Inflammation and oxidative stress in diabetic kidney disease

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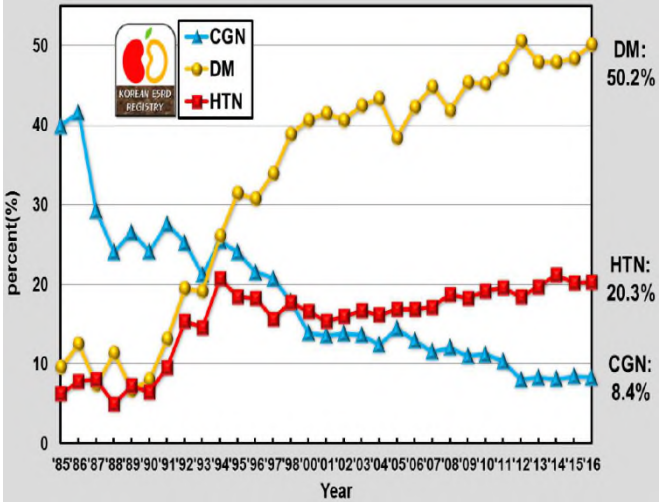
Epidemiology

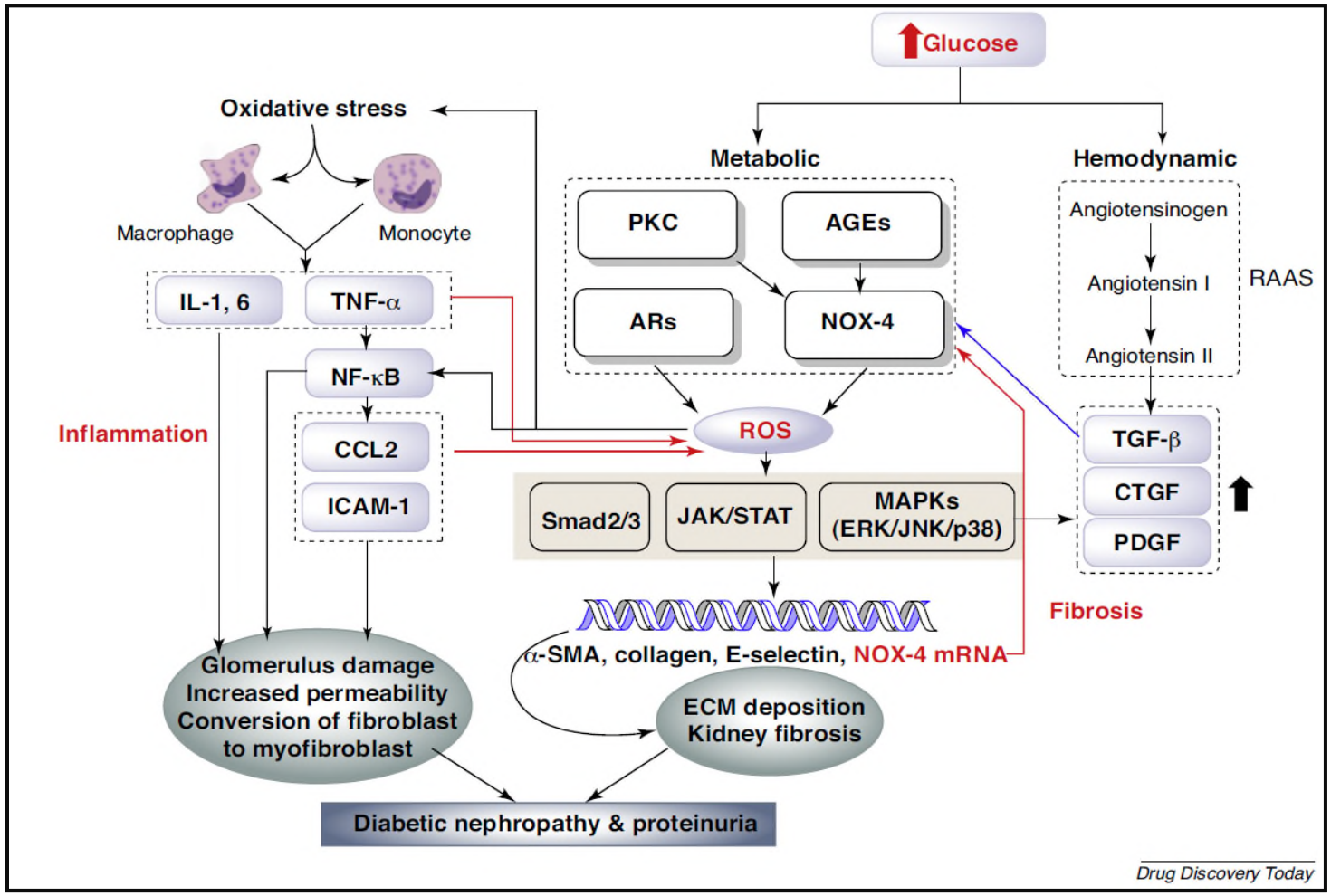


- One of the major **complications** associated with **type 2 diabetes** and a leading cause of **end stage renal disease**.
- Mortality in type 1 diabetes with normoalbuminuria: 2 times greater than normal controls
- Mortality in type 1 diabetes with overt proteinuria: 20-40 times greater than normal controls

Risk factors of DN

- Hypertension
- HbA1C > 8%
- Obesity
- Smoking
- Family history: diabetic nephropathy, dyslipidemia, hypertension
- Duration of diabetes > 5 years

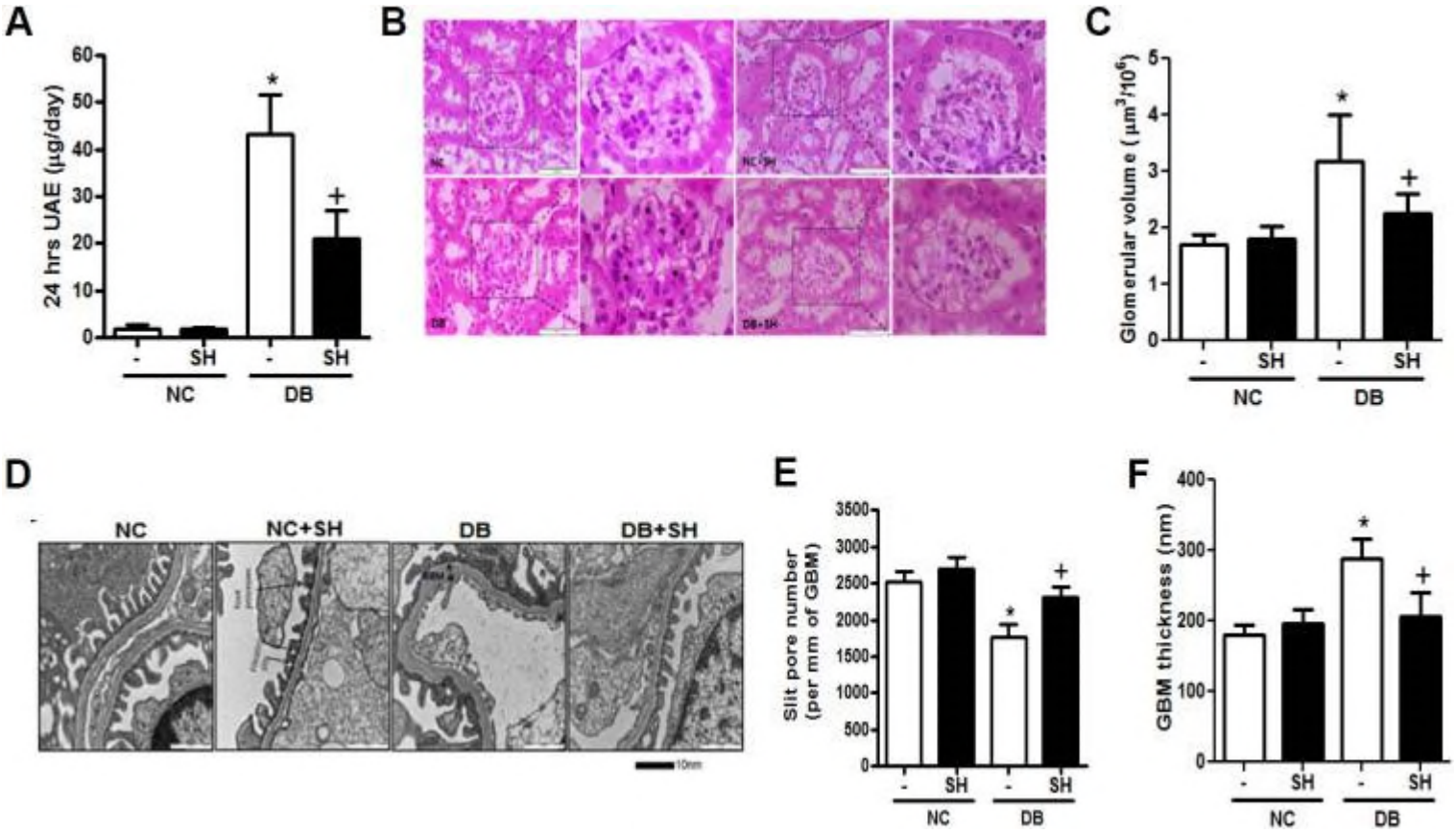
Pathogenesis



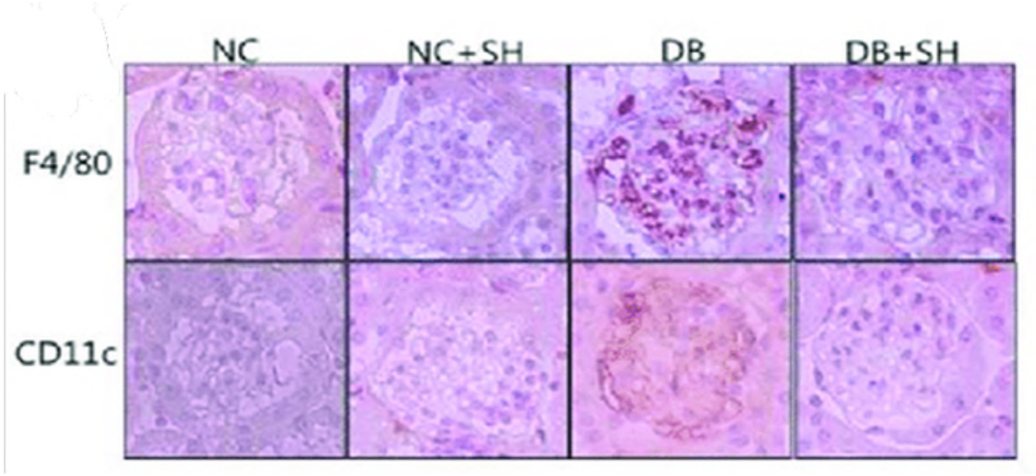
Therapeutic strategies

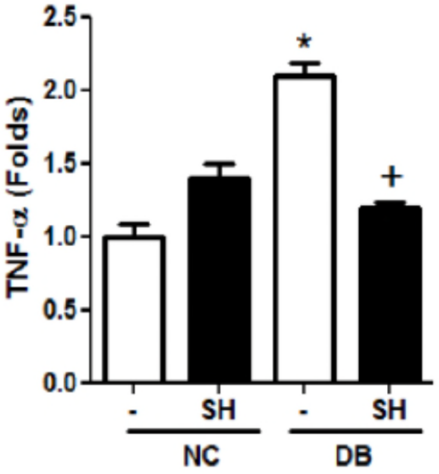
1. Glycemic control: Complete reversal of renal lesion in type 1 DM patients after pancreas transplantation
2. BP control: ACE inhibitors, ARB, renin inhibitor: aliskiren, aldosterone blockers: spironolactone and eplerenone
3. Aldose reductase inhibitors: fidarestat (Sung JK, et al, YMJ, 2010)
4. AGE inhibitors: aminoguanidine
5. PKC inhibitors: ruboxistaurin (improve retinopathy and macular edema; in case of nephropathy, effective in vitro but not effective in vivo)
6. VEGF inhibitors: pegaptanib, ranizumab, bevacizumab; effective in diabetic eye

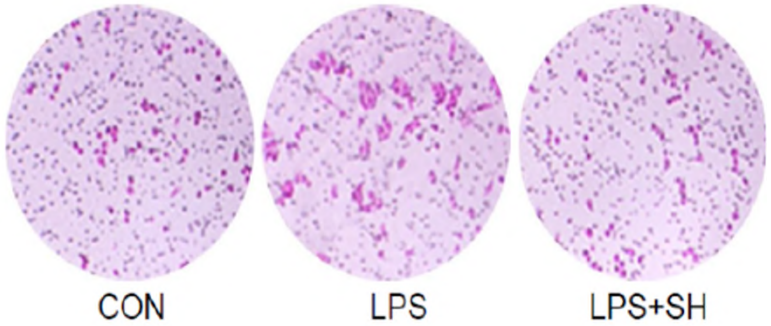
7. Serotonin 2A receptor antagonist: sarpogrelate hydrochloride



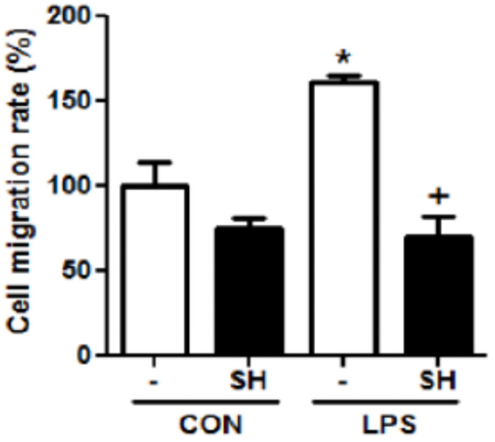
Lee ES et al. PLoS One. 2017 Jun 22;12(6):e0179221.







C



Lee ES et al. PLoS One. 2017 Jun 22;12(6):e0179221.

8. MCP-1/CCR2

- TGF-β type 1 receptor inhibitor: SB431542
- MCP-1 receptor (CCR2) antagonist: RS102895
- PI3 kinase inhibitor: LY294002
- TGF-β signaling inhibitor (ALK-5 inhibitor): EW-7197

Inflammation

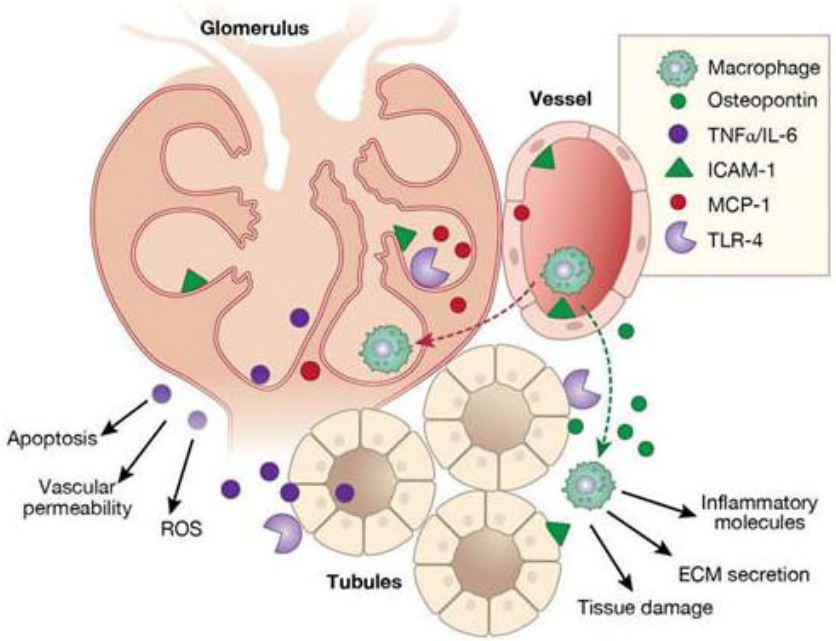
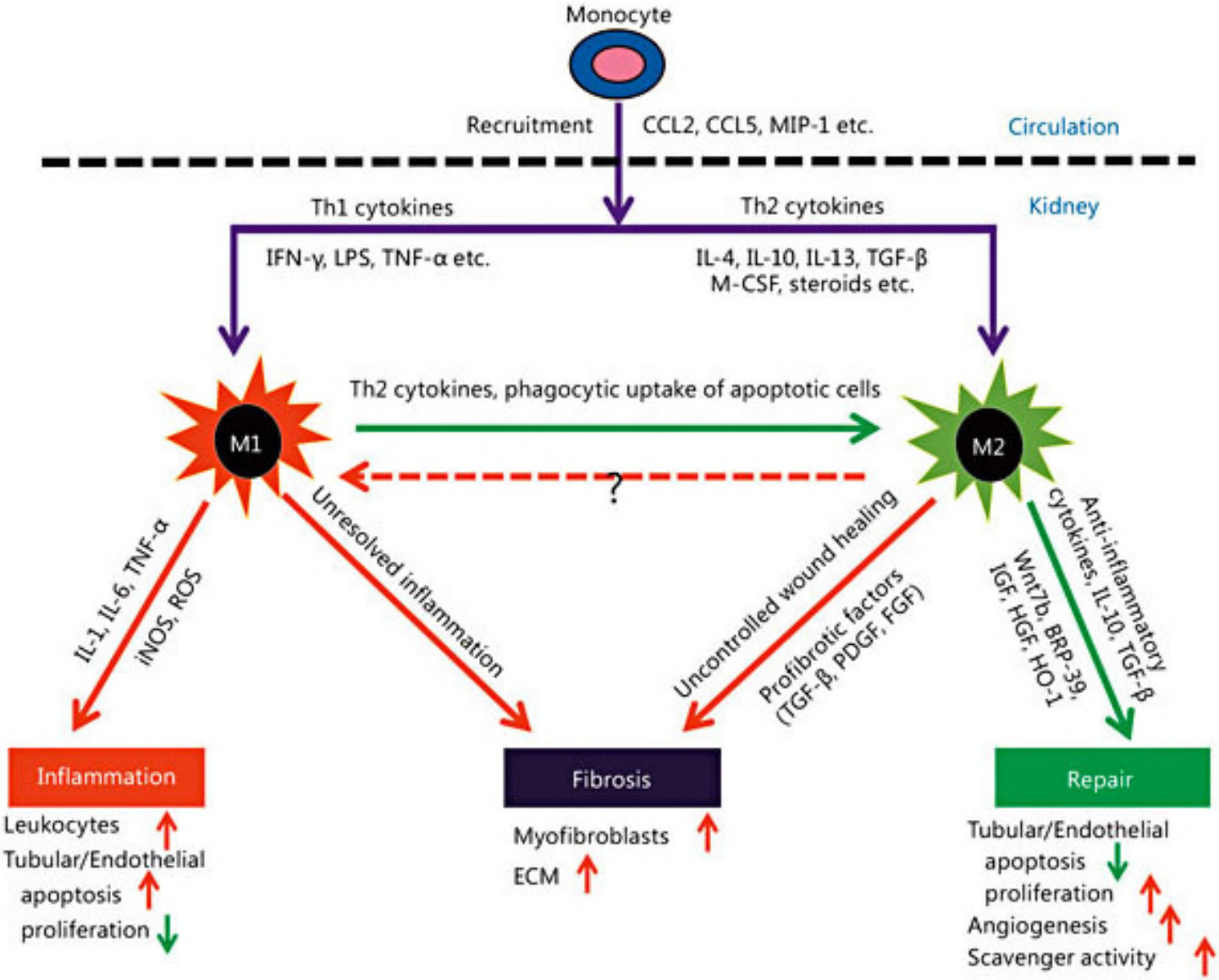
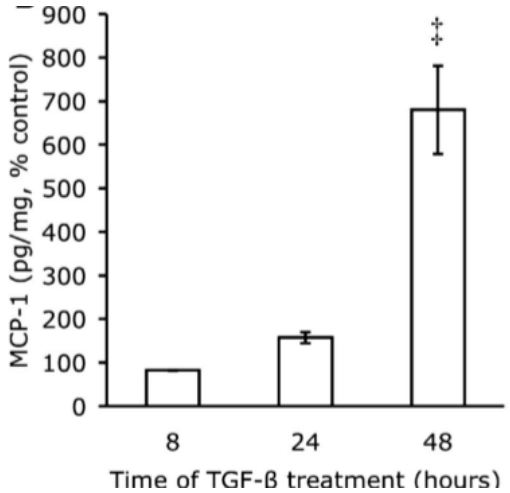


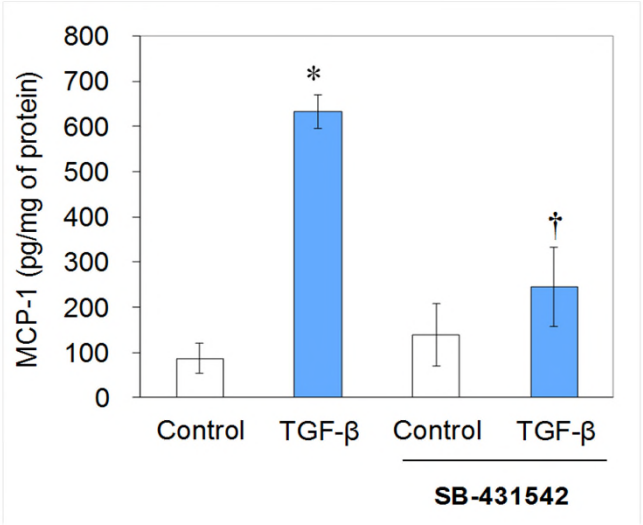
Figure 1: Key pathways of macrophage induced kidney damage.

