Role of FXR (Farnesoid X Receptor or Bile Acid Receptor) in Diabetic Nephropathy

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- Paul Wilson
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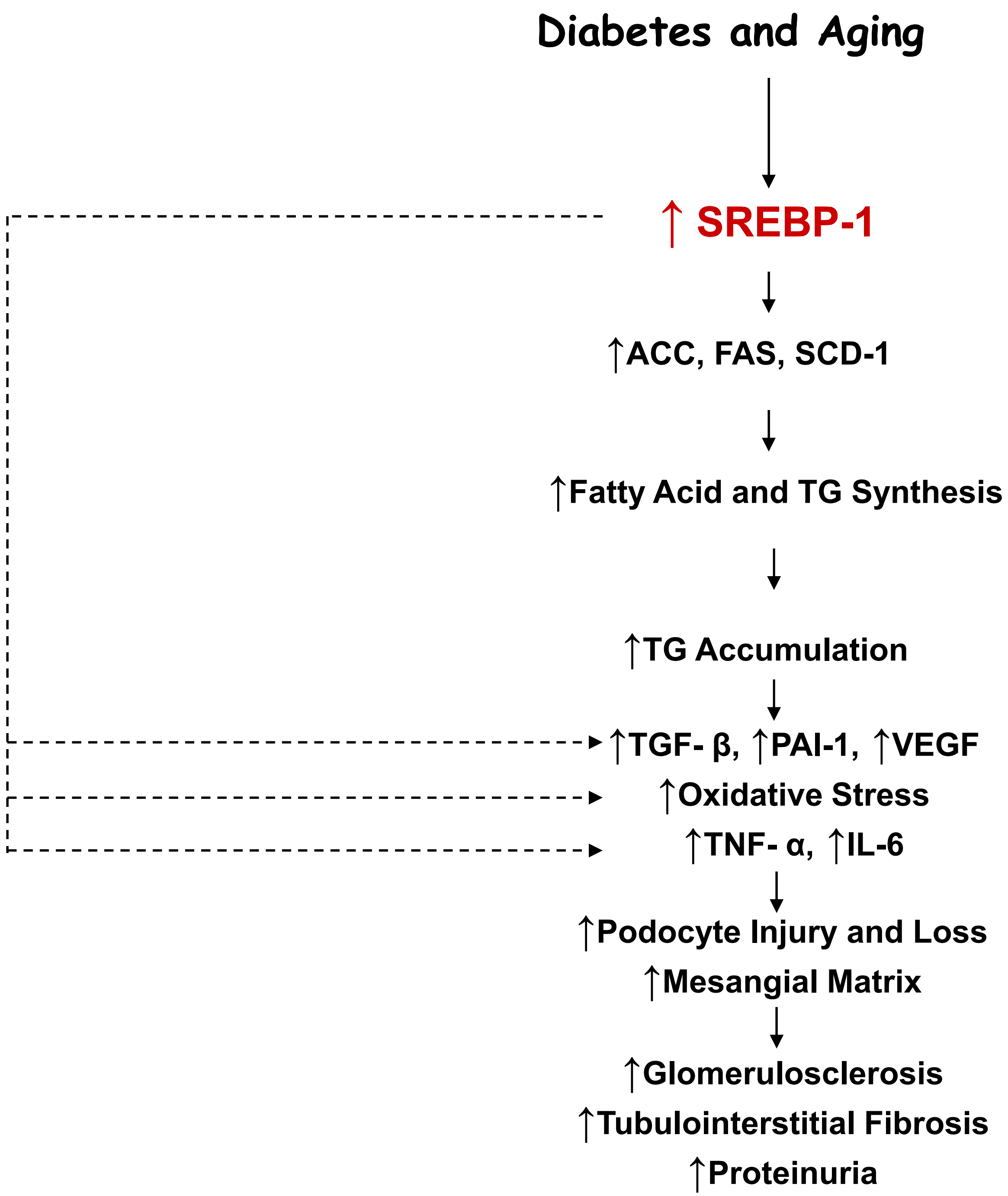
Why FXR?

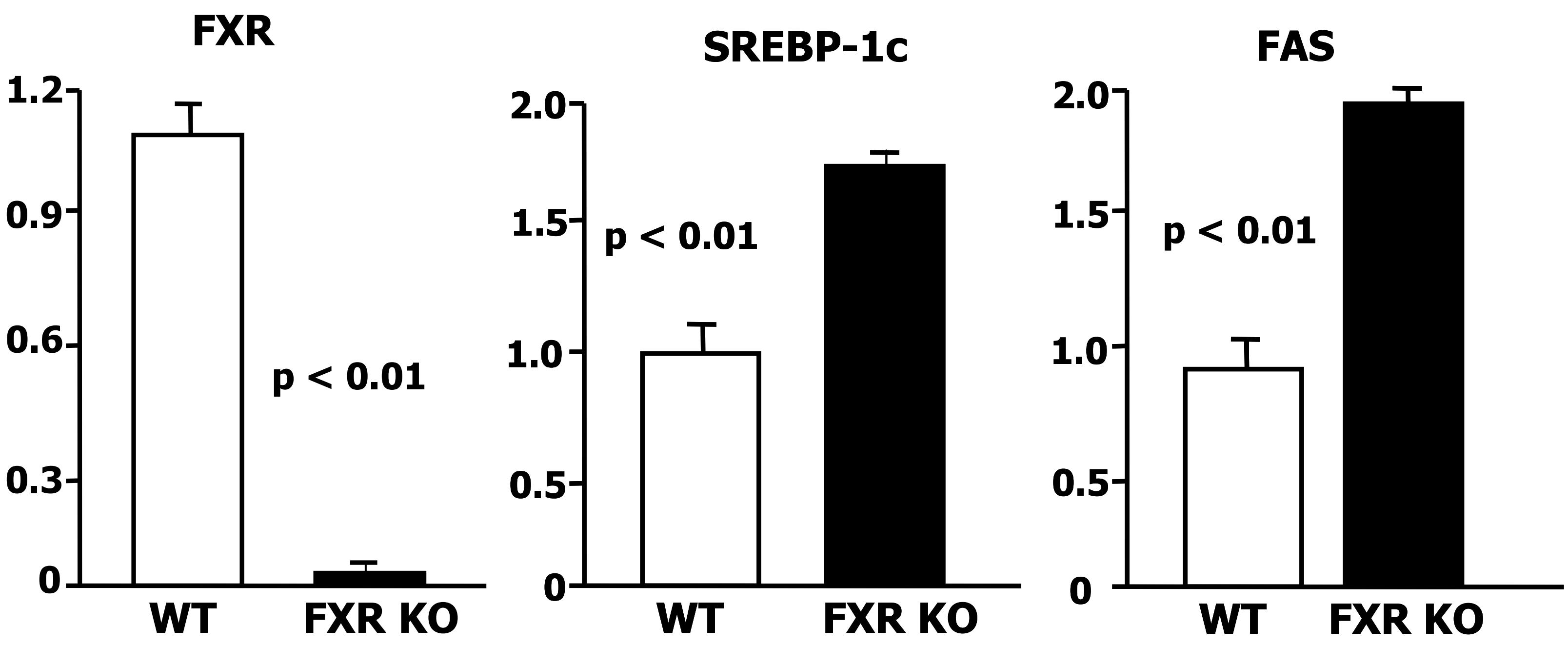
Renal SREBP-1 expression is increased in

- Type 1 Diabetes
- Diet Induced Obesity and Insulin Resistance (Metabolic Syndrome)
- Type 2 Diabetes
- Aging


Diabetes and Aging
↑ SREBP-1
↑ACC, FAS, SCD-1
↑Fatty Acid and TG Synthesis
↑TG Accumulation
↑TGF- β, ↑PAI-1, ↑VEGF
↑Oxidative Stress
↑TNF- α, ↑IL-6
↑Podocyte Injury and Loss
↑Mesangial Matrix
↑Glomerulosclerosis
↑Tubulointerstitial Fibrosis
↑Proteinuria

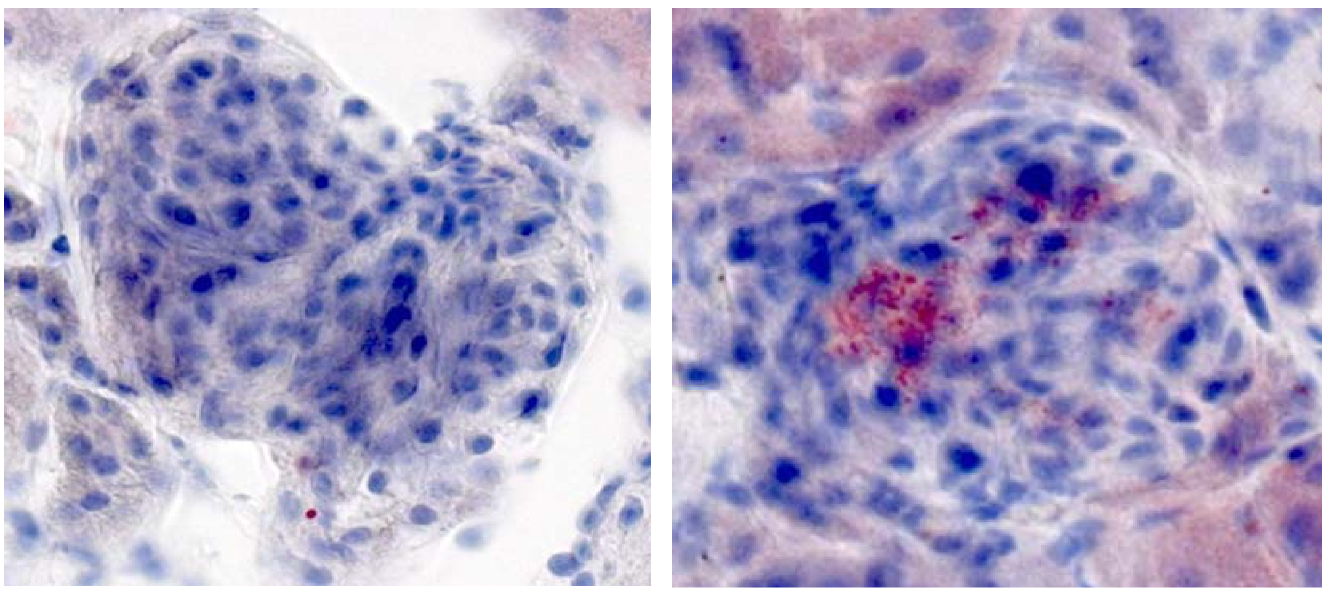
FXR KO Mice

FXR KO mice that have decreased FXR expression in the kidney have increased expression of SREBP-1c and its target enzyme FAS that mediates fatty acid synthesis

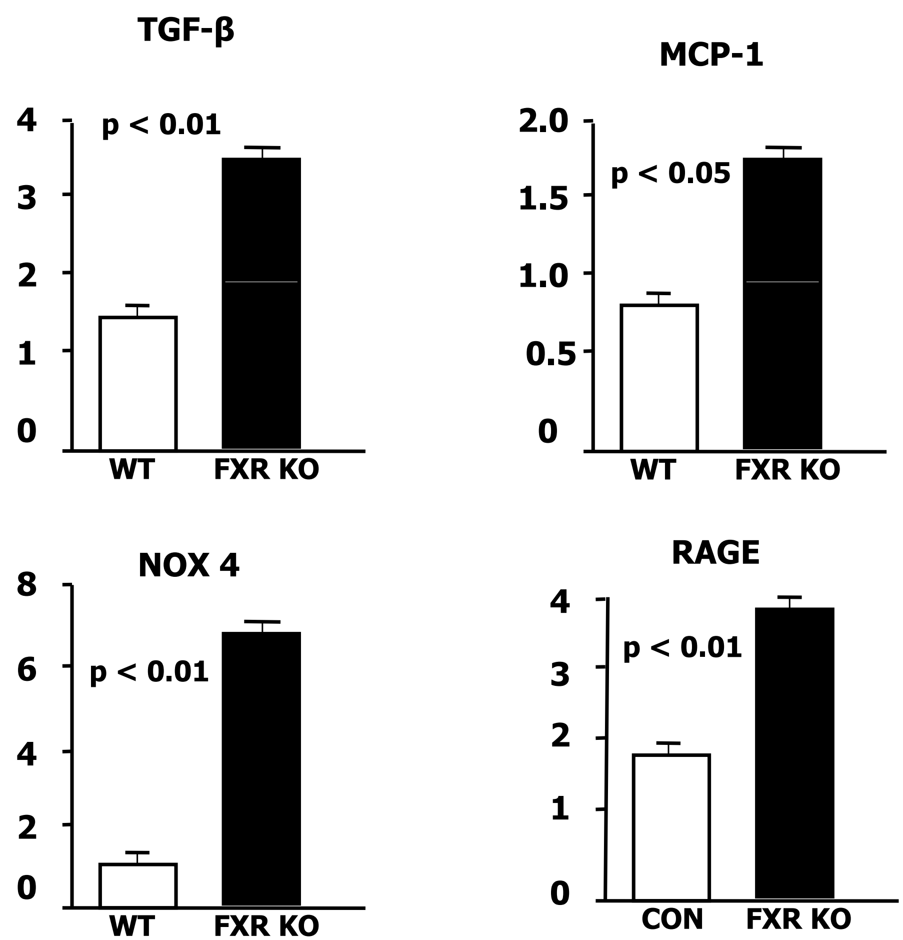


There is increased oil red O (neutral lipids) accumulation in the glomeruli of FXR KO mice

