

Vascular Inflammation
and
Peroxisome Proliferator-Activated Receptors
(PPAR)

Hyo-Soo Kim, MD, PhD

Cardiovascular Laboratory, Clinical Research Institute

Cardiovascular Center, Seoul National University Hospital

Contents

[1] Introduction

action mechanisms

vascular inflammation

[2] PPAR- γ

basic data

clinical data

mechanism to reduce neointima

[3] PPAR- α

[4] Perspective & Conclusion

PPAR

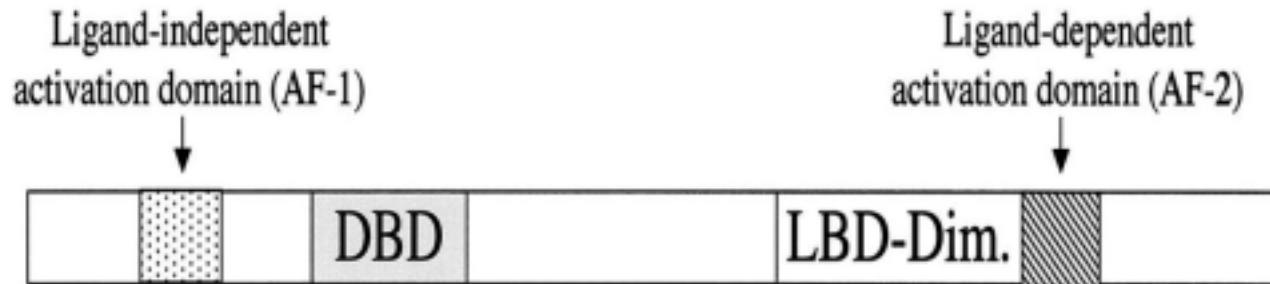
- originally cloned during identifying peroxisome proliferation in the liver of rodents.
(*Nature*. 1990)
- activate transcription factors
- belong to the **nuclear receptor** superfamily

Classification of PPAR

- PPAR family
 - , and /
 - 60~80% homology
 - mostly activated by **fatty acid**
- PPAR
 - agonist : oxidized phospholipid, **fibrate**, gemfibrozil...
- PPAR
 - agonist : 15d-PGJ₂, **thiazolidinedione**...
- PPAR /
 - involved in lipid metabolism, obesity, insulin resistance...

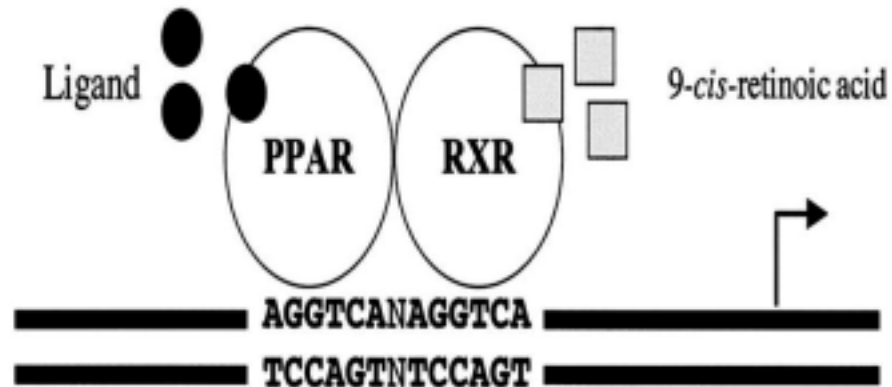
Action Mechanism (1)

- binding as heterodimer with RXR



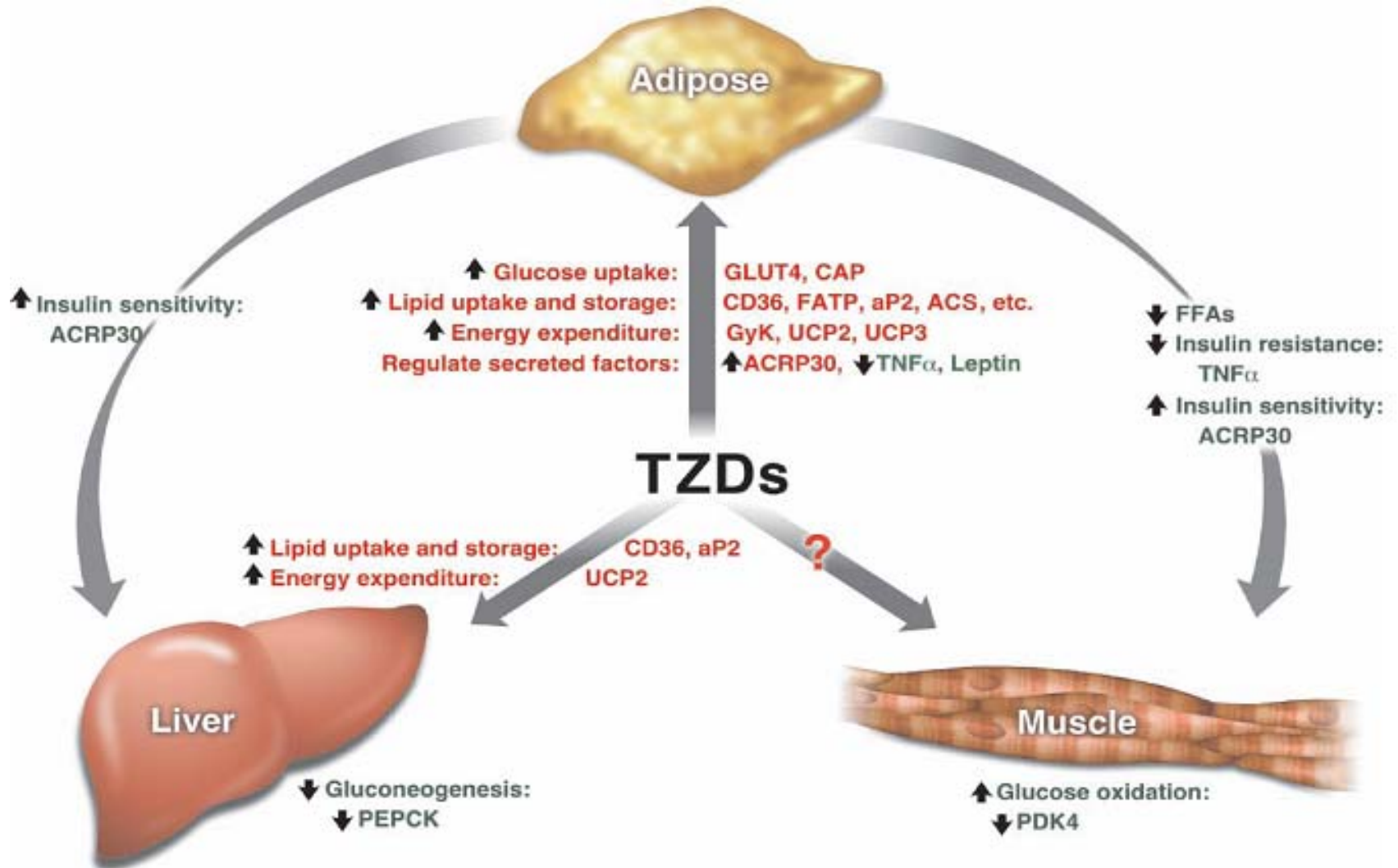
DNA
Binding Domain
(2 zinc fingers)