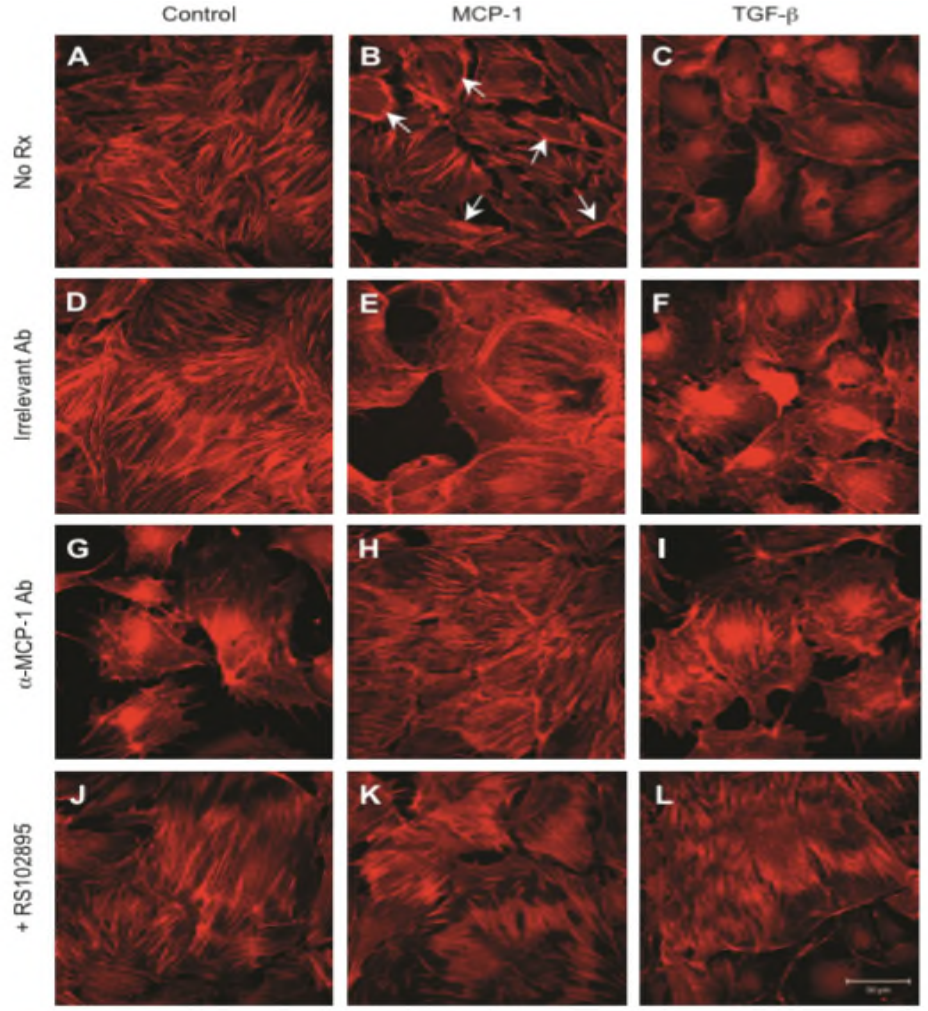
- Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages.


Monocyte
Recruitment
CCL2, CCL5, MIP-1 etc.
Circulation
Kidney
Th1 cytokines
IFN-γ, LPS, TNF-α etc.
Th2 cytokines
IL-4, IL-10, IL-13, TGF-β
M-CSF, steroids etc.
M1
M2
Th2 cytokines, phagocytic uptake of apoptotic cells
?
IL-1, IL-6, TNF-α
iNOS, ROS
Unresolved inflammation
Uncontrolled wound healing
Profibrotic factors
(TGF-β, PDGF, FGF)
Wnt7b, BRP-39,
IGF, HGF, HO-1
Anti-inflammatory
cytokines, IL-10, TGF-β
Inflammation
Leukocytes
Tubular/Endothelial
apoptosis
proliferation
Fibrosis
Myofibroblasts
ECM
Repair
Tubular/Endothelial
apoptosis
proliferation
Angiogenesis
Scavenger activity

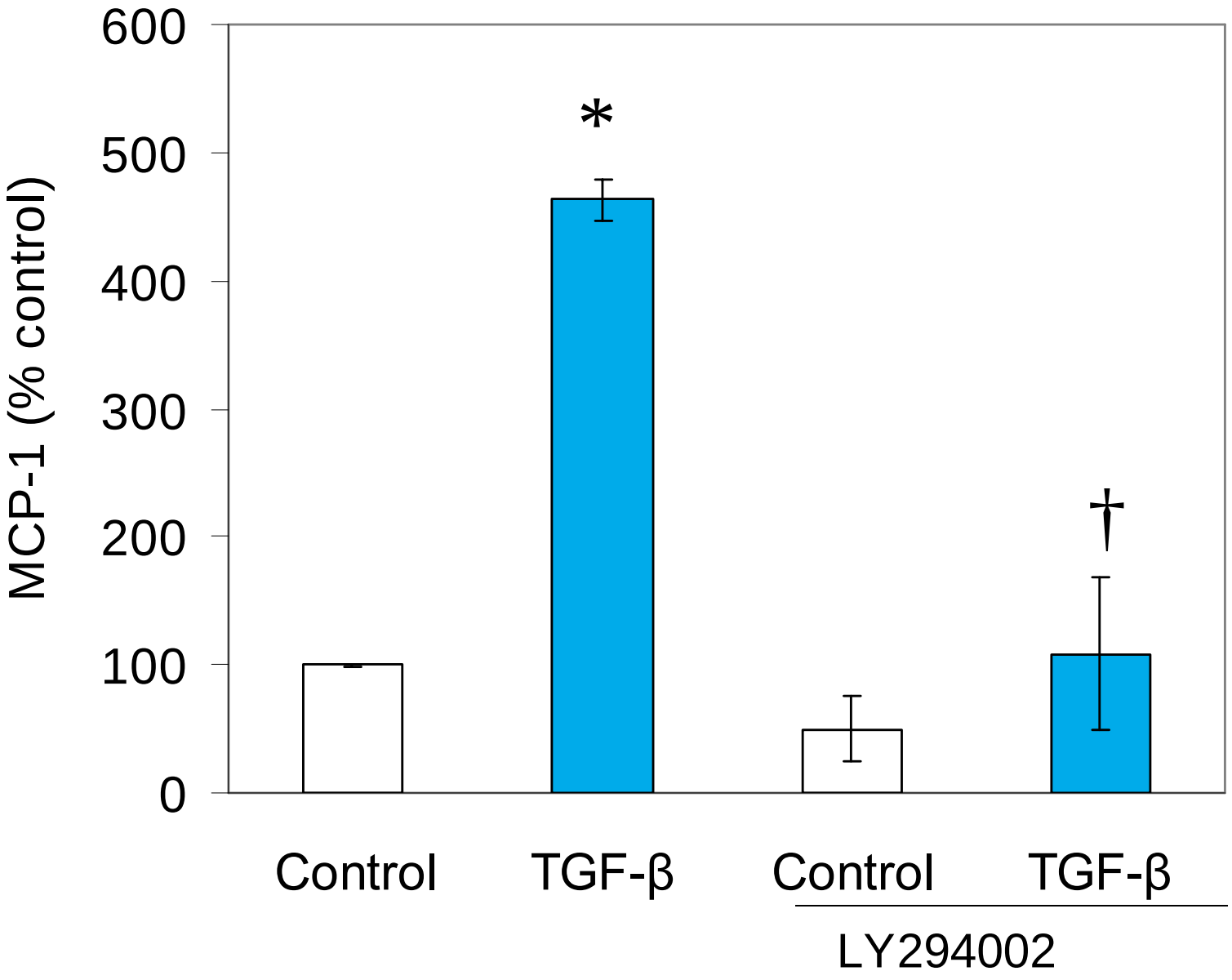
8-1) MCP-1/CCR2 loop, inducible by TGF-β, increases podocyte motility and albumin permeability



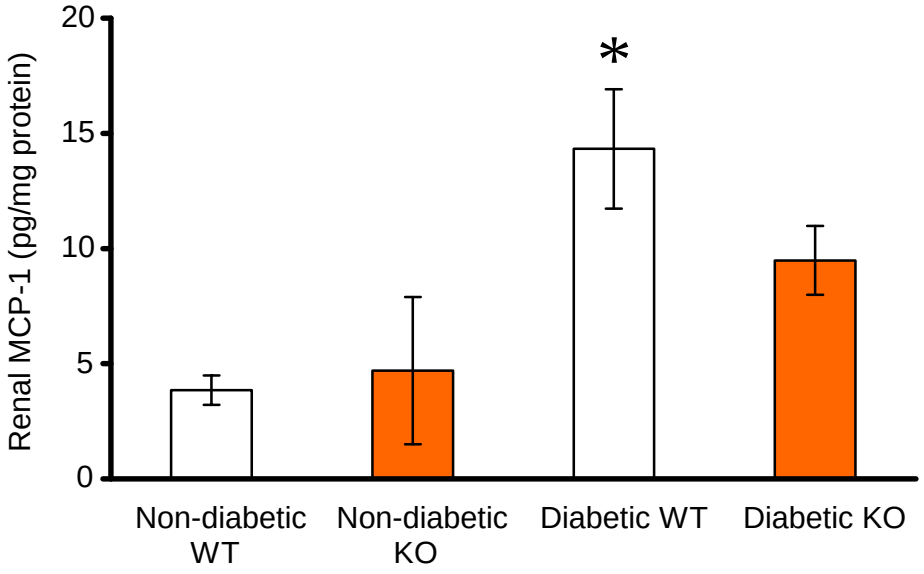


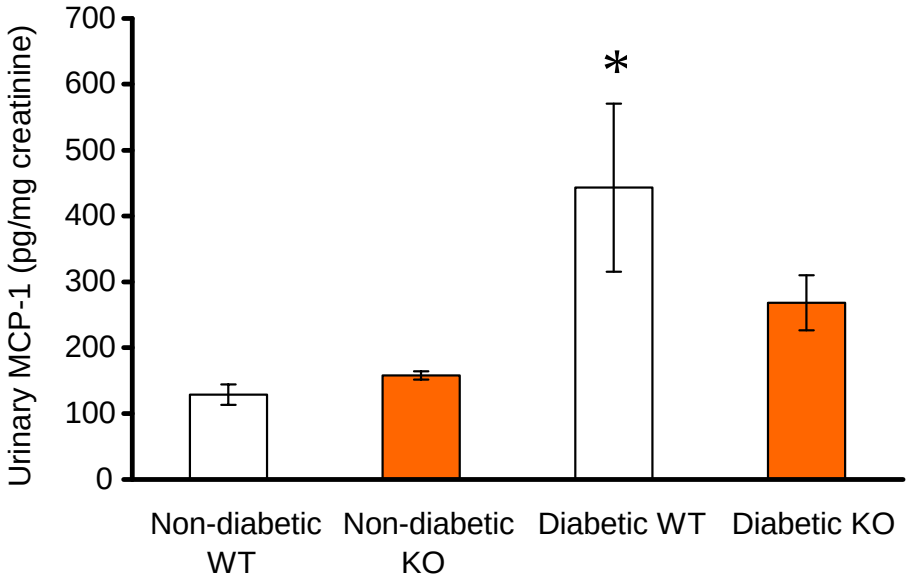


Lee EY and Chung CH et al, AJP Renal 297:F85-F94, 2009



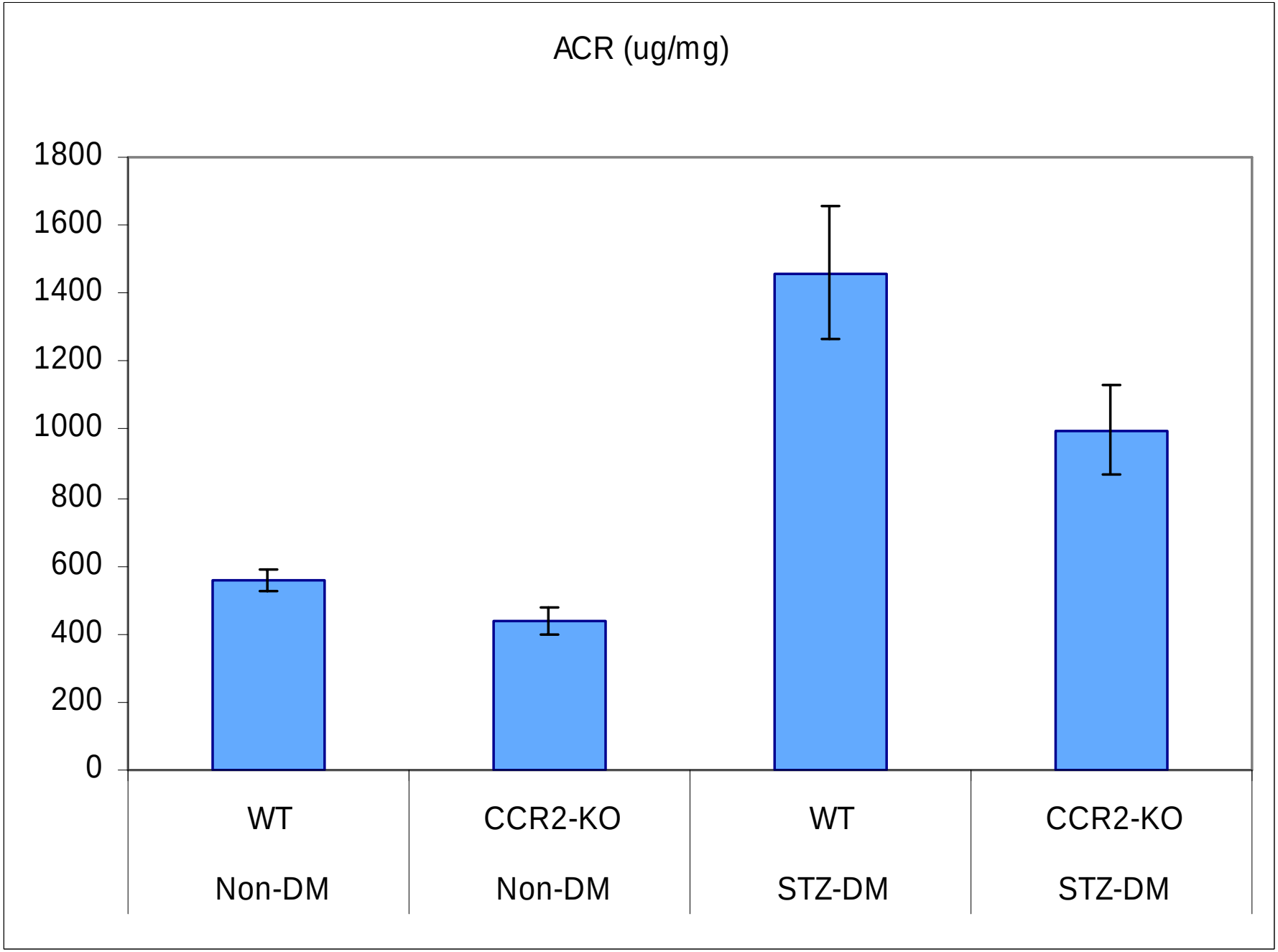
Lee EY and Chung CH et al, AJP Renal 297:F85-F94, 2009





* KO: smad3 KO mice

Lee EY and Chung CH et al, AJP Renal 297:F85-F94, 2009


ACR (ug/mg)
1800
1600
1400
1200
1000
800
600
400
200
0
WT
Non-DM
CCR2-KO
Non-DM
WT
STZ-DM
CCR2-KO
STZ-DM

Nephrol Dial Transplant (2013) 28: 1700–1710
doi: 10.1093/ndt/gfs555

ndt
Nephrology Dialysis Transplantation

Original Articles

Blockade of CCL2/CCR2 signalling ameliorates diabetic nephropathy in *db/db* mice

Su Jin Seok[1],
Eun Soo Lee[2],
Geun Tae Kim[1],
Miri Hyun[1],
Ji-Hye Lee[3],
Sheldon Chen[4],
Ran Choi[2],
Hong Min Kim[2],
Eun Young Lee[1]
and Choon Hee Chung[2]

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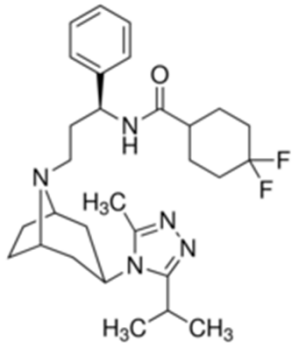
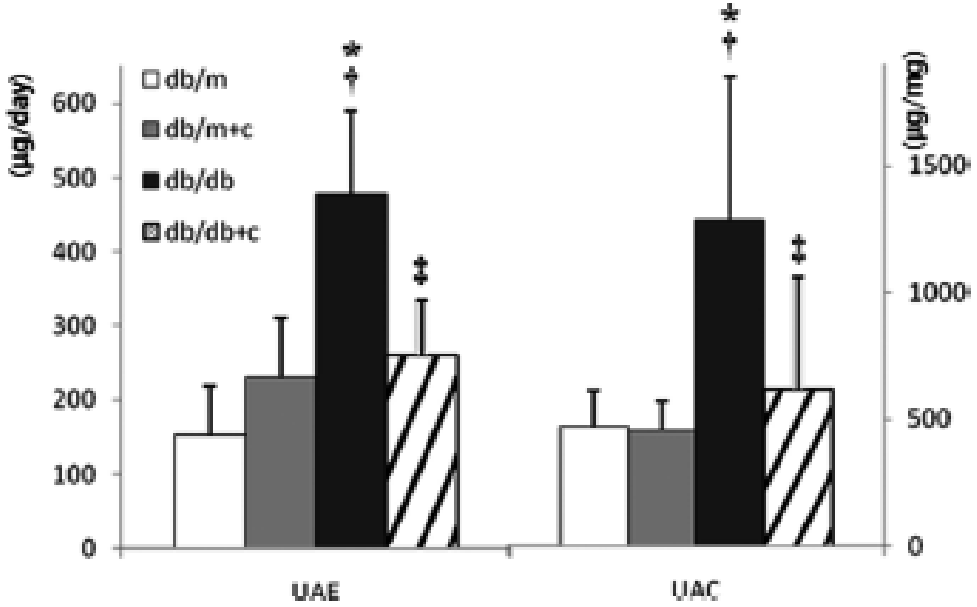
[2]Department of Internal Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea,
[3]Pathology, Soon Chun Hyang University Cheonan Hospital, Cheonan, Korea and
[4]Division of Nephrology/Hypertension, Northwestern University, Chicago, Illinois, USA

8-2) CCR2 inhibitor (RS102895)