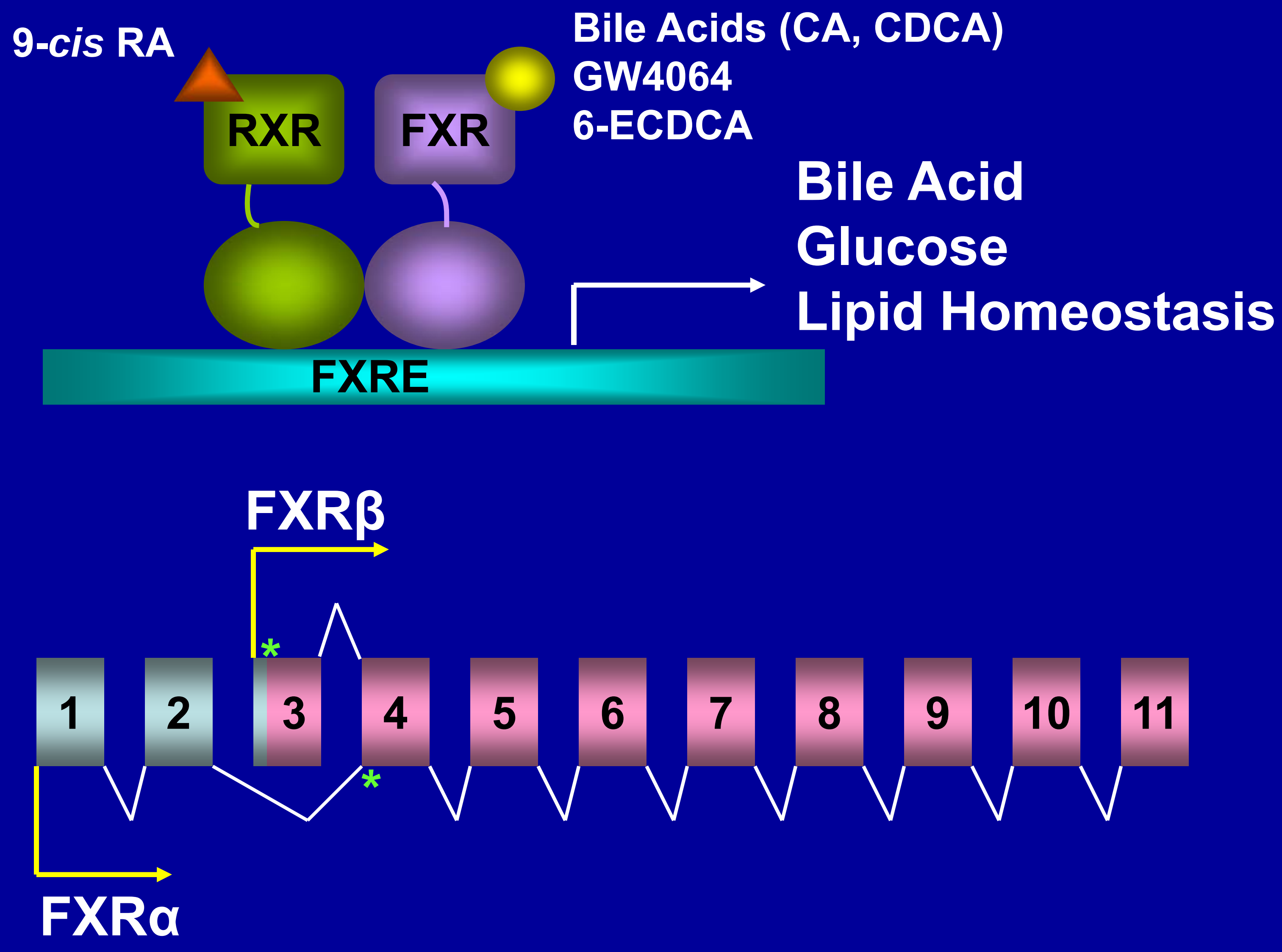
Wild Type FXR KO



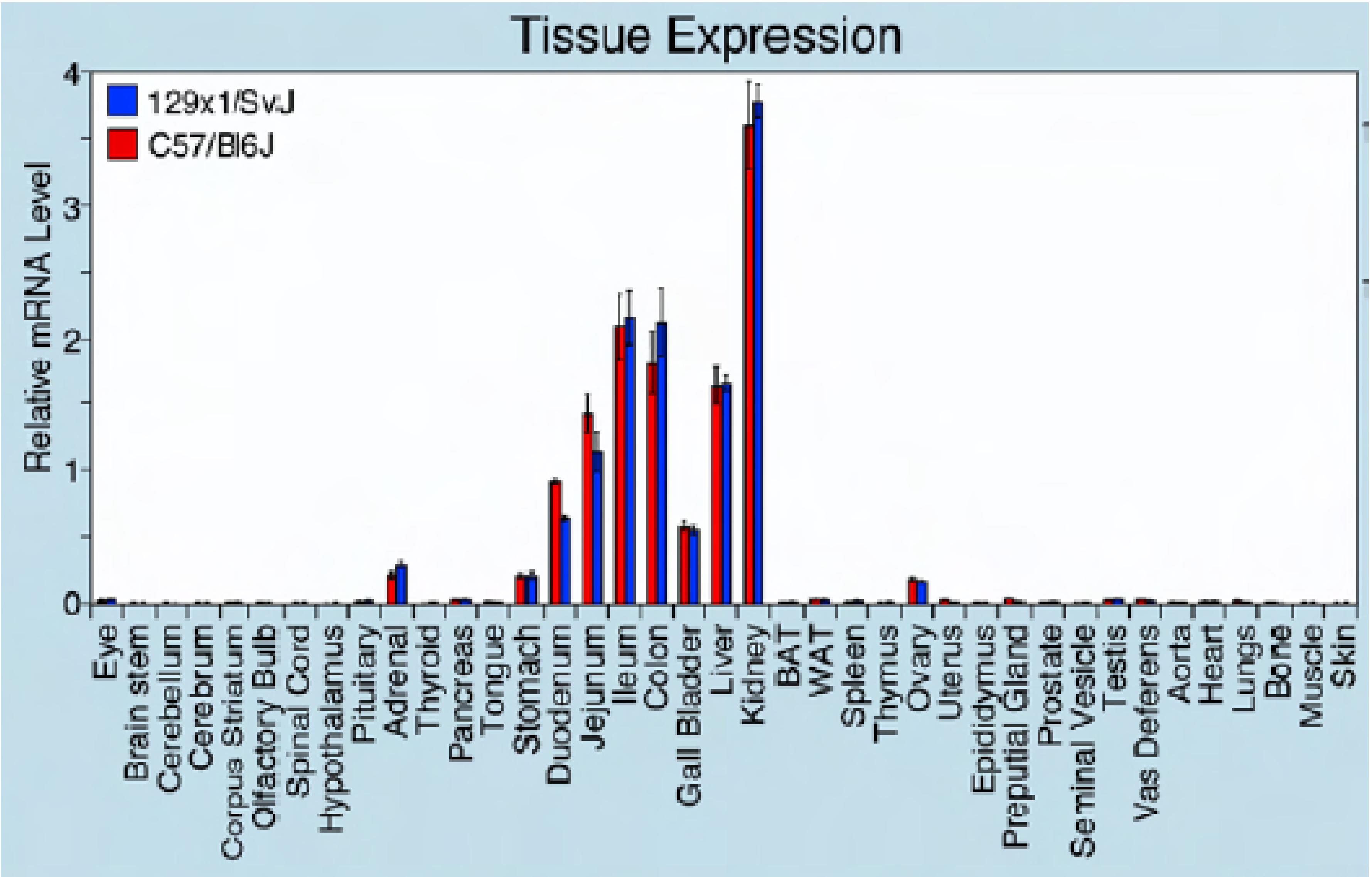
FXR KO mice have increased renal expression of TGF-β, MCP-1, Nox4 and RAGE



Farnesoid X Receptor (FXR)

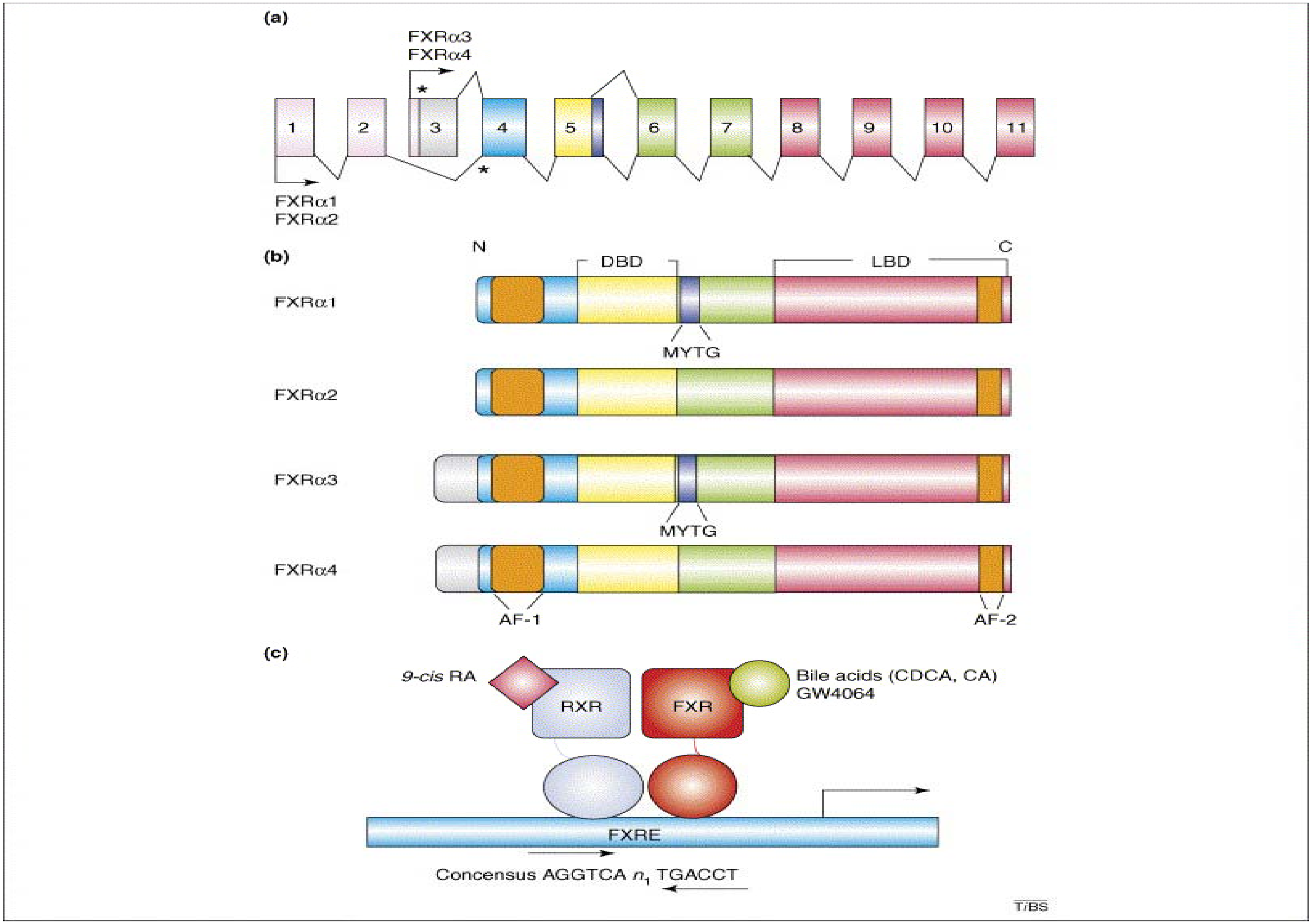


FXRα

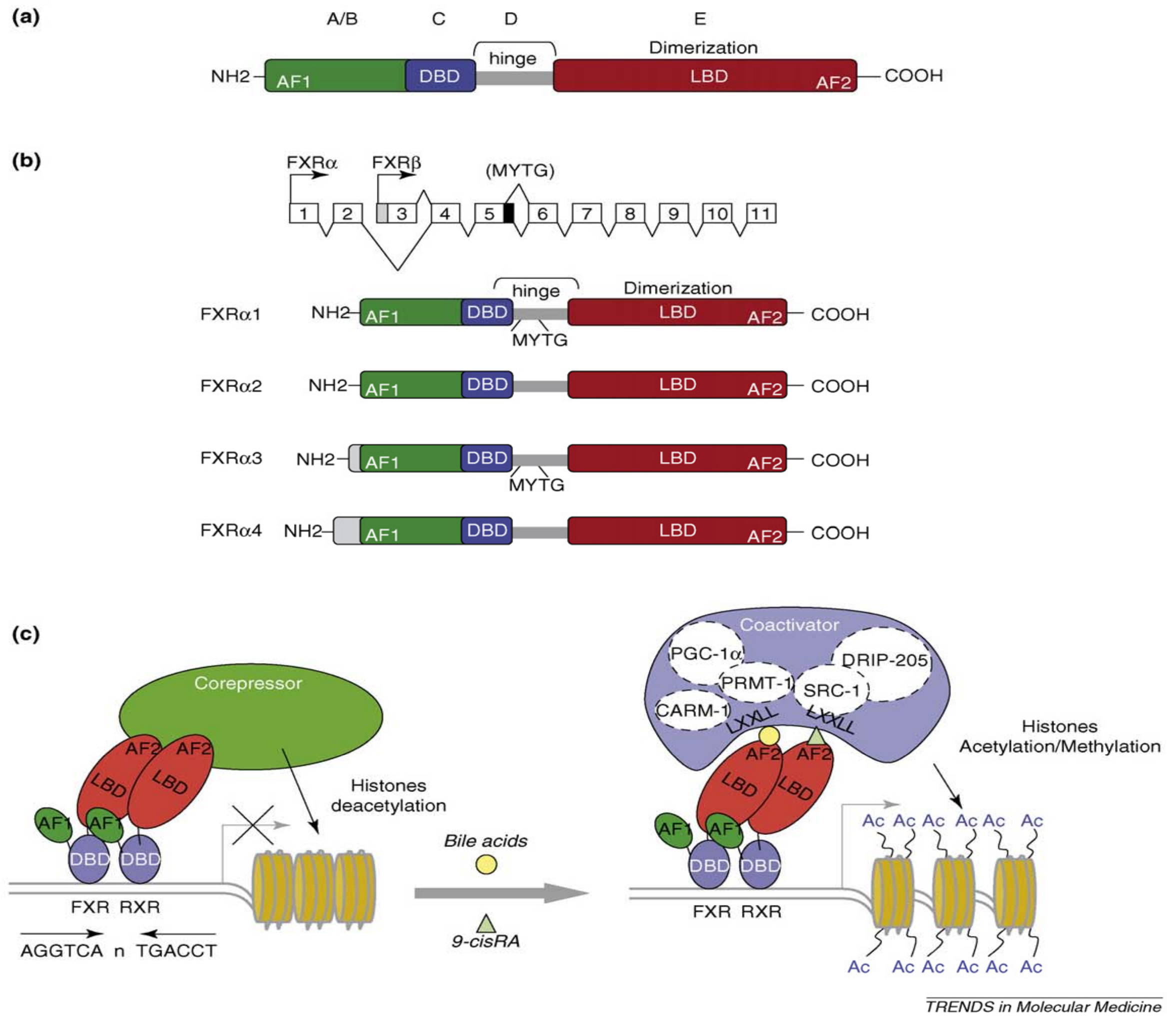


Tissue Expression
129x1/SvJ
C57/Bl6J
Relative mRNA Level
0
1
2
3
4
Eye
Brain stem
Cerebellum
Cerebrum
Corpus Striatum
Olfactory Bulb
Spinal Cord
Hypothalamus
Pituitary
Adrenal
Thyroid
Pancreas
Tongue
Stomach
Duodenum
Jejunum
Ileum
Colon
Gall Bladder
Liver
Kidney
BAT
WAT
Spleen
Thymus
Ovary
Uterus
Epididymus
Preputial Gland
Prostate
Seminal Vesicle
Testis
Vas Deferens
Aorta
Heart
Lungs
Bone
Muscle
Skin

