. (2014) J Diabetes Complications 28: 604-611

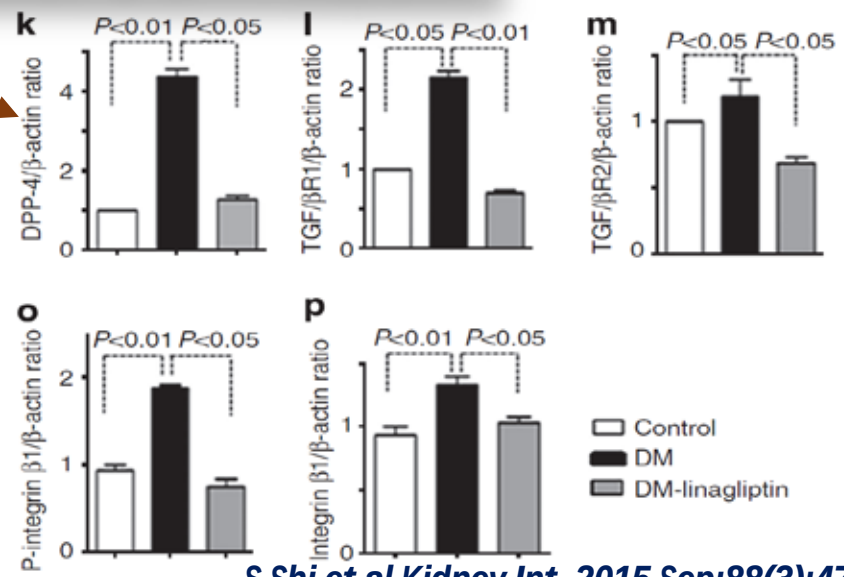
Ο ρόλος του microRNA29 & DPP4 στη ΔΝΝ

↑ DPP4

DM →↓MicroRNA-29 (miR29)

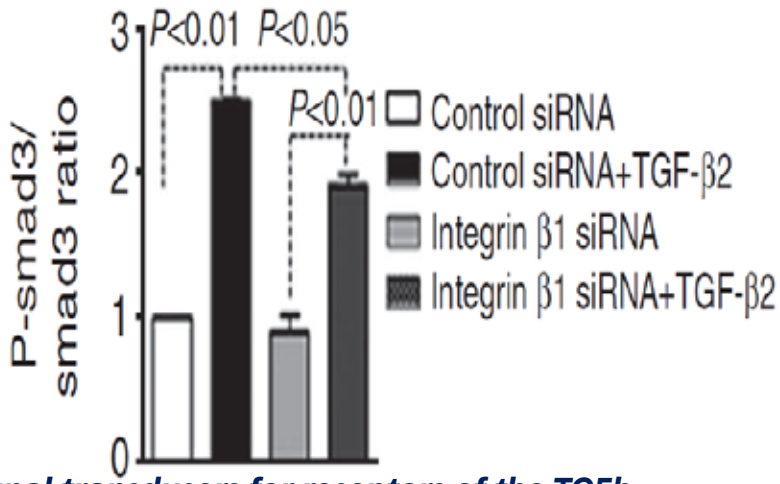


Kanasaki et al Diabetes Volume 63, June 2014

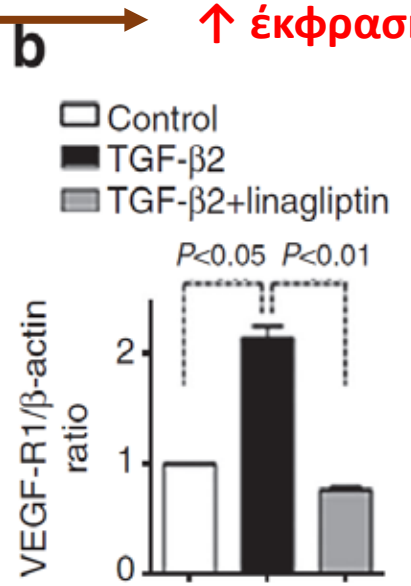


S Shi et al Kidney Int. 2015 Sep;88(3):479-89

↑ έκφραση Smad ← ↑ TGFb → ↑ έκφραση VEGF-R1



signal transducers for receptors of the TGFb



S Shi et al Kidney Int. 2015 Sep;88(3):479-89

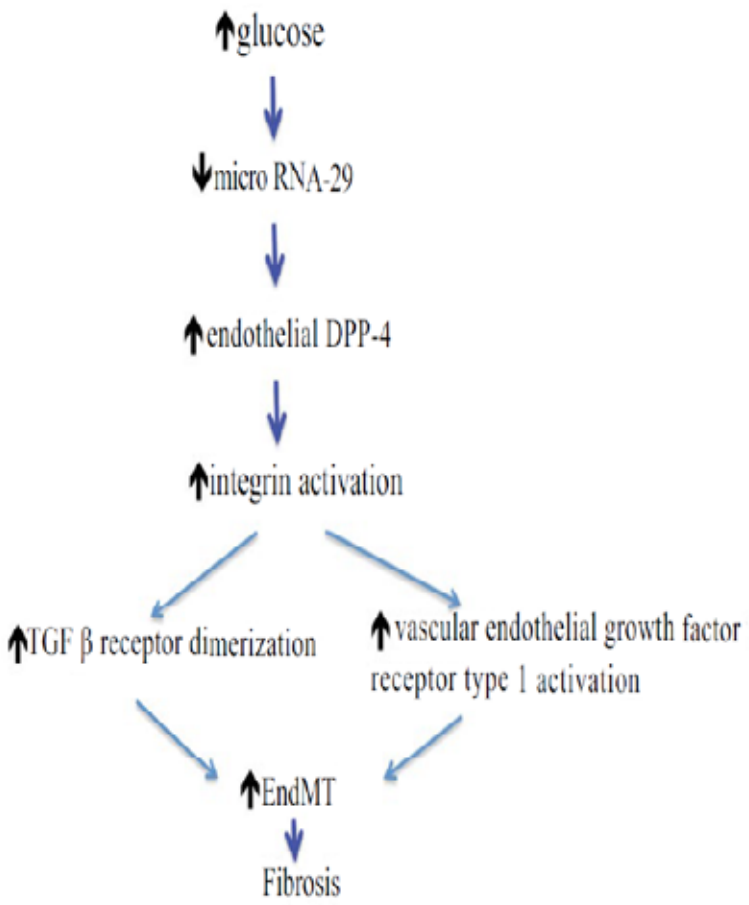
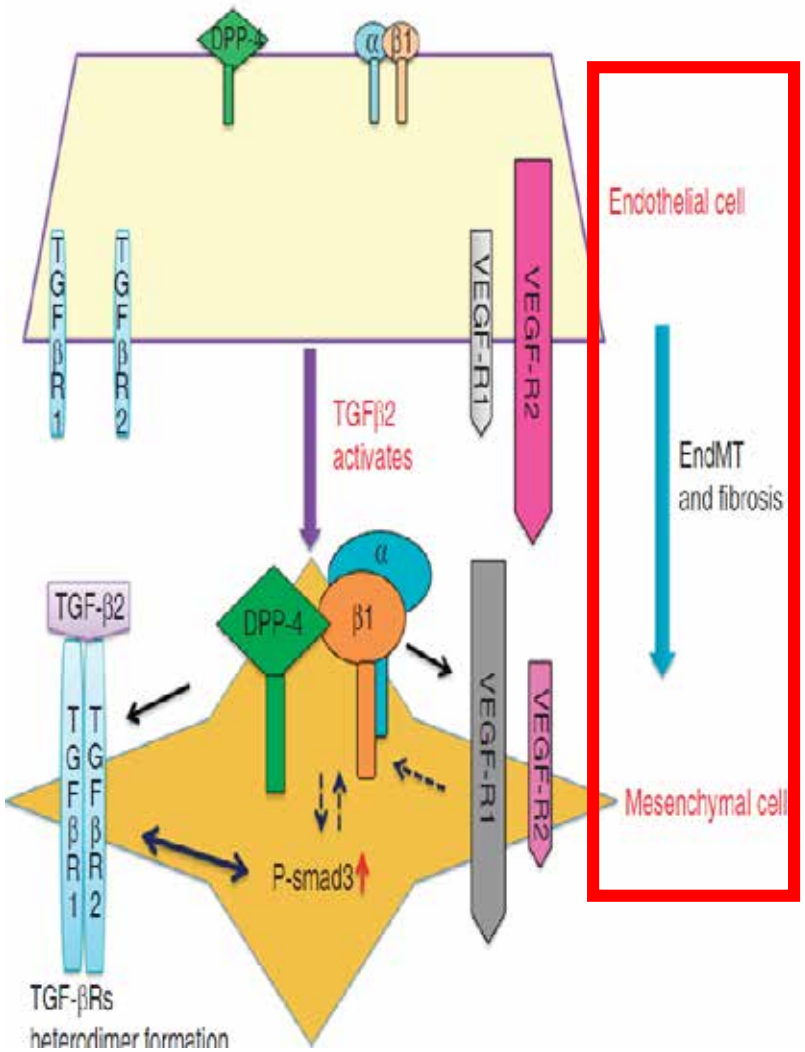


Figure 10 DPP-4 mediated renal fibrosis. DPP4= Dipeptyl peptidase-4; TGFβ= Transforming Growth Factor β; EndMT= Endothelial-mesenchymal transition.



Abdulazim DO (2017) Clin Exp Nephrol Vol 2 No 2: 35.