CCR2 inhibitor has anti-inflammatory effect on diabetic nephropathy

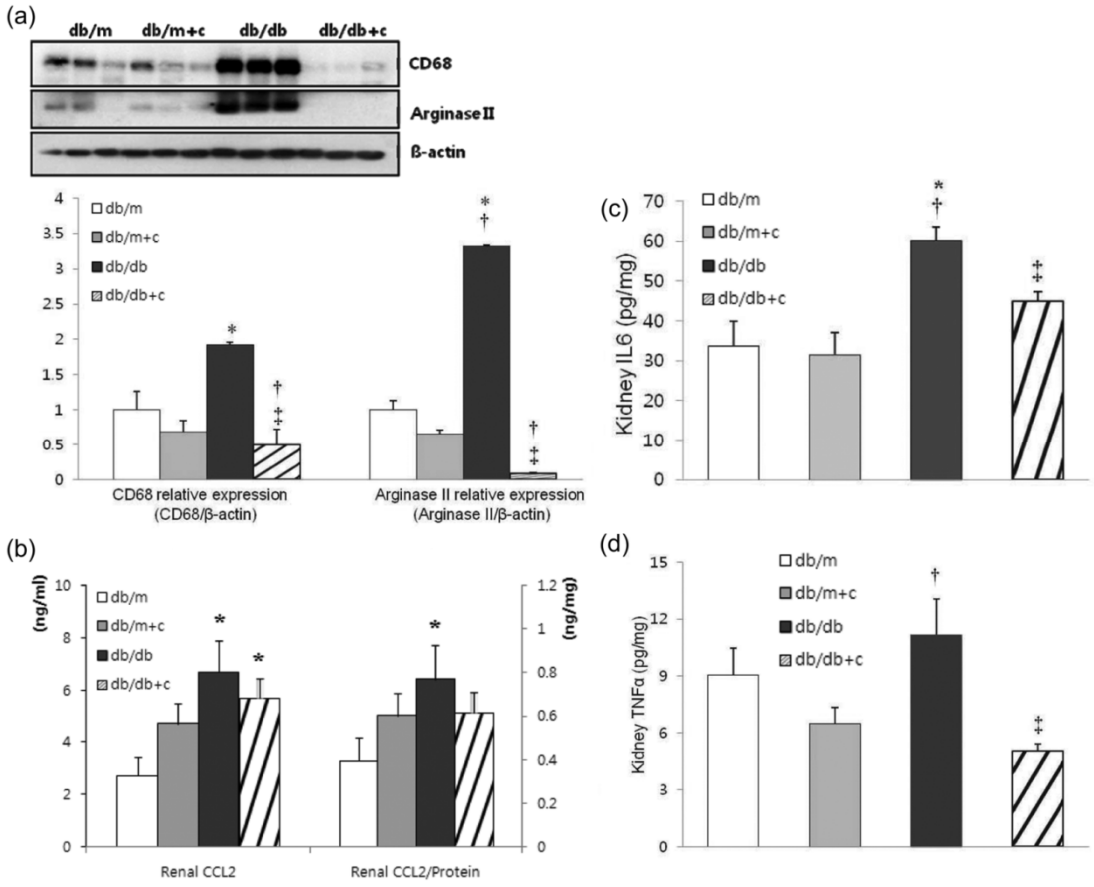
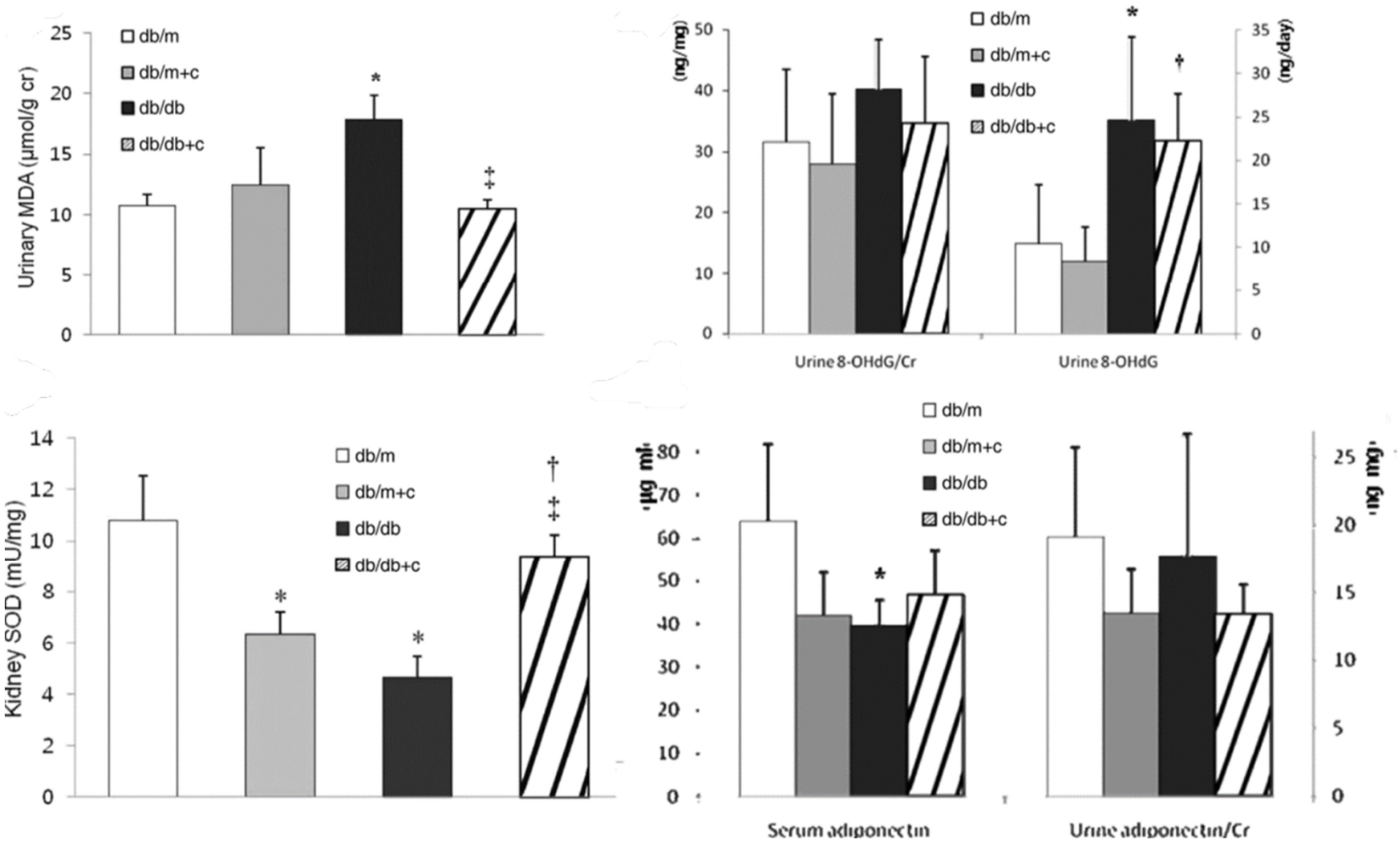


FIGURE 10. The effect of CCR2 blockade on renal macrophage accumulation and inflammatory cytokines.



Seok SJ et al., Nephrol Dial Transplant (2013) 28:1700-1710

8-3) EW-7197 (ALK-5 inhibitor)

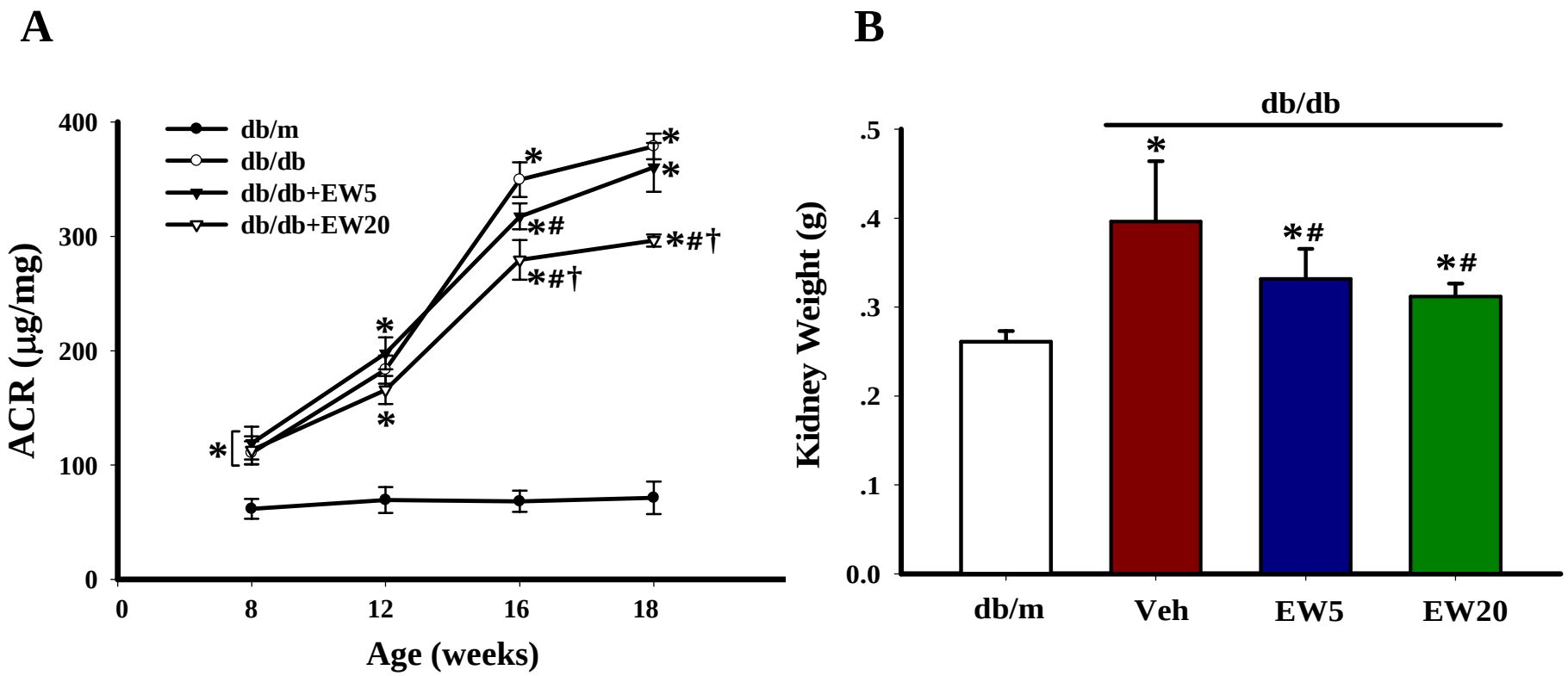


Fig 1. Urinary albumin/creatinine (ACR) ratios were monitored throughout experimental period (A). Kidney weight in all experimental groups (B). Each group n=6-8, $^{*}p$<0.01 vs. db/m controls; #p<0.01 vs. db/db controls; †p<0.01 vs. db/db+EW5 mg/kg/day

Unpublished data

EW-7197 down-regulates fibrotic mediators and infl ammatory markers in diabetic kidneys

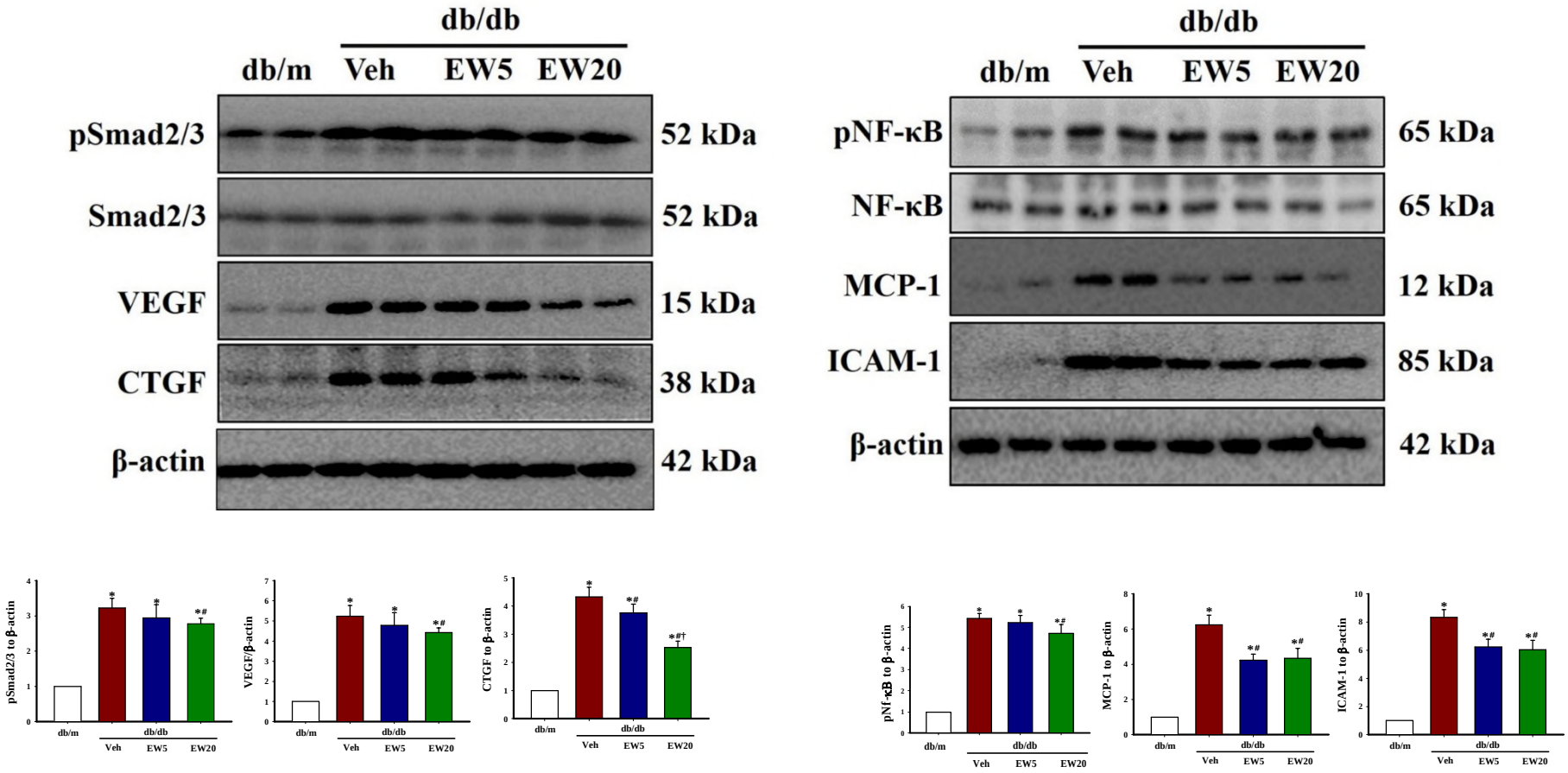
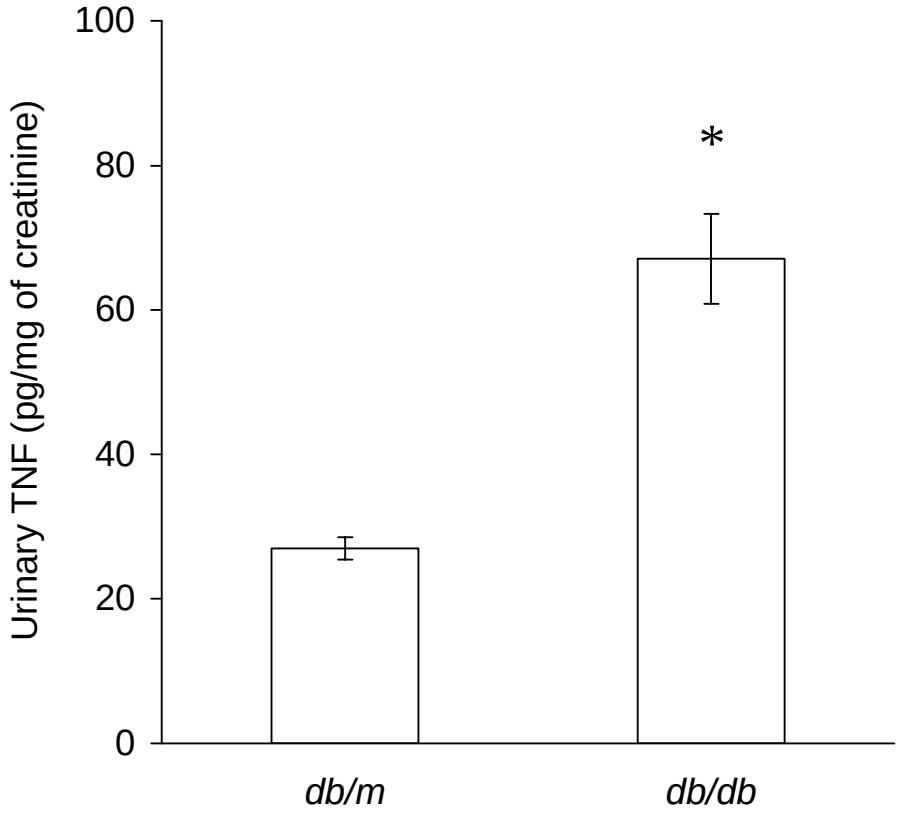
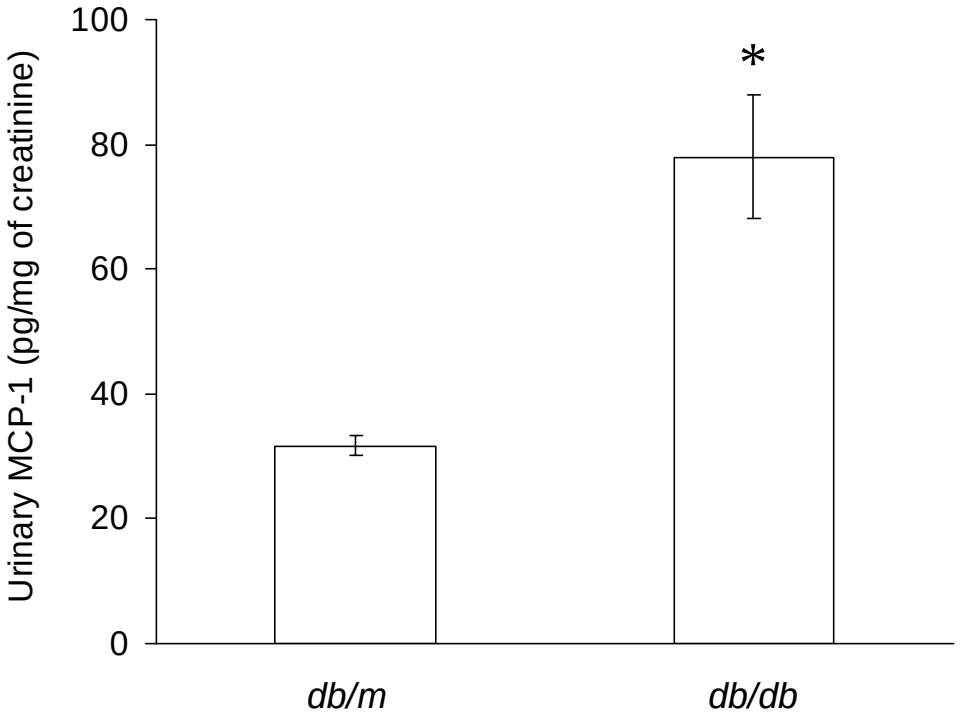


Fig 6. Protein expression related fibrosis and inflammation. Each group n=5, $^{*}p$<0.05 vs. db/m controls; #p<0.05 vs. db/db controls, †p<0.05 vs. db/db+EW5 mg/kg/day.

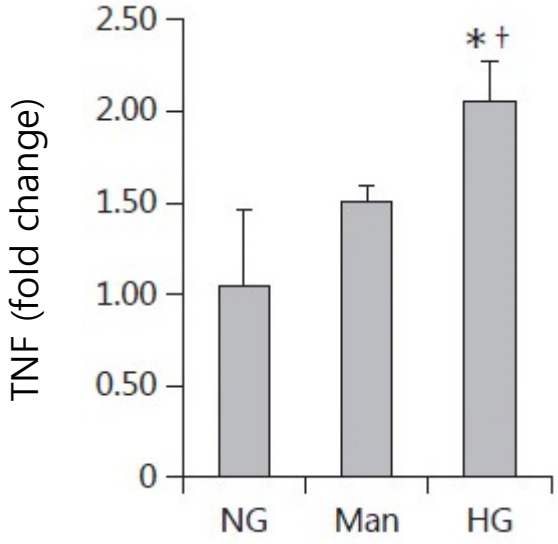
8-4) TNF-α induced MCP-1 via PI3 kinase may aggravate diabetic nephropathy

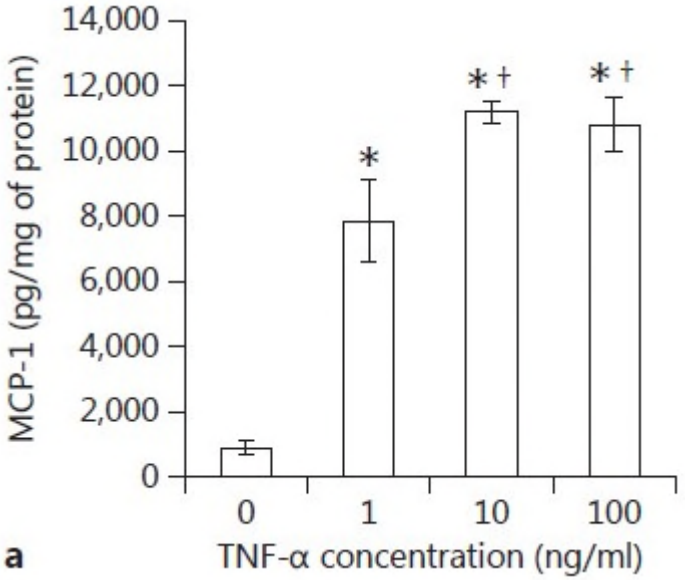
Chung CH et al, Nephron Extra. 2015 Feb 4;5(1):1-18