FXRα Isoforms

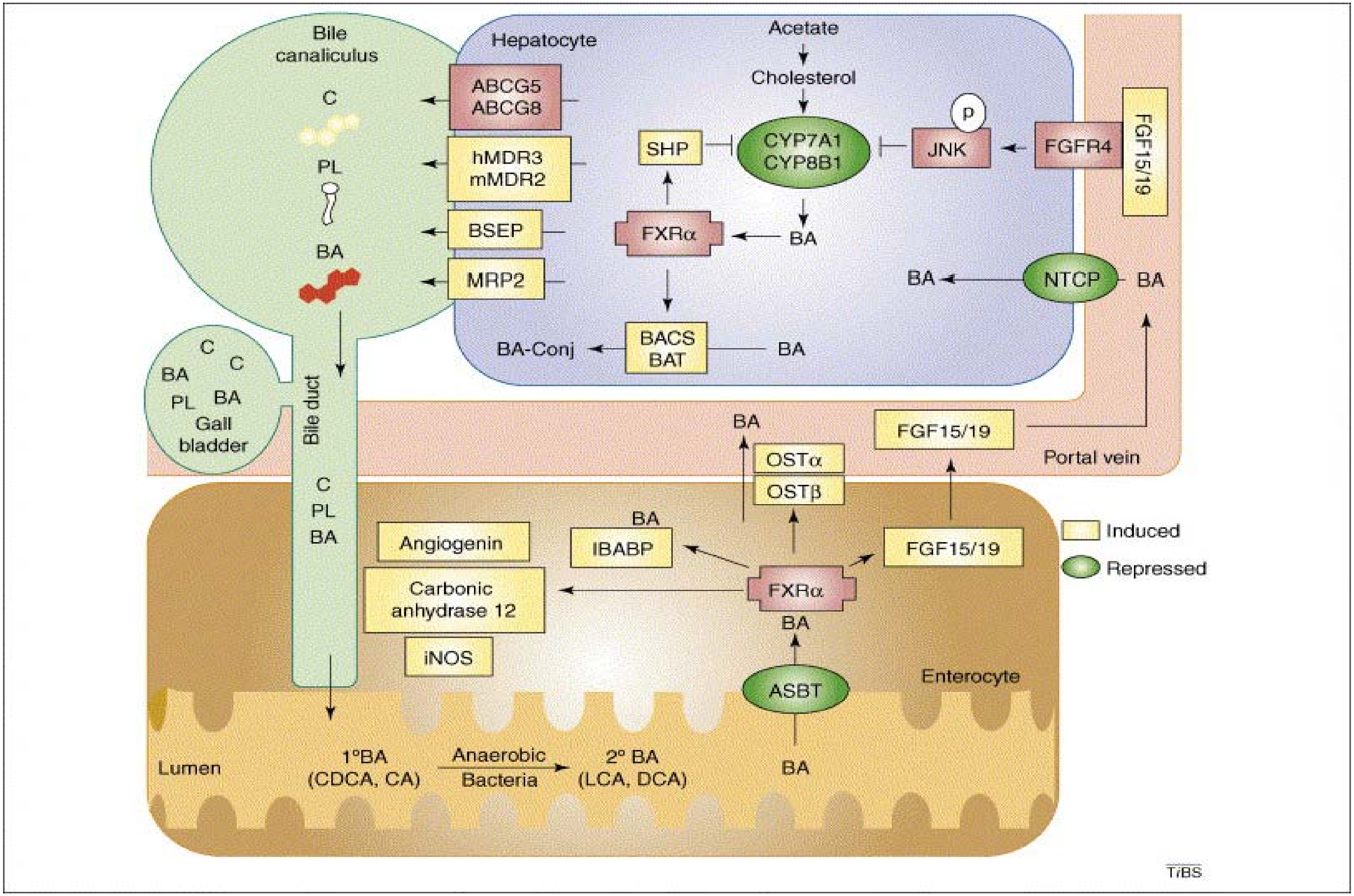


FXRα Isoforms

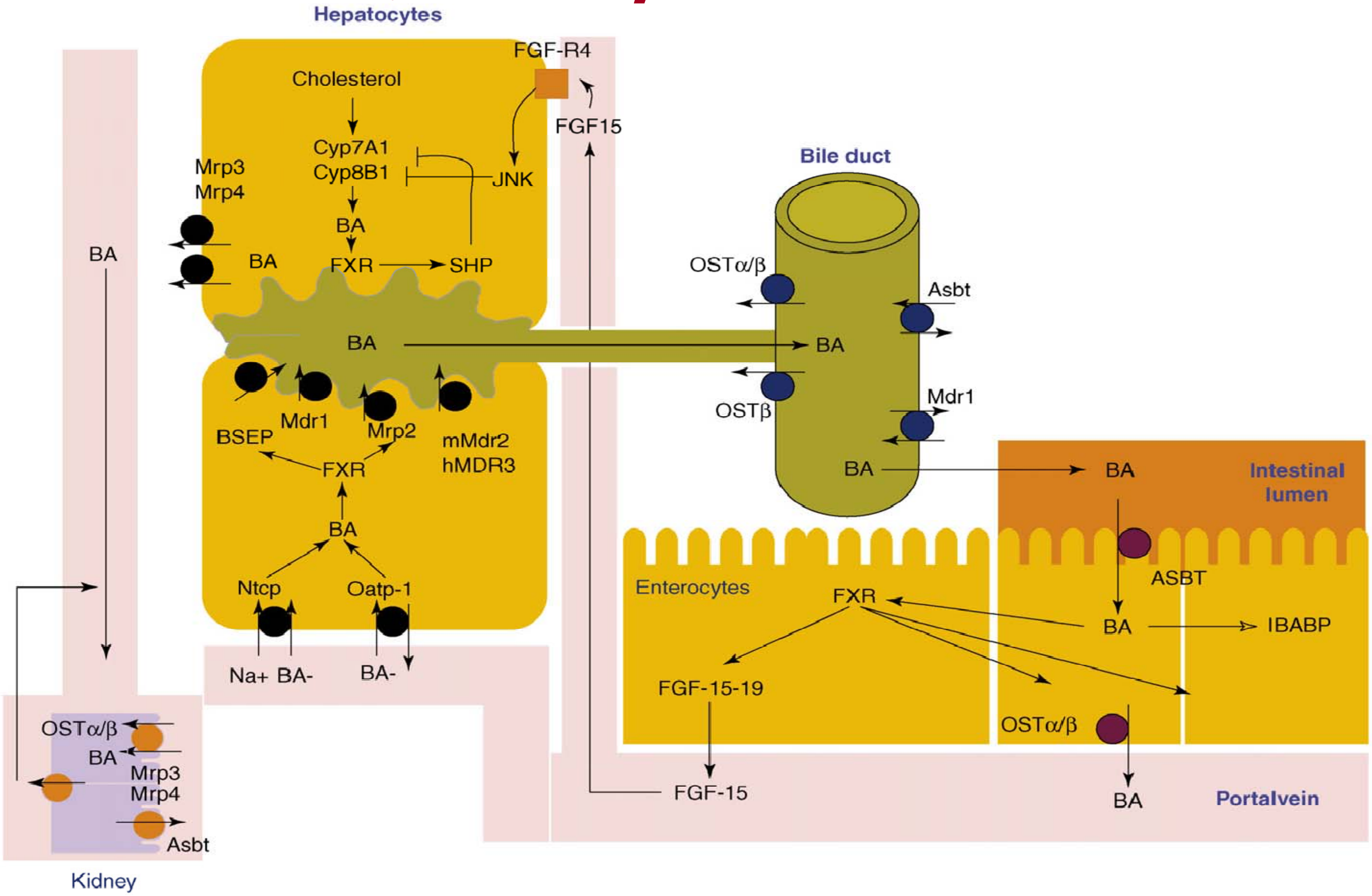


Fiorucci, et al. Trends in Molecular Medicine. 13(7):298-309, 2007.

FXR Regulates Bile Acid Metabolism

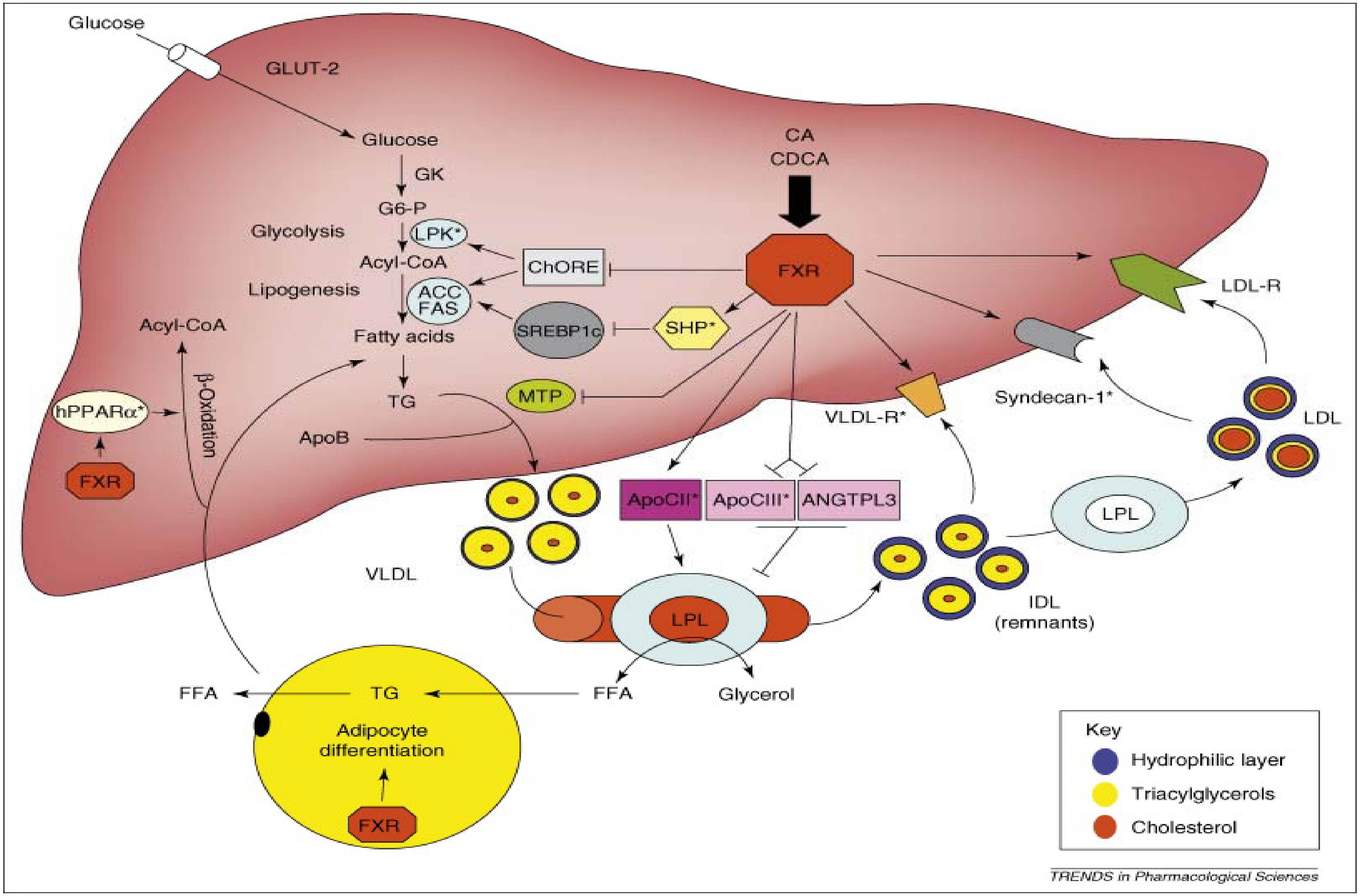


FXR regulates BA transporters in the liver, kidney and intestine

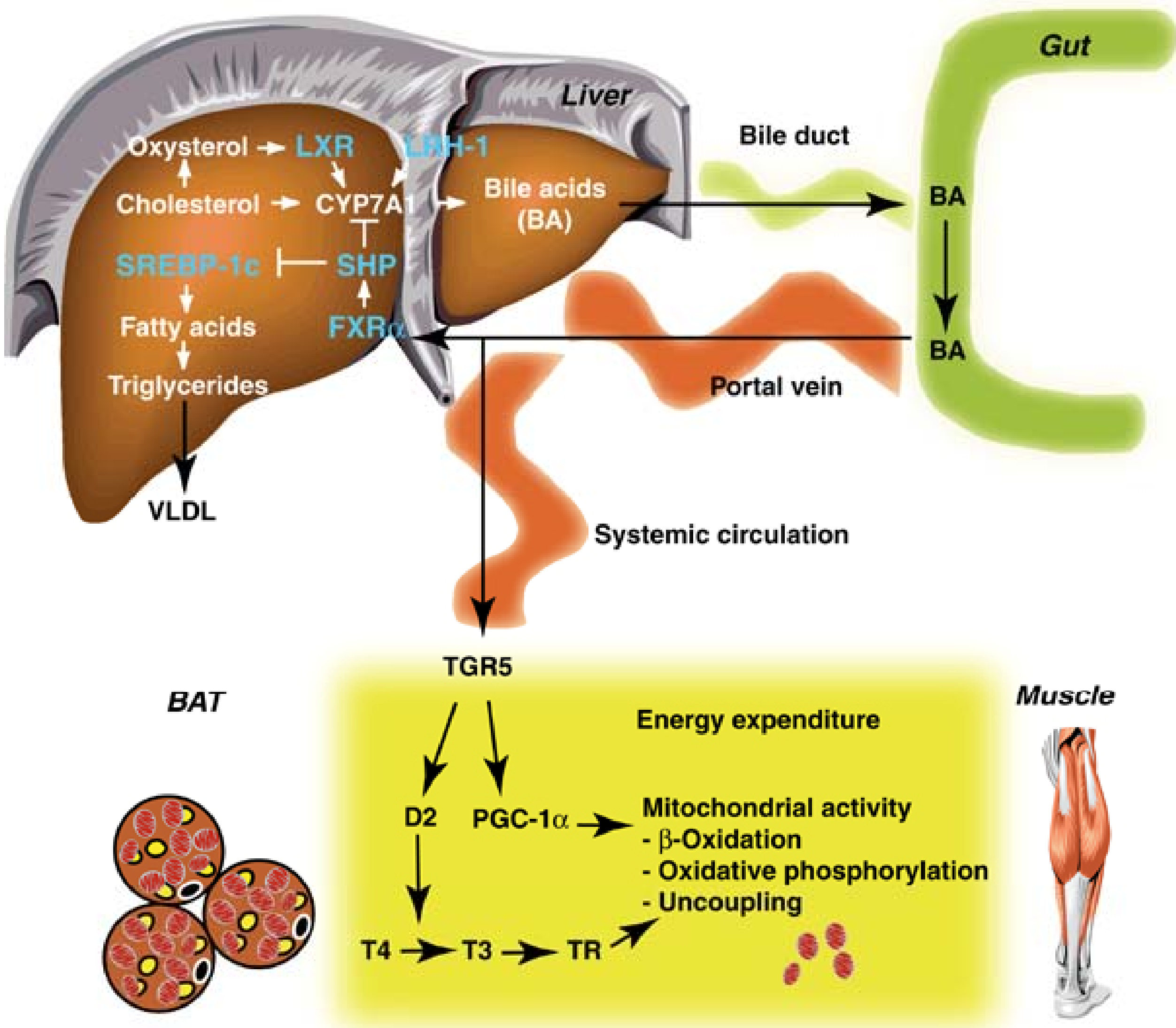


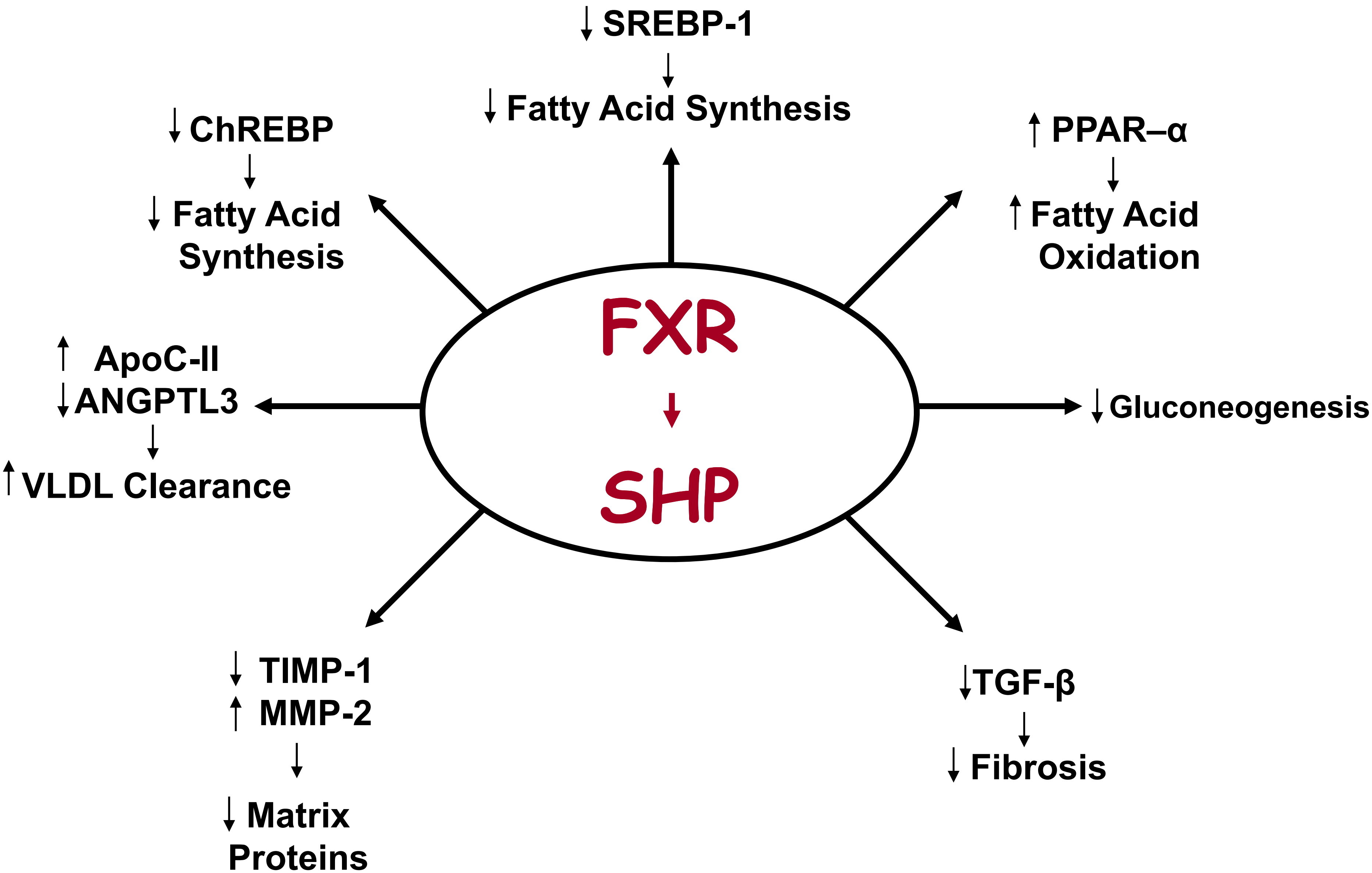
Fiorucci, et al. Trends in Molecular Medicine. 13(7):298-309, 2007.