Cross talk between FGF23 & RAS

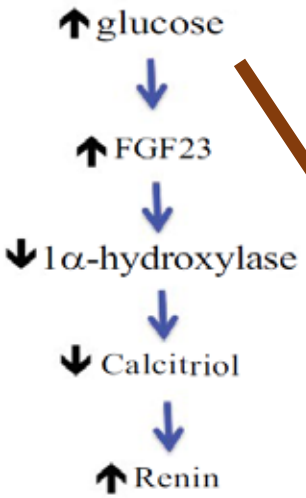
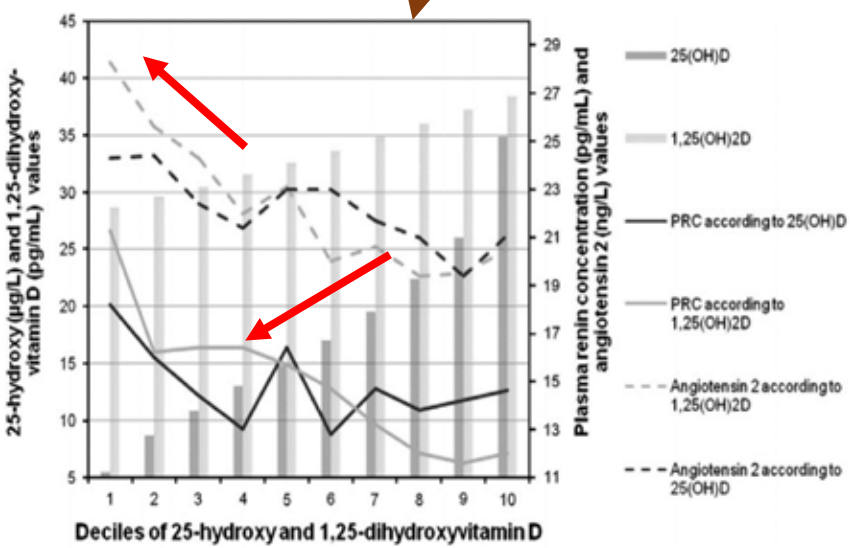


Figure 11 FGF23 mediated increased renin activity in diabetic patients. FGF23= Fibroblast growth factor 23.

n= 3296



A. Tomaschitz et al. Clinica Chimica Acta 411 (2010) 1354

Table 1 Baseline characteristics of patients with diabetic nephropathy, and healthy controls

Parameter	Diabetics (n=109)	Controls (n=32)	p Value
Age (years)	61.63±9.77	49.53±7.32	<0.001
Gender (F/M)	47/62	20/12	0.054
BMI (kg/m^2)	30.93±4.85	27.37±4.31	<0.001
Creatinine (mg/dL)	1.57±0.75	0.88±0.12	<0.001
GFR (mL/dak)-CKD-EPI (eGFR)	51.71±23.11	90.15±20.71	<0.001
UPCR (mg/d)	1625.43±2227.62	006.40±0.03	<0.001
UACR (mg/d)	263.30±329.72	4.28±6.37	<0.001
s-Klotho (ng/mL)	5.69±4.64	3.62±4.27	<0.001
FGF23 (pg/mL)	360.29±528.36	189.09±293.22	<0.001
25hD (ng/mL)	16.73±13.15	62.13±18.37	<0.001
PTH (pg/mL)	96.33±111.52	57.79±22.28	<0.001
Calcium (mg/dL)	9.36±0.55	9.37±0.39	0.974
Phosphorus (mg/dL)	3.43±0.64	3.28±0.67	0.136
ALP (U/L)	85.82±33.76	76.25±16.36	0.290
SBP (mm Hg)	140.40±25.48	11197±13.21	<0.001
DBP (mm Hg)	82.59±12.99	78.03±10.24	0.212

Inci A, et al. J Investig Med 2016;64:1128–1133

FGF-23 as a predictor of renal outcome in DN

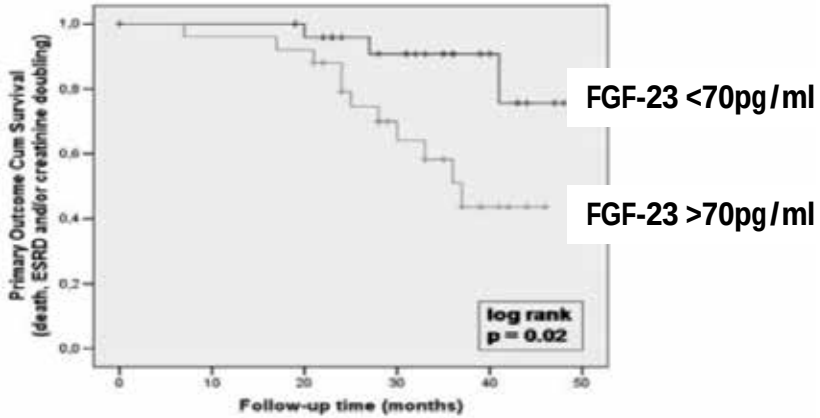
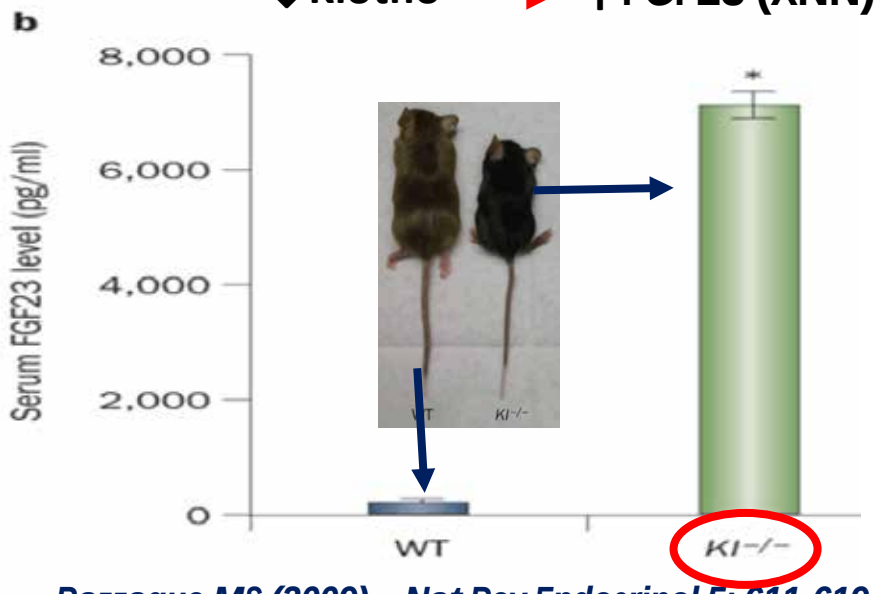


Figure 2. | Kaplan-Meier curves of the incidence of the composite primary outcome according to serum FGF-23 in 55 diabetic nephropathy patients.

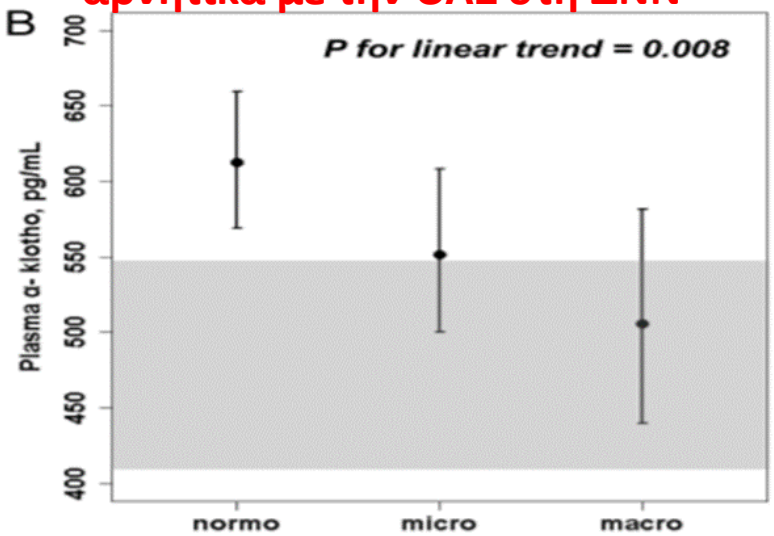
Titan SM et al. (2011) Clin J Am Soc Nephrol 6: 241-247

↓Klotho → ↑FGF23 (XNN)



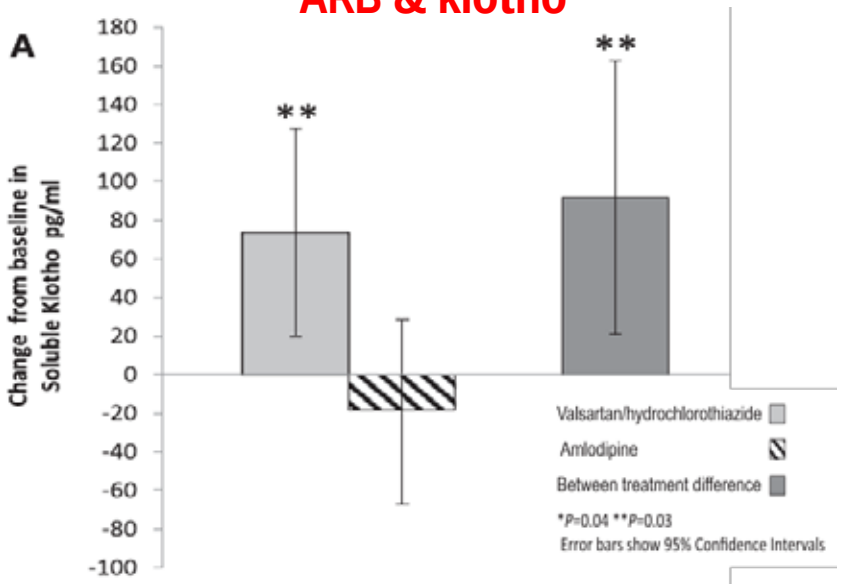
Razzaque MS (2009) . Nat Rev Endocrinol 5: 611-619.