Ligand Binding and
Dimerization Domains

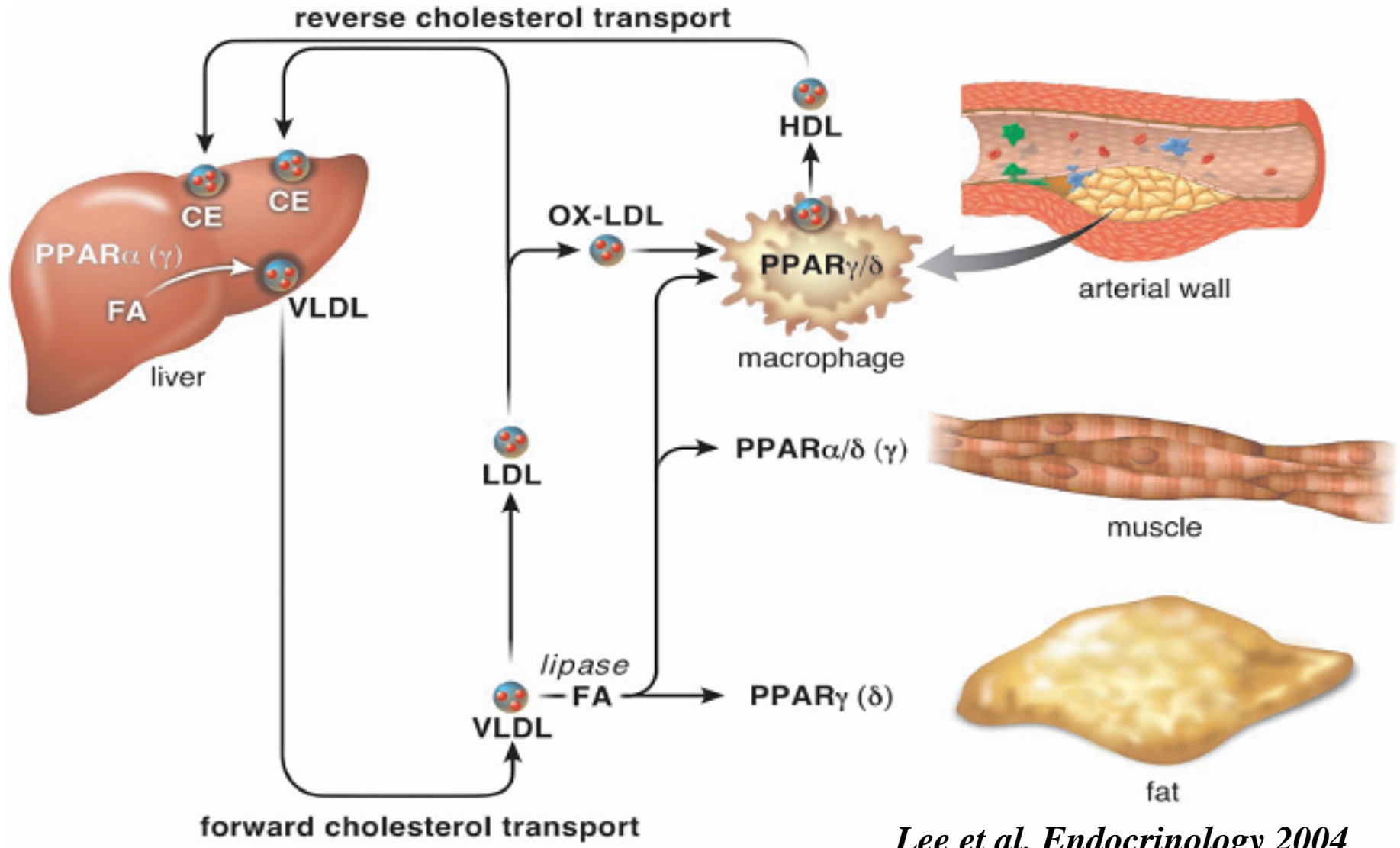


Action Mechanism (2)

- effects of PPAR agonist on glucose metabolism

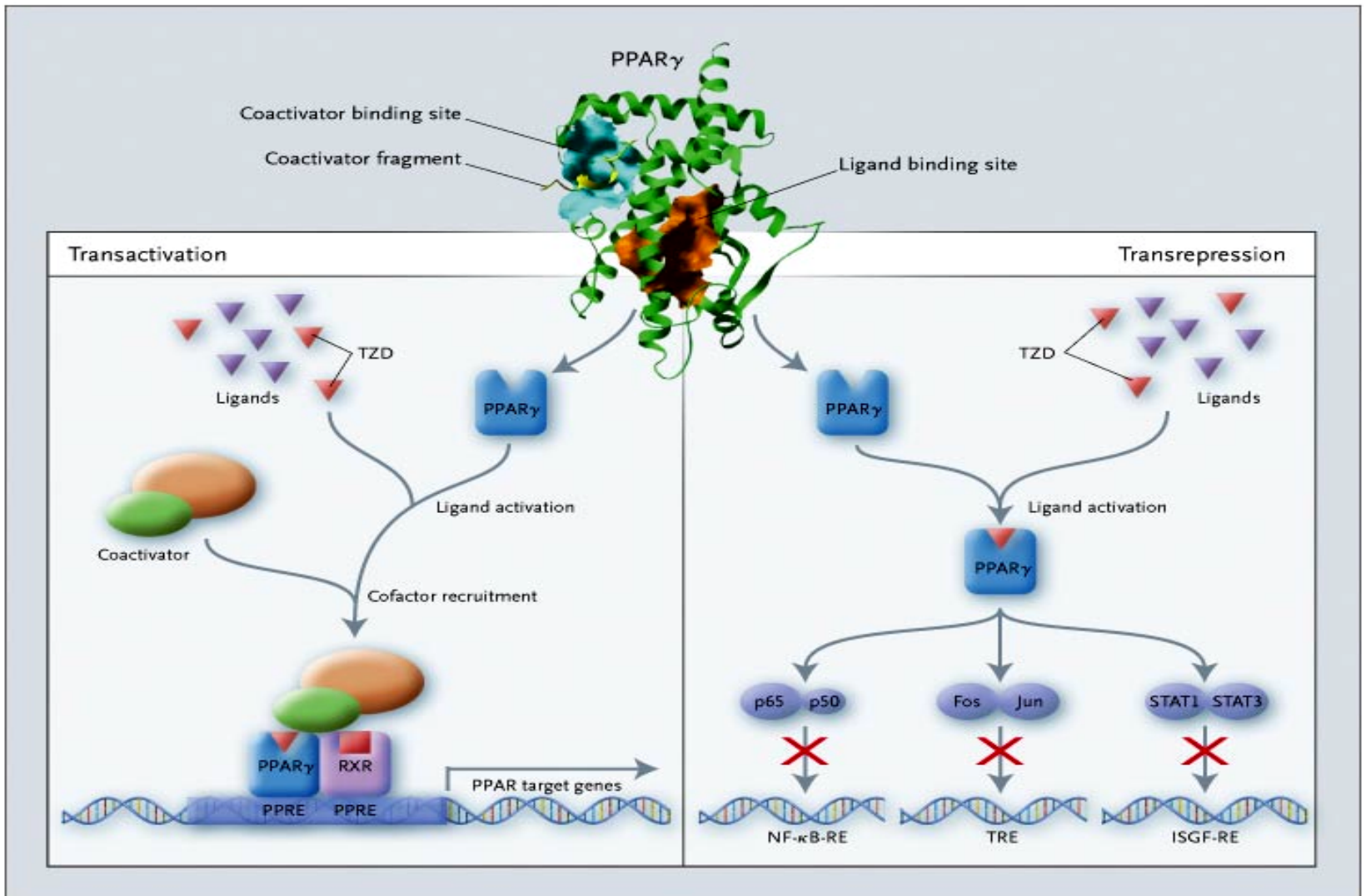


Action Mechanism (3) lipid metabolism



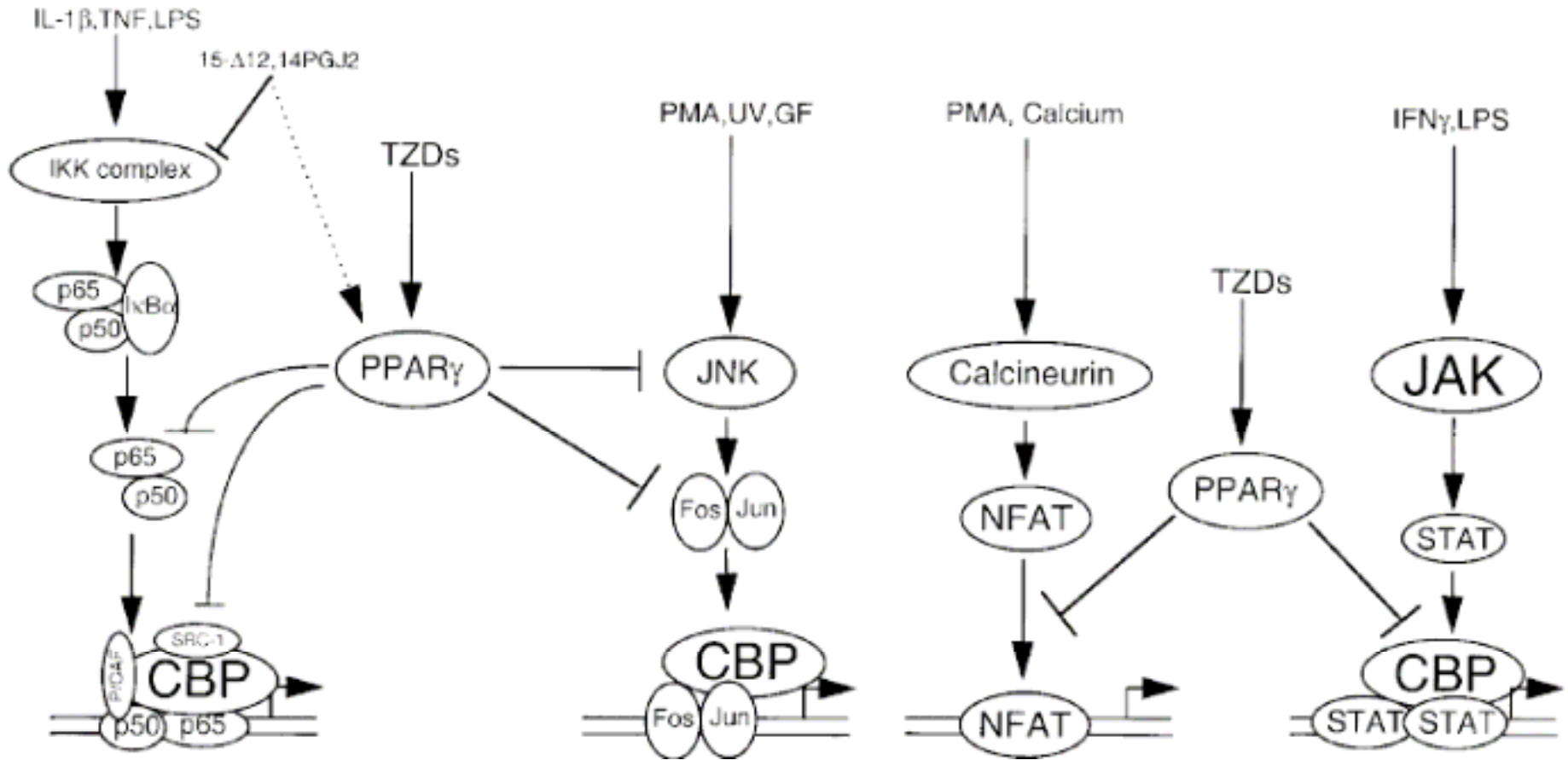
Action Mechanism (4)