FXR Regulates Lipid and Glucose Metabolism



FXR Regulates Energy Metabolism



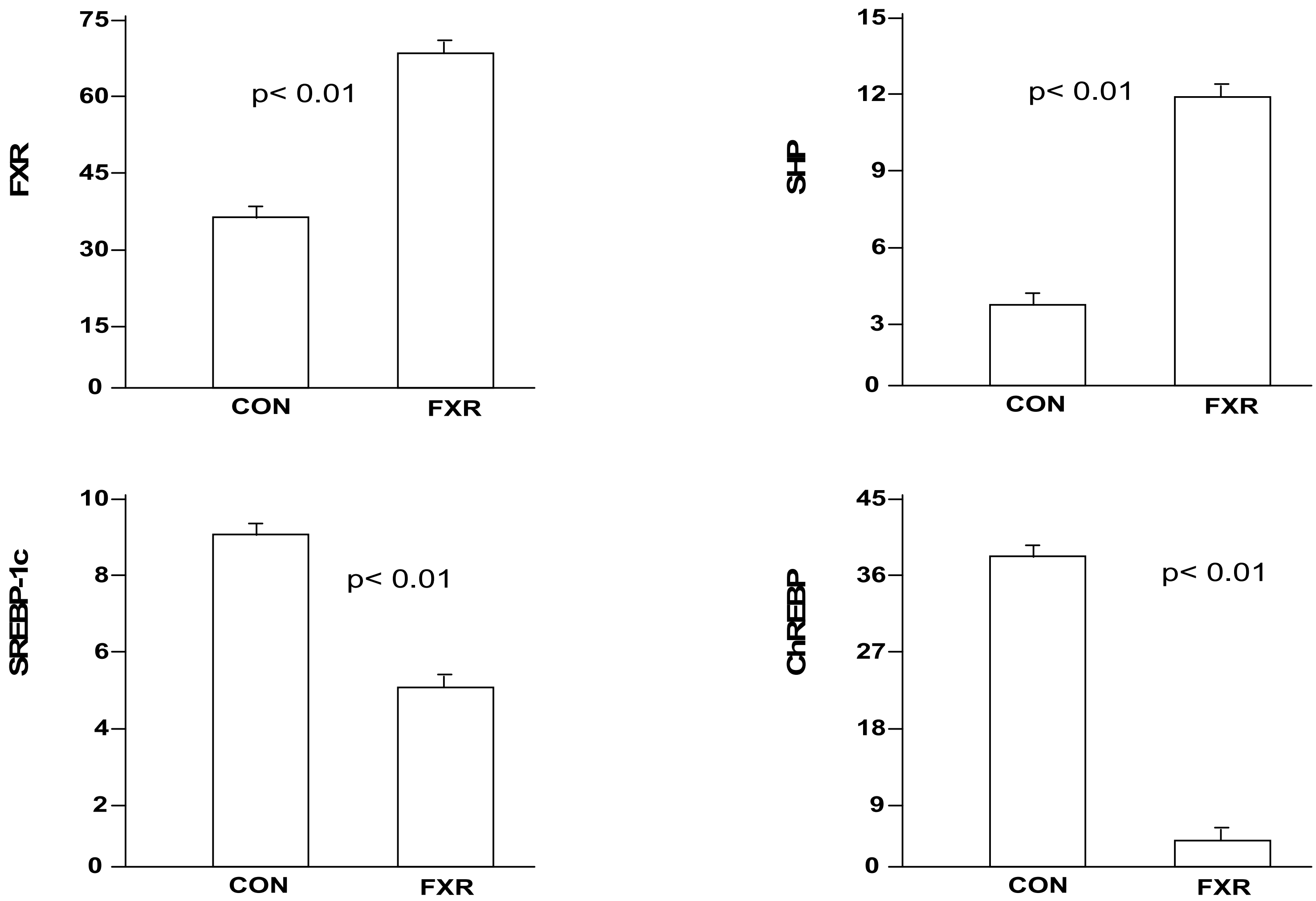

↓ SREBP-1
↓ Fatty Acid Synthesis
↓ ChREBP
↓ Fatty Acid Synthesis
↑ PPAR–α
↑ Fatty Acid Oxidation
↑ ApoC-II
↓ ANGPTL3
↑ VLDL Clearance
FXR
SHP
↓ Gluconeogenesis
↓ TIMP-1
↑ MMP-2
↓ Matrix Proteins
↓ TGF-β
↓ Fibrosis

Role of FXR in the Kidney

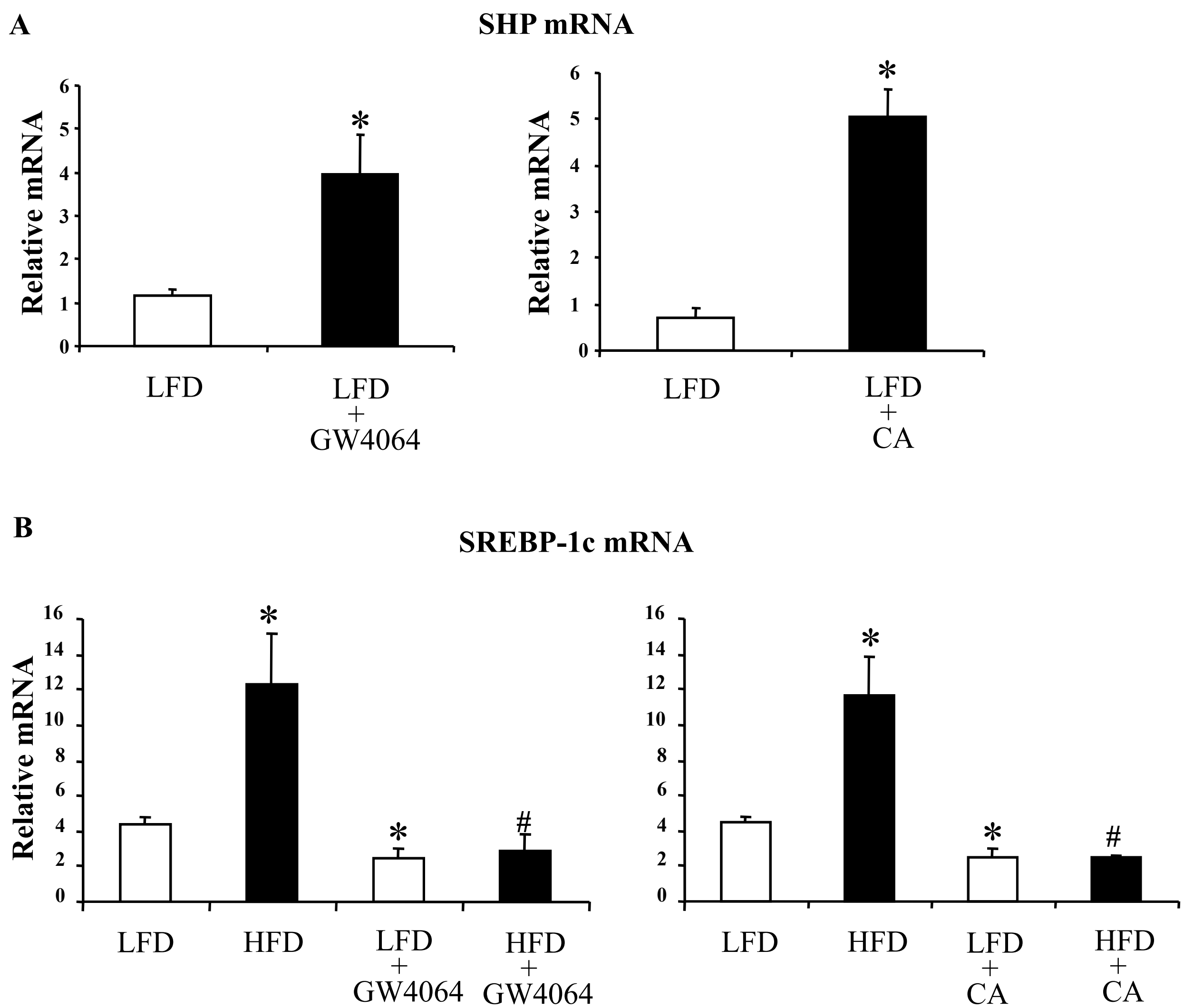
- Diet Induced Obesity Mice treated with FXR agonists
- Diabetic (db/db) Mice treated with FXR agonists

C57Bl/6 Mice fed a high fat diet (Diet Induced Obesity and Insulin Resistance) treated with FXR Agonists

Treatment of mice with FXR agonist GW4064 induces FXR and SHP and downregulates SREBP-1c and ChREBP mRNA



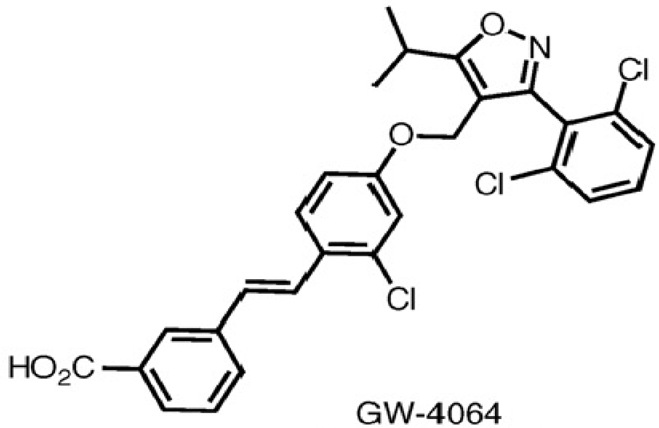
Treatment of mice with FXR agonists induce SHP and downregulate SREBP-1c



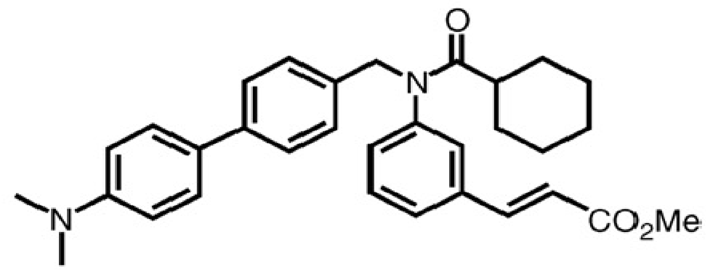
Type 2 diabetic db/db mice treated with FXR agonists

Short-term Studies with GW4064: a synthetic activating ligand of FXR

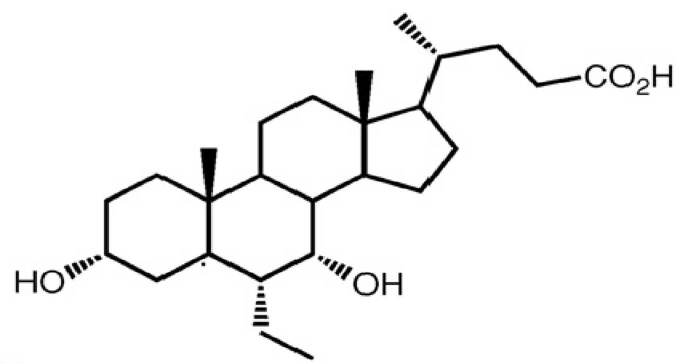
Synthetic FXR ligands



GW-4064
$EC_{50} = 0.037\ \mu M$



Fexaramine
$EC_{50} = 0.025\ \mu M$



6α-ECDCA
$EC_{50} = 0.078\ \mu M$

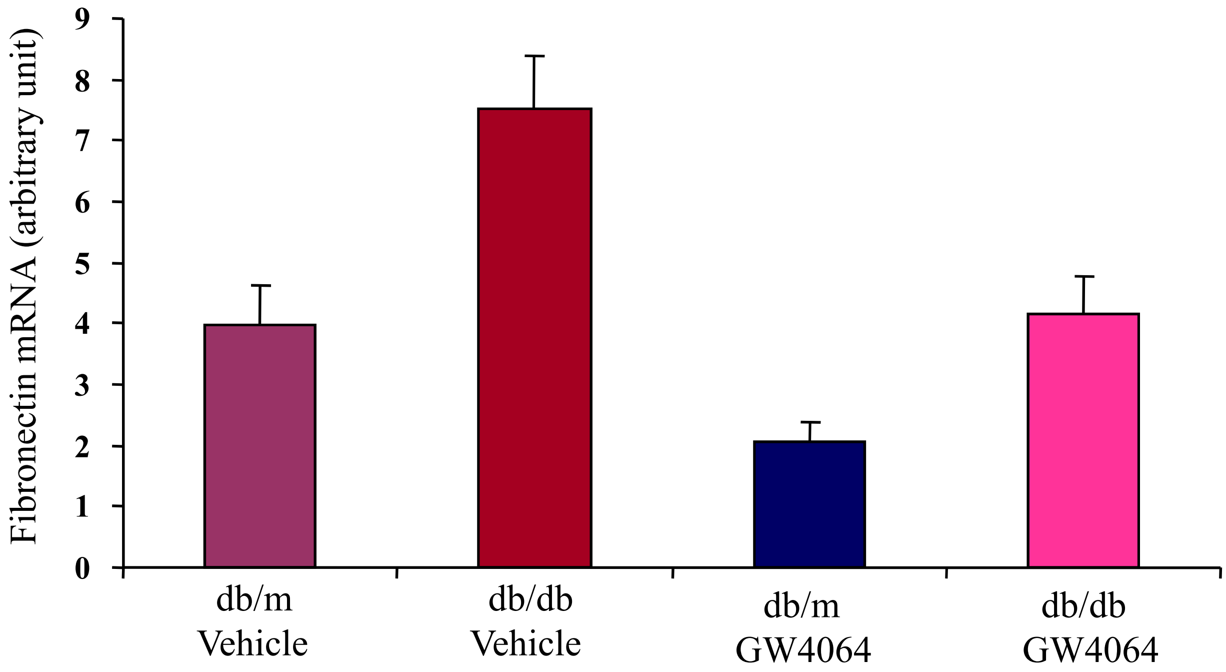
Fiorucci, et al. Trends in Molecular Medicine. 13(7):298-309, 2007.