Τα επίπεδα της α-klotho συσχετίζονται αρνητικά με την UAE στη ΔΝΝ



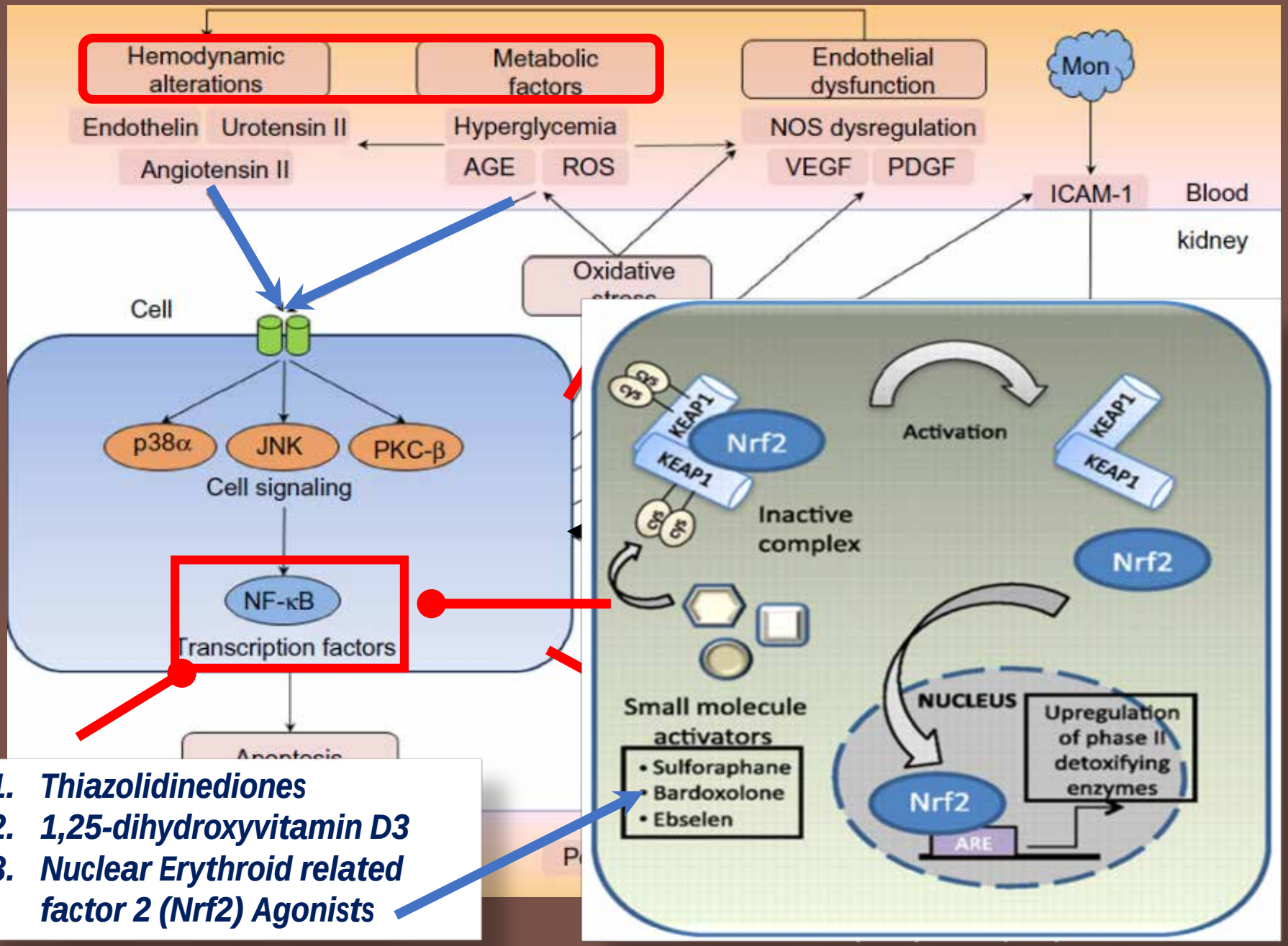
Lee et al PLoS One. 2014 Aug 1;9(8):e102984.

ARB & klotho



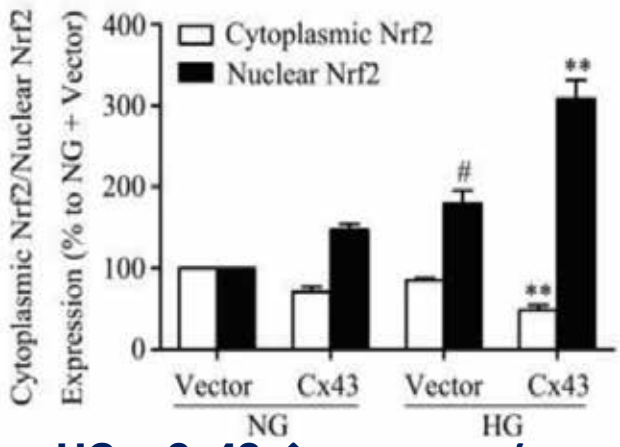
Karalliedde et al CJASN 8: 1899–1905, November, 2013 .

Φλεγμονή

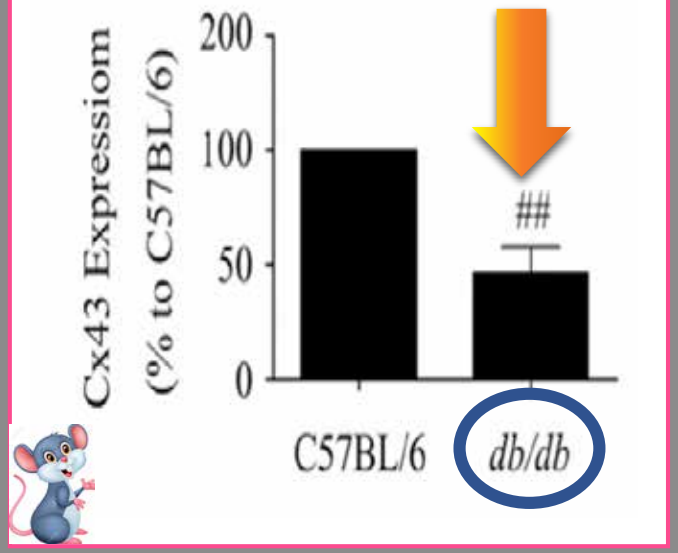


1. *Thiazolidinediones*
2. *1,25-dihydroxyvitamin D3*
3. *Nuclear Erythroid related factor 2 (Nrf2) Agonists*

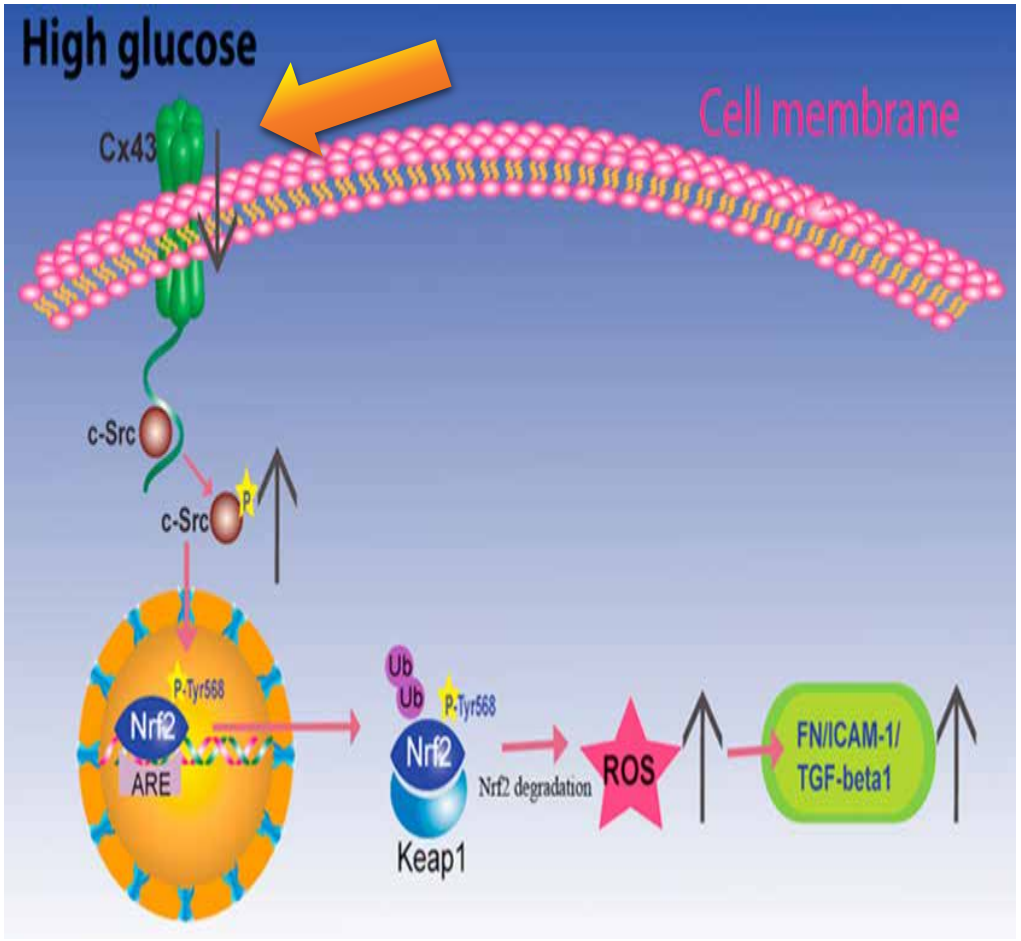
Nrf2 is activated in diabetic status but not enough to resist ROS



Σε HG η Cx43 ↑την μετακίνηση του Nrf2 στον πυρήνα



Connexin43 regulates high glucose-induced expression Nrf2



Chen Z et al. (2017). Free Radic Biol Med 102: 77-86.

JAK-STAT, Janus kinase/signal transducer and activator of transcription

The Phase 2 clinical trial of the efficacy of baricitinib in the treatment of diabetic kidney disease (129 pts multicentre, randomised, double-blind, placebo-controlled study →↓UAER)

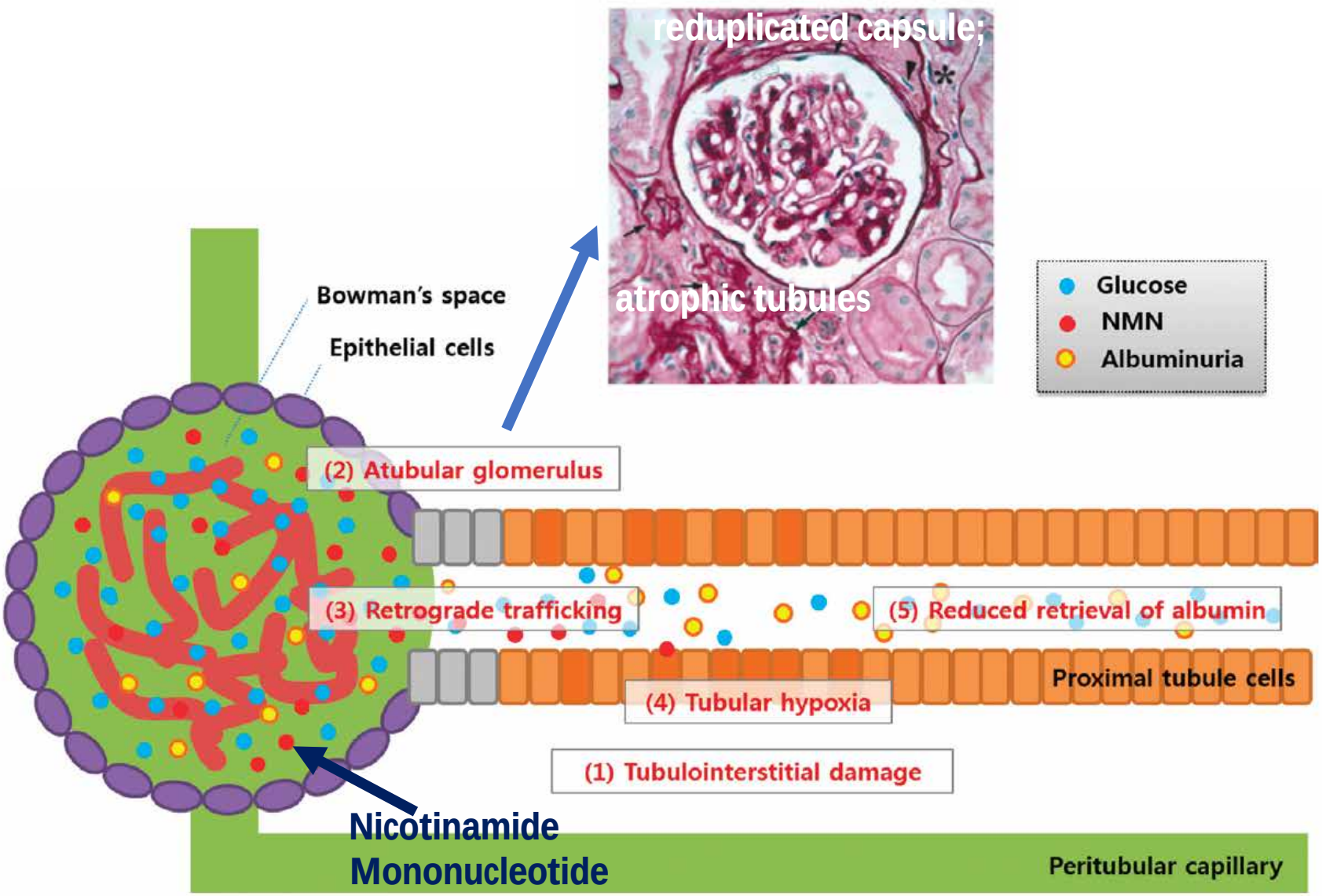
Gene	Glomerular compartment			Tubulointerstitial compartment		
	Controls	Early DKD	Prog. DKD	Controls	Early DKD	Prog. DKD
JAK1	0.13 ± 0.08	0.69 ± 0.43 (0.0007)**	0.43 ± 0.42 (0.003)**	0.81 ± 0.73	0.59 ± 0.14 (0.878)	1.29 ± 0.86 (0.023)
JAK2	0.83 ± 1.42	5.14 ± 2.84 (0.0006)**	1.81 ± 1.89 (0.016)*	0.29 ± 0.19	0.33 ± 0.13 (0.340)	0.66 ± 0.34 (0.0014)**
JAK3	0.14 ± 0.39	0.44 ± 0.35 (0.032)	0.71 ± 1.10 (0.003)**	0.01 ± 0.01	0.02 ± 0.01 (0.056)	0.27 ± 0.32 (0.0001)**
STAT1	0.57 ± 0.67	3.05 ± 1.61 (0.001)**	1.14 ± 1.14 (0.204)	0.26 ± 0.27	0.25 ± 0.18 (0.601)	0.86 ± 0.80 (0.0014)**
STAT3	0.26 ± 0.17	0.90 ± 0.37 (0.001)**	0.57 ± 0.38 (0.019)	0.74 ± 0.71	0.54 ± 0.18 (0.644)	0.71 ± 0.18 (0.175)