– transactivation & transrepression



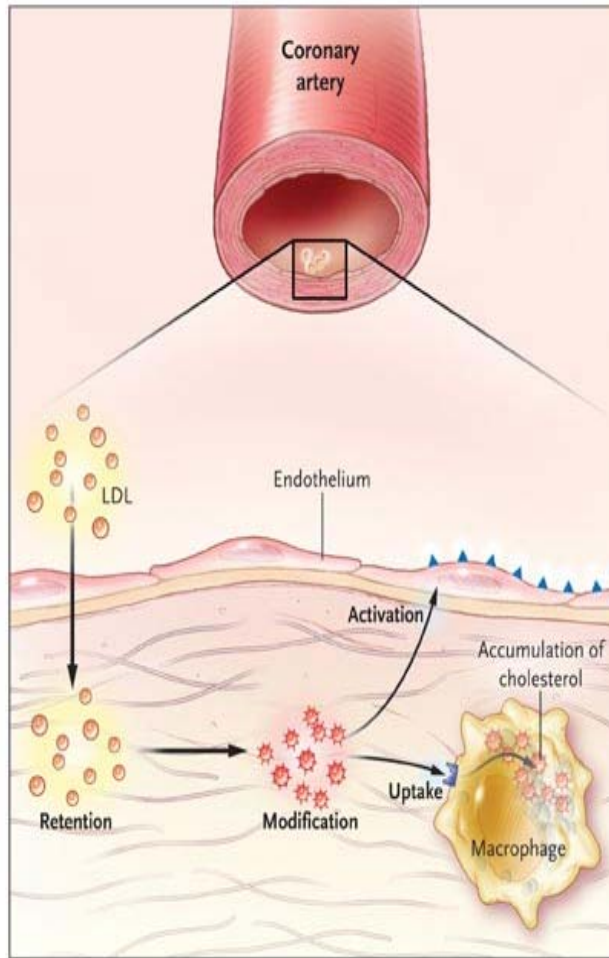
PPAR = strong **anti-inflammatory** molecule

blocking various inflammatory signaling pathways

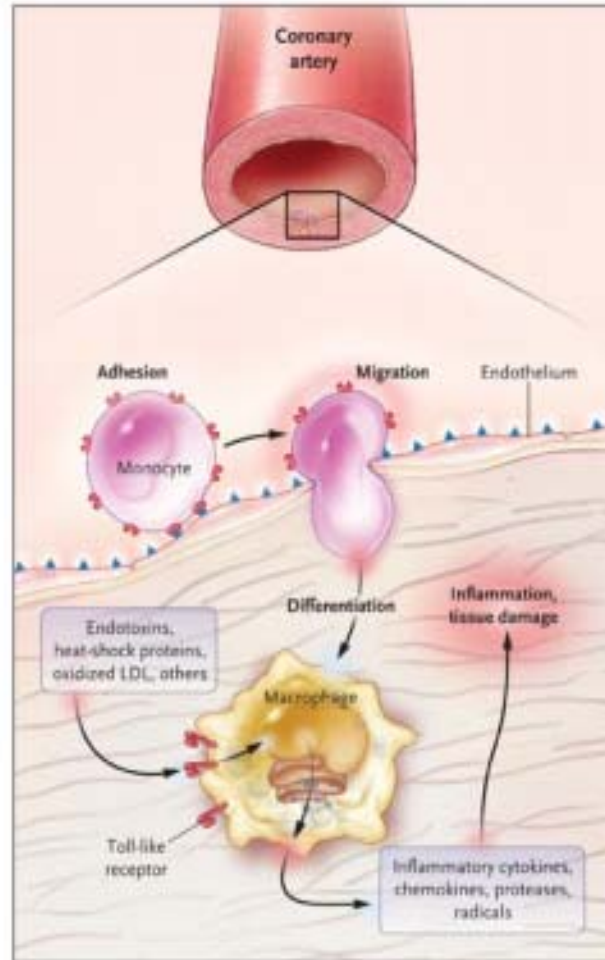


Delerive et al. Journal of Endocrinology (2001) 169, 453–459

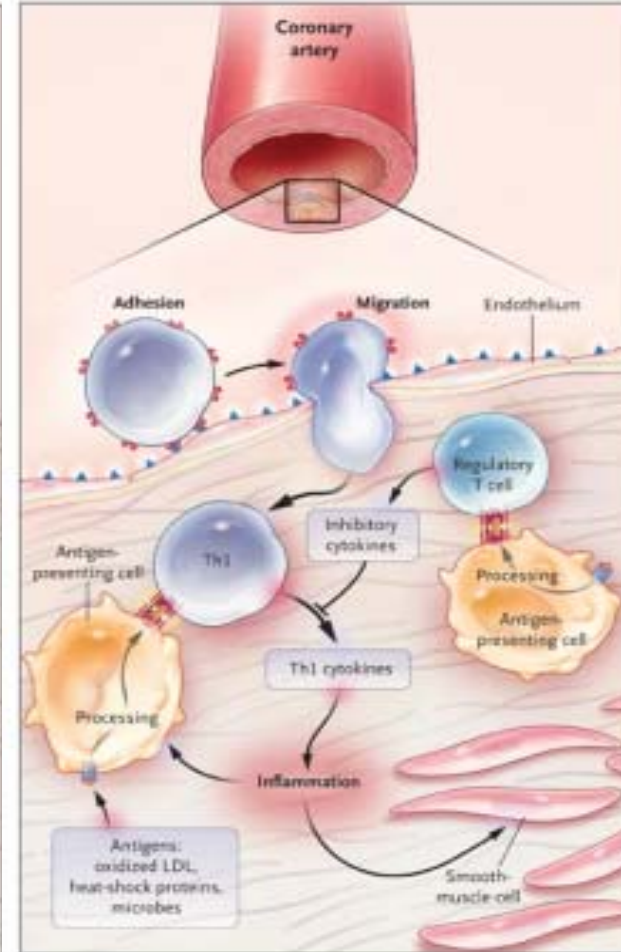
Atherosclerosis = Vascular Inflammation mechanism



Effect of **LDL** infiltration

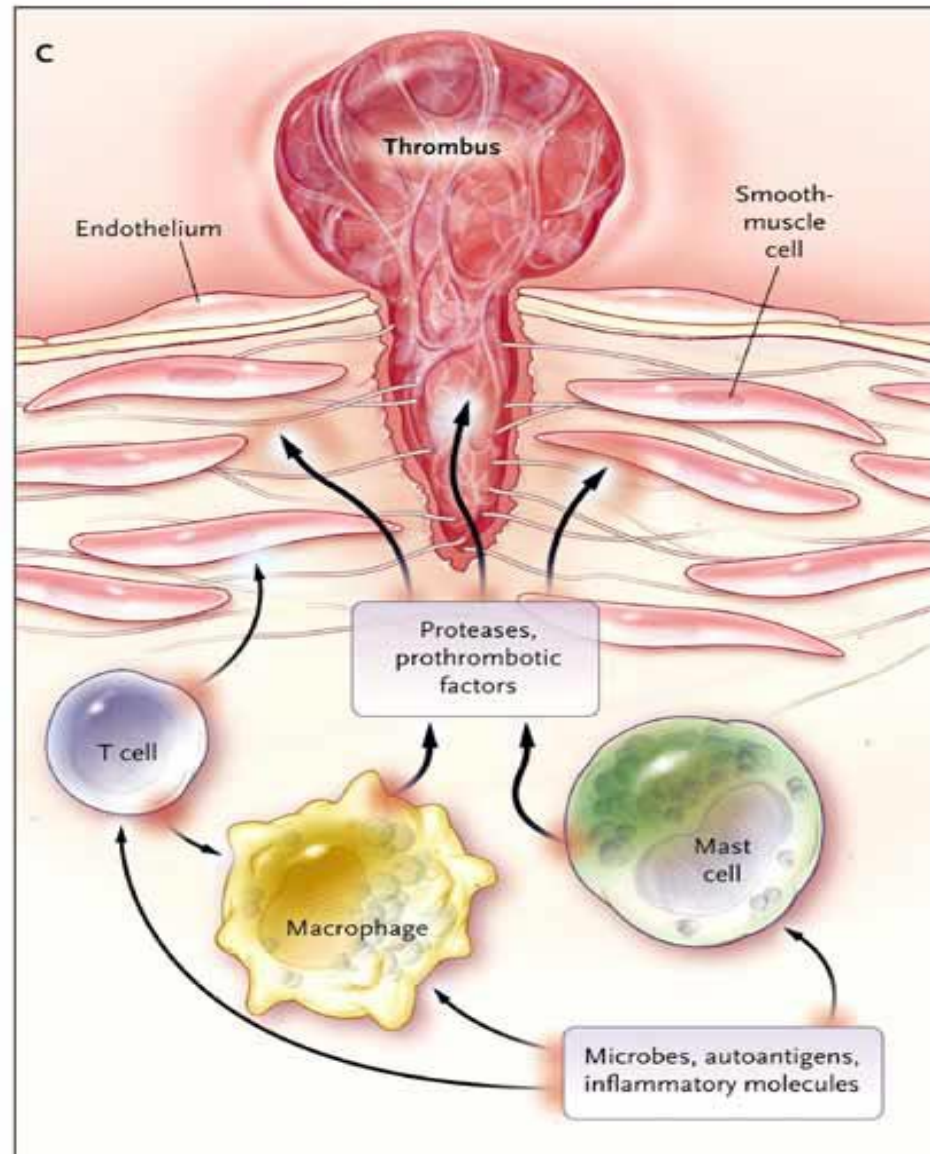
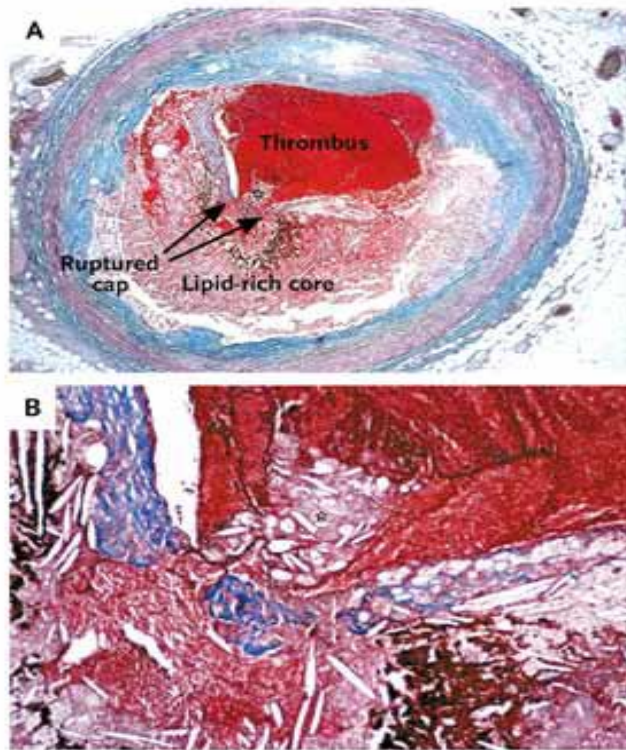