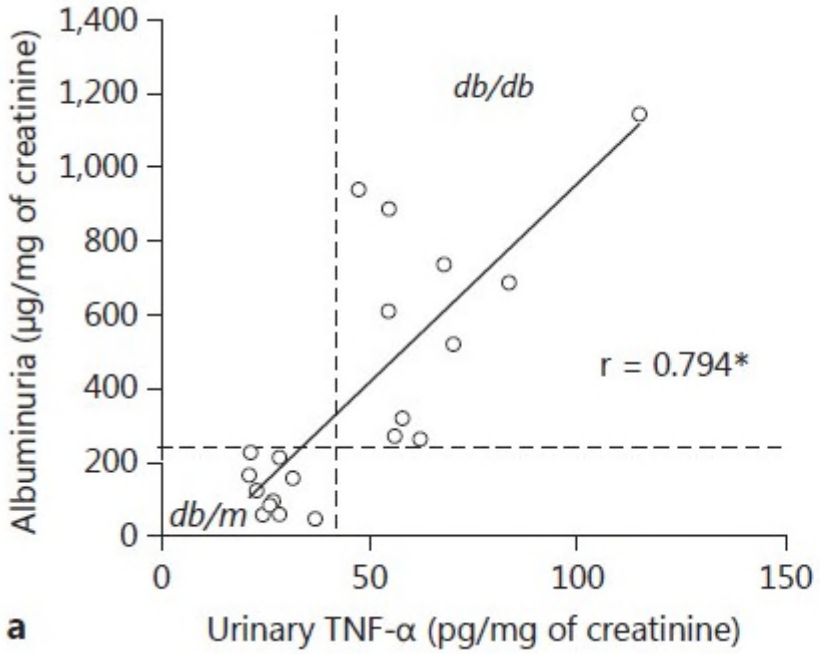


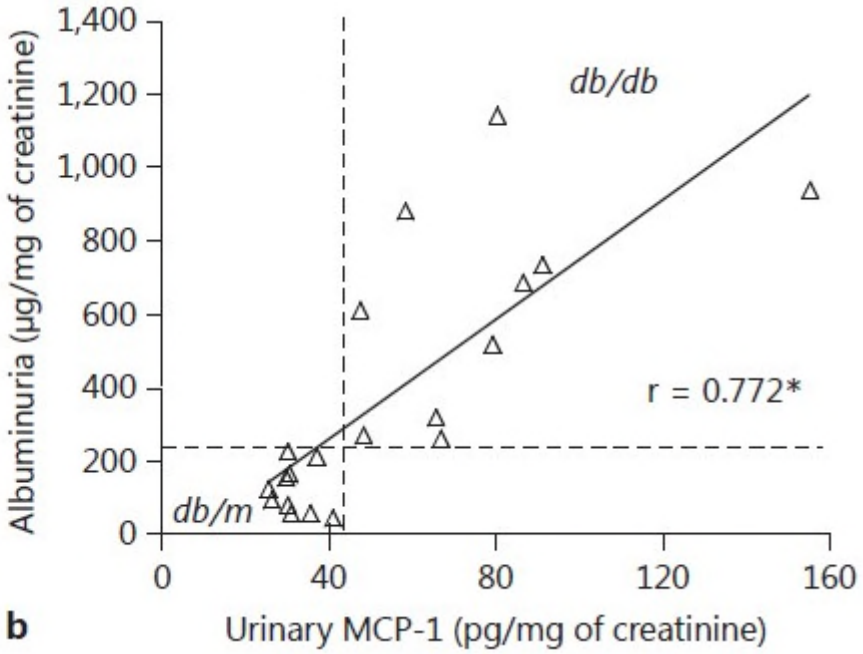


Chung CH et al, Nephron Extra. 2015 Feb 4;5(1):1-18

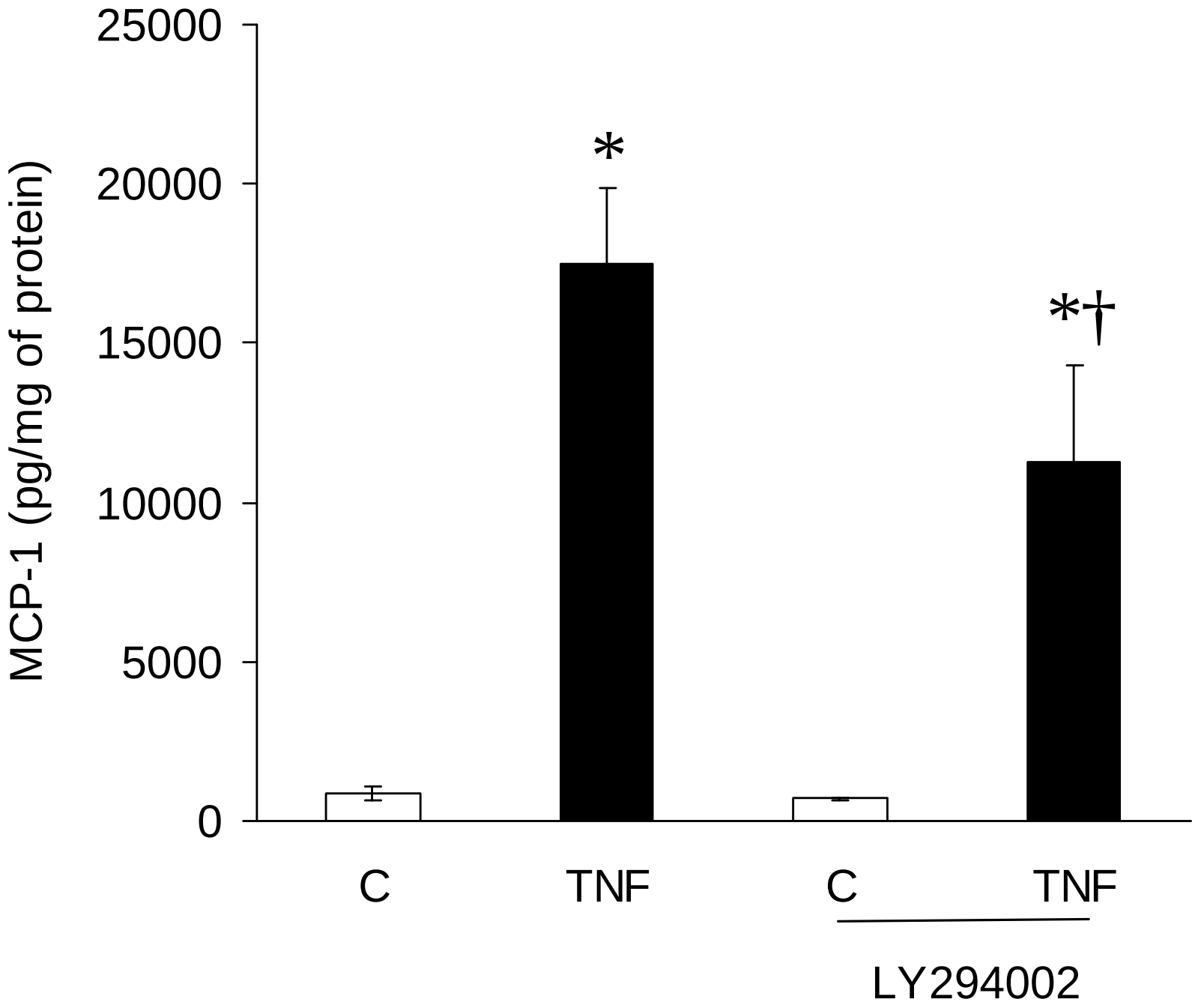


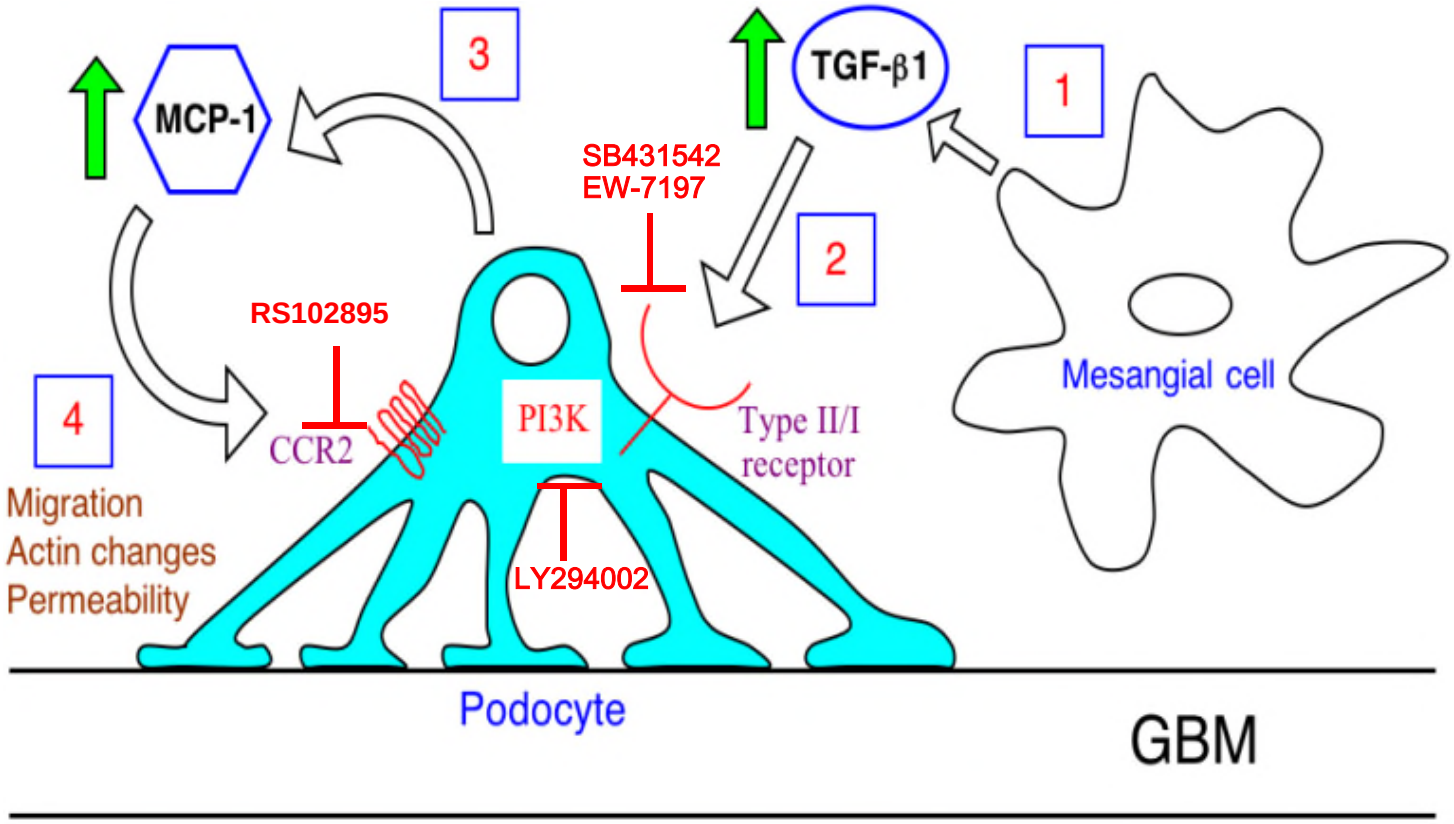






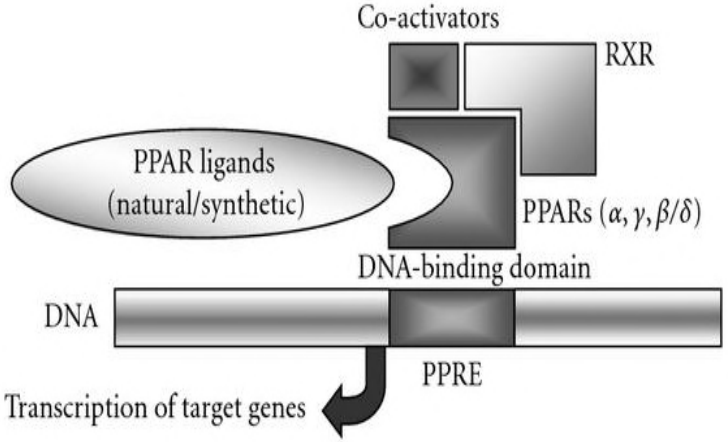
Nephron Extra 2015;5:1–18


25000
20000
15000
10000
5000
0
MCP-1 (pg/mg of protein)
*
*†
C
TNF
C
TNF
LY294002


MCP-1
3
TGF-β1
1
SB431542
EW-7197
2
RS102895
4
CCR2
PI3K
Type II/I
receptor
Mesangial cell
Migration
Actin changes
Permeability
LY294002
Podocyte
GBM

9. Peroxisome proliferator-activated receptors (PPARs)

- It is generally known that peroxisome proliferator-activated receptors (PPARs) heterodimerize with the retinoid X-receptor (RXR) and bind to a specific DNA sequence [a sequence termed peroxisome proliferators response element (PPRE), that is found in a variety of genes involved in lipid and carbohydrate metabolism, inflammation, and cell proliferation and differentiation].



Kliewer SA et al. Science. 1999

9-1) Thiazolidinediones: 24hrs urine protein

Protein (mg/day)	22nd	30th	40th	50th
Control LETO	12.50±3.6	9.38±0.73	12.70±5.18*	12.56±16.56*
Control OLETF	19.23±5.53	30.16±21.35	108.26±75.54	108.37±28.09
Pioglitazone	17.44±8.29	24.84±12.93	29.98±11.74*	63.15±24.57*
Rosiglitazone	16.86±5.29	21.34±9.39	27.78±13.93*	56.08±38.14*

Data express mean ± SD *: $p<0.05$ compared with control OLETF

Lee MY and Chung CH et al, YMJ 2007

9-2) PPAR-delta

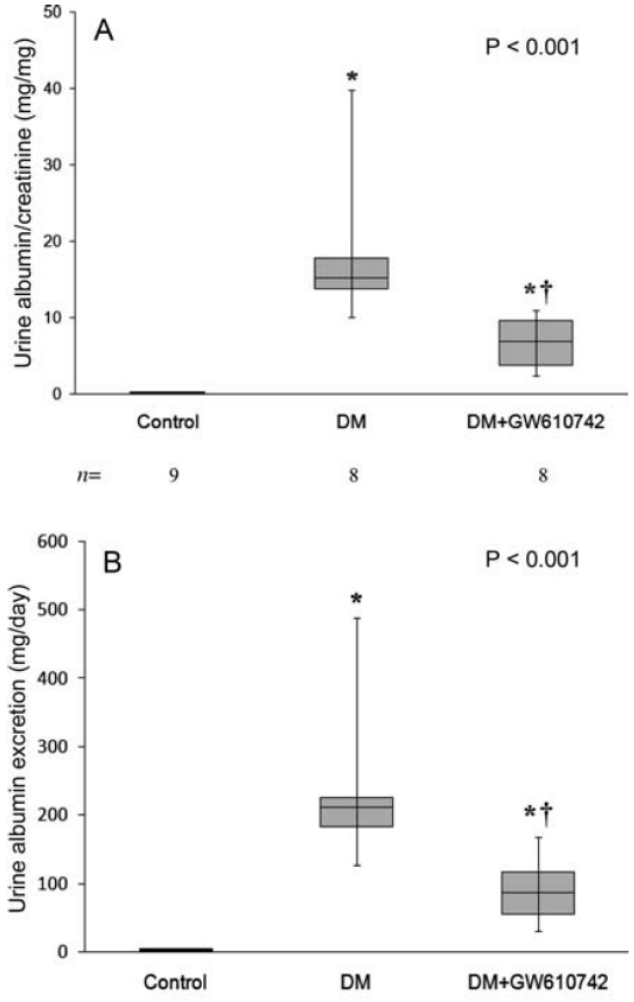
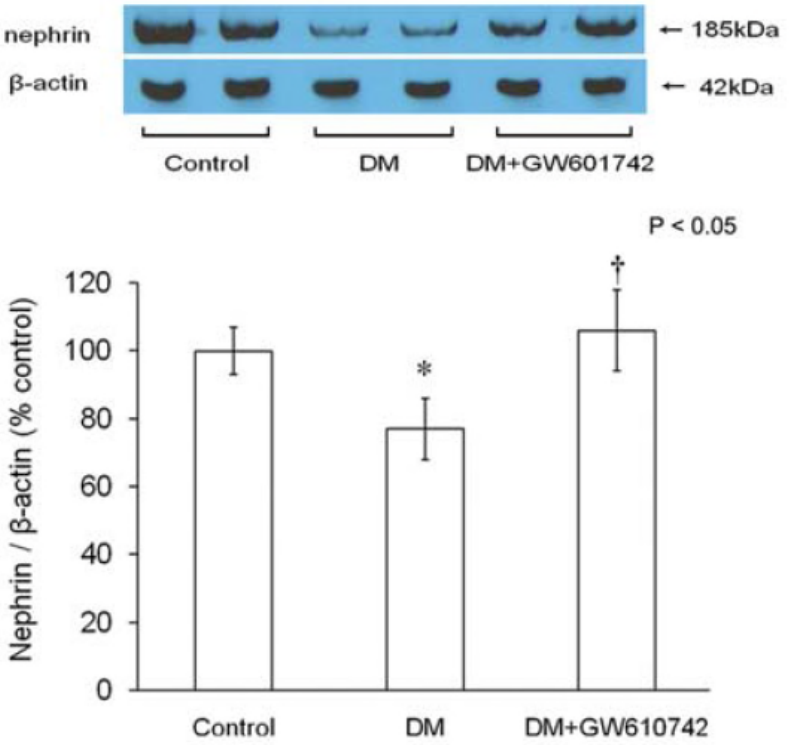


Fig. 1. Albuminuria. The albumin excretion rate, measured using ELISA on 24-h urine samples



PPAR-δ agonist: GW610742

Fig. 4. Nephrin mRNA and protein expression.

Lee EY, Kim GT, Hyun M, Kim S, Seok S, Choi R, Lee MY, Chung CH. Nephrol Dial Transplant. 2012 Nov;27(11):4069-79.

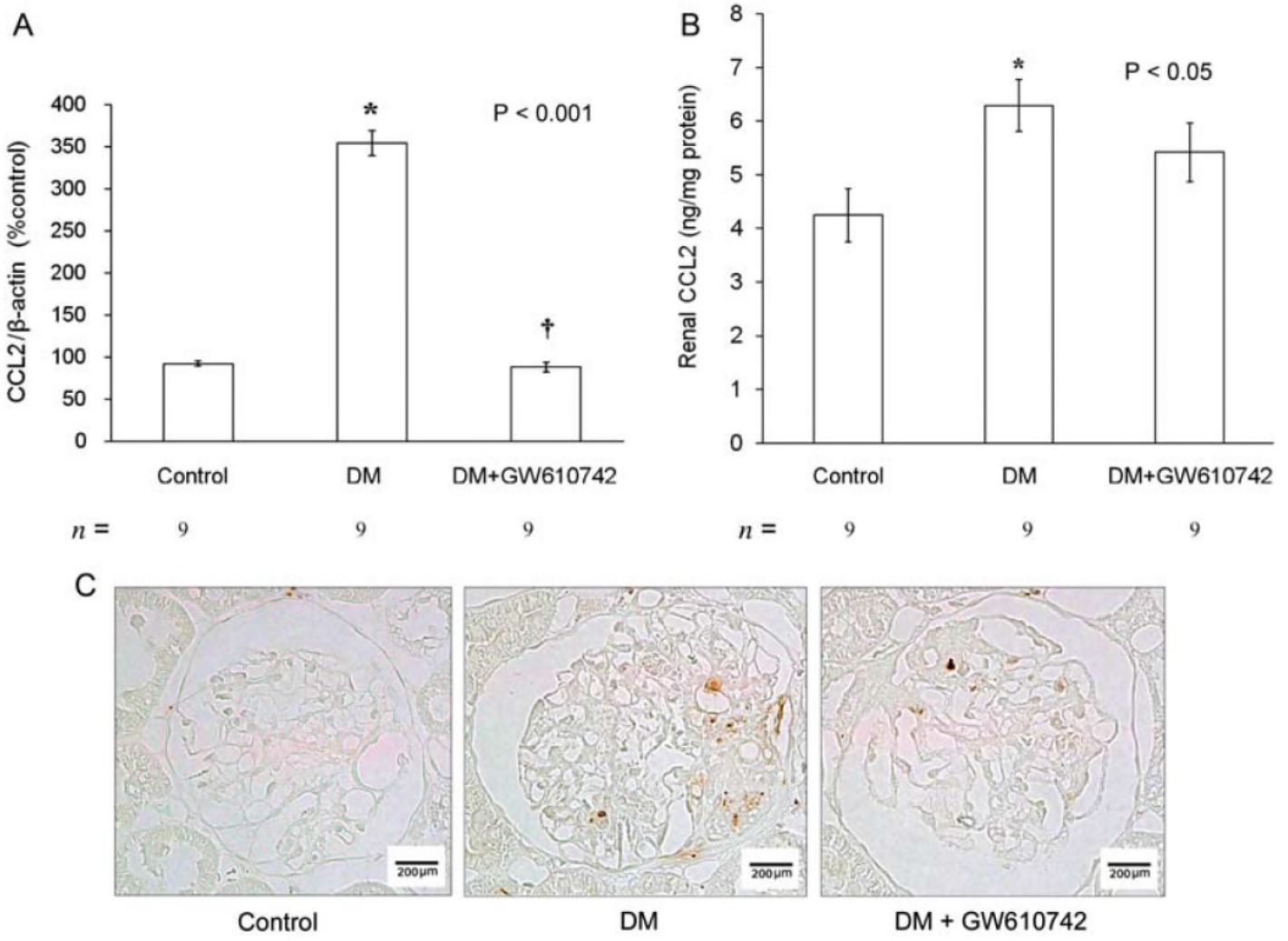
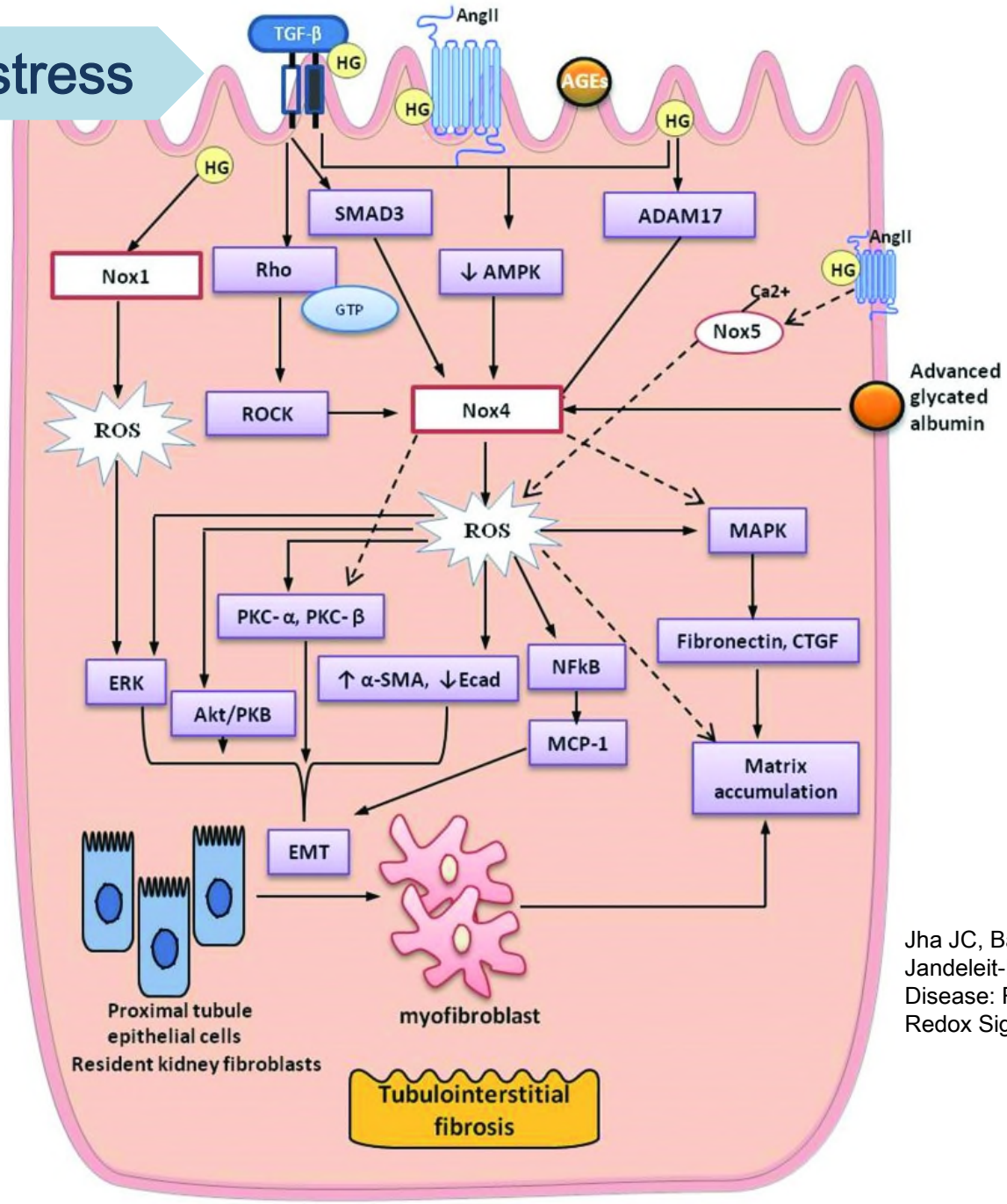


Fig. 5. Renal CCL2 expression and macrophage accumulation

Oxidative stress



Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K. Diabetes and Kidney Disease: Role of Oxidative Stress. Antioxid Redox Signal. 2016 Oct 20;25(12):657-684.