Brosius et al. Diabetologia. 2016 August ; 59(8): 1624–1627 -------Tuttle et al NTD(2018) 33: 1950–1959

Radica Z. Clin J Am Soc Nephrol 12: 2032–2045, December, 2017

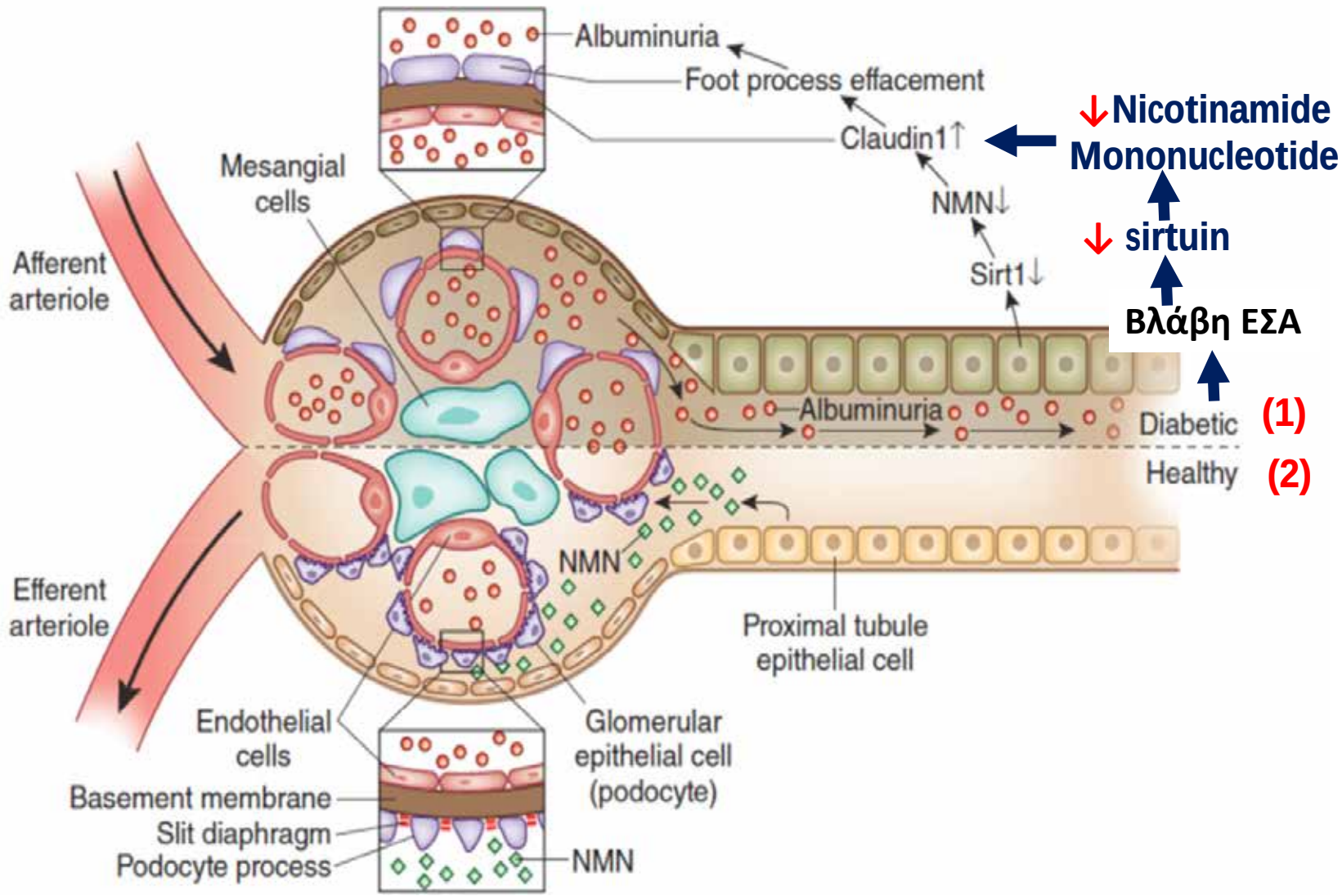
DCeline C et al DIABETES, VOL. 58, 2/2009

Pathophysiology of diabetic nephropathy: tubule versus glomerulus



Sirt1-Claudin-1 crosstalk regulates renal function

Proximal tubules & podocytes communication by releasing NMN

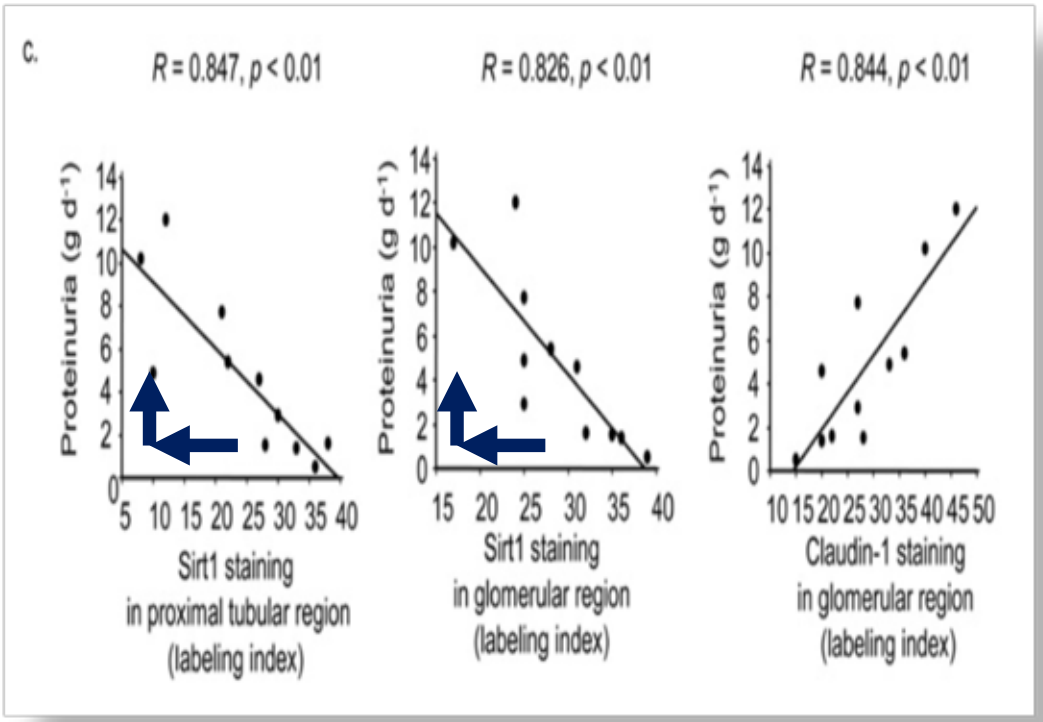


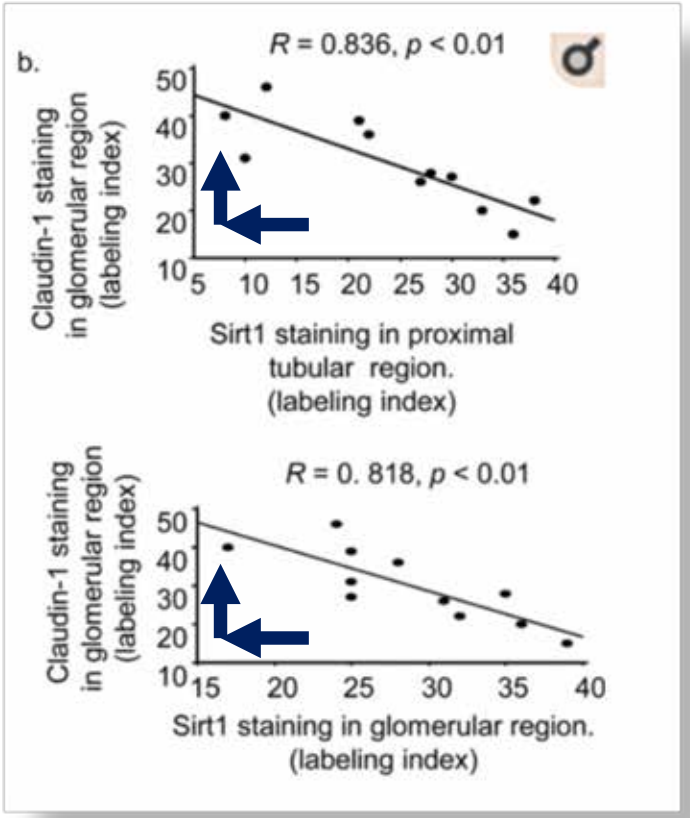
Nihalani D et al Nature Medicine. 2013;19:1371-1372