Role of **macrophage**



Effect of **T cell** activation

Vascular Inflammation ; significance



Coronary inflammation

→ plaque rupture

→ sudden death

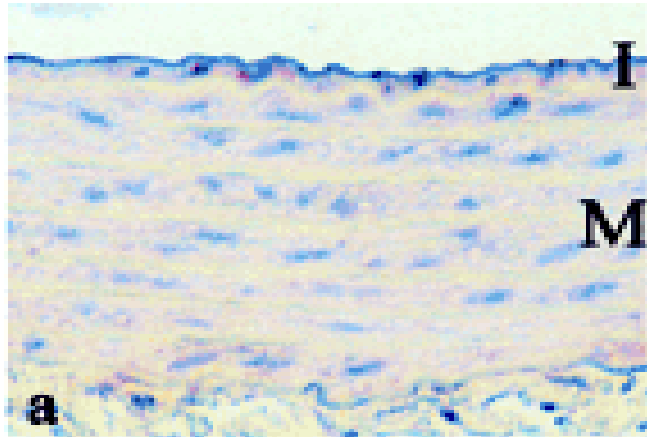
PPAR

Classical **metabolic** effect of PPAR- activation

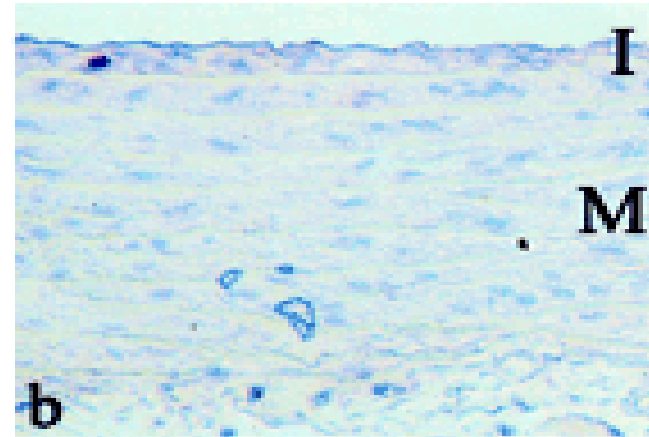
- Induce **fat cell** differentiation
(large, insulin resistant → small, sensitive)
- Reduce free fatty acids and **adipocytokines**
(TNF- , leptin, resistin)
- Increase anti-atherogenic **adiponectin**

PPAR- γ expression in **neointima** after injury to rat aorta

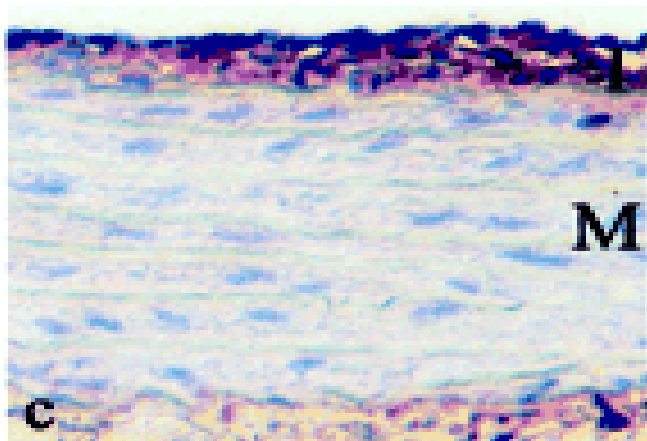
Control (uninjured)



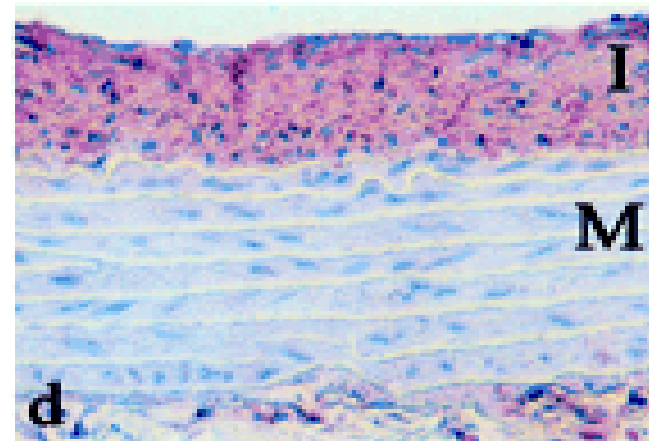
2 d



7 d

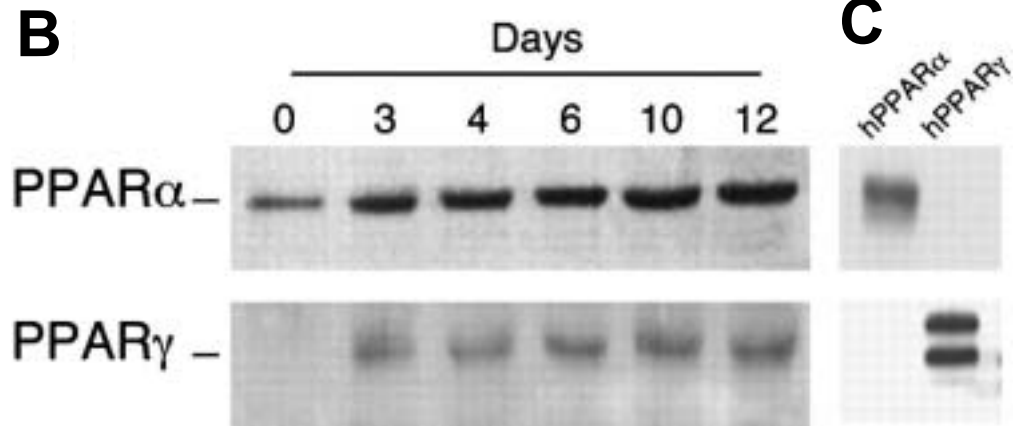
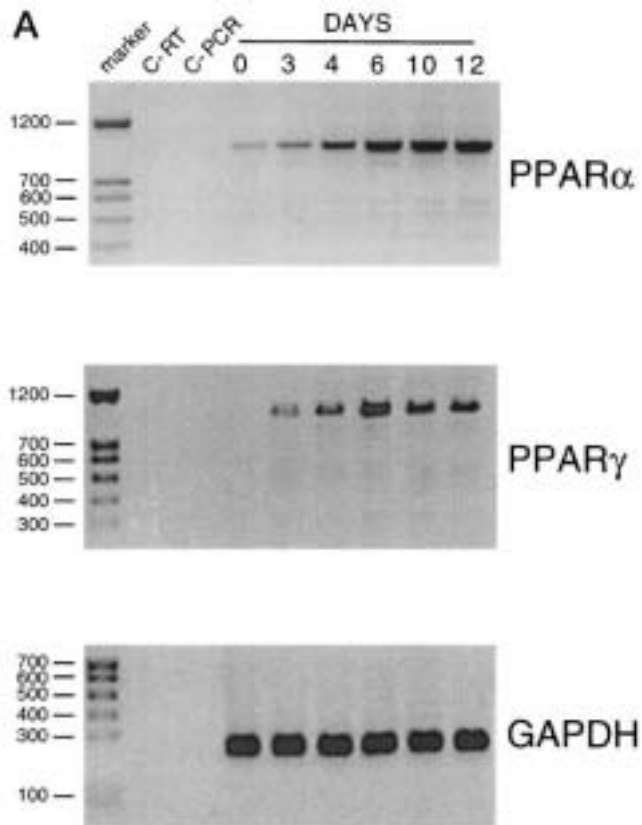