Effects of 1-wk GW4064 treatment in C57BLKS/J db/db mice

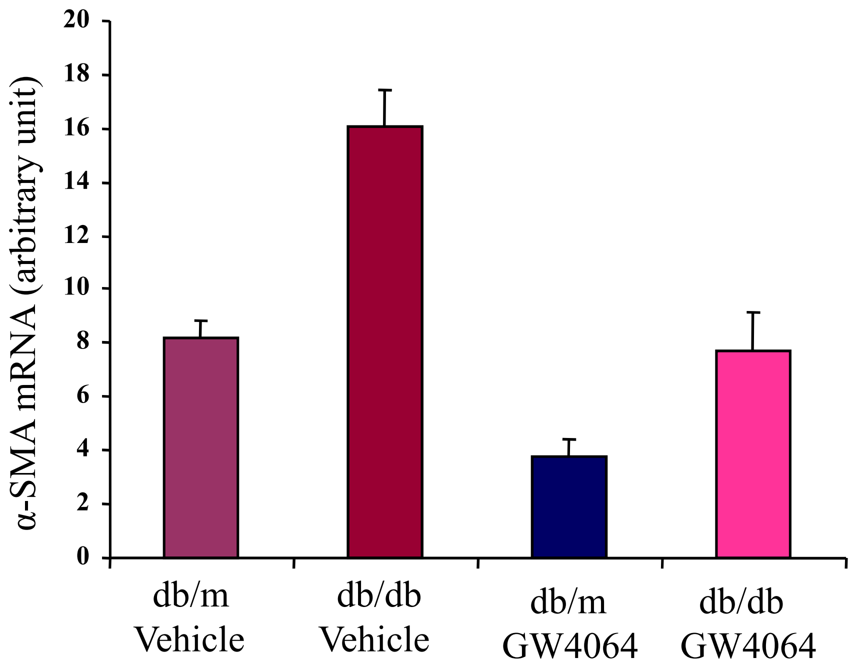
	db/m	db/db	db/m + GW4064	db/db+ GW4064
Body weight (g)	**23.45±0.53**	**34.45±0.95[a]**	**22.19±0.42**	**33.64±1.48**
Blood glucose (mg/dl)	**139.2±9.0**	**475.7±11.5[a]**	**126.5±9.1**	**447.8±22.9**
Plasma triglyceride (mg/dl)	**41.76±8.41**	**47.03±4.45**	**36.94±6.47**	**48.34±4.49**
Plasma cholesterol (mg/dl)	**92.26±6.88**	**105.28±5.49**	**53.51±2.39[a]**	**73.52±2.57[b]**

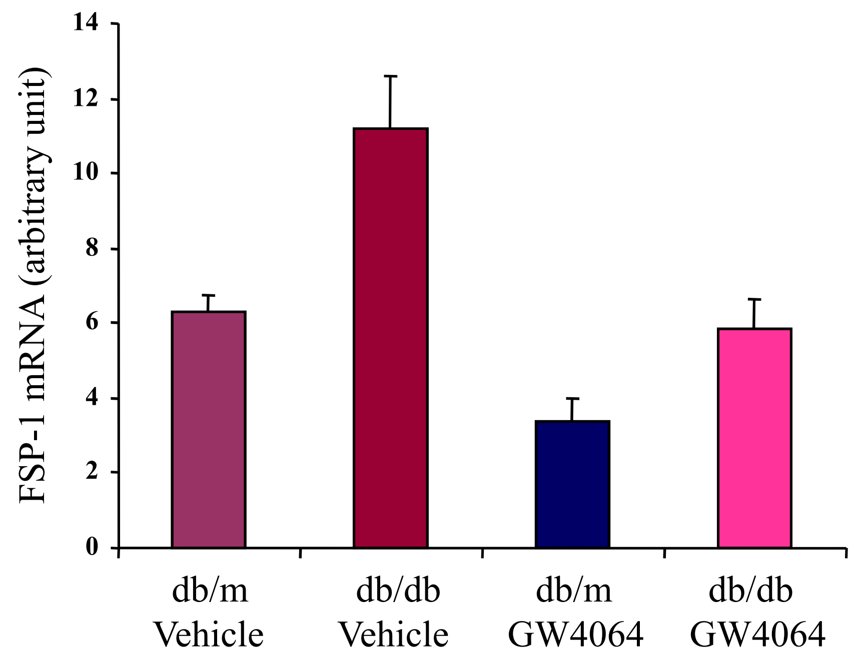
Data are means ± SE (n=8 mice in each group): a= $p < 0.01$ vs. db/m; b= $p < 0.01$ vs. db/db.

Treatment of db/db mice with GW4064 resulted in significant attenuation of extracellular matrix protein

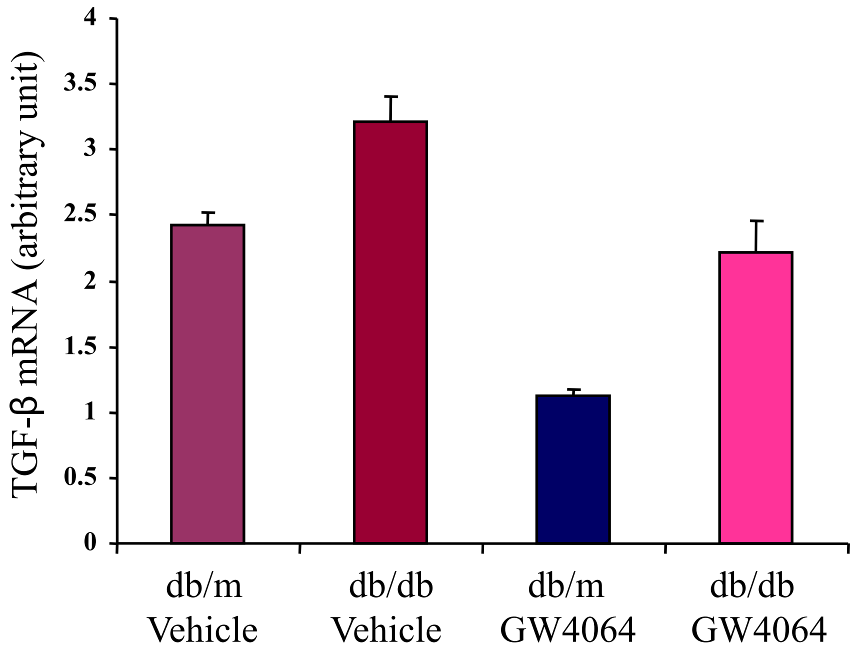


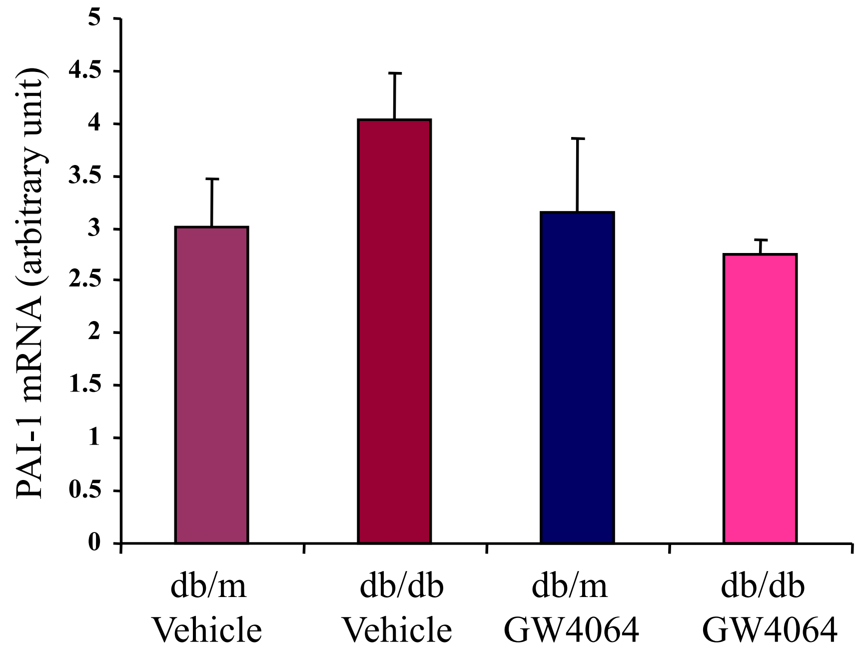
Treatment of db/db mice with GW4064 resulted in decrease in accumulation of kidney fibrosis markers α-SMA and FSP-1



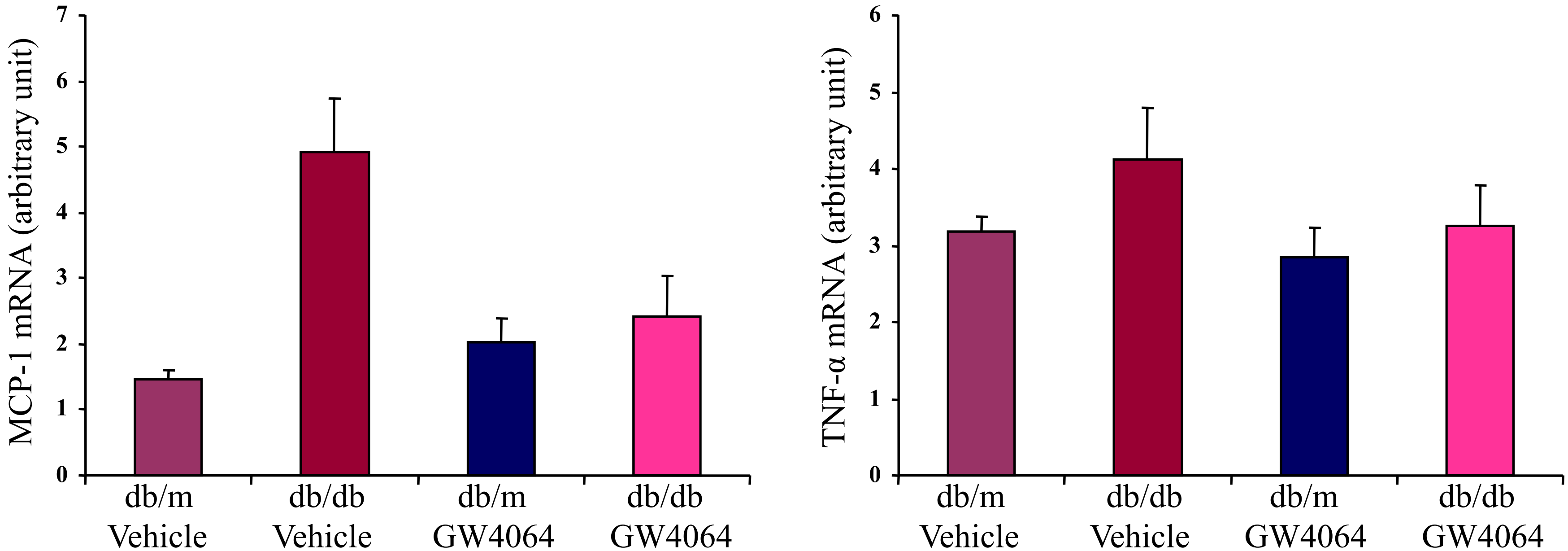


Treatment of db/db mice with GW4064 resulted in decrease in profibrotic growth factors including TGF-β and PAI-1

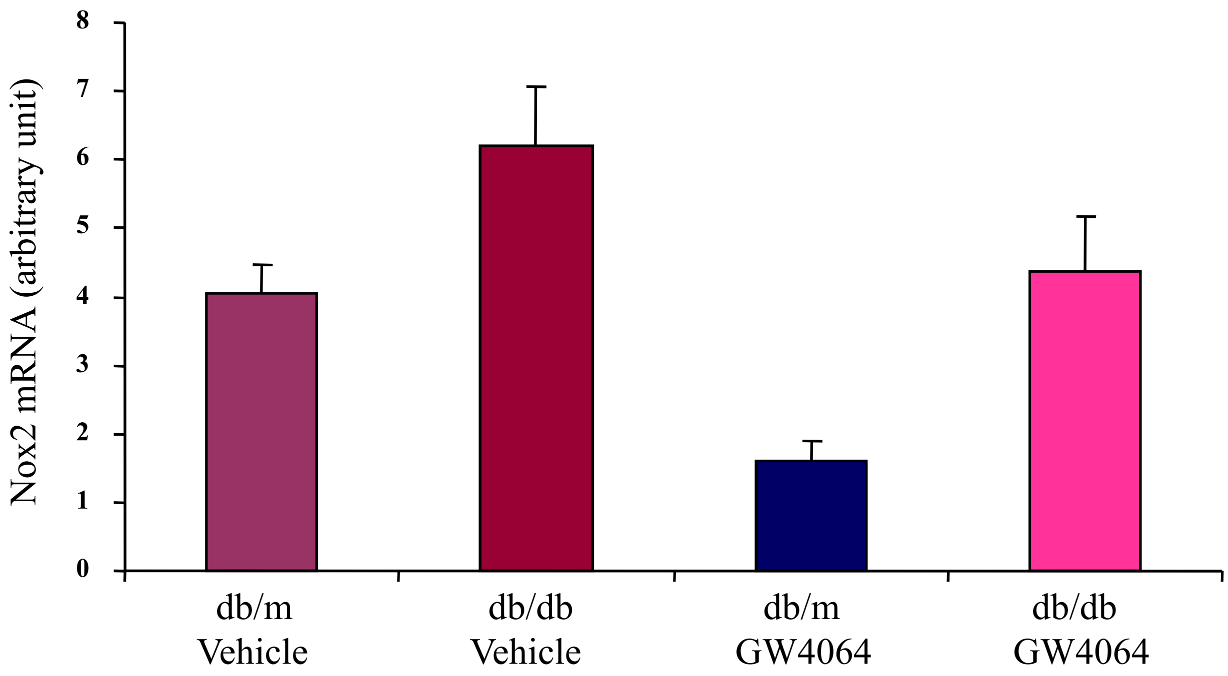




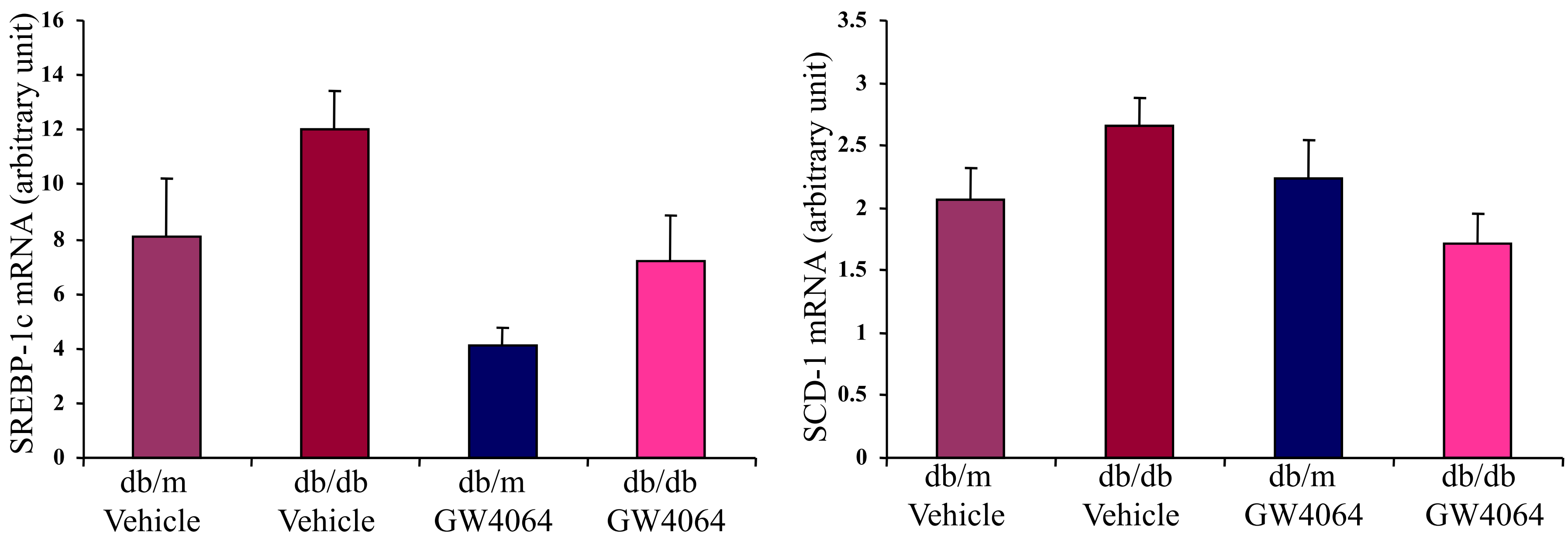
Treatment of db/db mice with GW4064 resulted in decrease in proinflammatory cytokines including MCP-1 and TNF-α



Treatment of db/db mice with GW4064 resulted in decrease in NADPH Oxidase expression including Nox2