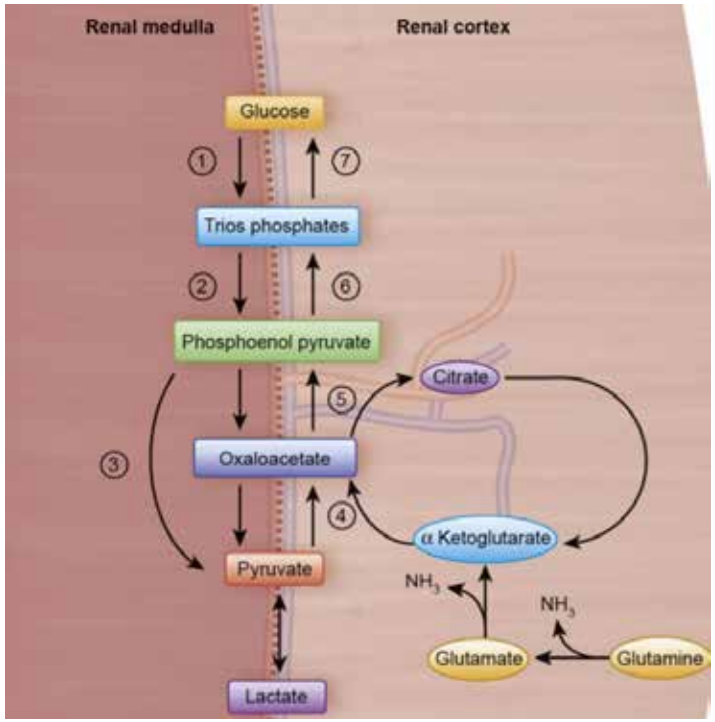
Nat Med. 2013 Nov;19(11):1496-504. doi: 10.1038/nm.3363. Epub 2013 Oct 20.

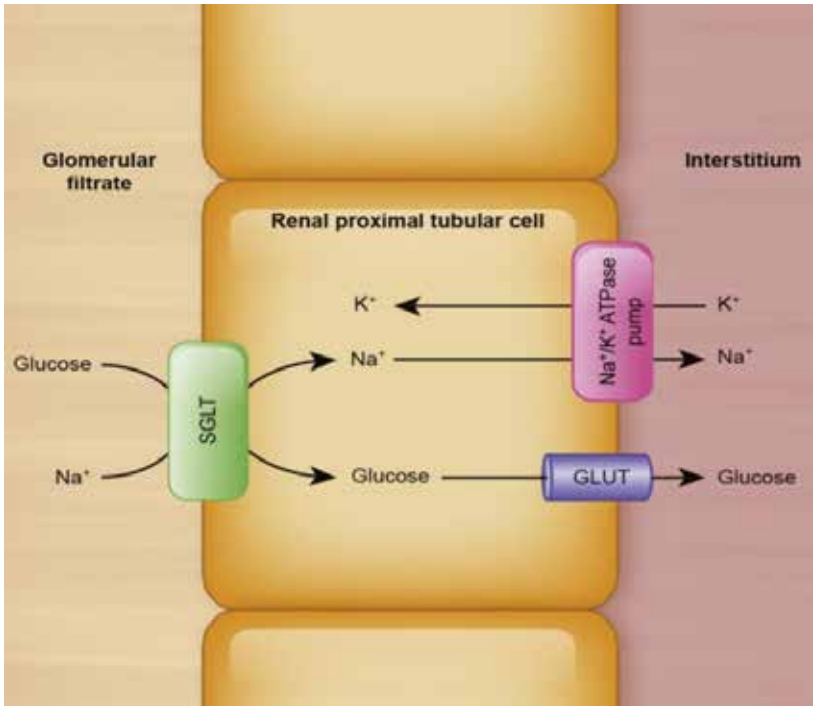
Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes.

Hasegawa K[1], Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, Hosoya K, Komatsu M, Kaneko Y, Kanda T, Kubota E, Tokuyama H, Hayashi K, Guarente L, Itoh H.









Γλυκονεογένεση

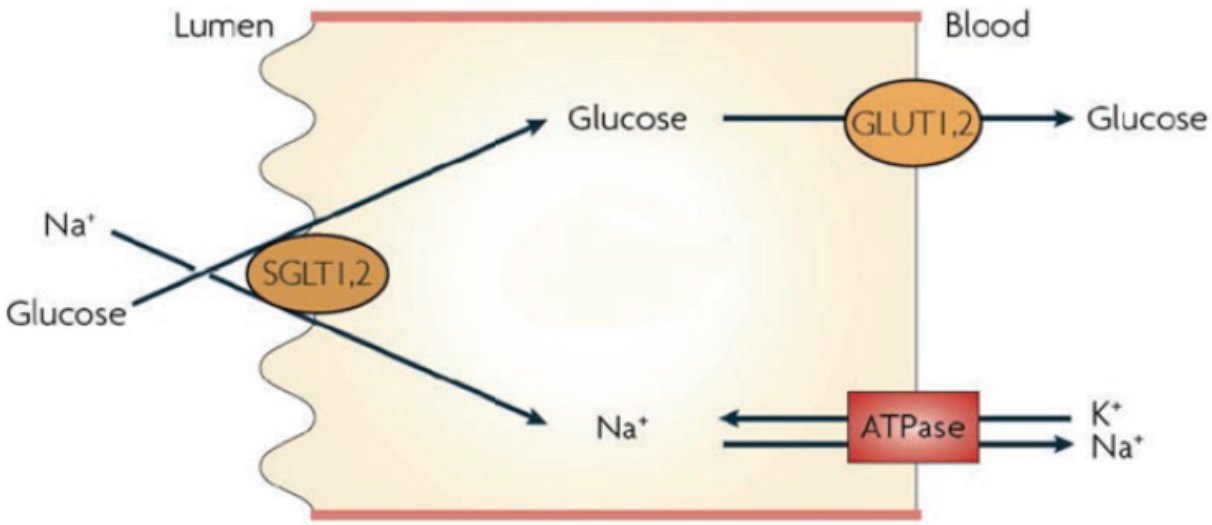
↑SGLT2 (ΣΔ)