14 d



Ronald *et al.* *Circulation*. 2000

PPAR are expressed upon differentiation of human monocytes to macrophage



Western blot analysis

RT-PCR

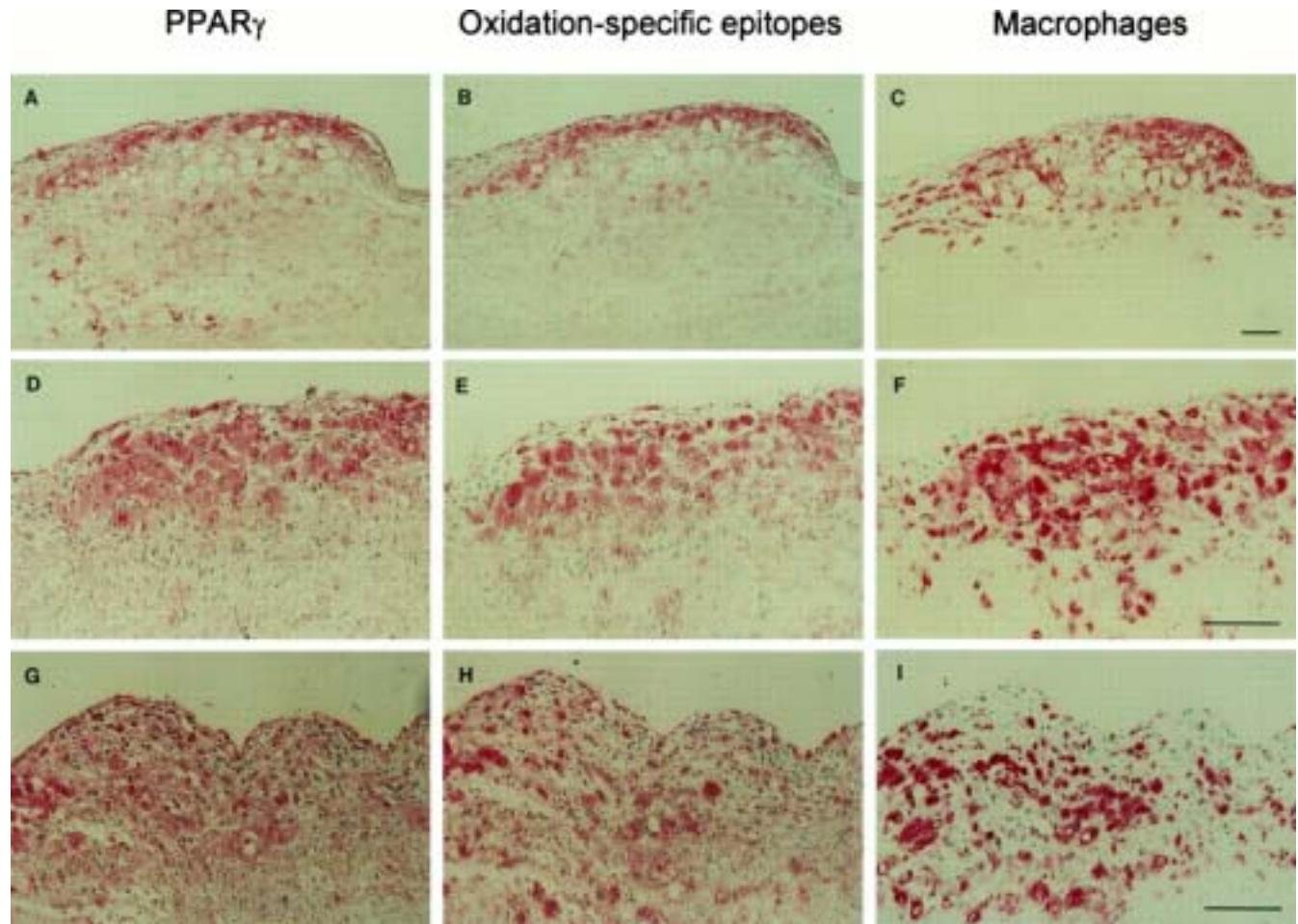
G. Chinetti et al. *J Biol Chem* **273** (1998), pp. 25573–25580.

PPAR express in **macrophage** and **atherosclerotic lesion** of human coronary a.