10. Antioxidants

- Vitamin C & E: decrease ROS, but does not prevent or improve microvascular complications in clinical studies
- ACE inhibitors, ARBs, statins
- α-lipoic acid: Thioctacid®
- NADPH oxidase inhibitor: apocynin
- Taurine, oleanolic acid, ferulic acid, curcumin, dibenzoylmethane (DBM), dehydrozingerone (DHZ)

1) Apocynin

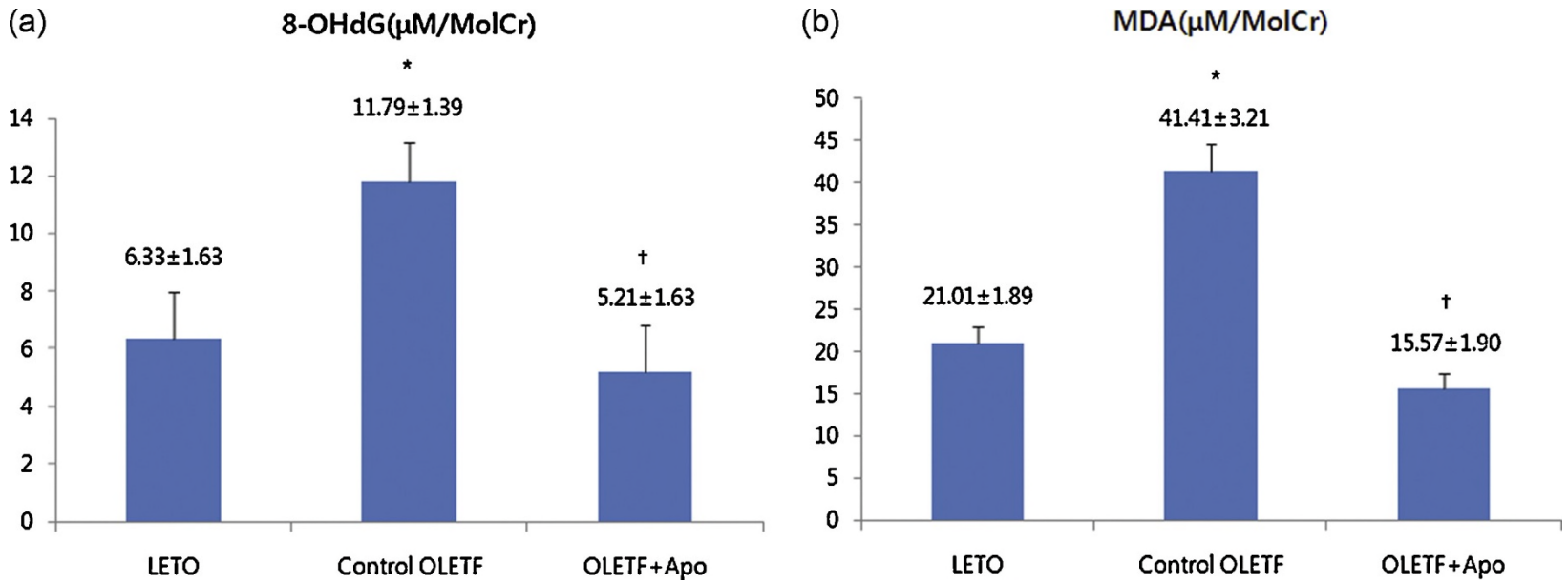
Changes in 24 hrs urine ACR and protein levels

	Albumin-creatinine ratio (mg/mgCr)		Protein-creatinine ratio (mg/mgCr)	
	25wk	50wk	25wk	50wk
LETO	0.004±0.001	0.014±0.025†	0.10±0.03	0.07±0.06†
OLETF	0.093±0.091	2.519±3.311	0.37±0.28	3.15±4.30
OLETF+Apo	0.189±0.204*	**0.285±0.245†**	0.40±0.24	**0.67±0.48†**

The values are mean±S.D. *;p<0.01 vs. LETO group, †;p<0.05 vs. OLETF group, ACR; albumin-creatinine ratio

Nam SM and Chung CH et al, DRCP 2009

24hrs urine 8-OHdG & MDA at 50 weeks



Nam SM and Chung CH et al, DRCP 2009

2) Ferulic acid

Table 2. Changes of 24 h urinary albumin (mg/day) and ACR (mg/mgCr)

	24 h urinary albumin (mg/day)		
	25 week	37 week	45 week
CON	1.05 ± 0.57	1.92 ± 1.08	0.85 ± 0.40
DM	12.35 ± 6.97*	24.11 ± 5.98*	26.76 ± 9.46*
DM + FA	11.98 ± 12.35*	21.14 ± 14.15*	16.39 ± 9.69*,†
	ACR (mg/mgCr)		
CON	0.09 ± 0.05	0.13 ± 0.07	0.07 ± 0.04
DM	0.92 ± 0.59*	1.79 ± 0.66*	2.51 ± 1.04*
DM + FA	0.88 ± 0.67*	1.64 ± 1.04*	1.49 ± 0.90*,†

CON, control; DM, diabetes; FA, ferulic acid; ACR, albumin creatinine ratio. The values are mean ± S.D. *$P < 0.05$ compared with CON, †$P < 0.05$ compared with DM.

Choi R et al, Exp Mol Med. 2011 Dec 31;43(12):676-83

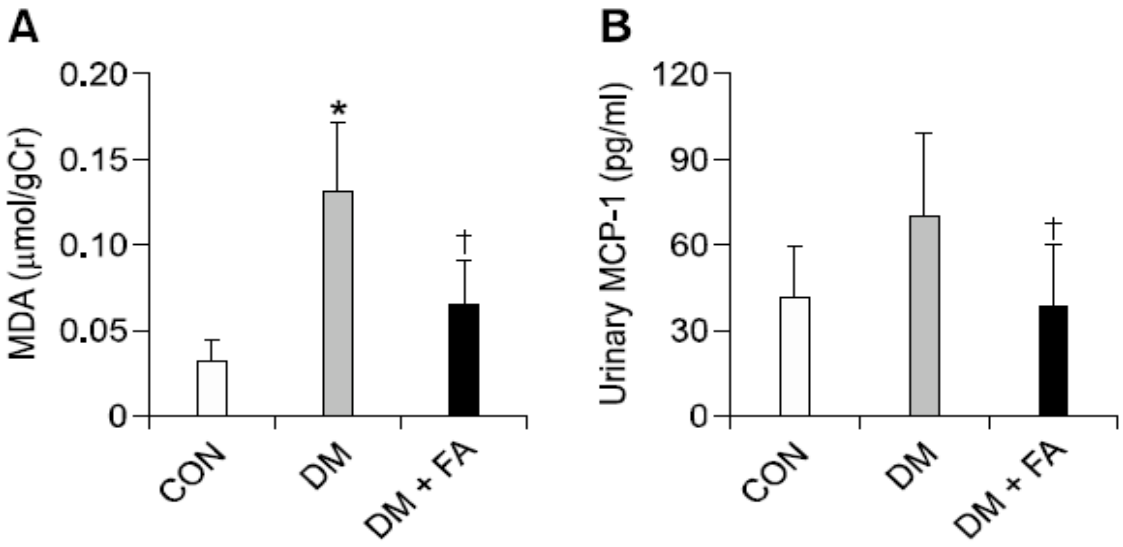
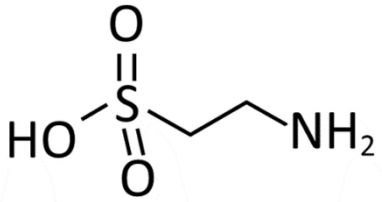


Figure 2. Changes of 24 h urinary malondialdehyde (MDA) and monocyte chemoattractant protein-1 (MCP-1) levels.

3) Taurine

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 397307, 11 pages
http://dx.doi.org/10.1155/2014/397307



Research Article

Taurine Alleviates the Progression of Diabetic Nephropathy in Type 2 Diabetic Rat Model

Jang Hyun Koh,[1] Eun Soo Lee,[2] Miri Hyun,[3] Hong Min Kim,[2] Yoon Jung Choi,[4] Eun Young Lee,[3] Dhananjay Yadav,[2] and Choon Hee Chung[2]

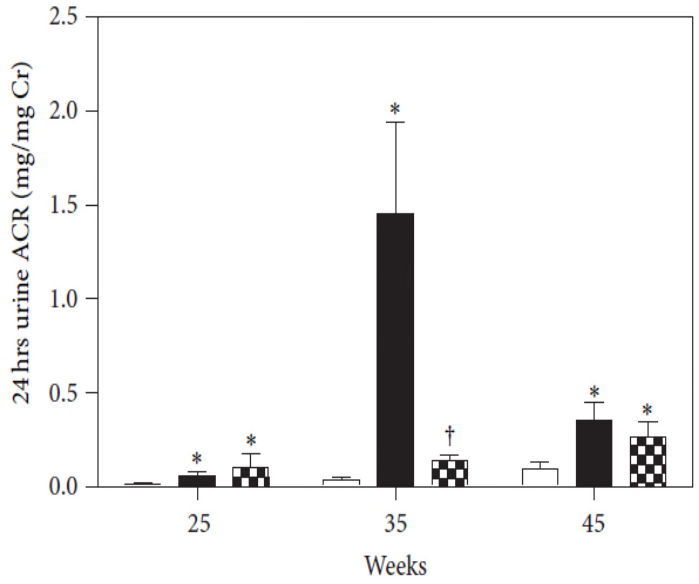


Figure 1: Changes of 24 hours urine albumin in control, OLETF and taurine treated groups

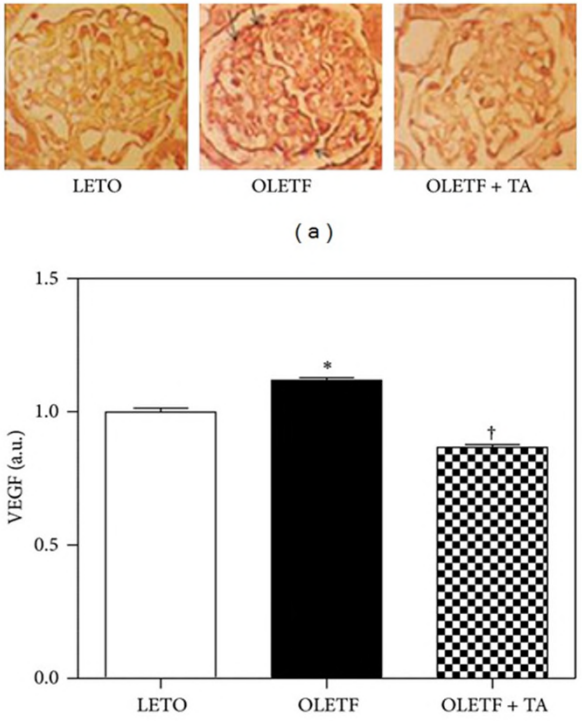


Figure 5: Effects of taurine on the expression of VEGF and nephrin in renal cortex.

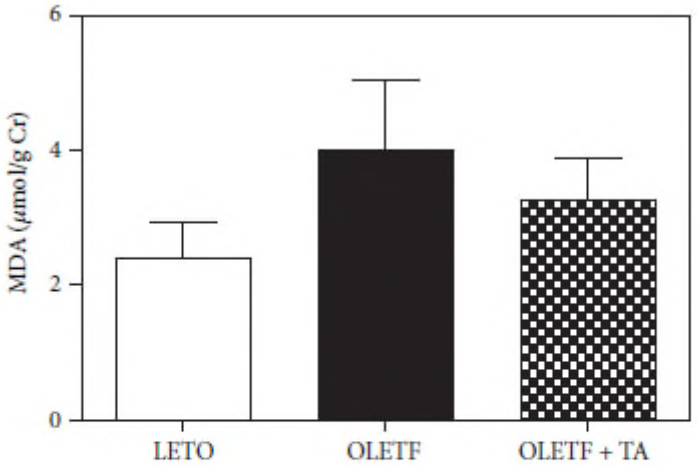


FIGURE 6: Changes in 24 hours urinary MDA levels at 45 weeks of age. In the diabetic control group, MDA increased compared to the normal control group. MDA decreased in the taurine-treatment group compared to the diabetic control group. However, there was no statistical significance in this difference. MDA, malondialdehyde; LETO, normal control group; OLETF, diabetic control group; OLETF + TA, taurine-treated diabetic group.

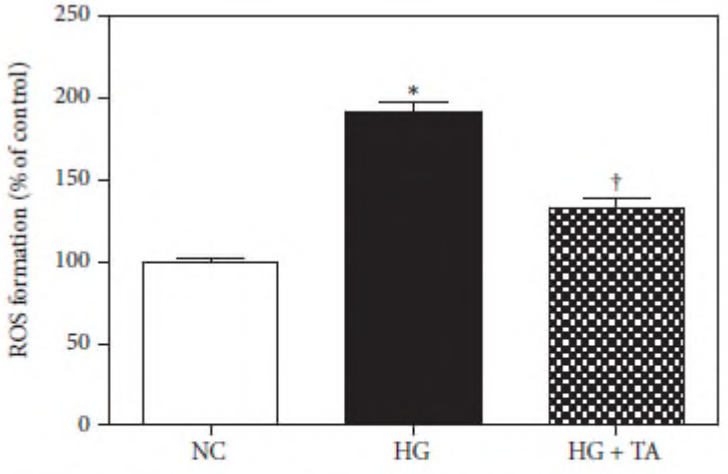
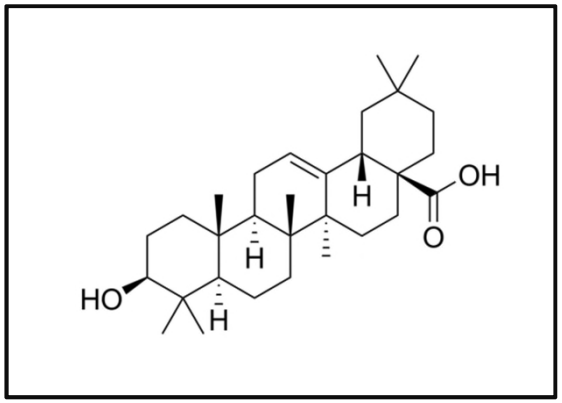


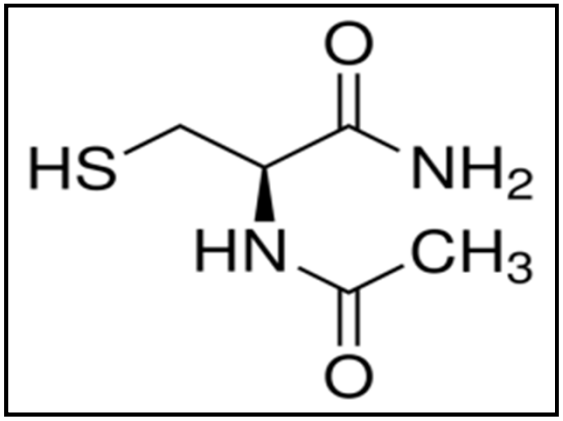
FIGURE 7: Changes in ROS formation in podocytes among the three groups. The taurine-treated high glucose group demonstrated a significantly decreased ROS production. NC, normal glucose; HG, high glucose; HG + TA, taurine-treated high glucose. Data are expressed as mean ± SEM. $^{*}P < 0.05$ compared with NG; $^{\dagger}P < 0.05$ compared with HG.

4) Oleanolic acid



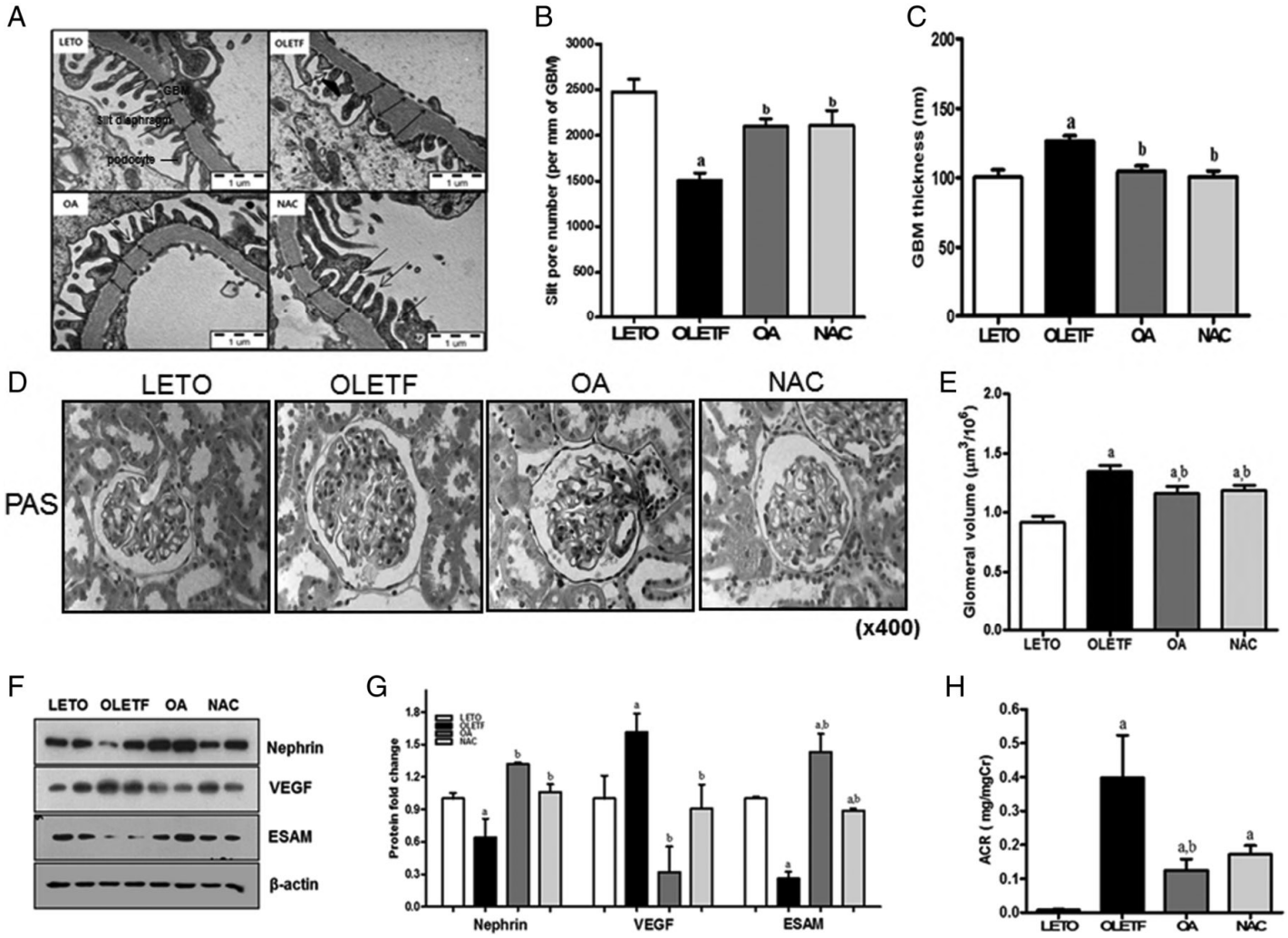
- Naturally occurring triterpenoid
- Anti-inflammation, anti-oxidant, anti-tumor and anti-viral properties
- Hypoglycemic, hypolipidemic efficacy in diabetic rats