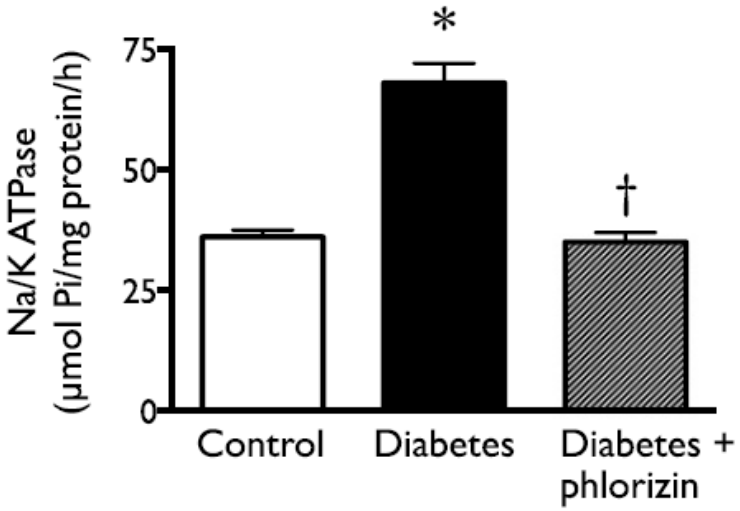
↑ανάγκες κατανάλωσης ATP και O_2

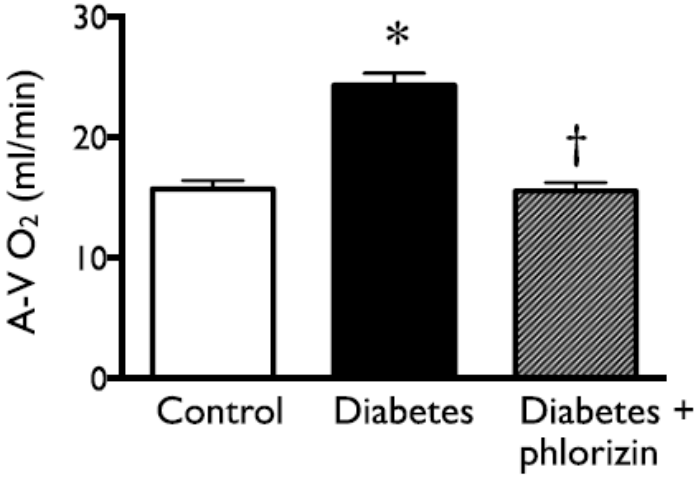
Μεταβολική απορρύθμιση

Βλάβες αγγείων

Υποξία Σωληναρίων

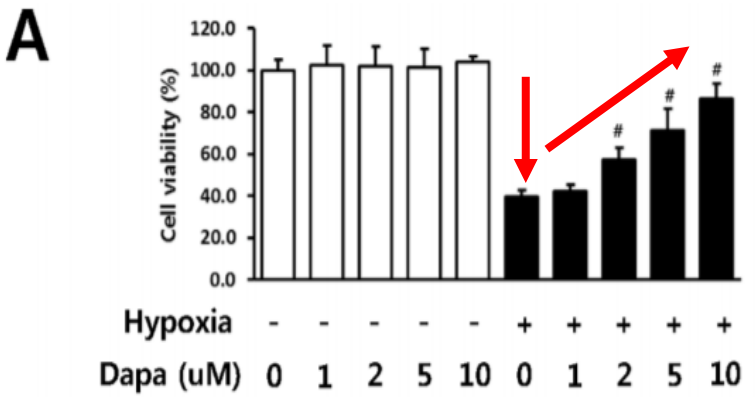




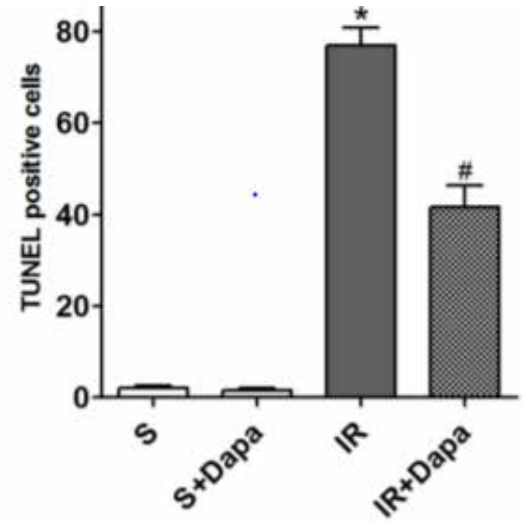


Körner A et al Diabetes 1994;43:629–633

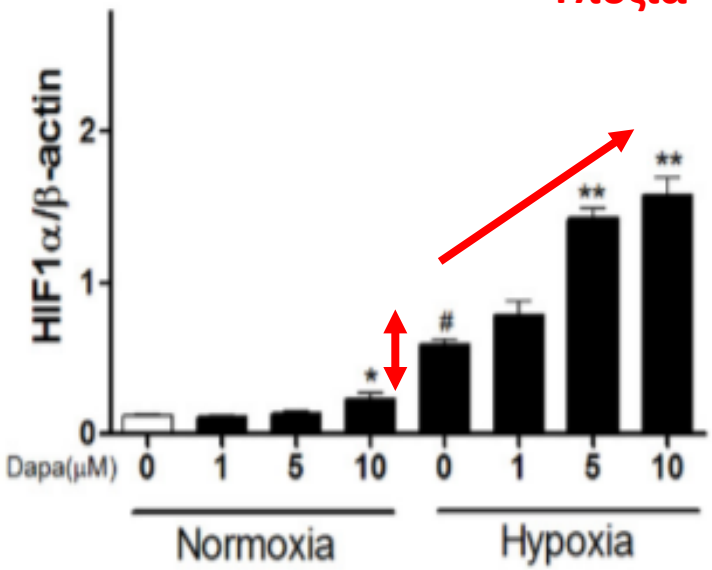
Βιωσιμότητα σωληναριακών κυττάρων

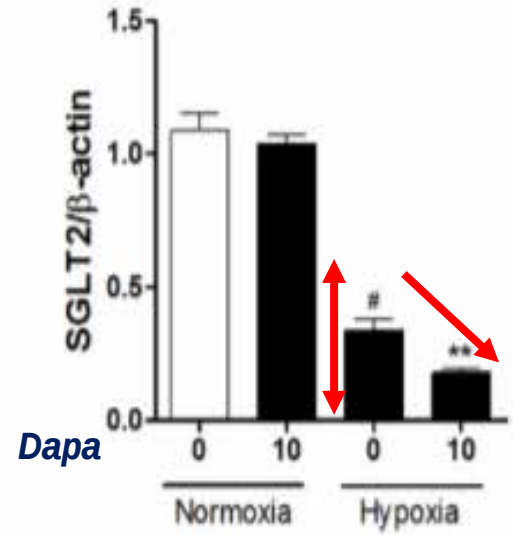


Απόπτωση σωληναριακών κυττάρων



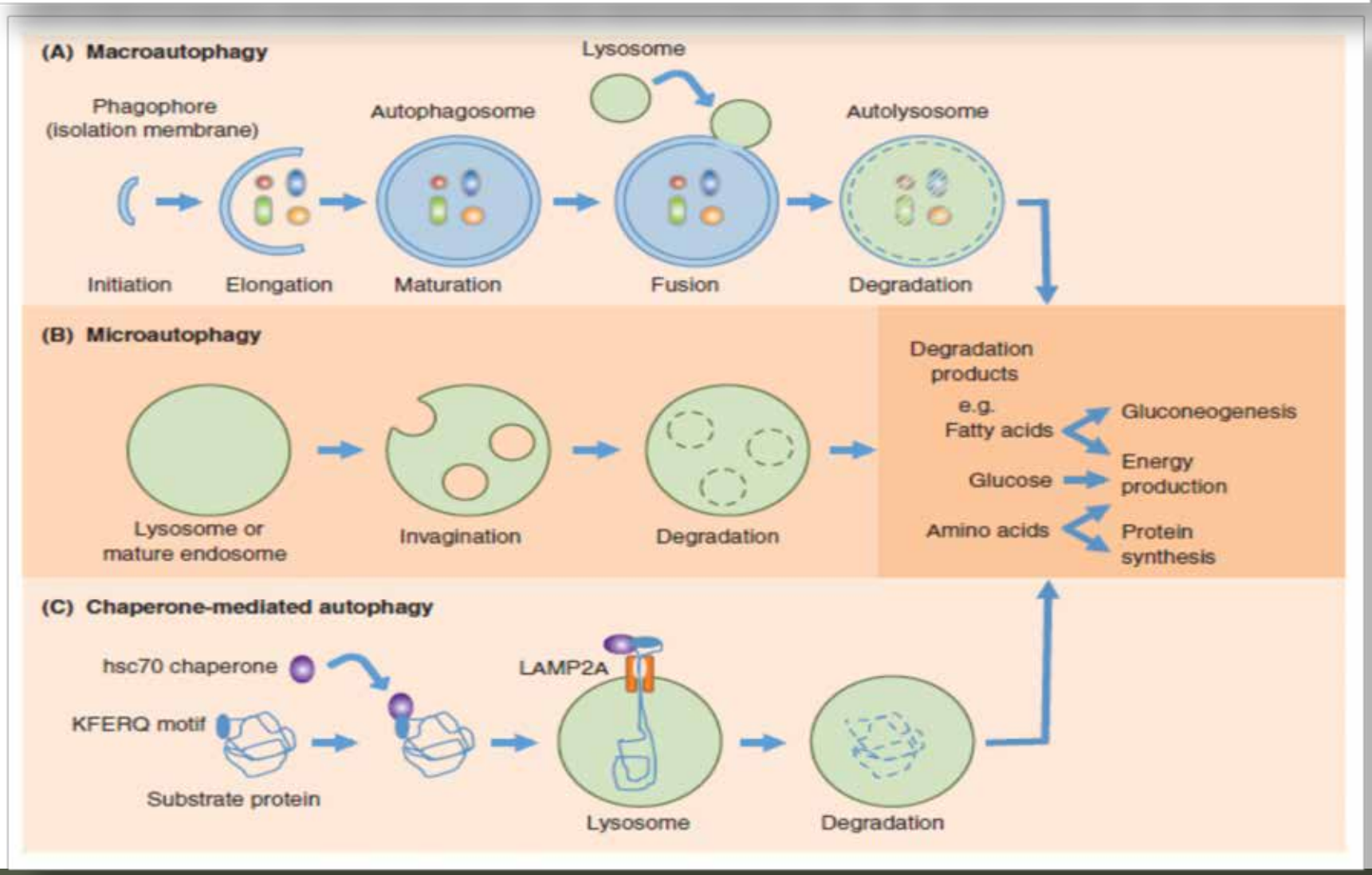
Υποξία – HIF – SGLT2



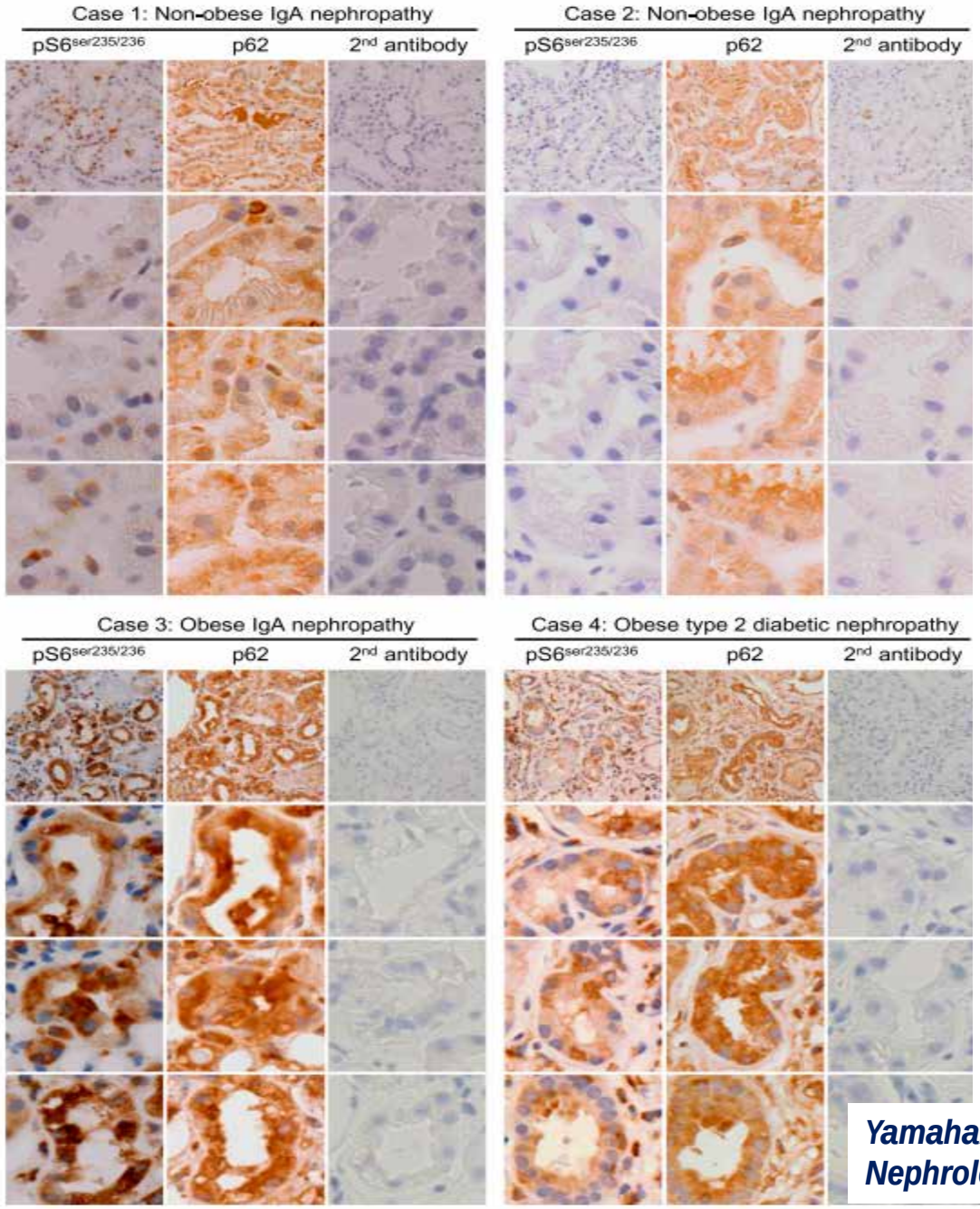


Chang YK et al PLoS One. 2016;11:e0158810.