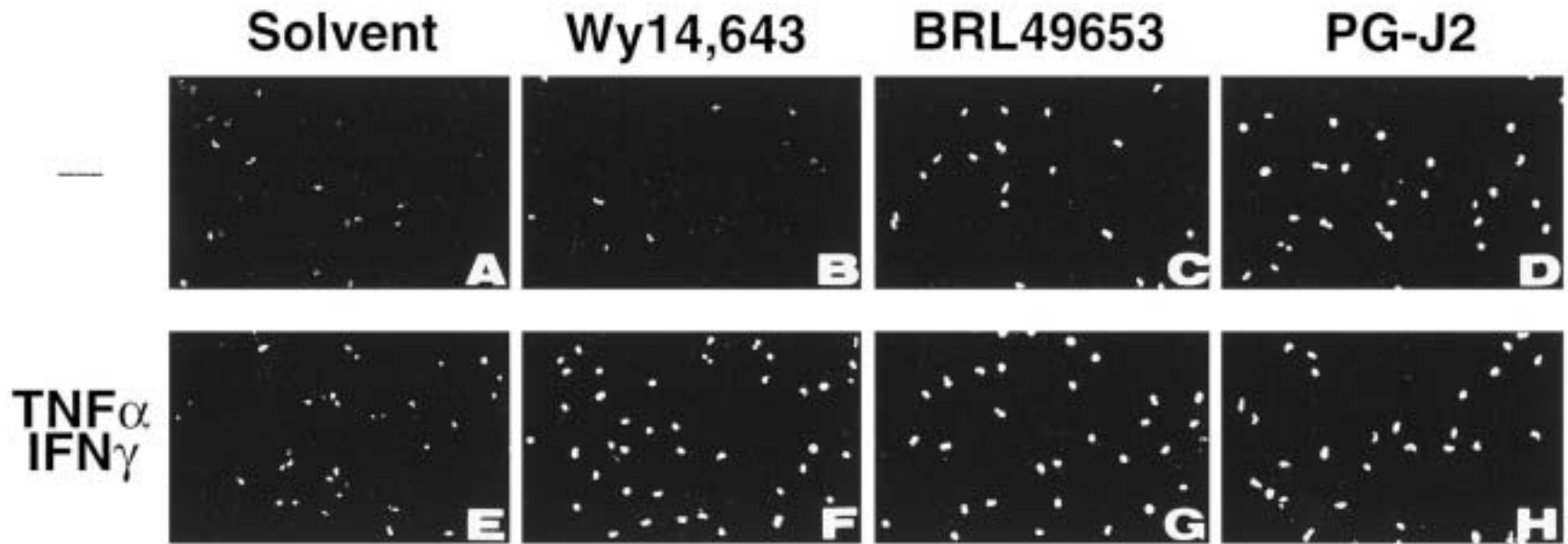
Early
atherosclerotic
lesion

Shoulder
Region of
atheroma

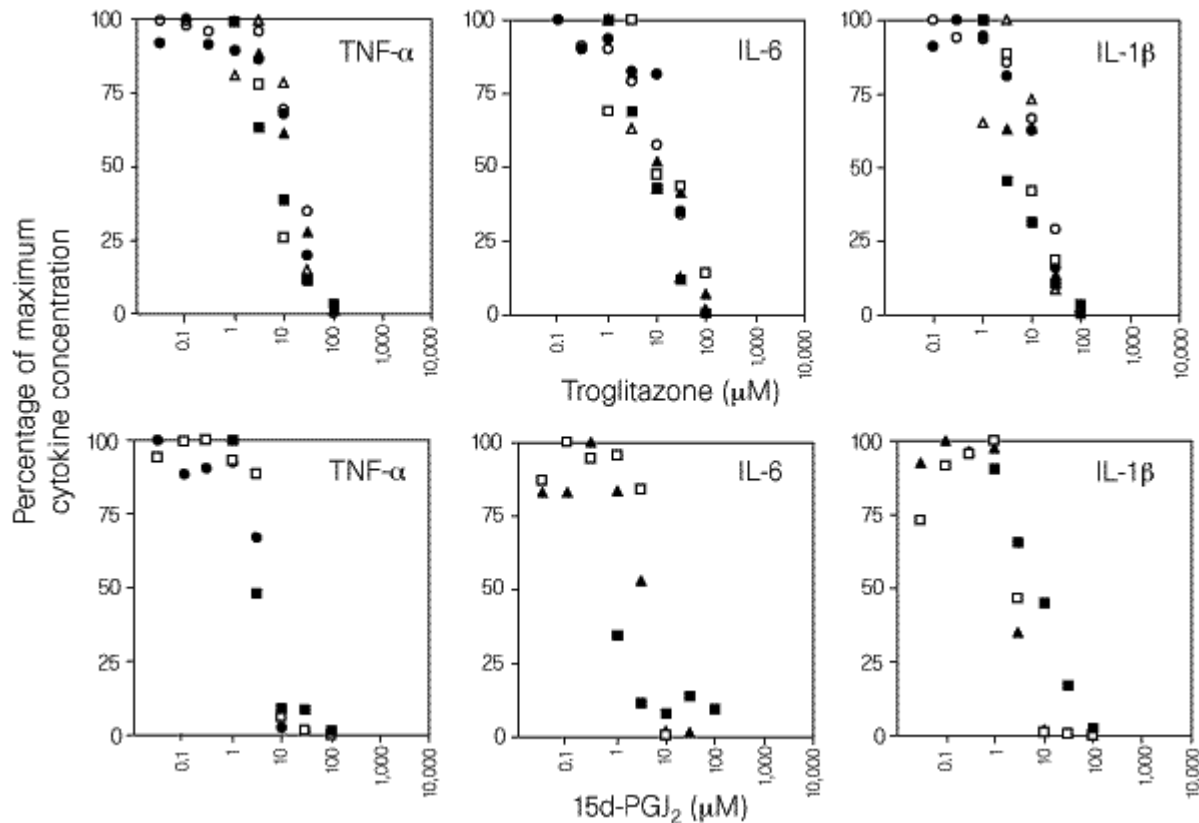
Fatty streak



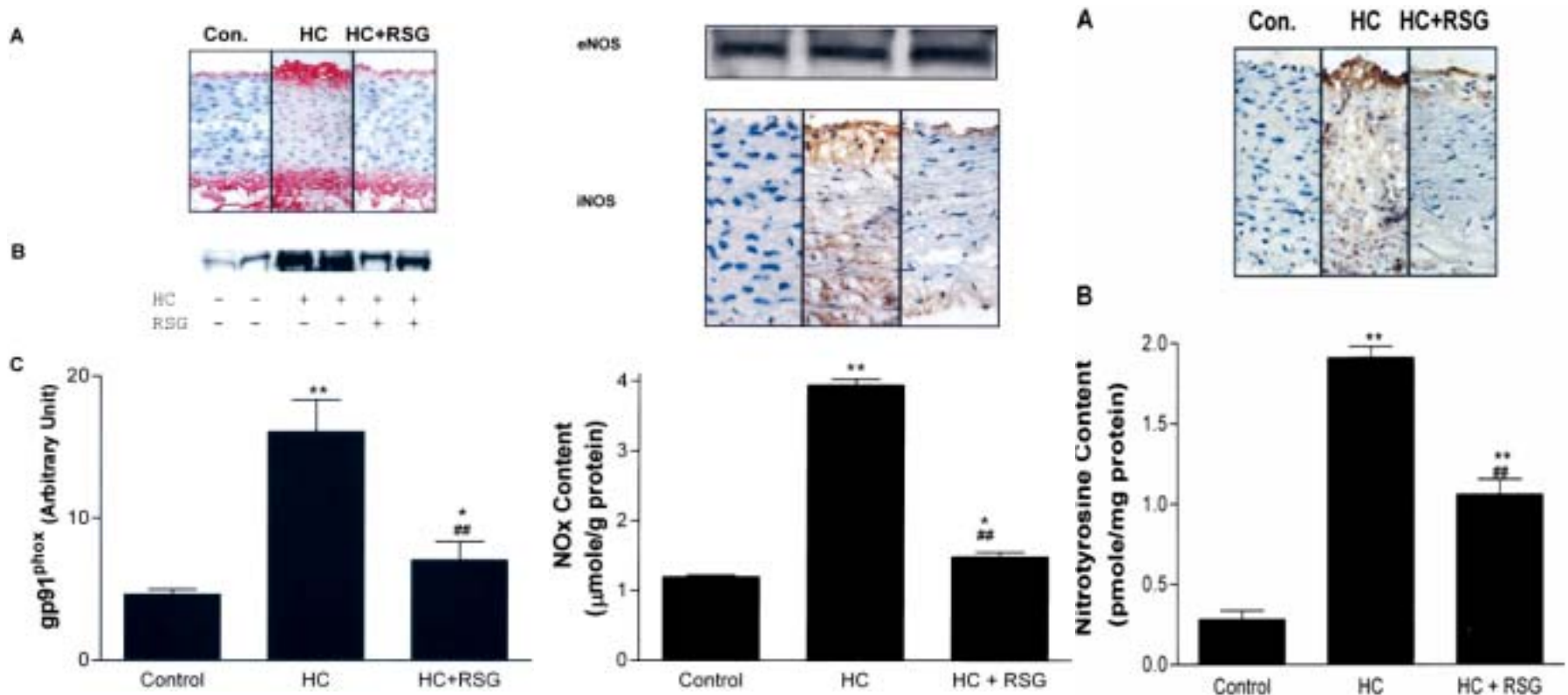
PPAR- activators also induce apoptosis in macrophages



PPAR agonist **decreased** inflammatory cytokine
(TNF- α , IL-1 β , IL-6) released from **human monocytes**

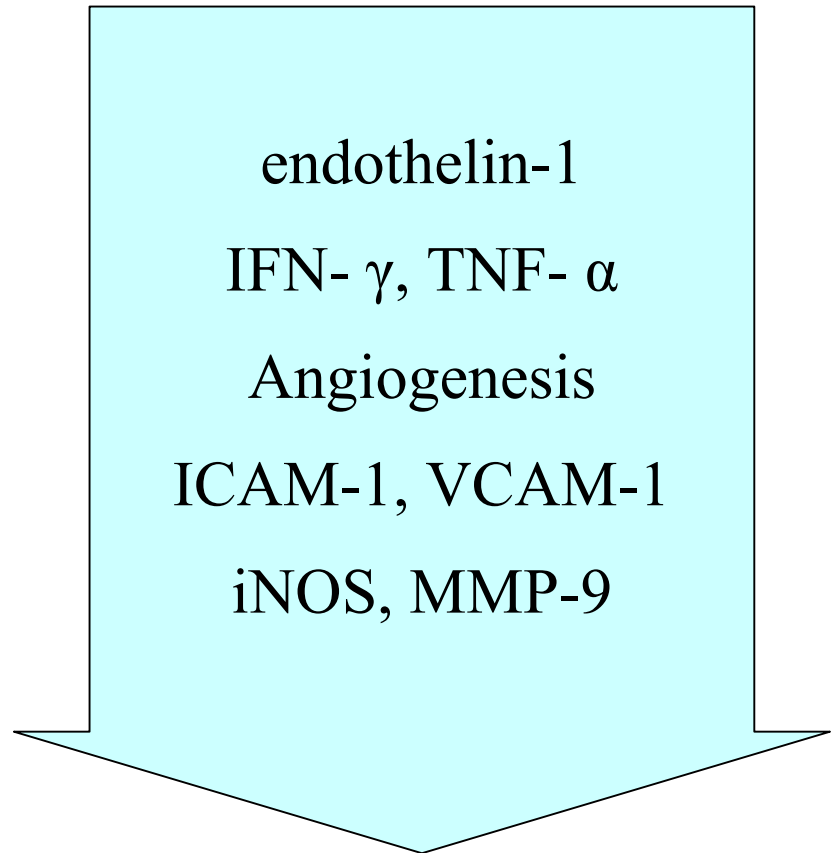
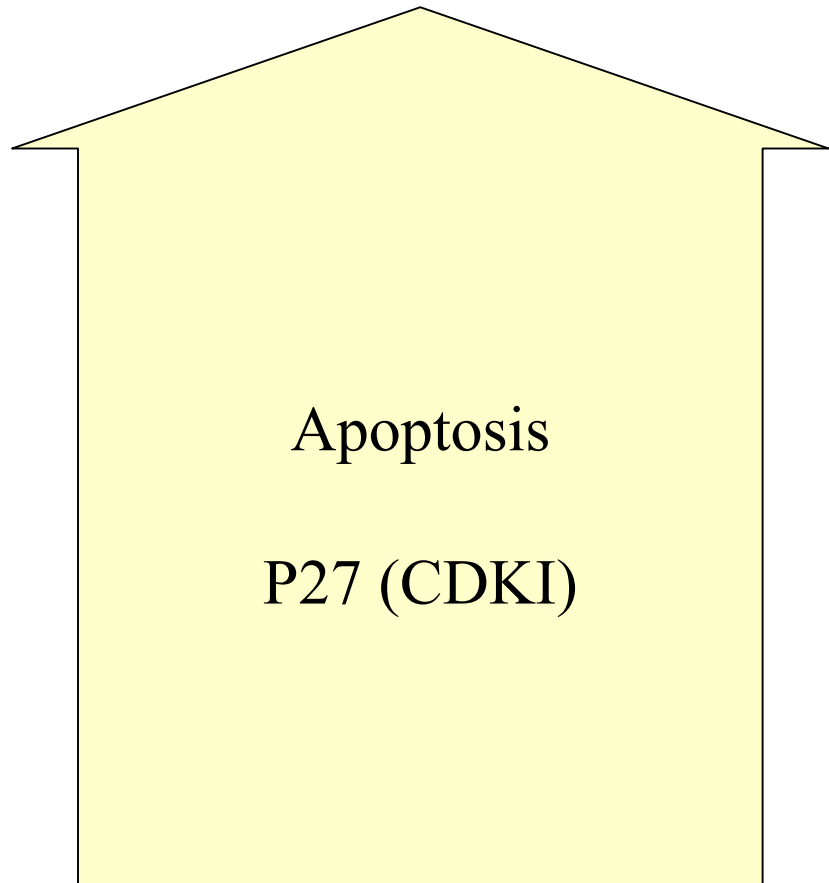