N-acetylcysteine



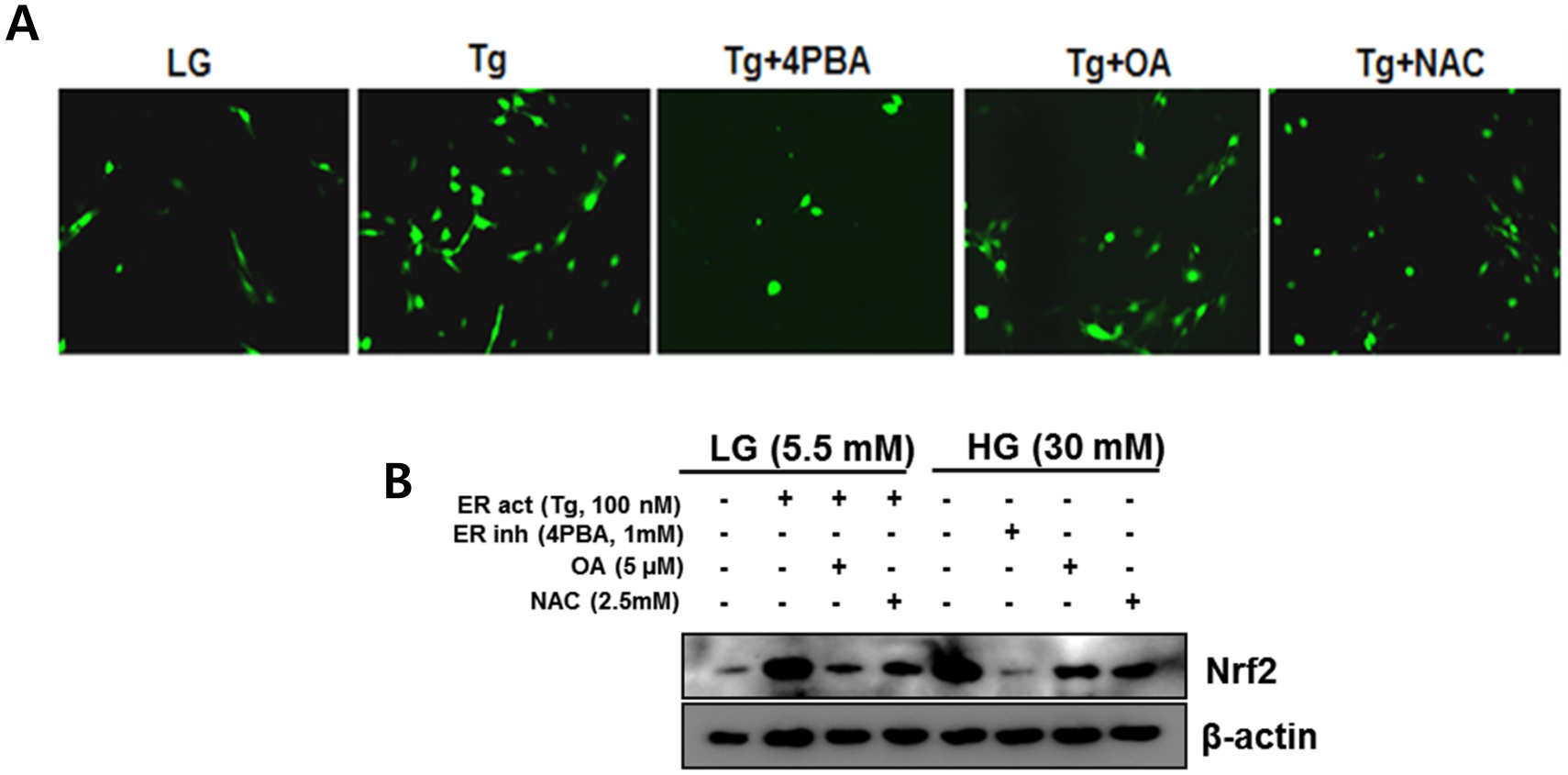
- N-acetyl cysteine comes from the amino acid L-cysteine
- Pharmacologic antioxidant
- Protective effect on the β-cells of diabetic mouse model
- Improve endothelial function

OA & NAC repaired damaged renal structures and urinary ACR



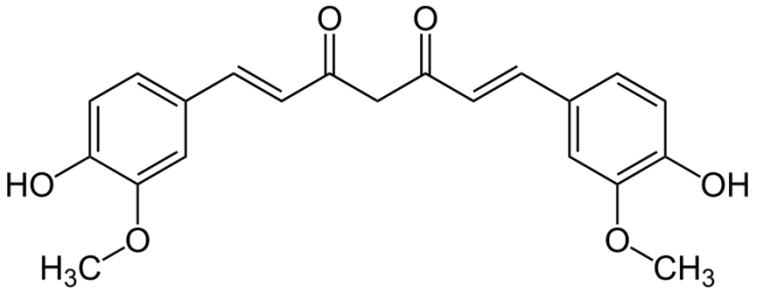
Eun Soo Lee et al. Nephrol. Dial. Transplant. 2016 Mar;31(3):391-400

OA & NAC suppress ROS formation induced by ER stress activator

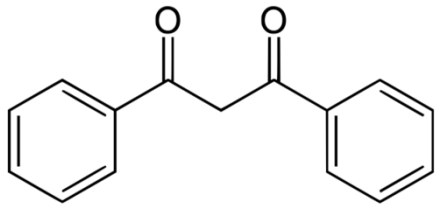


Eun Soo Lee et al. Nephrol. Dial. Transplant. 2016 Mar;31(3):391-400

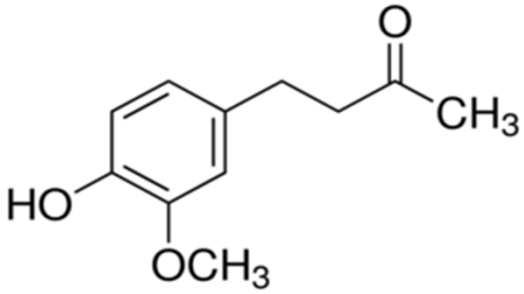
5) CURCUMIN



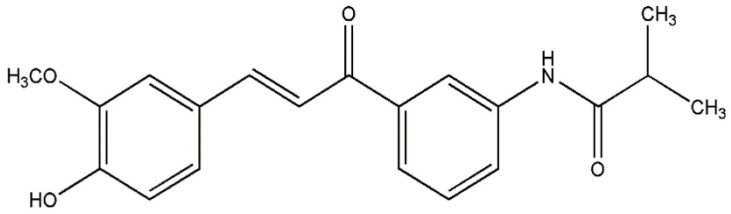
- Debenzoylmethane (DBM)



- Dihydrozingerone (DHZ)



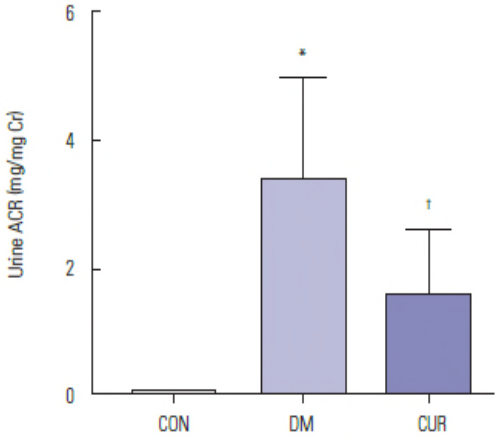
- Curcumin 2004-8

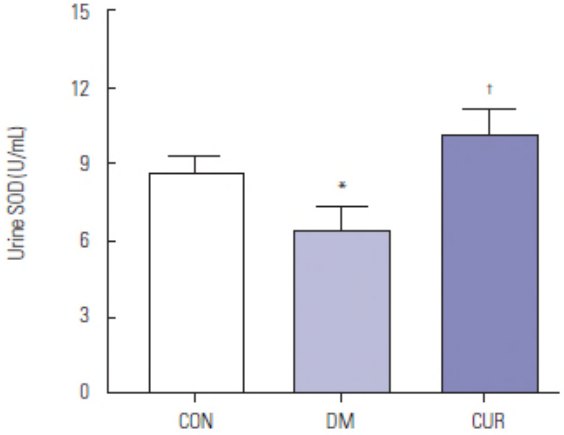


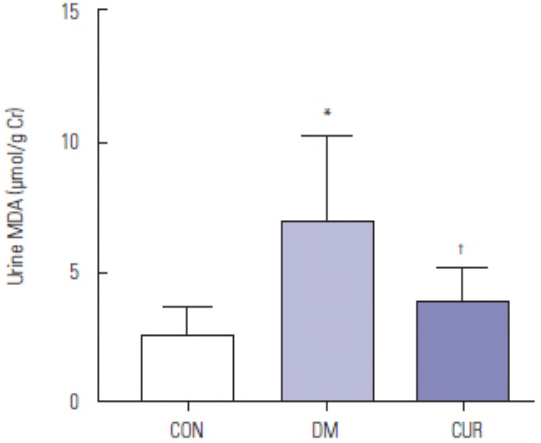
Curcumin

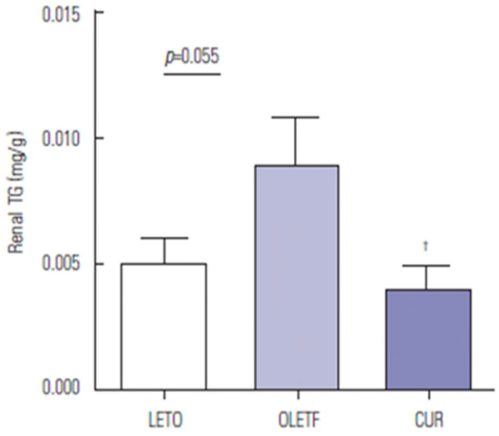
Protective Effects of Curcumin on Renal Oxidative Stress
and Lipid Metabolism in a Rat Model of Type 2 Diabetic Nephropathy.

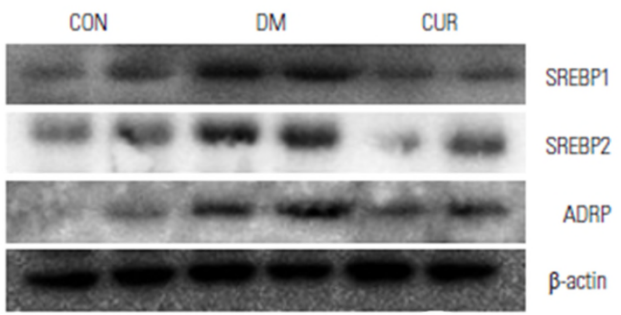
Yonsei Med J. 2016 May;57(3):664-73.

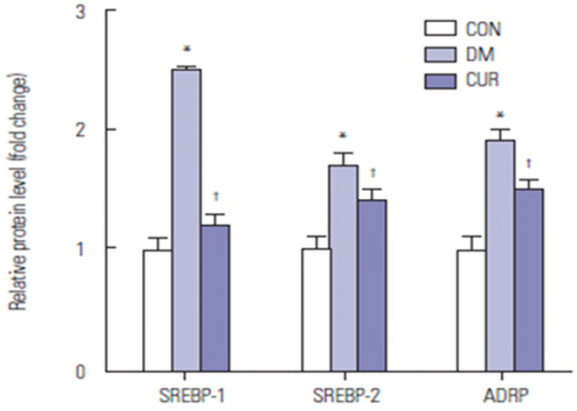







0.015
0.010
0.005
0.000
Renal TG (mg/g)
p=0.055
†
LETO
OLETF
CUR


CON
DM
CUR
SREBP1
SREBP2
ADRP
β-actin


3
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1
0
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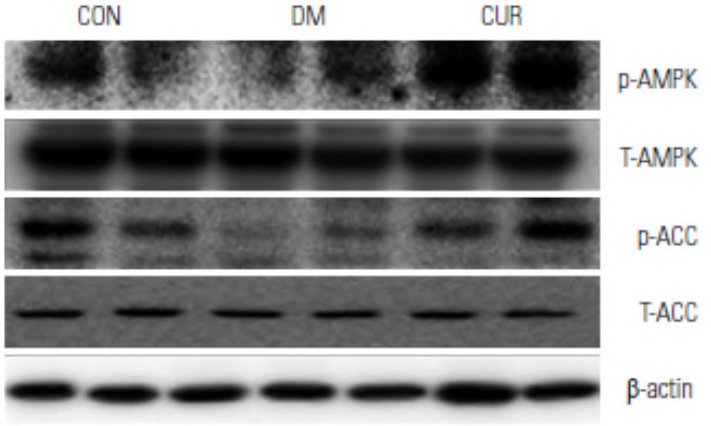
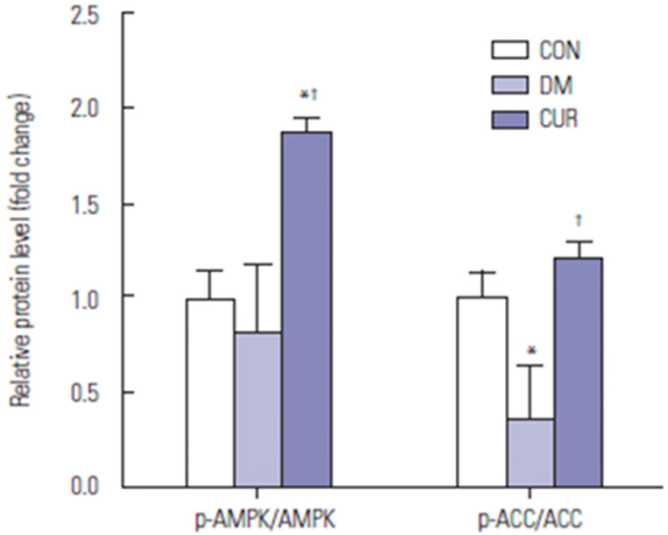
†
SREBP-1
SREBP-2
ADRP


CON
DM
CUR
p-AMPK
T-AMPK
p-ACC
T-ACC
β-actin


2.5
2.0
1.5
1.0
0.5
0.0
Relative protein level (fold change)
CON
DM
CUR
*†
*
†
p-AMPK/AMPK
p-ACC/ACC

CURCUMIN

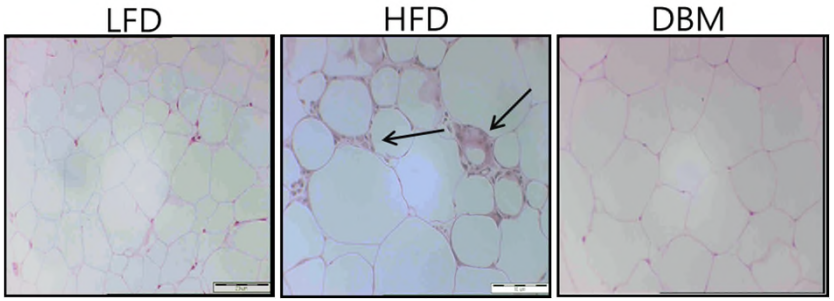
Dibenzoylmethane (DBM)

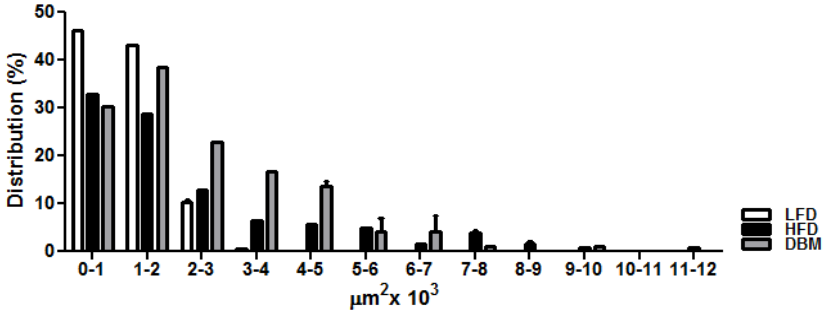
- Hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*
- anti-inflammatory, anti-tumor, anti-diabetic, neuroprotective, cell cycle regulation

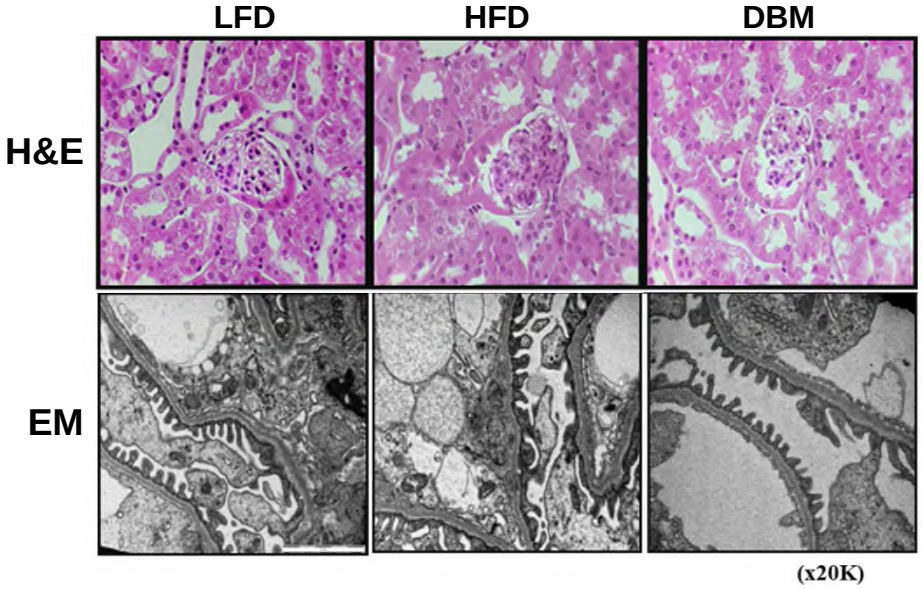
Dehydrozingerone (DHZ)

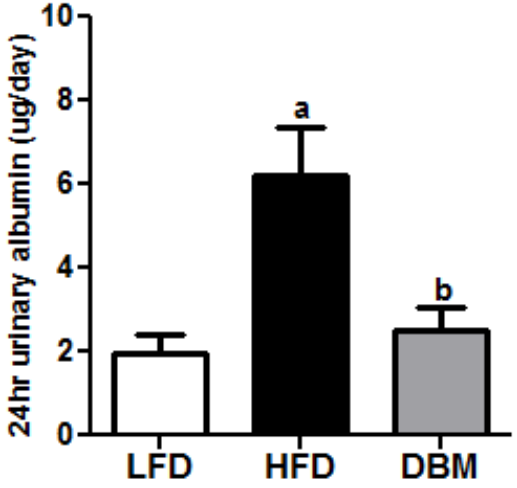
- anti-oxidant
- anti-tumor
- inhibition of lipid peroxidation activity

Diabenzoylmethane









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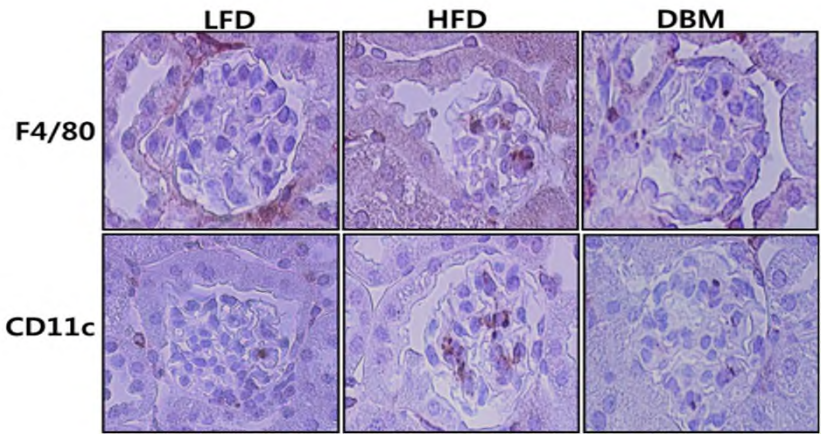

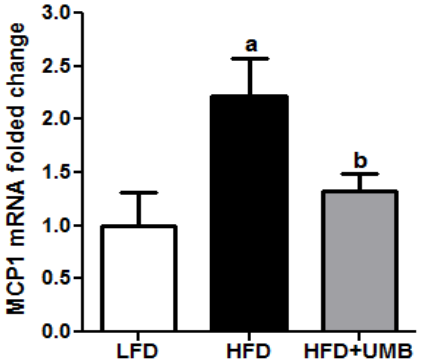

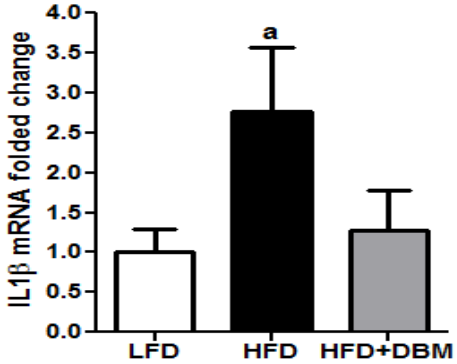

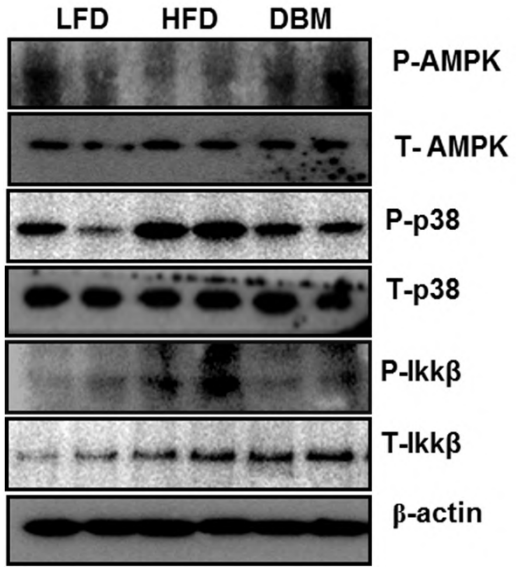

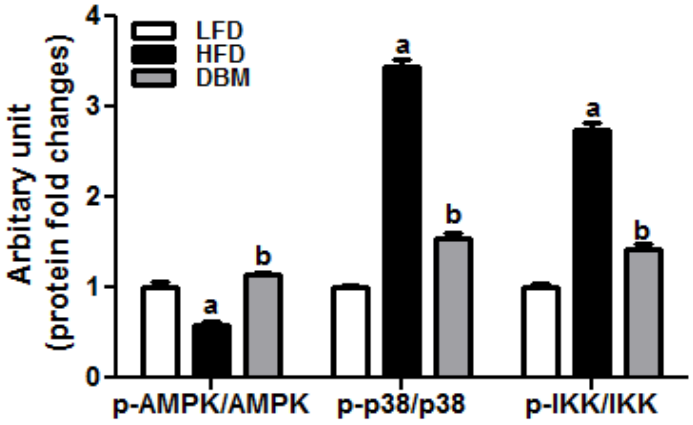

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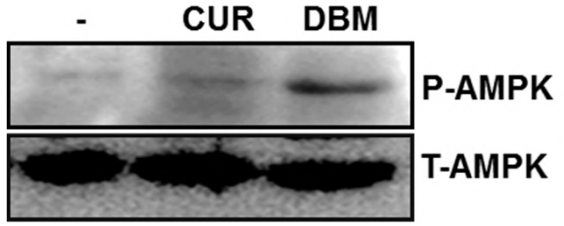