Η Autophagy αποτελεί μια διαδικασία ανακύκλωσης ενδοκυττάριων προϊόντων μεταβολισμού απαραίτητη για την ομοιοστασία και την ακεραιότητα του κυττάρου



Yan Ding Journal of Endocrinology (2015) 224, R15–R30



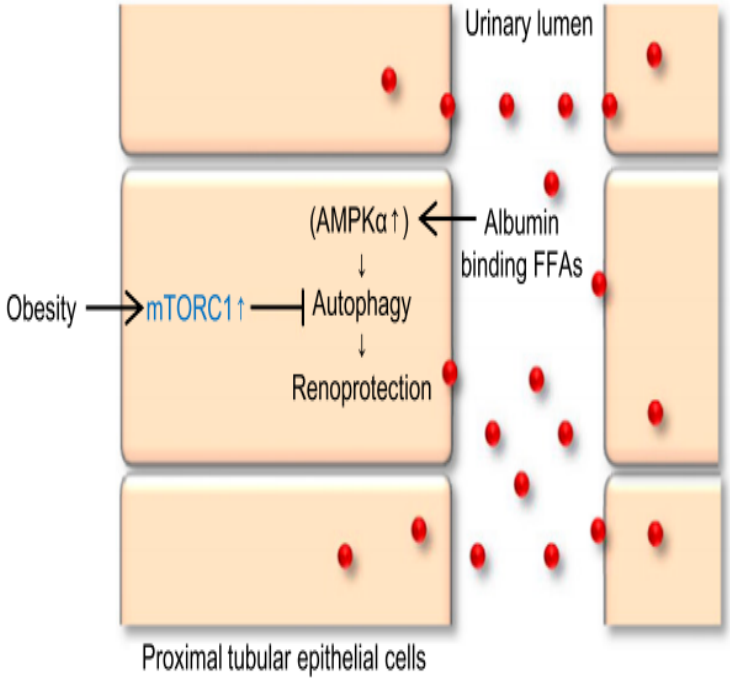


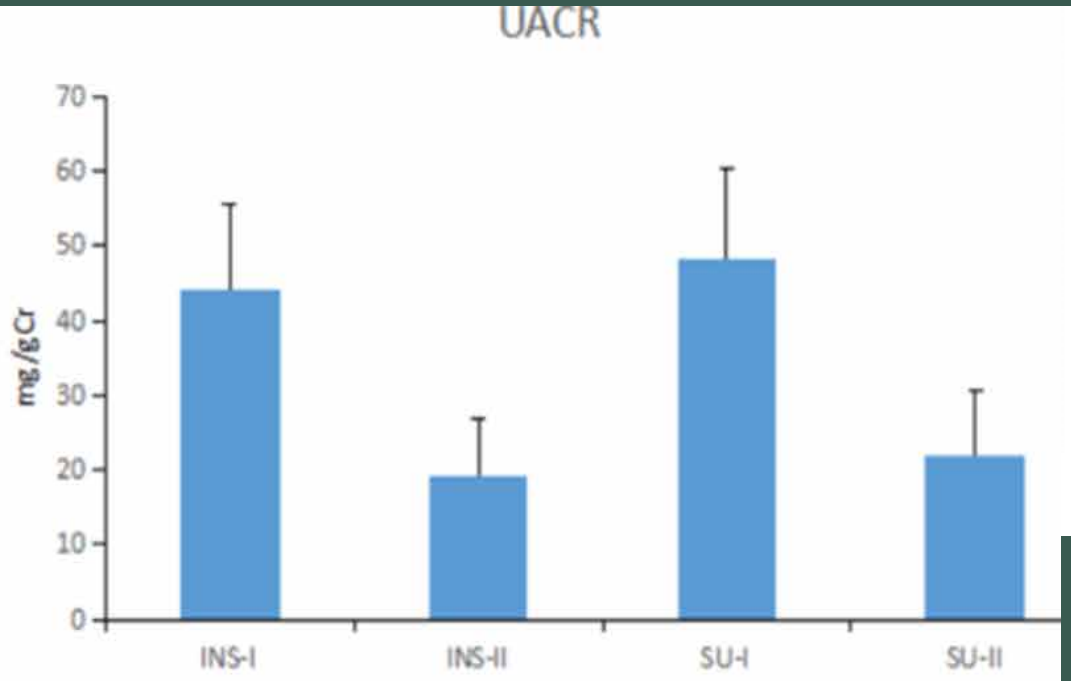
Figure 7. Proposed mechanisms of proteinuria-induced autophagy in proximal tubular cells. Proteinuria renoprotectively elicits autophagy, as well as activates AMPKα in proximal tubular cells. Obesity suppresses proteinuria-induced autophagy by hyperactivating mTORC1 in proximal tubular cells, leading to the obesity-mediated exacerbation of proteinuria-induced tubulointerstitial damage.

Yamahara K et al . Journal of the American Society of Nephrology 24, 1769–1781- 2013

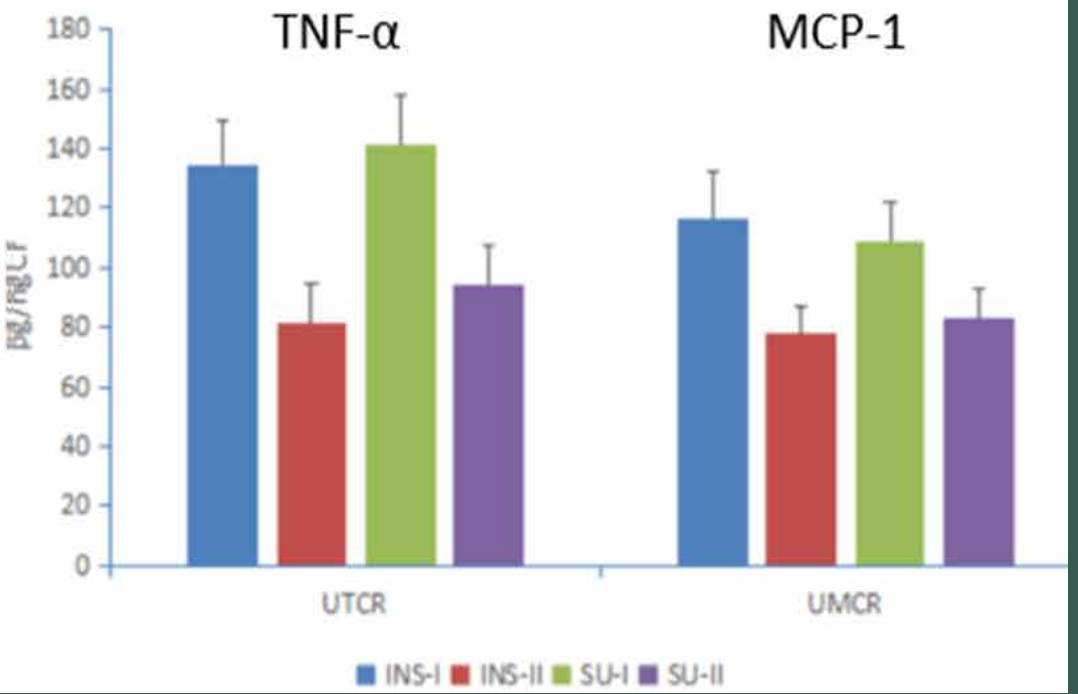
Research Article

Protective Effect of Metformin on Glomerular Podocyte in Patients with Type 2 Diabetes and its Mechanism

Yang Lin[1,2], Shandong Ye[3]*, and Yan Chen[4]

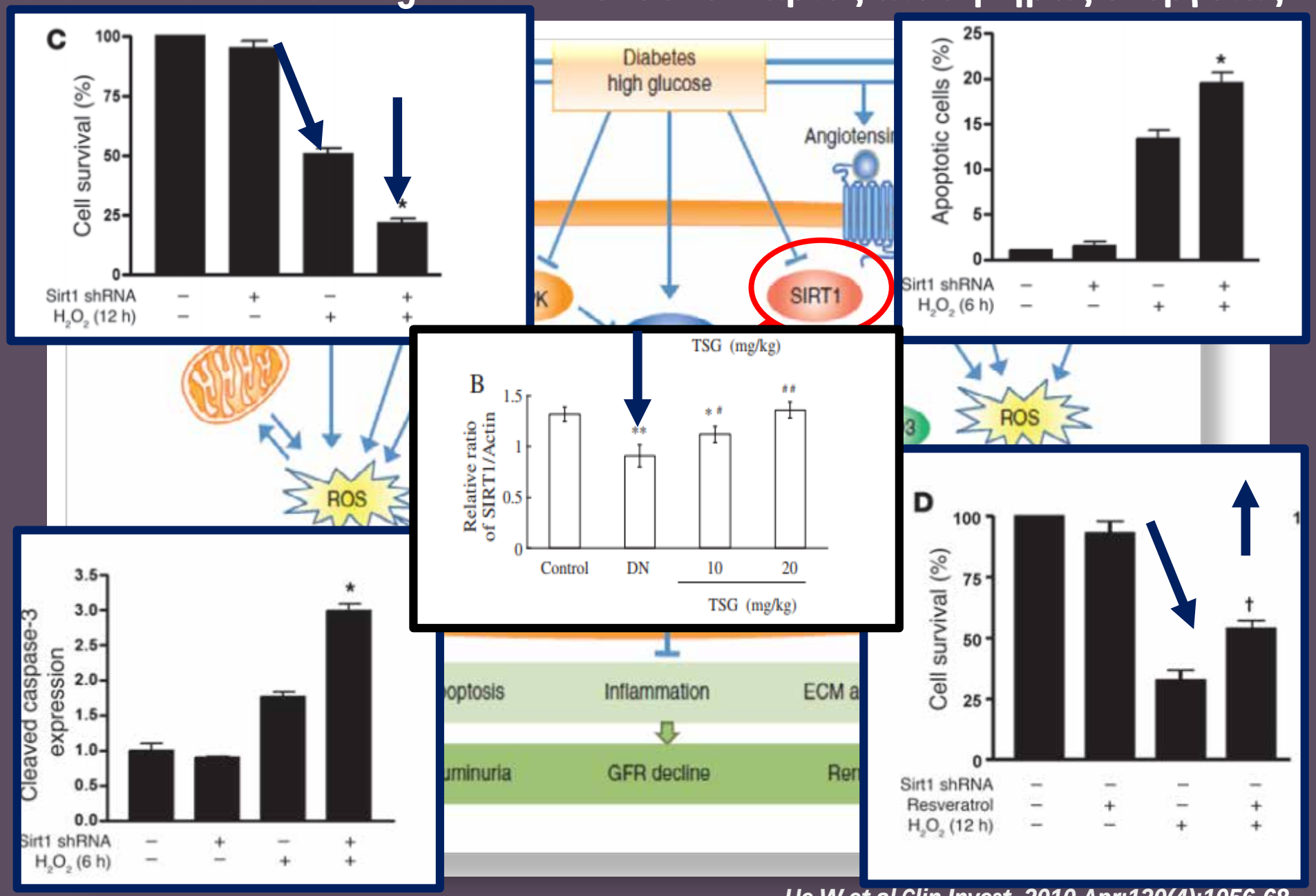


180 DMT2 pts were divided into insulin group(INS group, n=93) and sulfonylurea group(SU group, n=87), and then the two subgroups were divided according to whether or not combined with metformin , 2 years