Rosiglitazone **reduces oxidative** stress in hyper-cholesterolemic rabbit

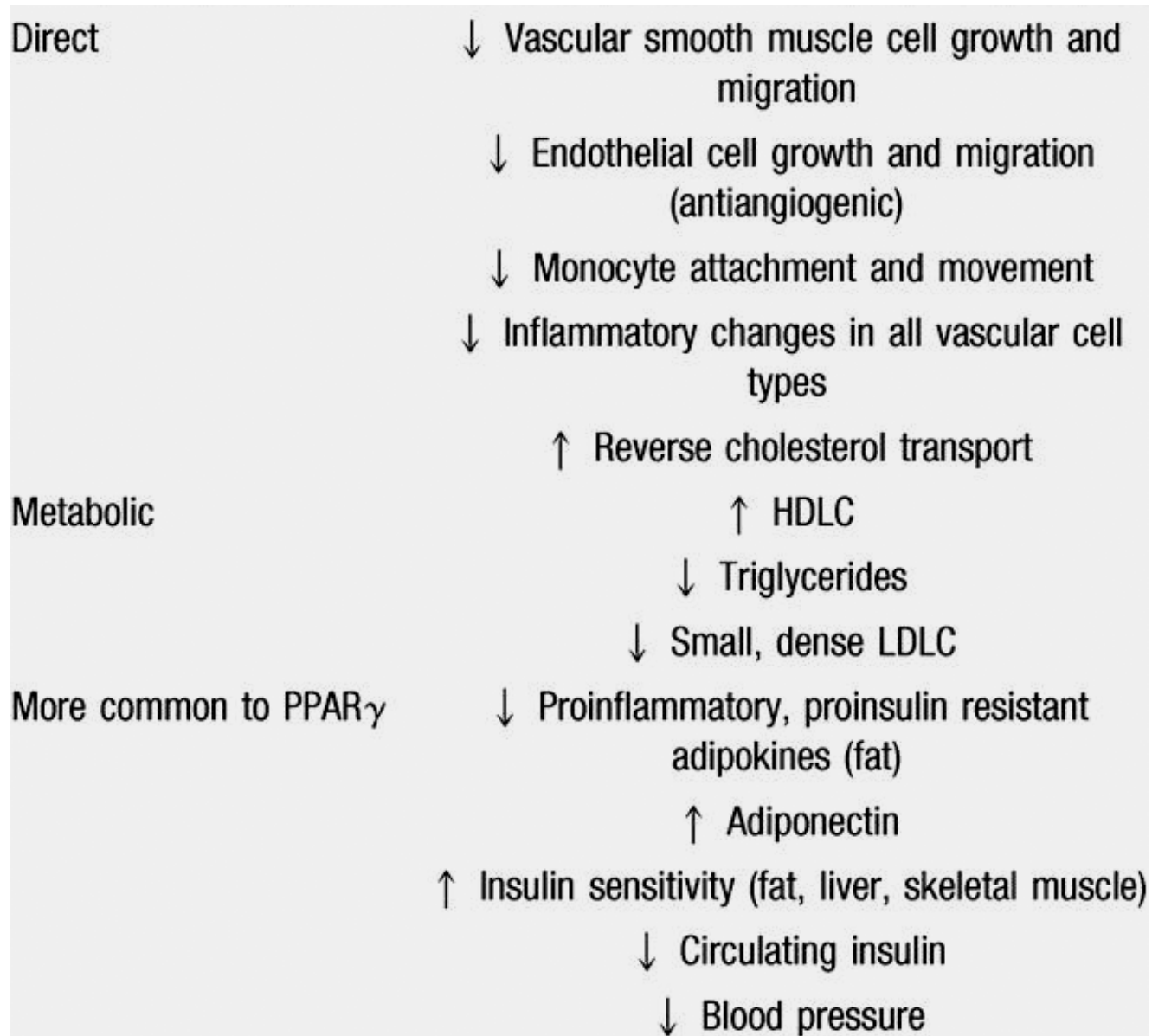


PPAR γ - cellular actions

- in ECs, VSMCs, Macrophages and T cell.



Mechanism of PPAR- to prevent cardiovascular ds.

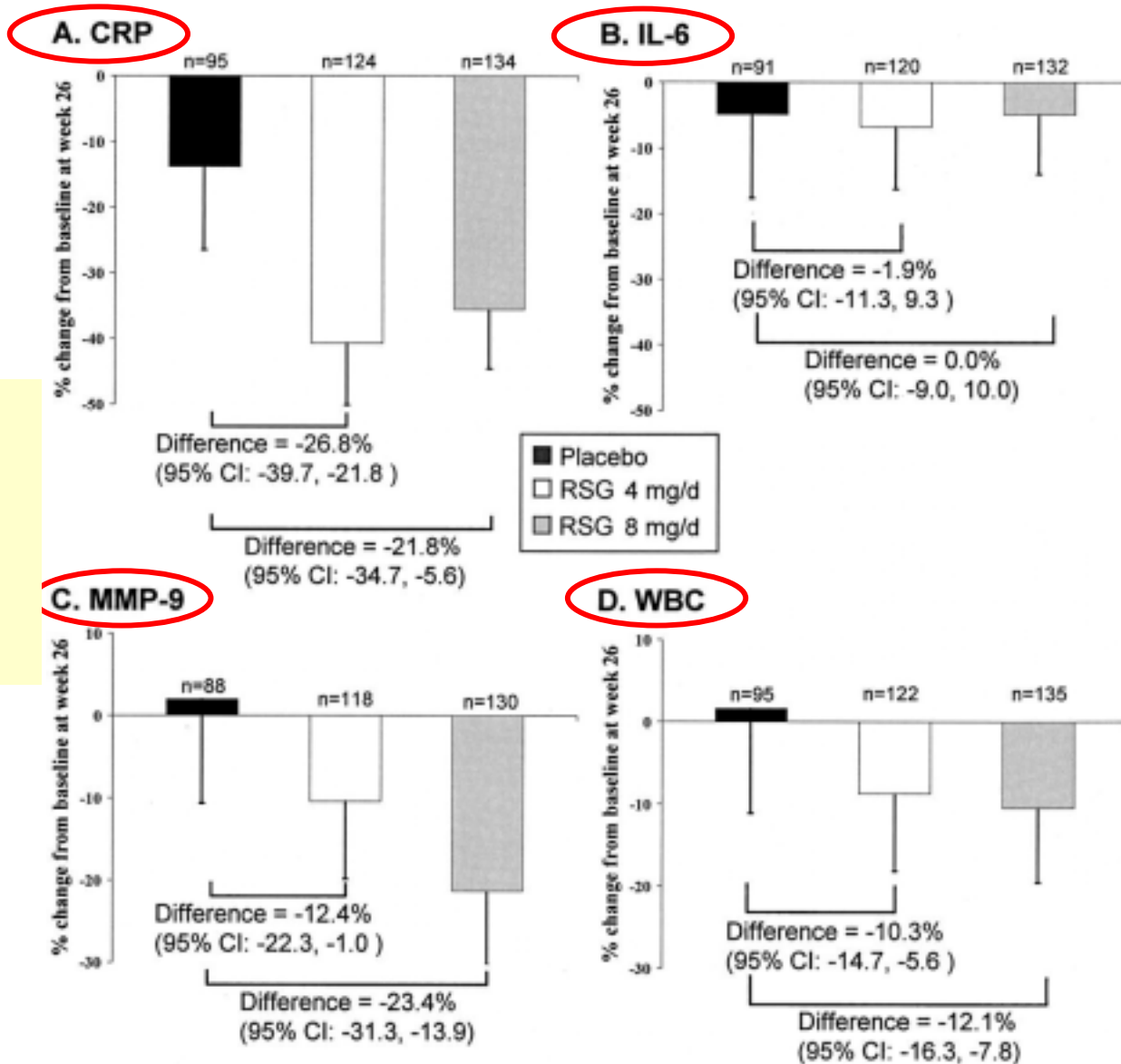


PPAR- γ – clinical data

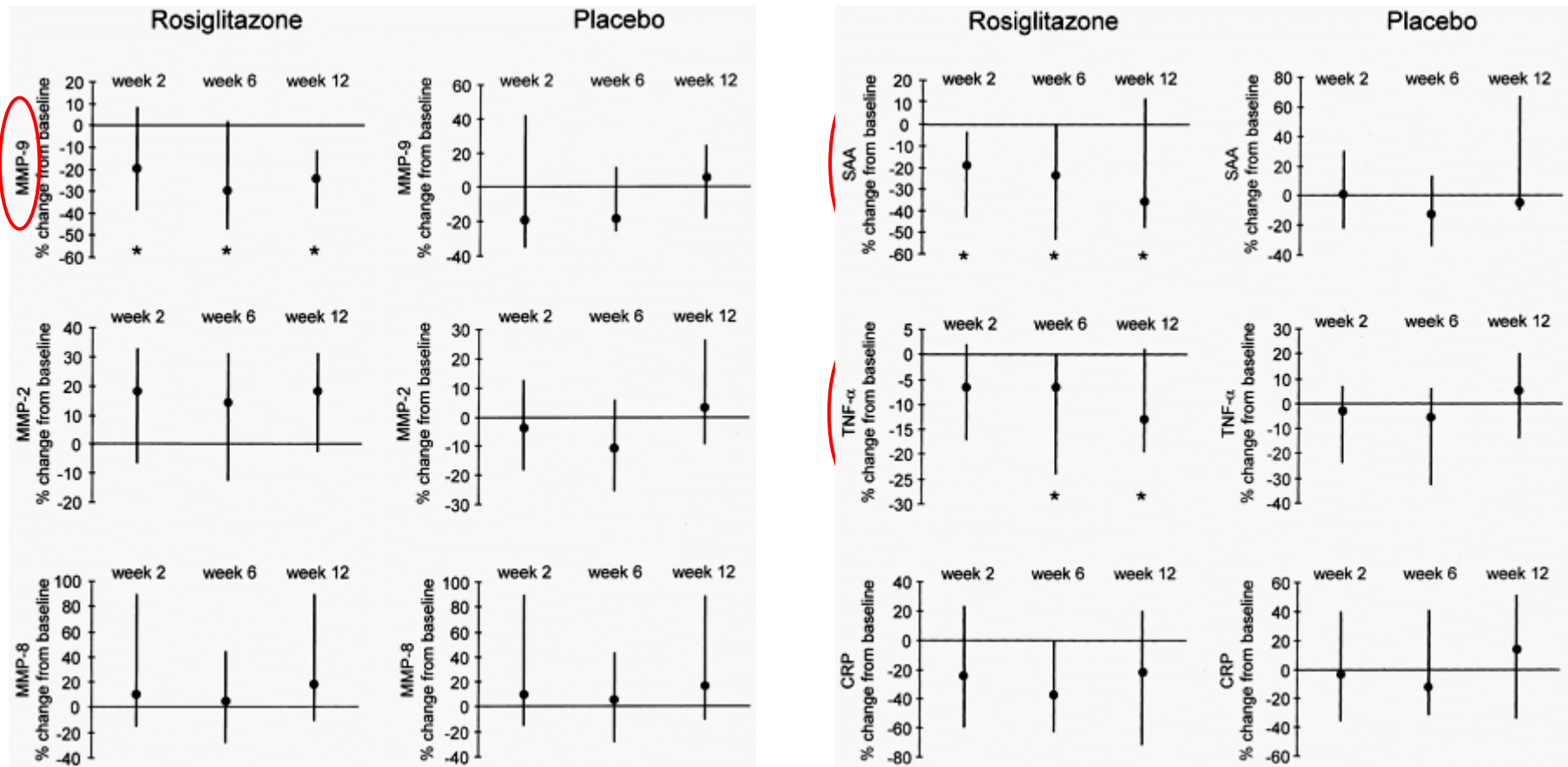
- decrease **inflammatory** biomarkers
CRP, MMP-9, sCD40L, TNF- α ,
serum amyloid A, E-selectin.
- improve **endothelial** function
- decrease human **carotid** IMT
- decrease human aortic **atheroma**
- decrease late loss at 6 month after **stenting**