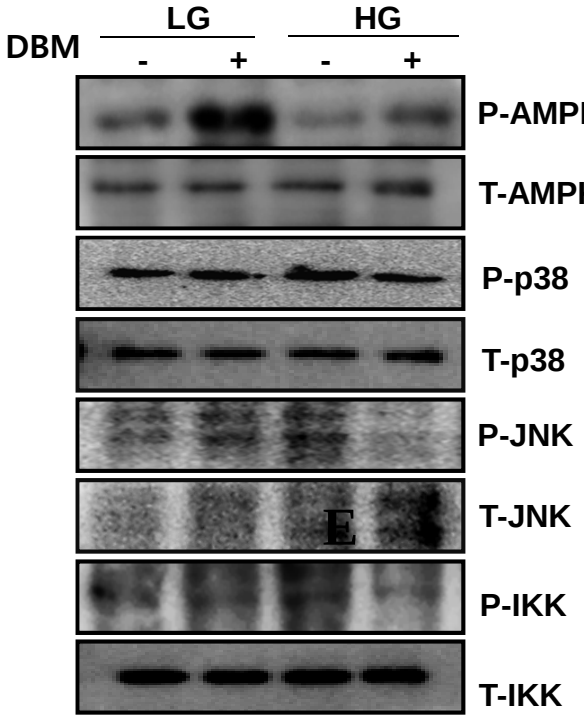

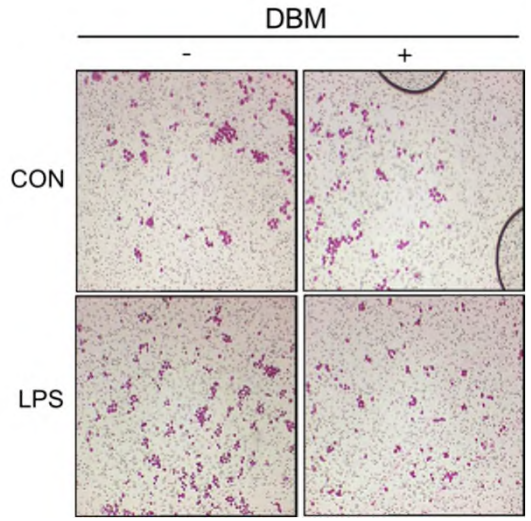

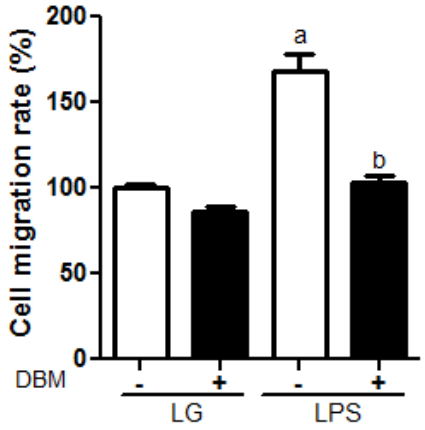

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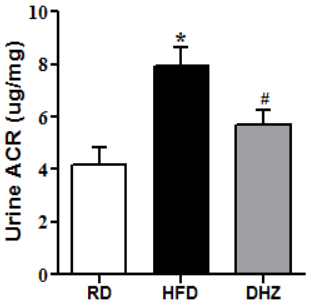

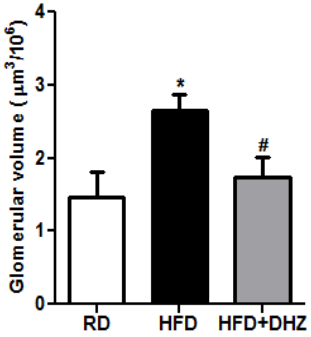

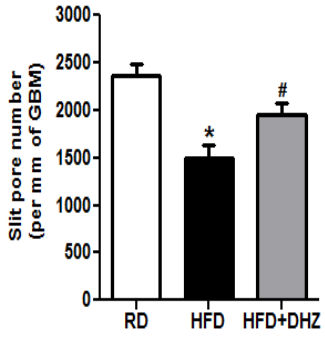

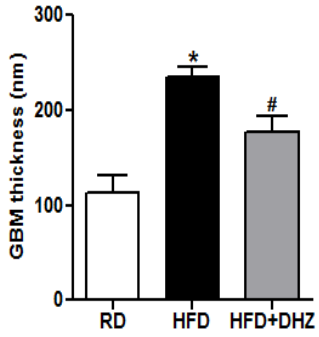

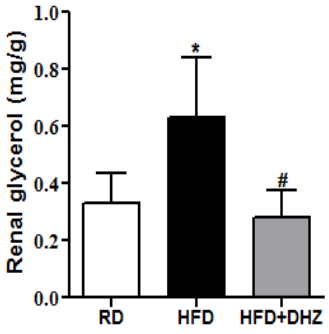

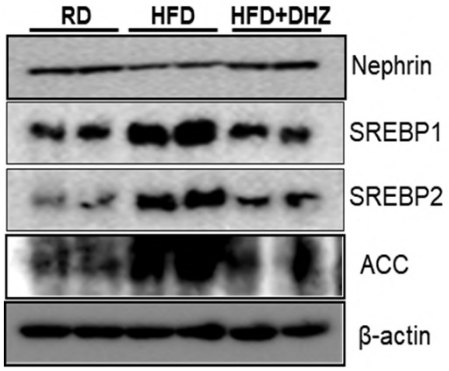

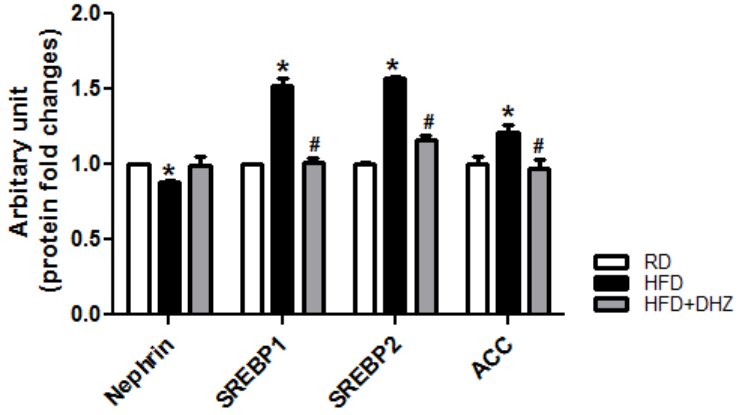

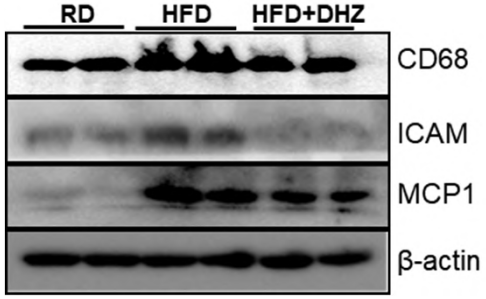

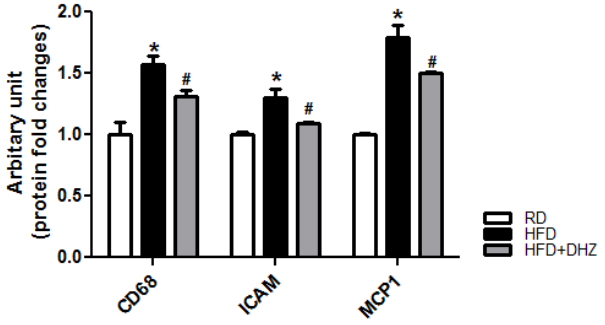

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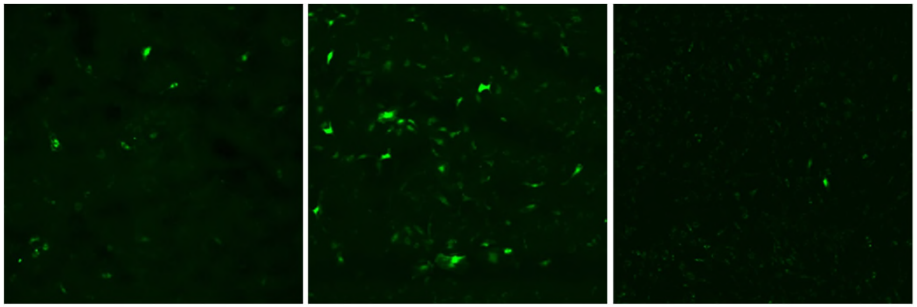
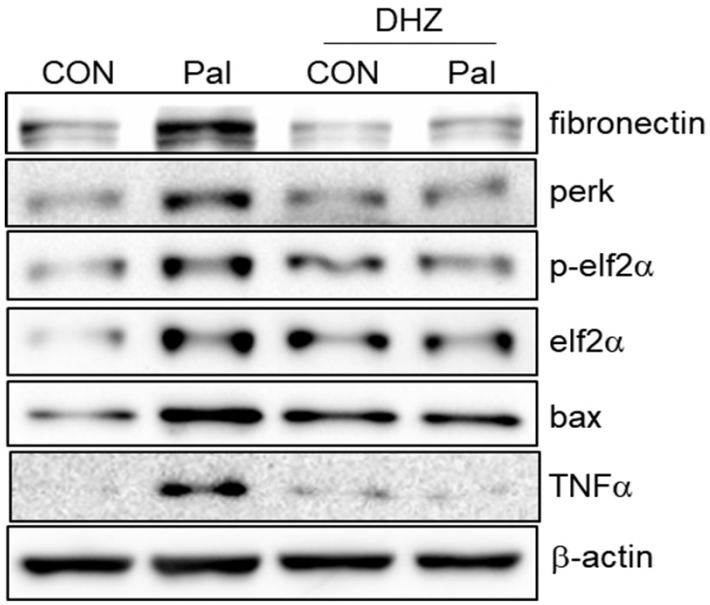
Mouse podocytes





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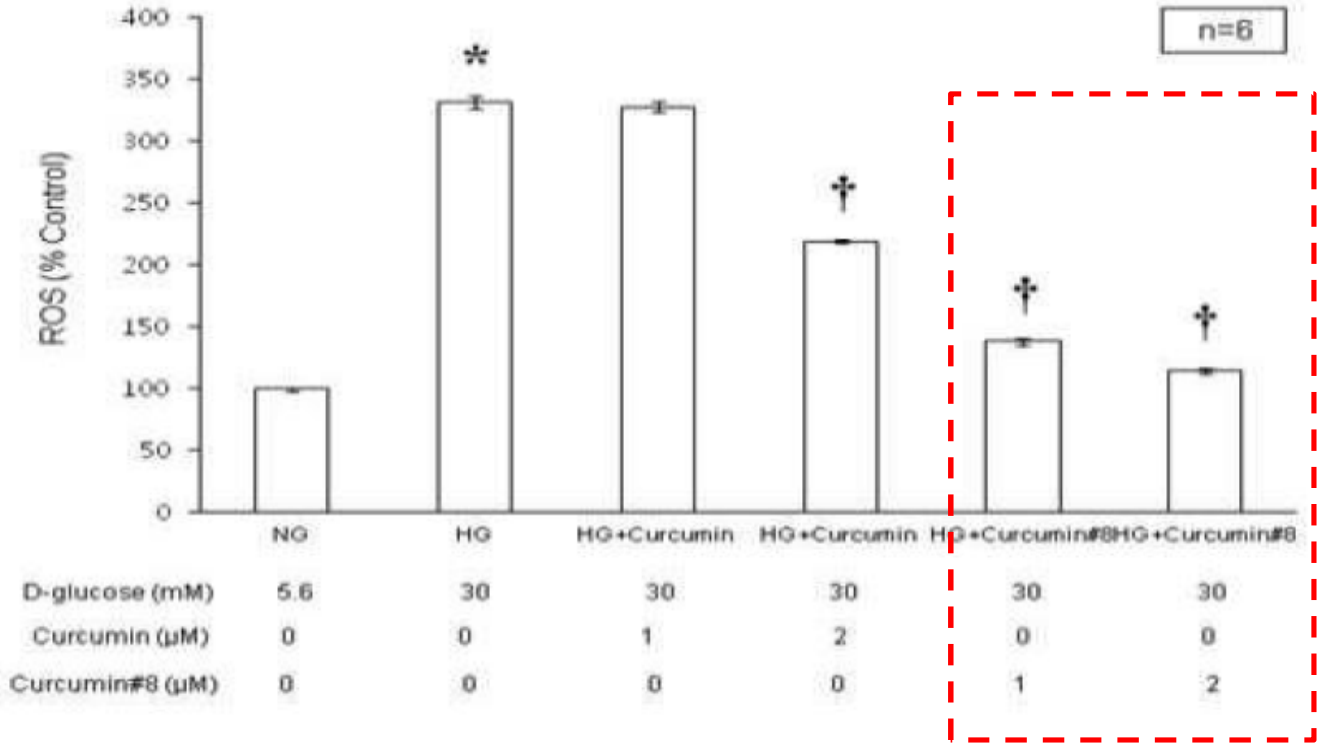
On going study

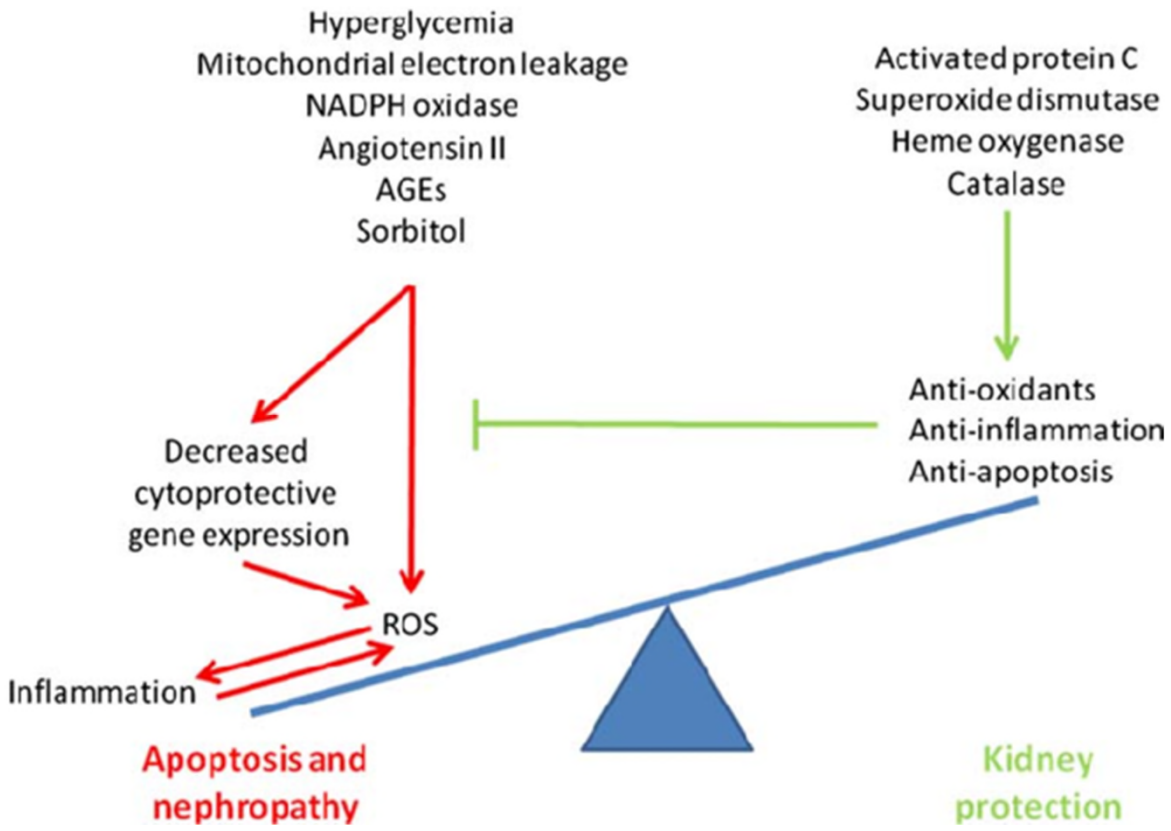
Curcumun 2004-8

Curcumin 8



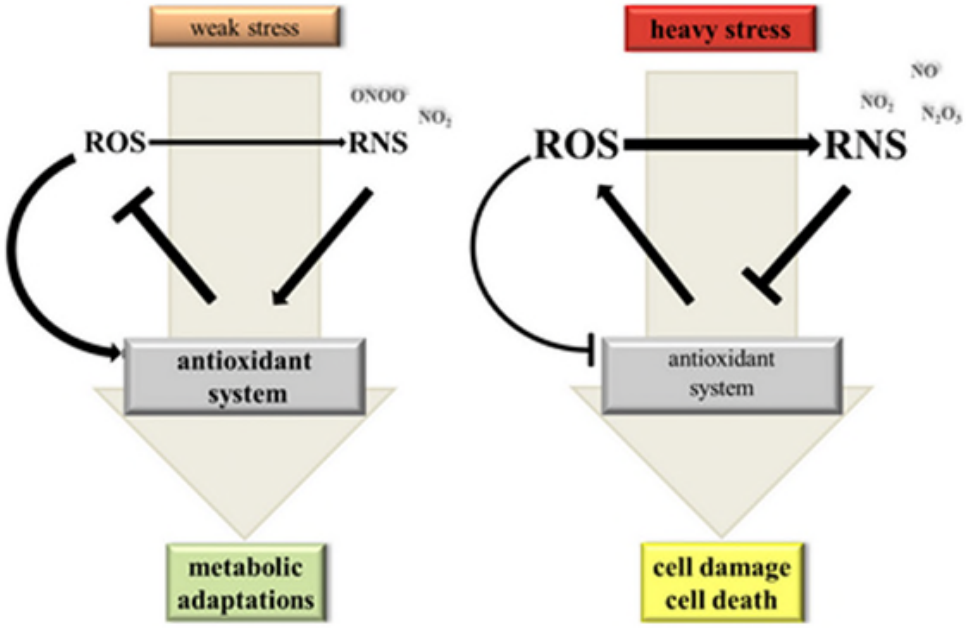
The Importance of ROS Regulation

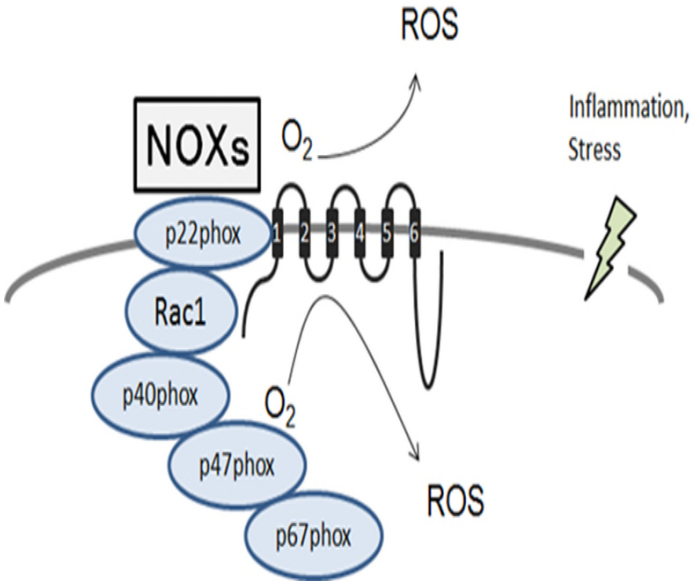
Fig. 1 Excess ROS leads to apoptosis and diabetic nephropathy. Model demonstrating the different causes of ROS formation that result in the induction of inflammation by pro-inflammatory genes, leukocyte infiltration, and aggravation of ROS formation. Ultimately, this may lead to apoptosis and kidney dysfunction. We postulate that in humans induction of cytoprotective proteins, such as HO-1, SOD or catalase, will restore this ROS-mediated skewing of the redox balance by generating products that protect against oxidative stress, and/or inflammation and apoptosis, or a combination