Ye et al Ann Clin Exp Metabol 2(2): 1020 (2017)

Silent information regulator T1 ενδοκυττάριος αισθητήρας ενέργειας

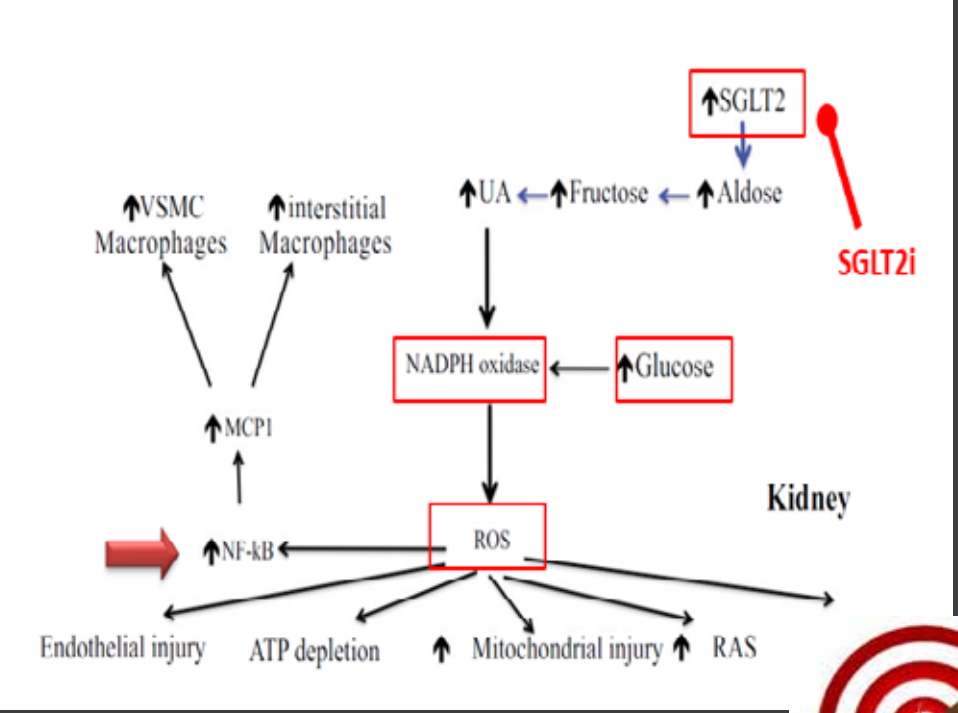


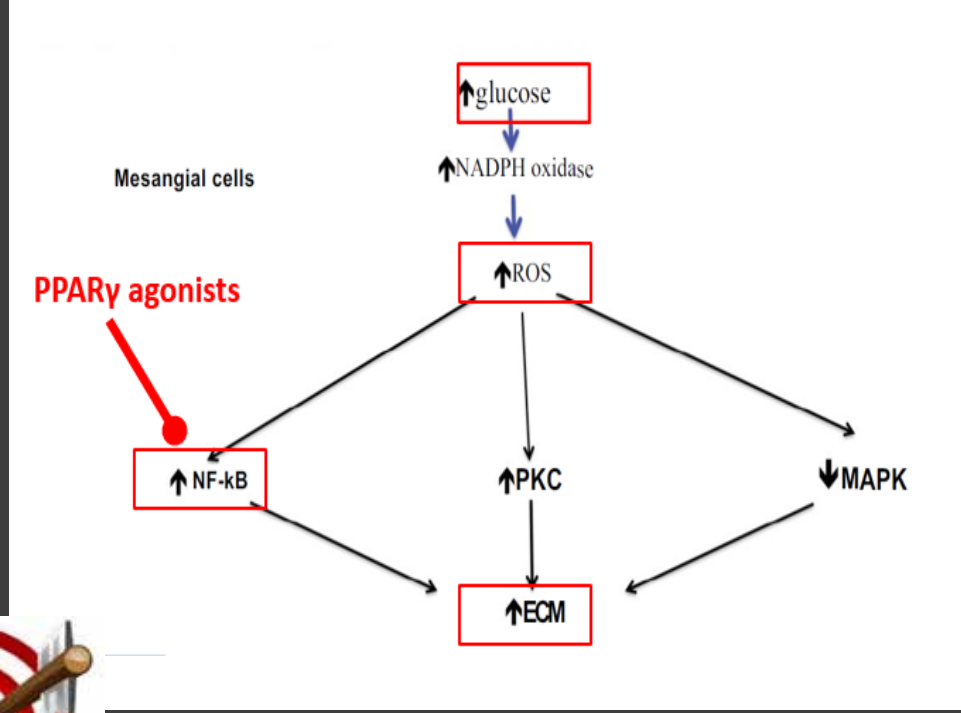
He W et al Clin Invest. 2010 Apr;120(4):1056-68.
Li C, et al 2010a European Journal of Pharmacology 649.

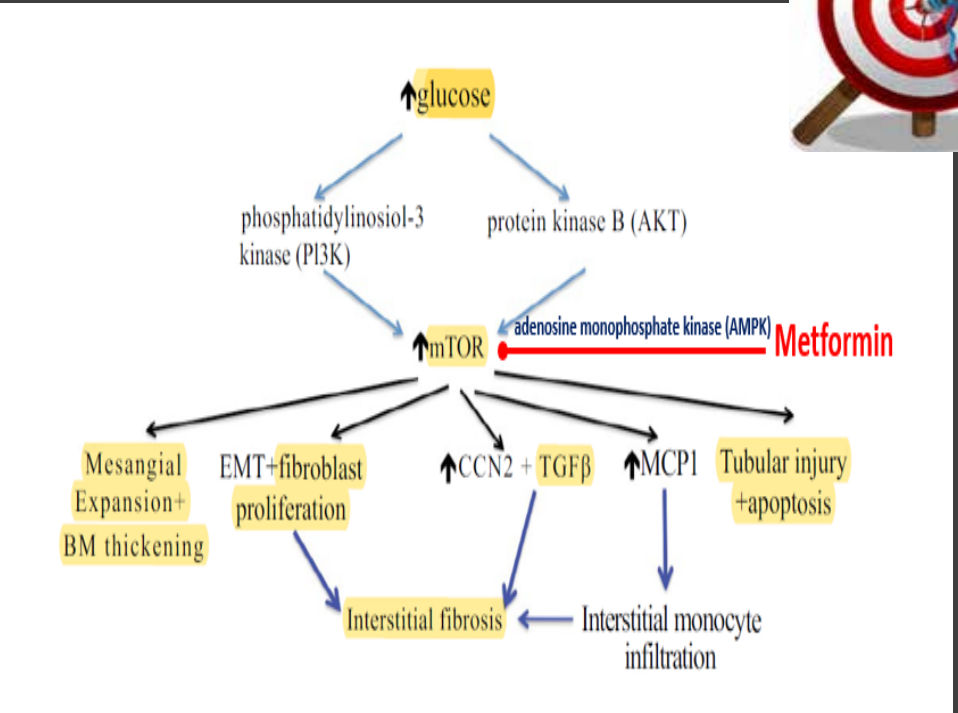
Παθογένεια – Θεραπεία ΔΝΝ

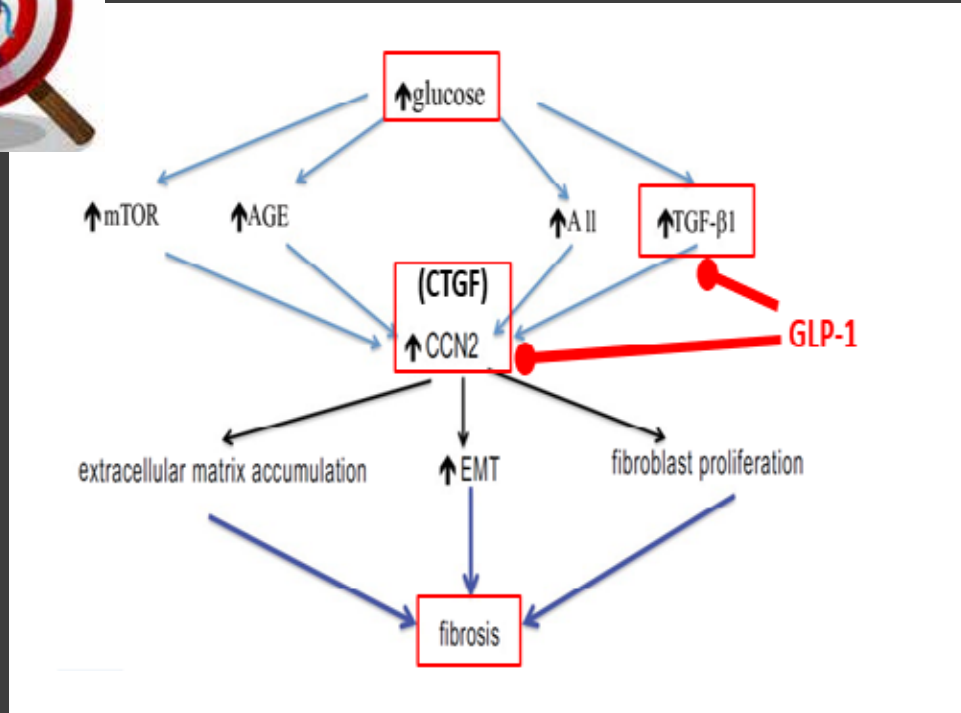
“Manage the disease from stage 1 not stage 3 of DKD…”

It takes 2 to Tango….


↑SGLT2
SGLT2i
↑UA
↑Fructose
↑Aldose
↑VSMC Macrophages
↑interstitial Macrophages
NADPH oxidase
↑Glucose
↑MCP1
Kidney
↑NF-kB
ROS
Endothelial injury
ATP depletion
↑ Mitochondrial injury
↑ RAS


↑glucose
Mesangial cells
↑NADPH oxidase
PPARγ agonists
↑ROS
↑ NF-kB
↑PKC
↓MAPK
↑ECM


↑glucose
phosphatidylinosiol-3 kinase (PI3K)
protein kinase B (AKT)
↑mTOR
adenosine monophosphate kinase (AMPK)
Metformin
Mesangial Expansion+ BM thickening
EMT+fibroblast proliferation
↑CCN2 + TGFβ
↑MCP1
Tubular injury +apoptosis
Interstitial fibrosis
Interstitial monocyte infiltration


↑glucose
↑mTOR
↑AGE
↑A II
↑TGF-β1
(CTGF)
↑CCN2
GLP-1
extracellular matrix accumulation
↑EMT
fibroblast proliferation
fibrosis

THANK YOU!