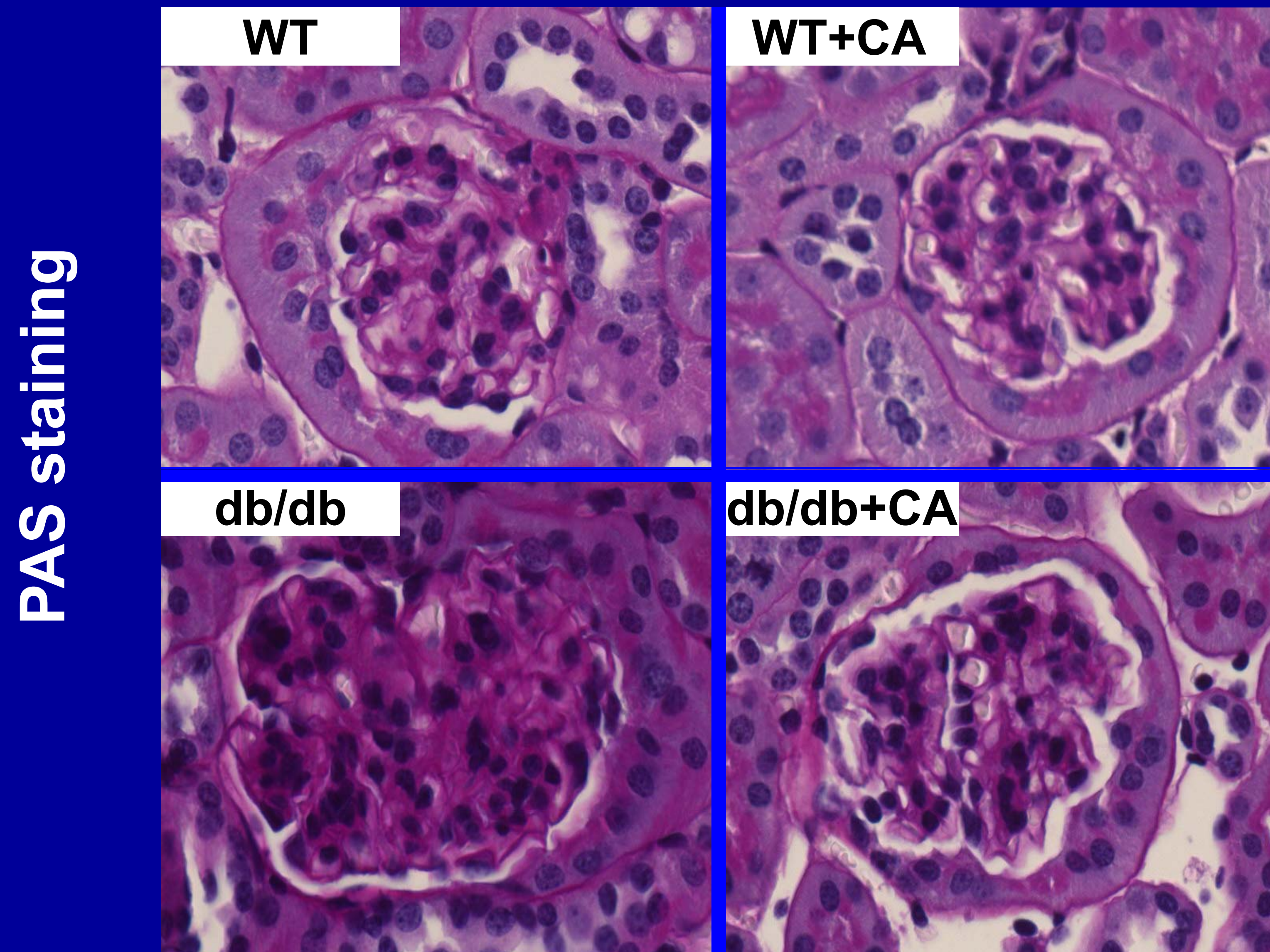


Treatment of db/db mice with GW4064 resulted in decrease fatty acid synthesis pathways including SREBP-1c and SCD-1



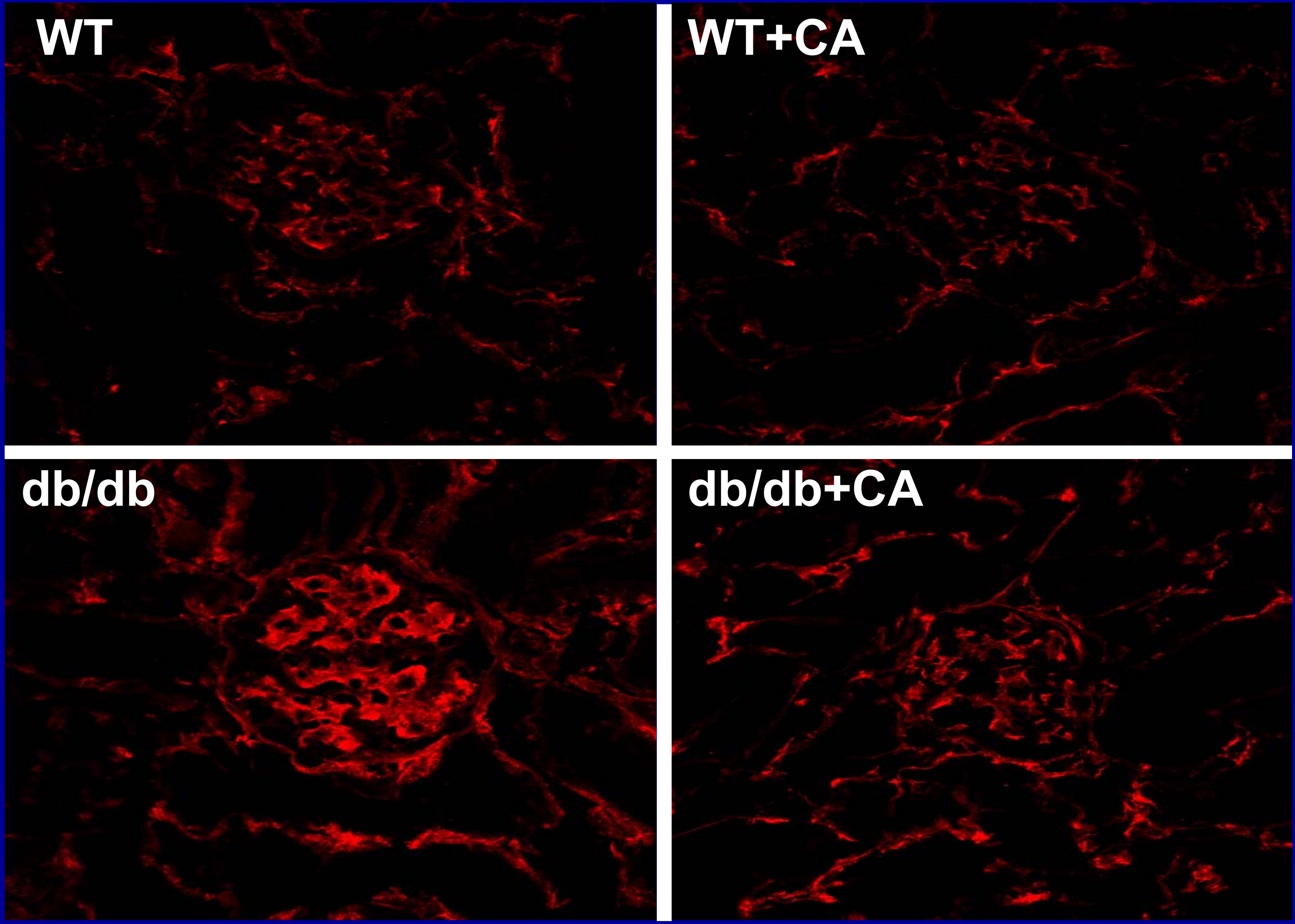
Long-term Studies with cholic acid: a natural activating ligand of FXR

Treatment of db-db mice with cholic acid resulted in inhibition of mesangial expansion

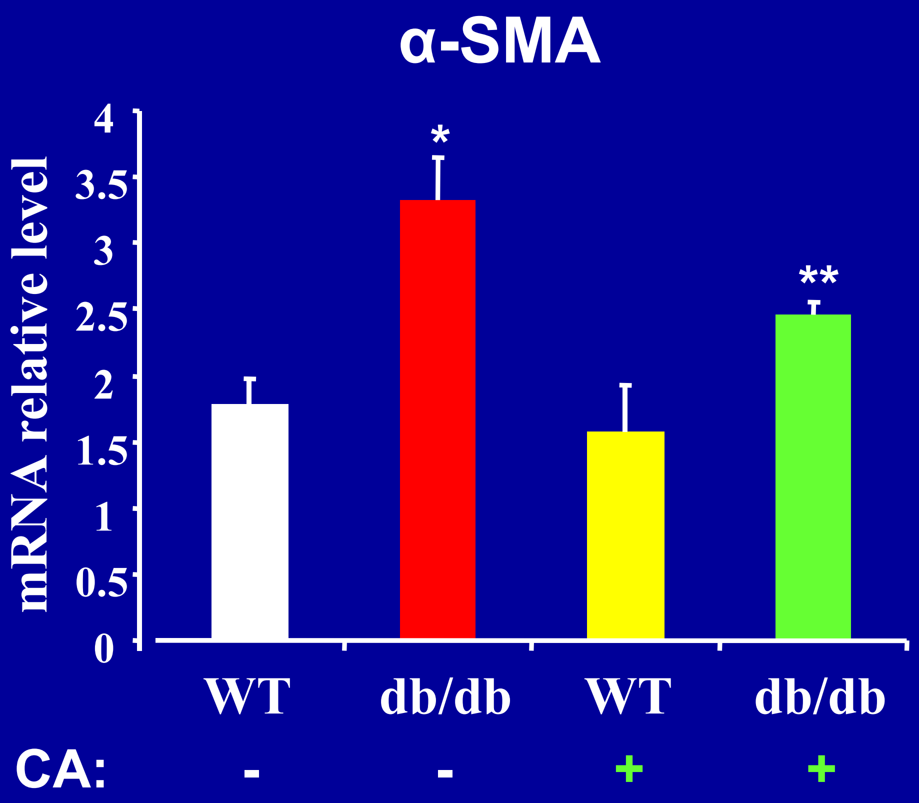


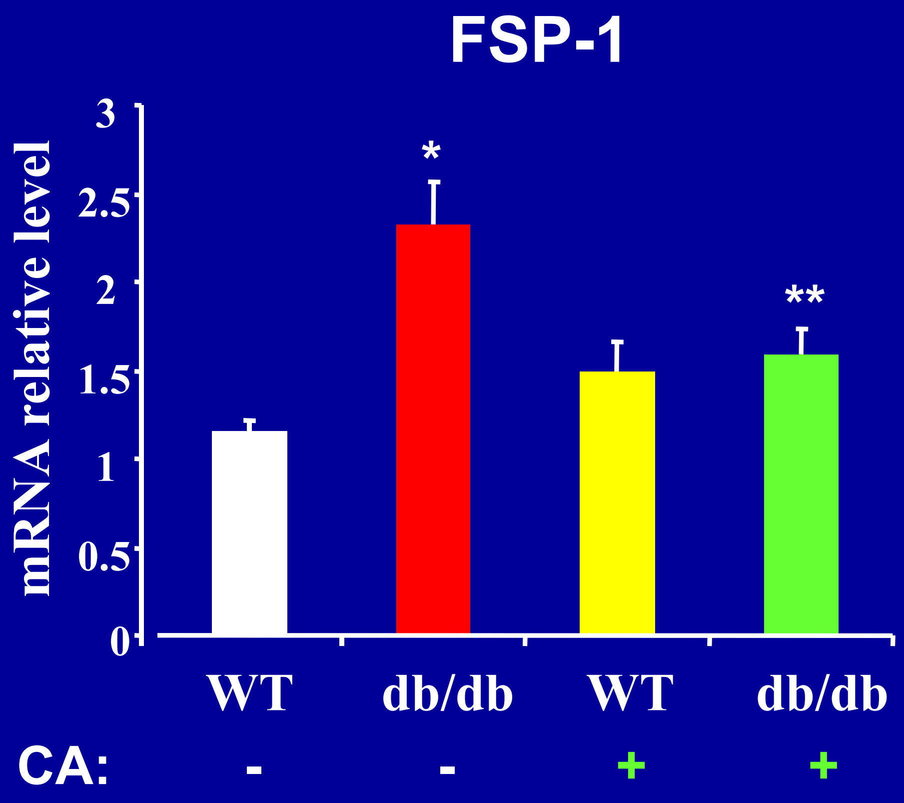
Treatment of db/db mice with cholic acid resulted in decreased accumulation of extracellular matrix proteins

Fibronectin Immunostaining



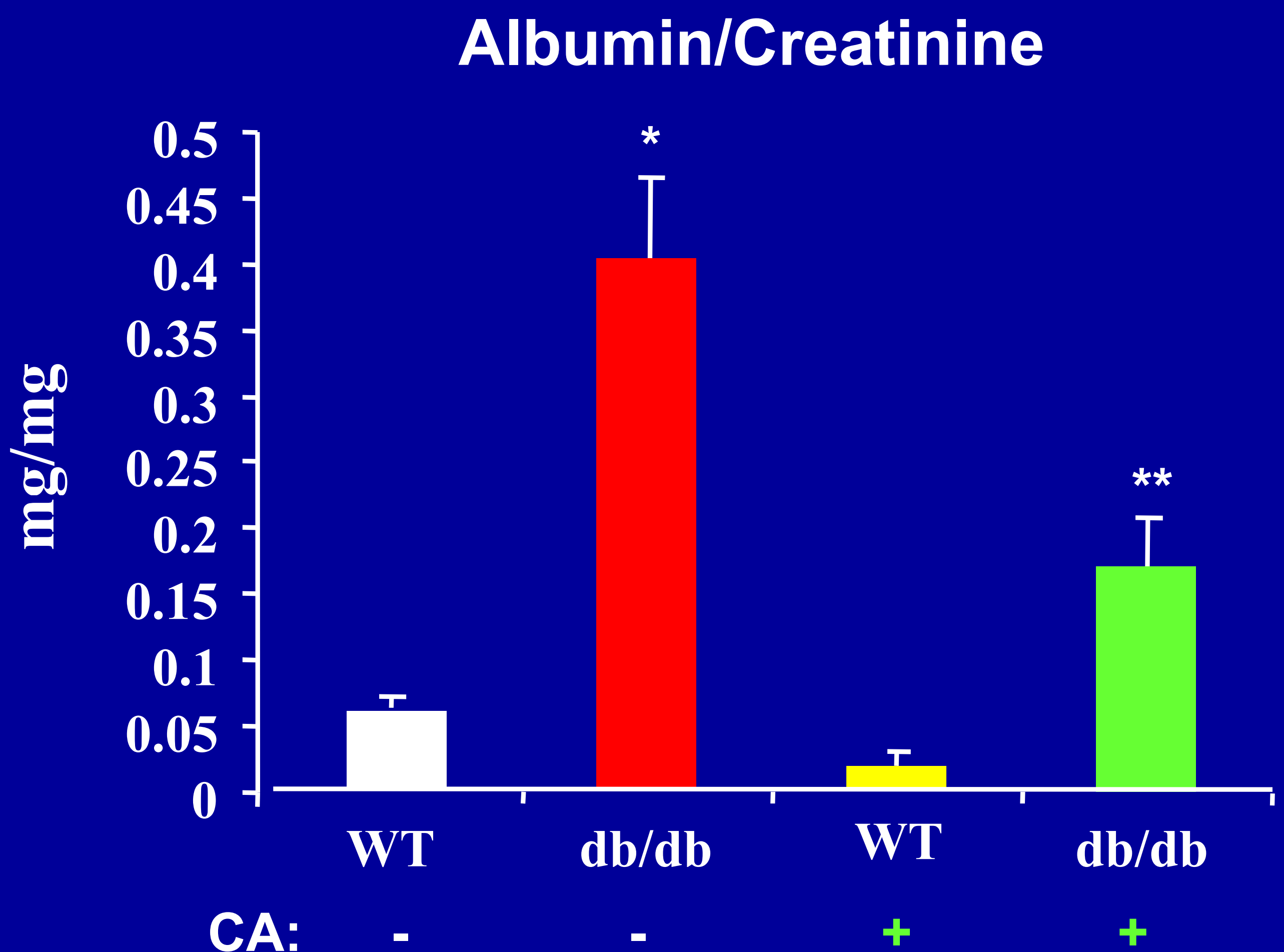
Treatment of db/db mice with cholic acid resulted in decreased expression of kidney fibrosis markers