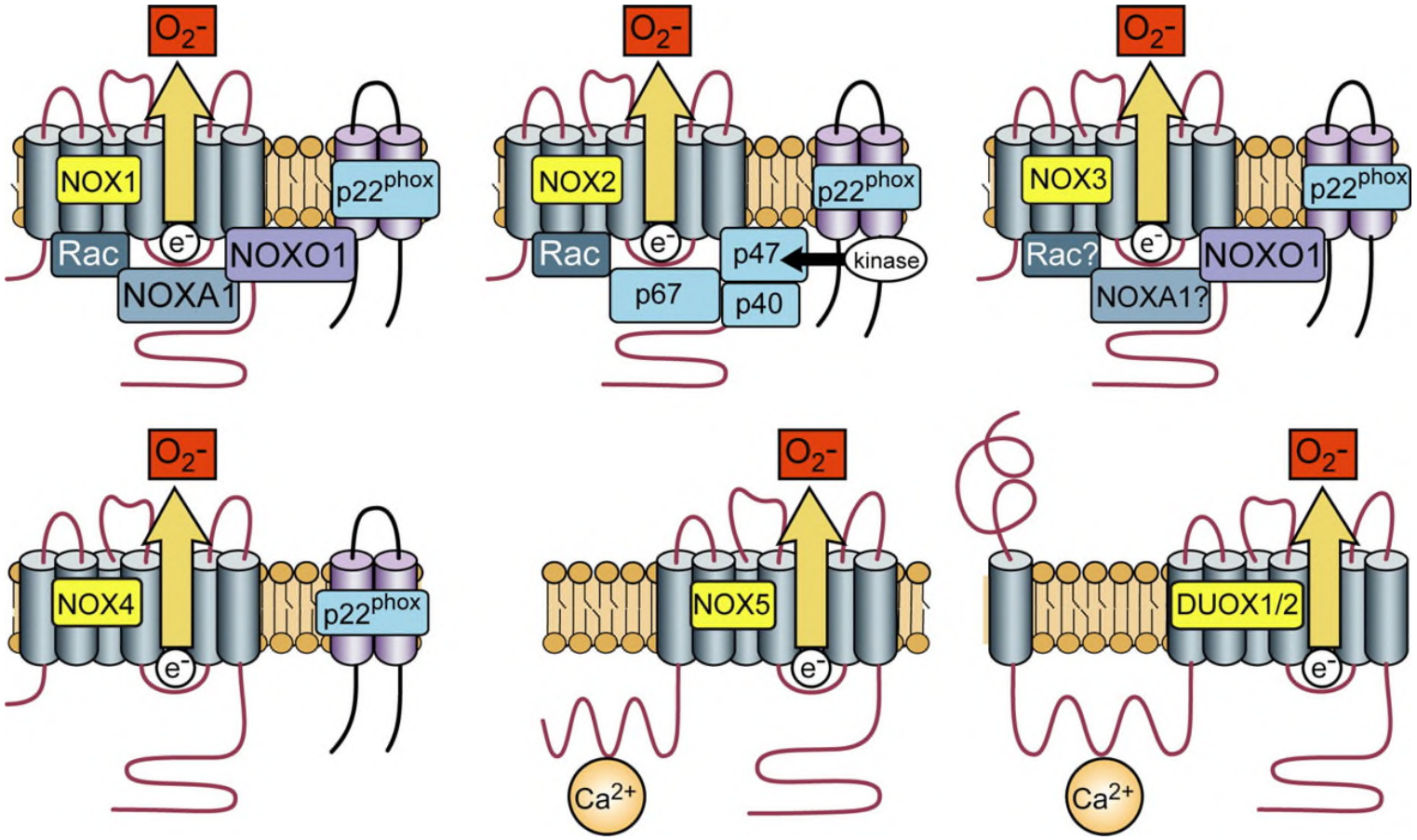
Wagener FA et al., Apoptosis 14(12):1451-8

Discovery of new therapeutic target for DKD





Activation of NADPH oxidase isoforms



Physiological Reviews Published 1 January 2007 **Vol.** 87 **no.** 1, 245-313

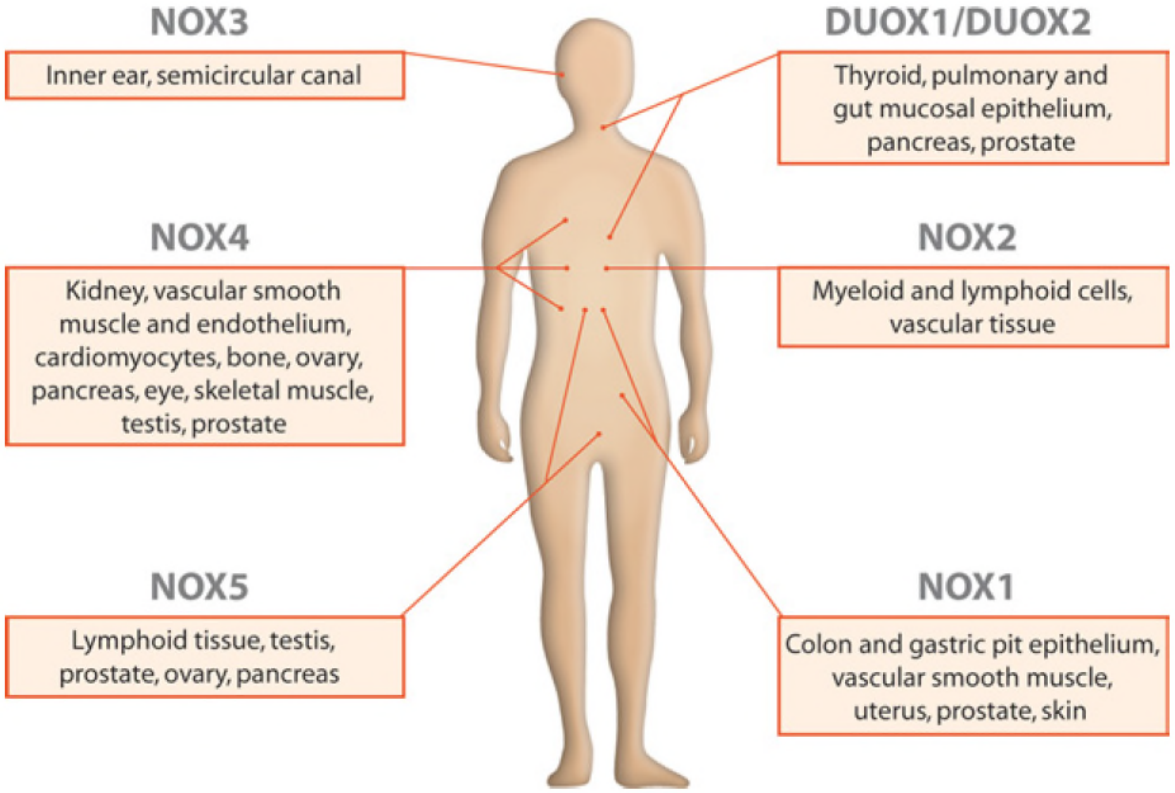


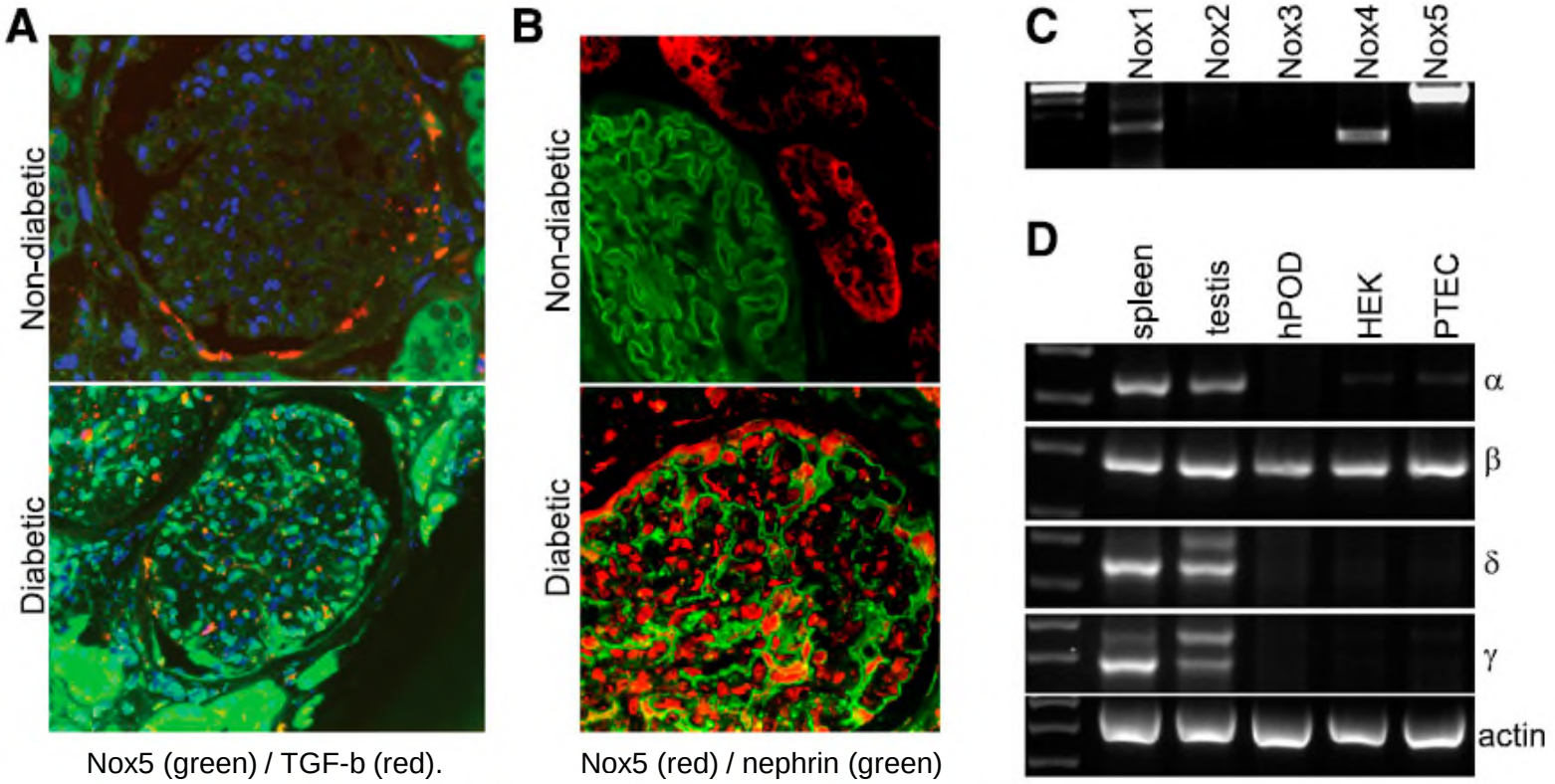
Figure 2 **Locations of NOX proteins in the body**

Tissues reported to express NOX homologues throughout the human body are indicated. See text for further details.

NOX inhibitors

- Small molecule inhibitors: Diphenylene iodonium (DPI), Apopcynin, ML171
- Triazolo purimidine derivatives: VAS2870, VAS3947
- Pyrazolopyridine derivatives: GKT136901, GKT137831

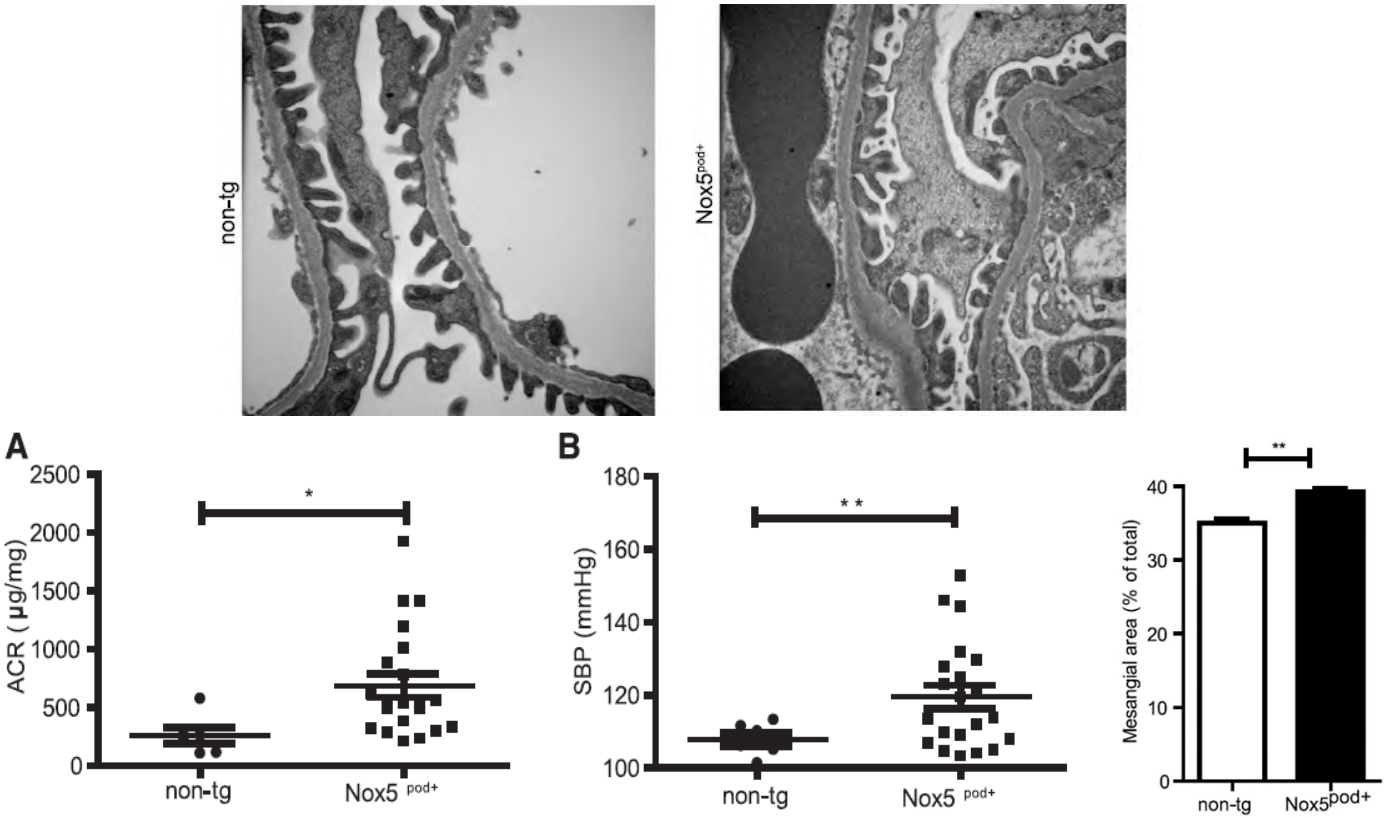
Nox5 is expressed in human diabetic kidney biopsies and podocytes



Nox5 (green) / TGF-b (red).

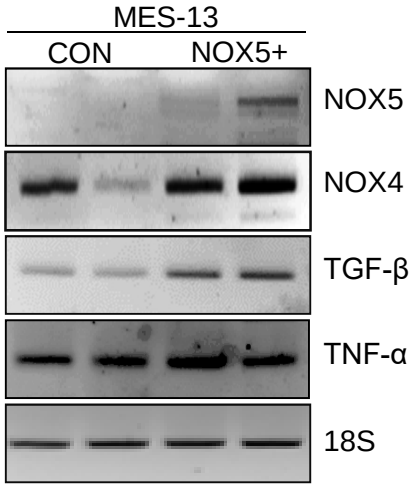
Nox5 (red) / nephrin (green)

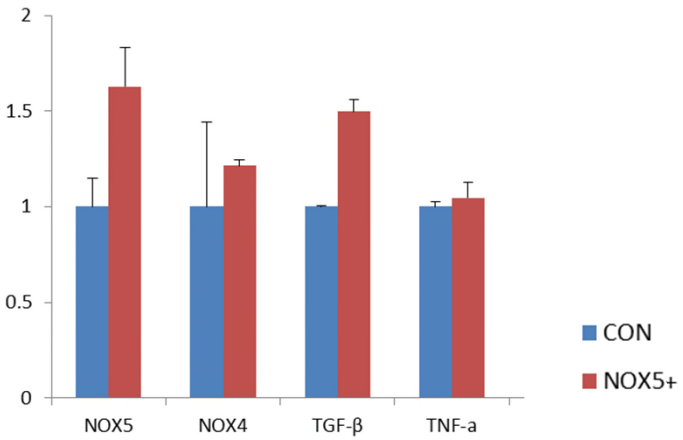
J Am Soc Nephrol 25: 784–797, 2014

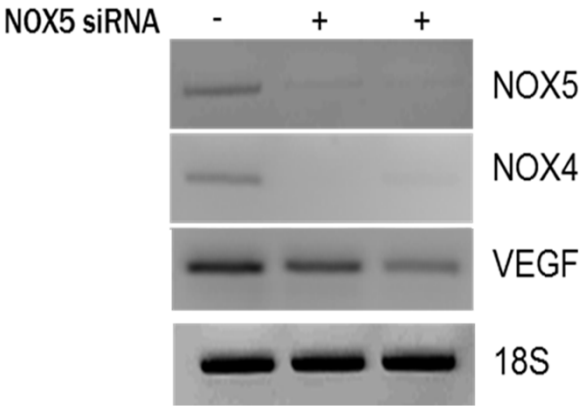


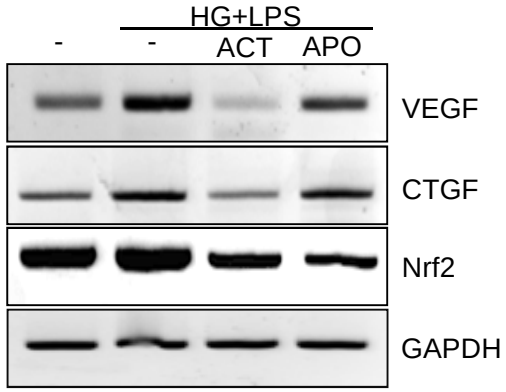
J Am Soc Nephrol 25: 784–797, 2014

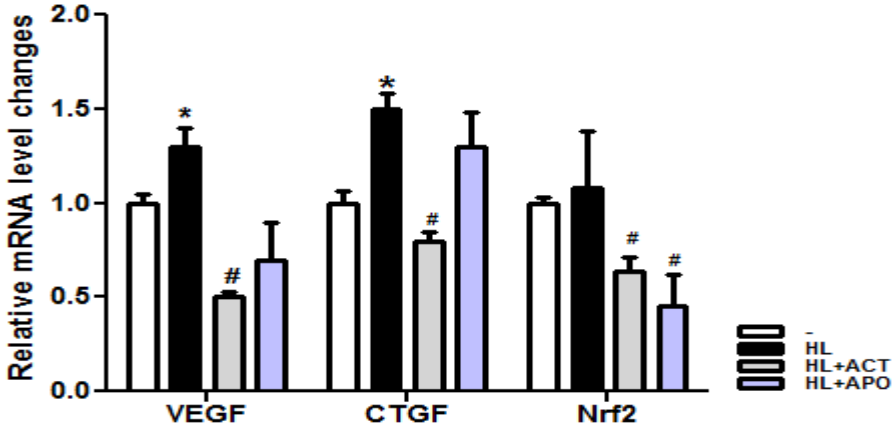
On going study









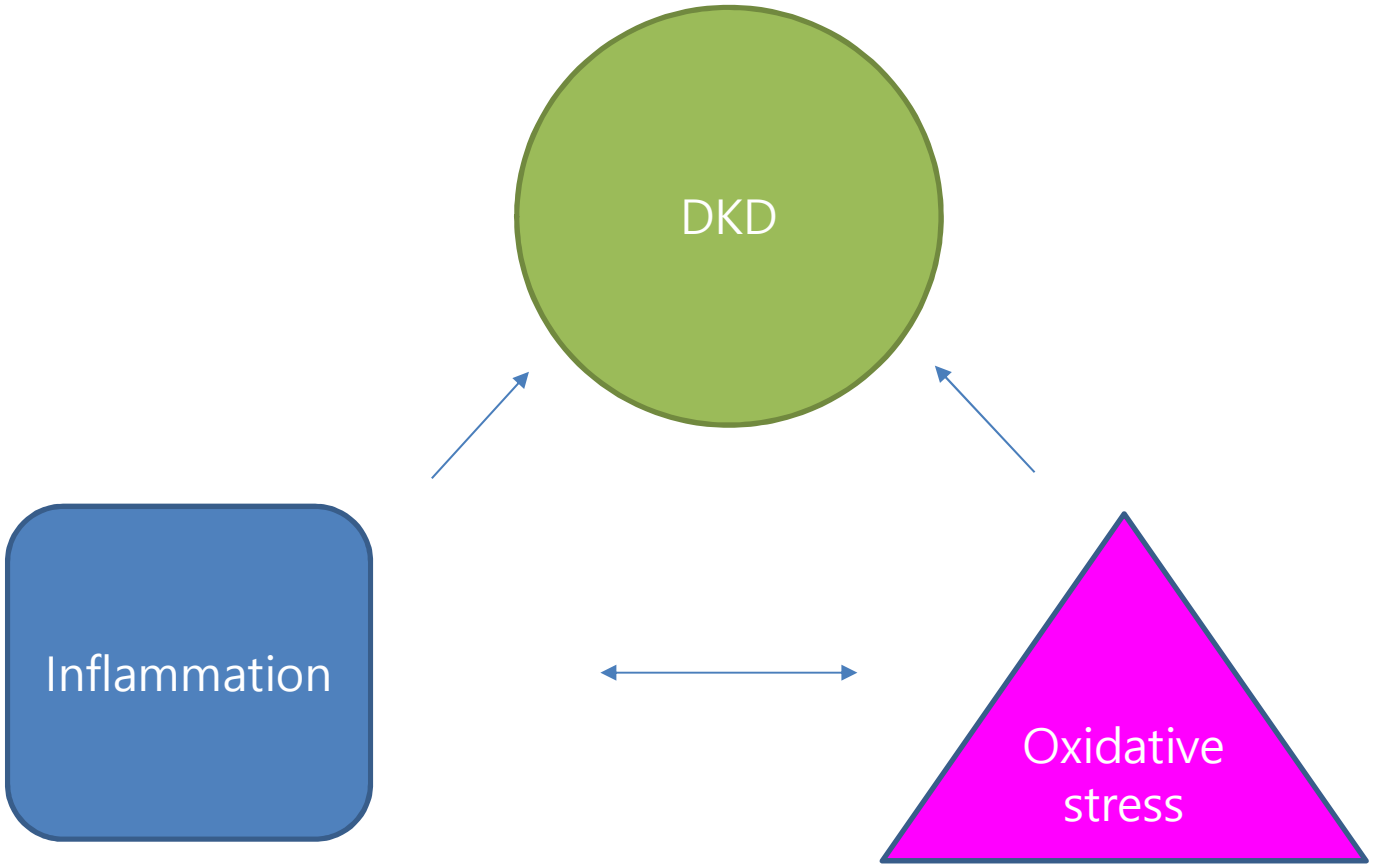


ACT, NOX1/4 inhibitor
APO, Apocynin

Take Home Message

In the pathogenesis of DKD, inflammation and oxidative stress are very important. We suggest that the blockade of MCP-1 and ROS in the progression of DKD would be important therapeutic strategies.

Oxidative stress and inflammation in DKD



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