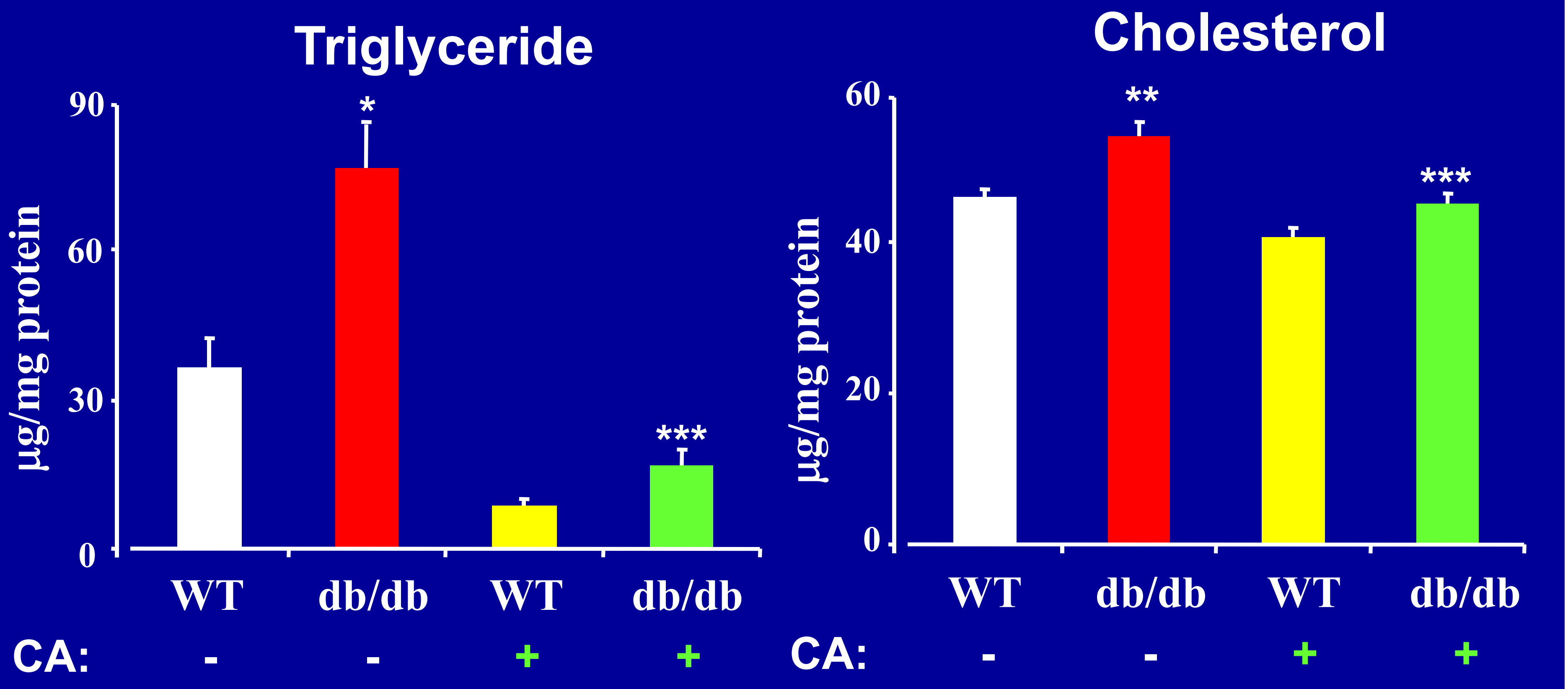
* $p<0.05$ vs. WT; ** $p<0.05$ vs. db/db

Treatment of db/db mice with cholic acid resulted in decreased proteinuria



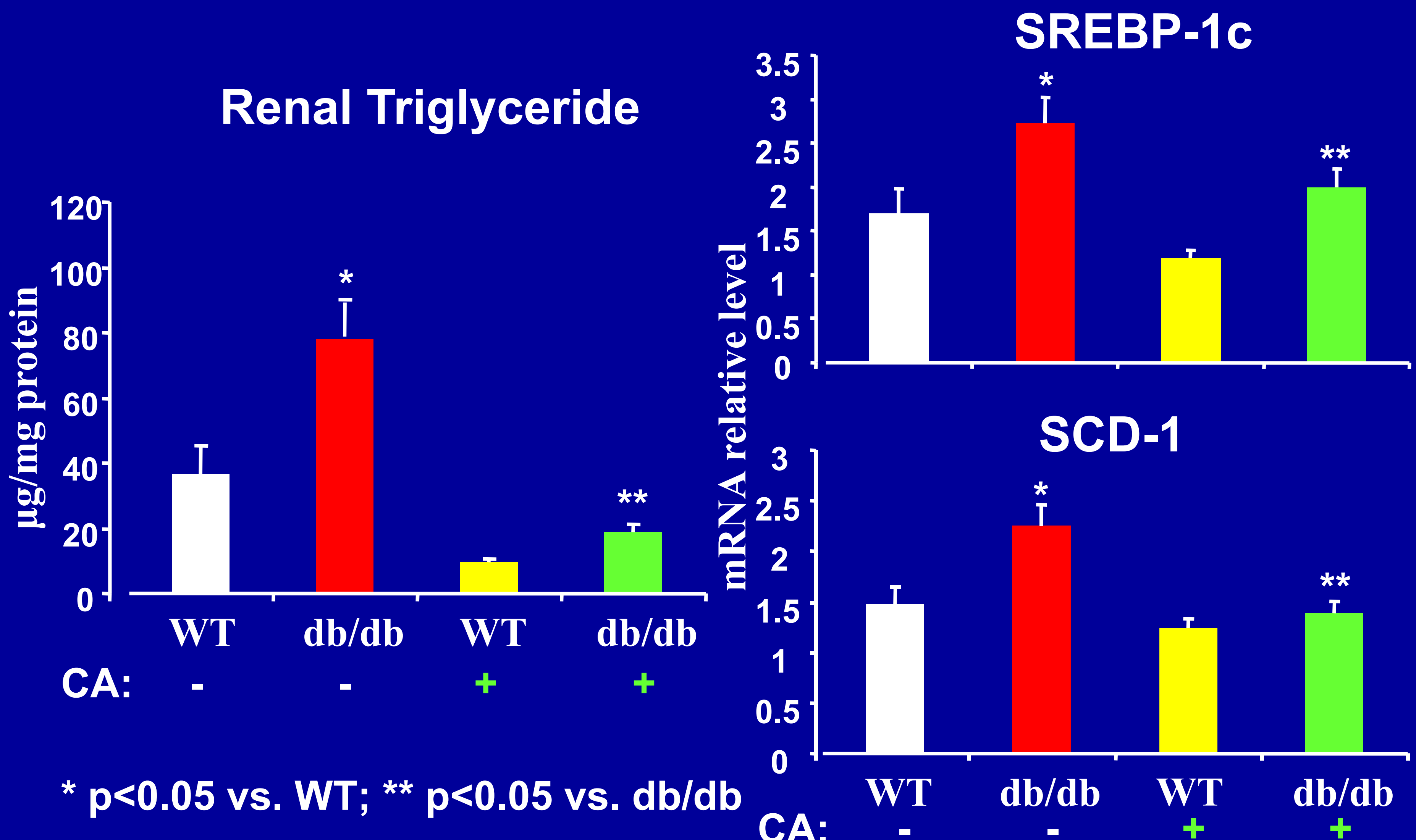
* p<0.05 vs. WT; ** p<0.05 vs. db/db

Treatment of db/db mice with cholic acid resulted in decreased kidney triglyceride and cholesterol accumulation



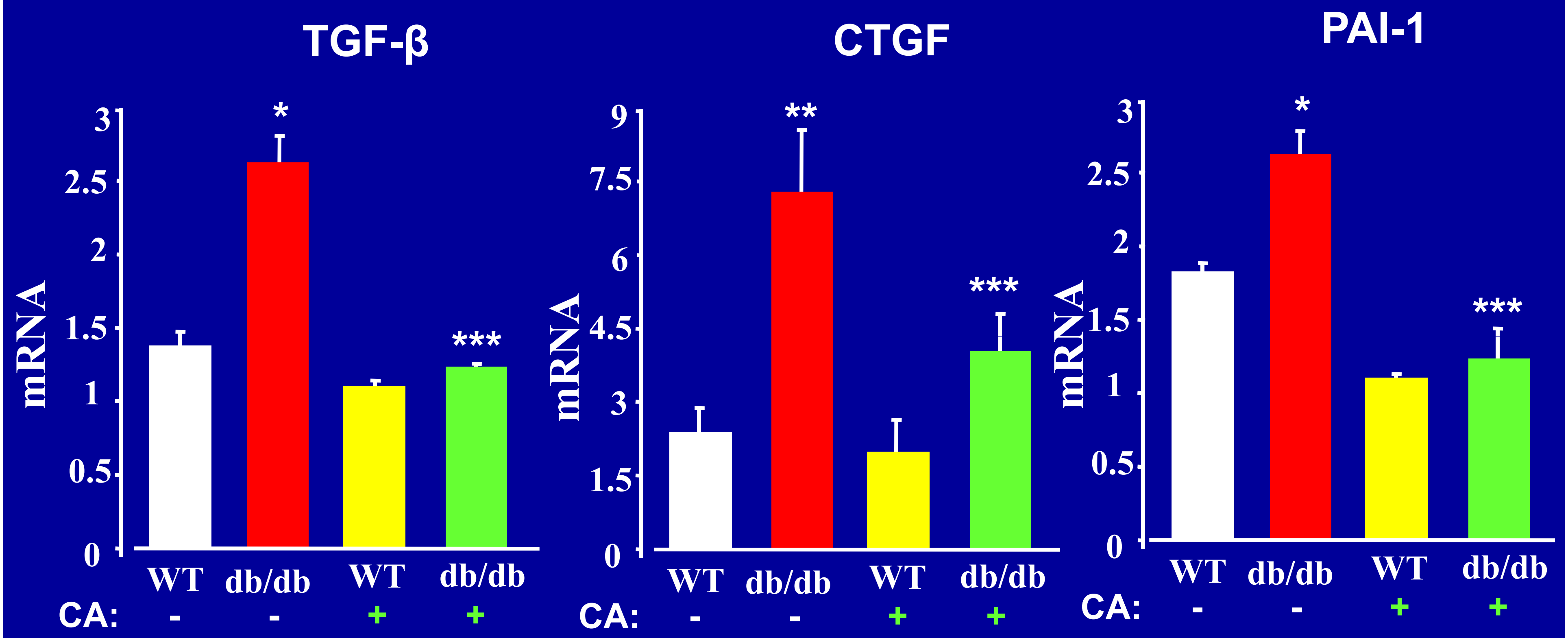
* p<0.05 vs. WT; ** p<0.01 vs. WT; *** p<0.05 vs. db/db

Treatment of db/db mice with cholic acid resulted in inhibition of pathways that mediate fatty acid and triglyceride synthesis



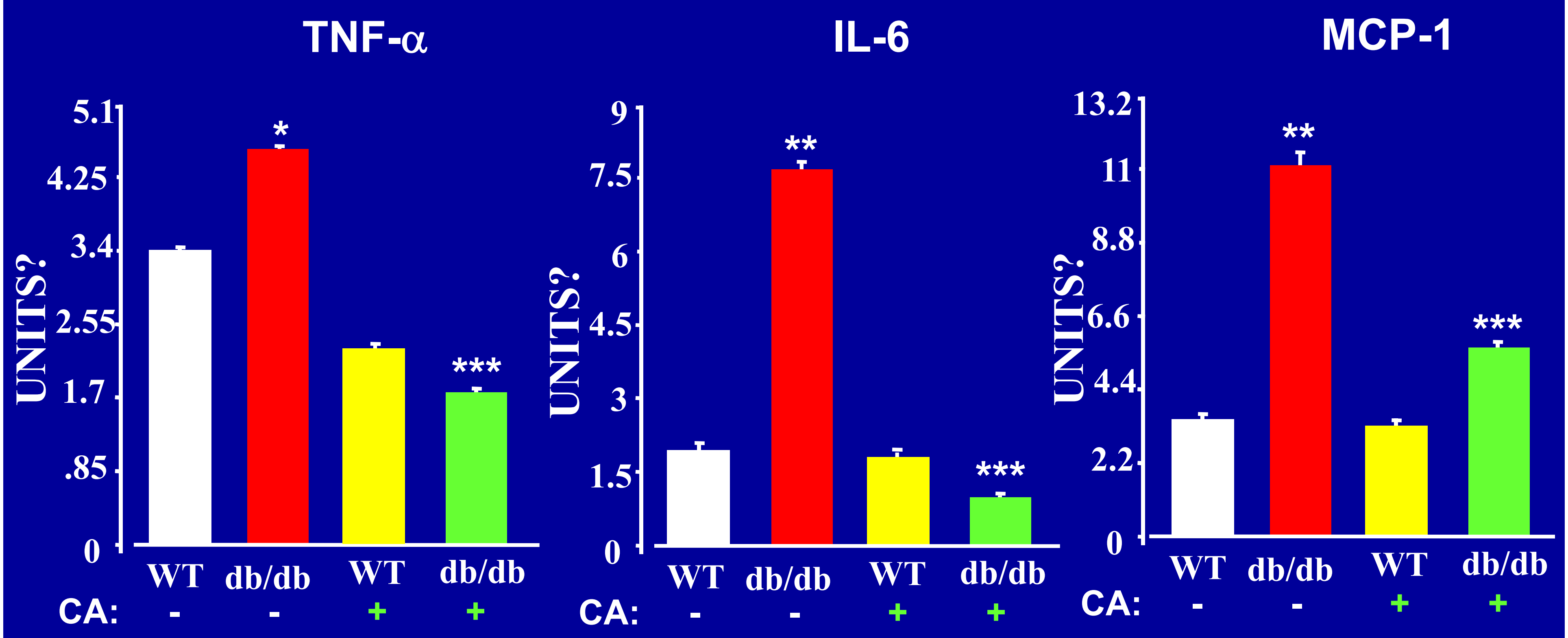
* p<0.05 vs. WT; ** p<0.05 vs. db/db

Treatment of db/db mice with cholic acid resulted in decreased renal expression of profibrotic growth factors



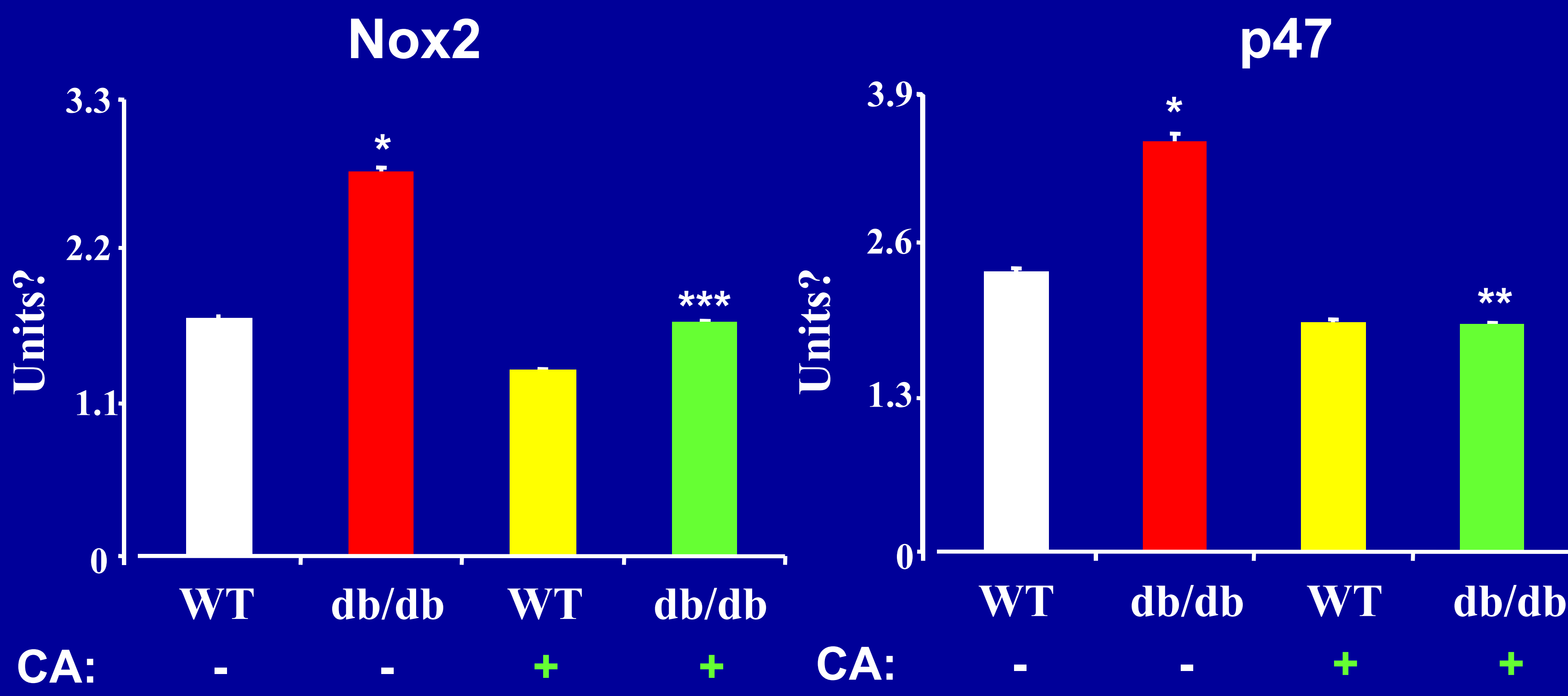
* p<0.05 vs. WT; ** p<0.01 vs. WT; *** p<0.01 vs. db/db

Treatment of db/db mice with cholic acid resulted in decreased renal expression of proinflammatory cytokines



* p<0.05 vs. WT; ** p<0.01 vs. WT; *** p<0.01 vs. db/db

Treatment of db/db mice with cholic acid resulted in decreased renal expression of NADPH Oxidase

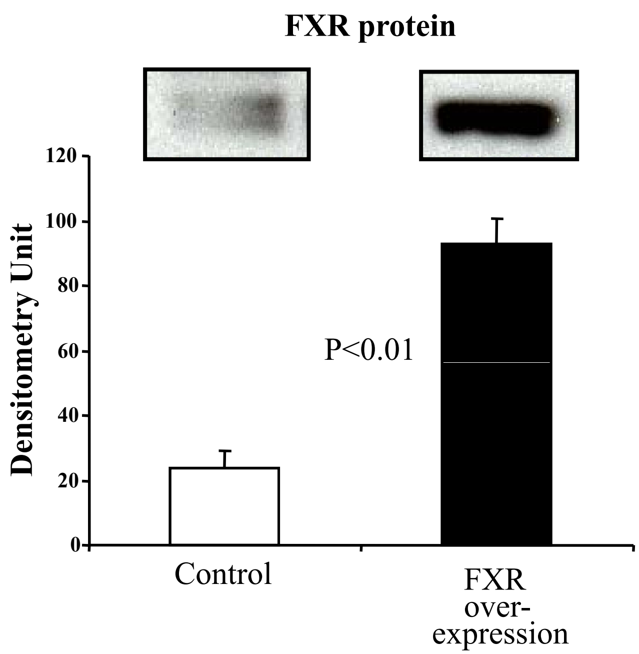


* p<0.05 vs. WT; ** p<0.01 vs. db/db; *** p<0.05 vs. db/db

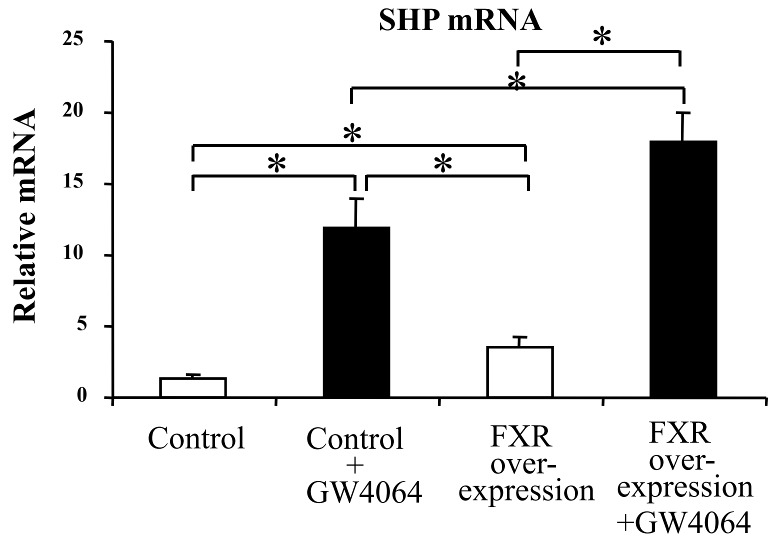
Is there a direct effect of FXR independent of systemic effects to modulate renal response to diabetes?

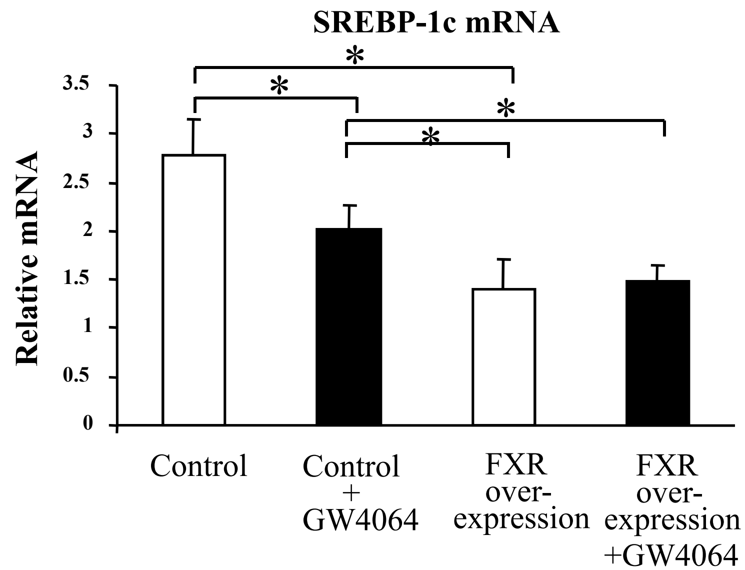
Mesangial Cells overexpressing FXR

Increased expression of FXR in mouse mesangial cells



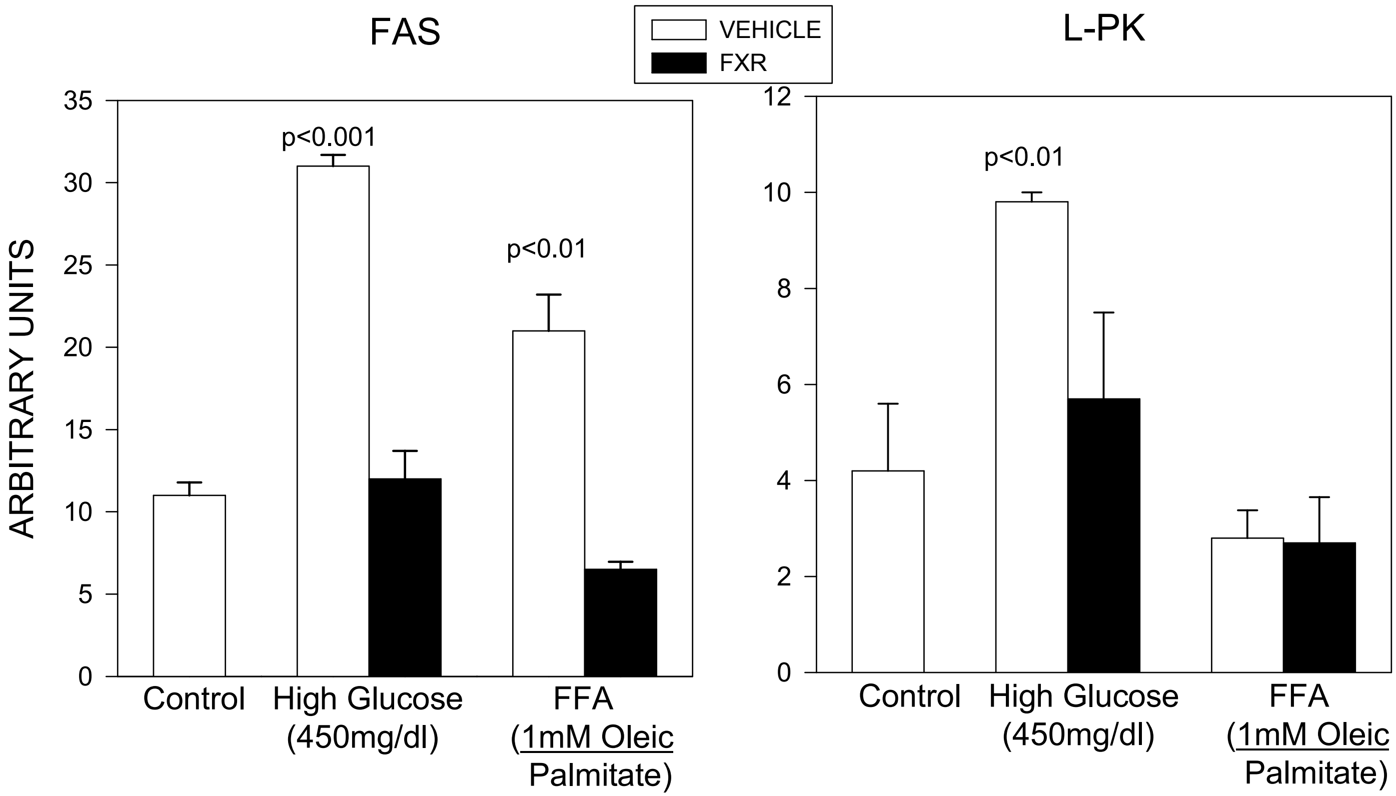
Increased expression of FXR in mouse mesangial cells induce SHP and inhibit SREBP-1c



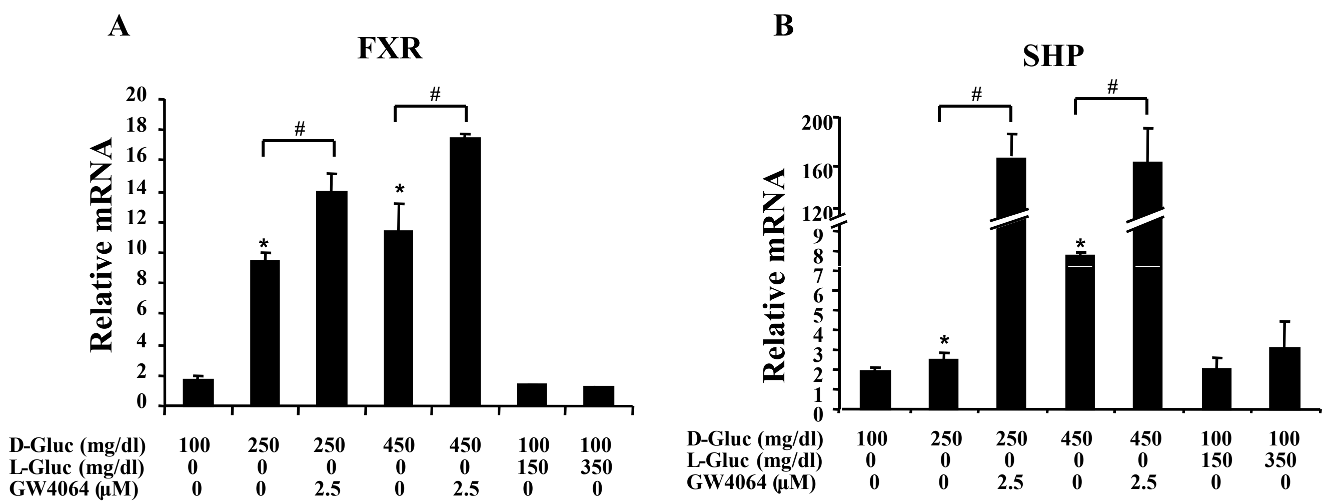


Mesangial Cells treated with FXR agonists

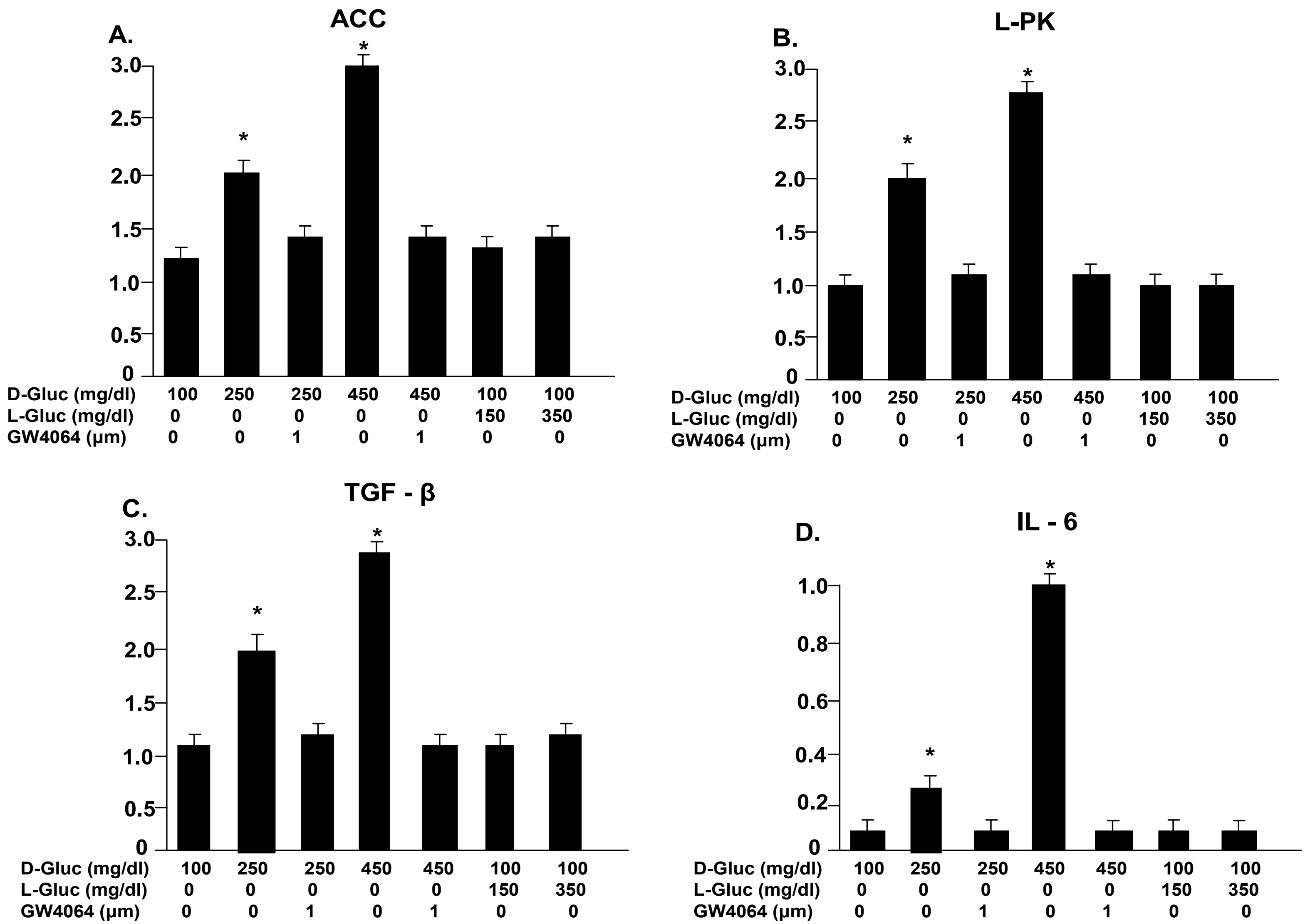
Treatment of mesangial cells with a selective FXR agonist prevents glucose and FFA induced upregulation of FAS and glucose induced upregulation of L-PK



In mouse mesangial cells glucose causes upregulation of SHP and FXR: treatment with GW4064 induces further upregulation of SHP and FXR



FXR agonist prevents glucose induced upregulation of fatty acid synthesis, TGF-β, and IL-6



Summary

Diabetes

FXR agonist

Lipids

Profibrotic growth factors

Proinflammatory cytokines

Oxidative stress

Fibrosis

Proteinuria

Diabetic Kidney Disease

Future Studies

- Generalized FXR knockout or transgenic mice on the FVB or DBA/2J genetic background
- Cross breed with Akita mice or db/db mice on the FVB or DBA/2J background

Future Studies

- Kidney podocyte or proximal tubule specific FXR knockout or transgenic mice on the FVB or DBA/2J genetic background
- Cross breed with Akita mice or db/db mice on the FVB or DBA/2J background

Future Studies: FXR agonist studies in

- Type 1 Diabetes
- Diet Induced Obesity and Insulin Resistance (Metabolic Syndrome)
- Type 2 Diabetes
- Aging

Thank you