Rosiglitazone reduces inflammatory markers

- type 2 DM
- N= 353
- RSG vs placebo
- 6m F/U



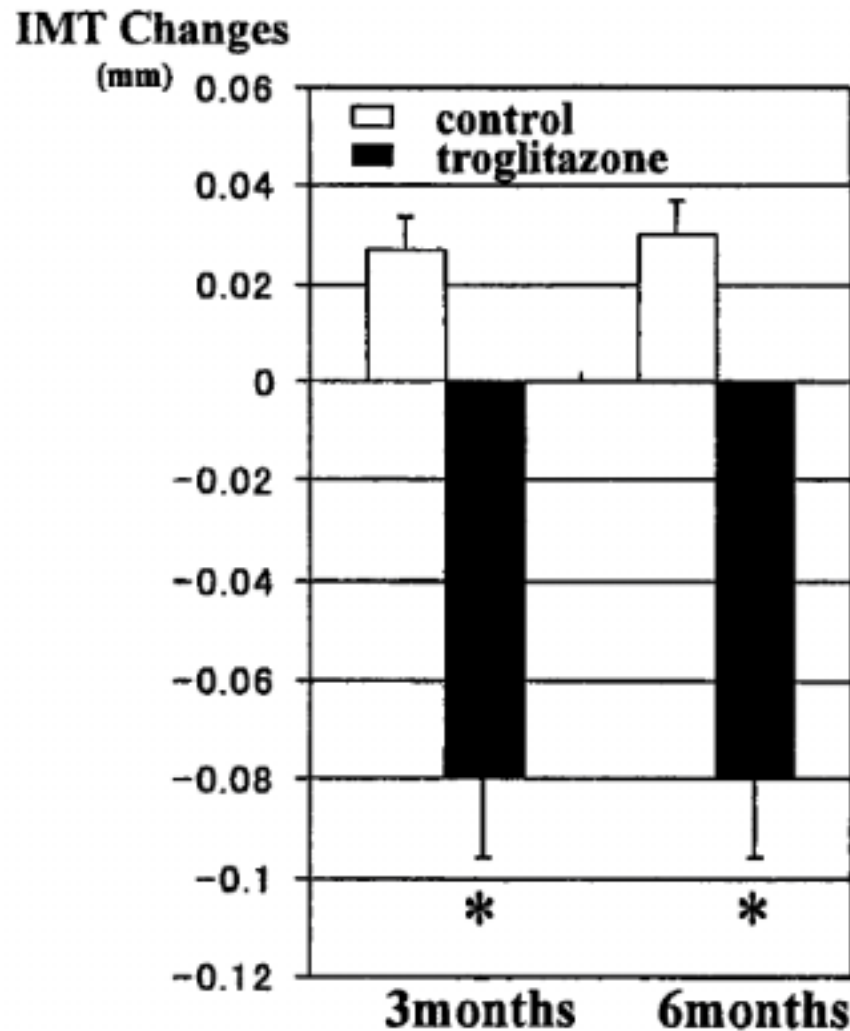
Rosiglitazone reduce inflammatory marker ; MMP9, SAA, TNF- α



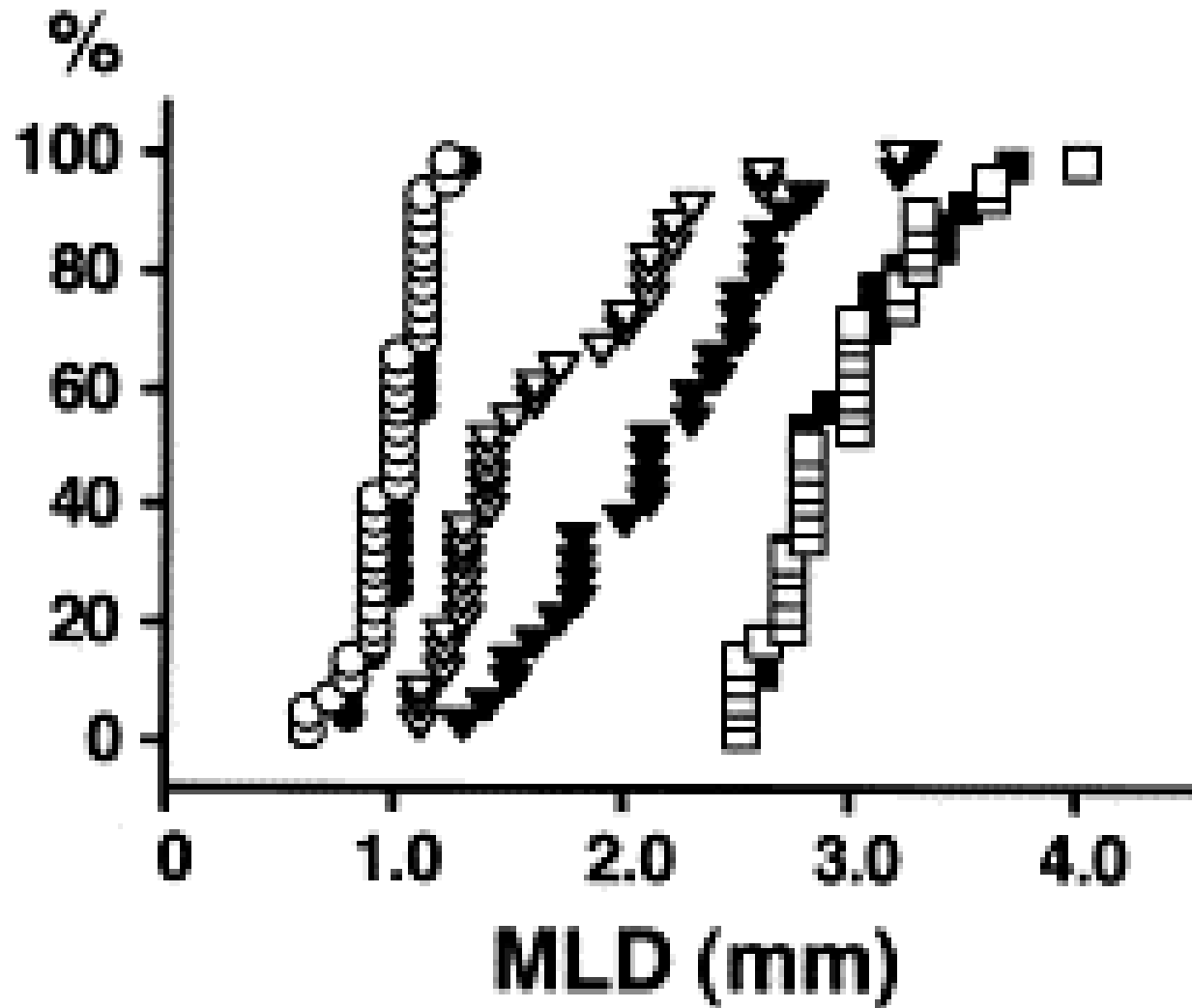
CAD & DM patients, N= 39, RSG vs placebo, 3m F/U

Troglitazone reduce carotid atheroma

- type 2 DM
- N= 132
- troglitazone vs control
- 6m F/U
- carotid IMT by US



Troglitazone **reduce late loss** after stenting at 6m F/U



○ control-pre

□ control-post

control-F/U

● TRG-pre

■ TRG-post

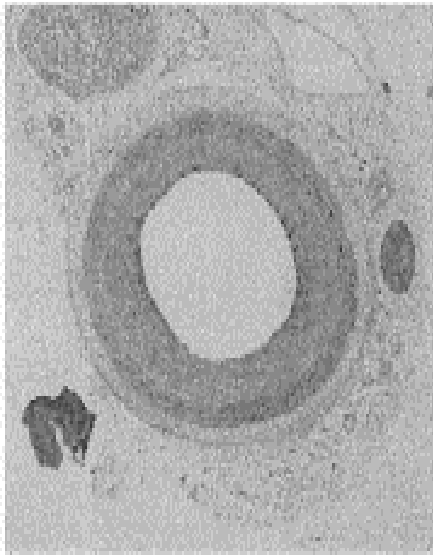
▼ TRG-F/U

•DM

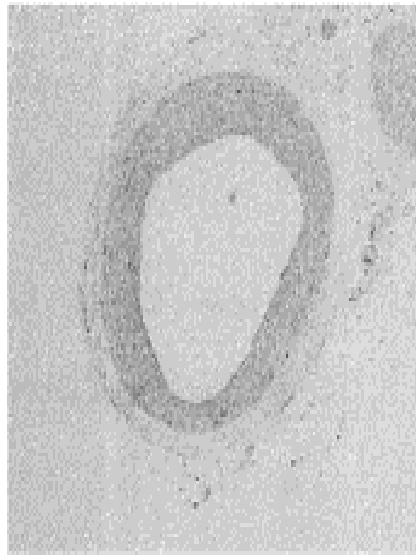
•Control = 27

•TGZ = 25

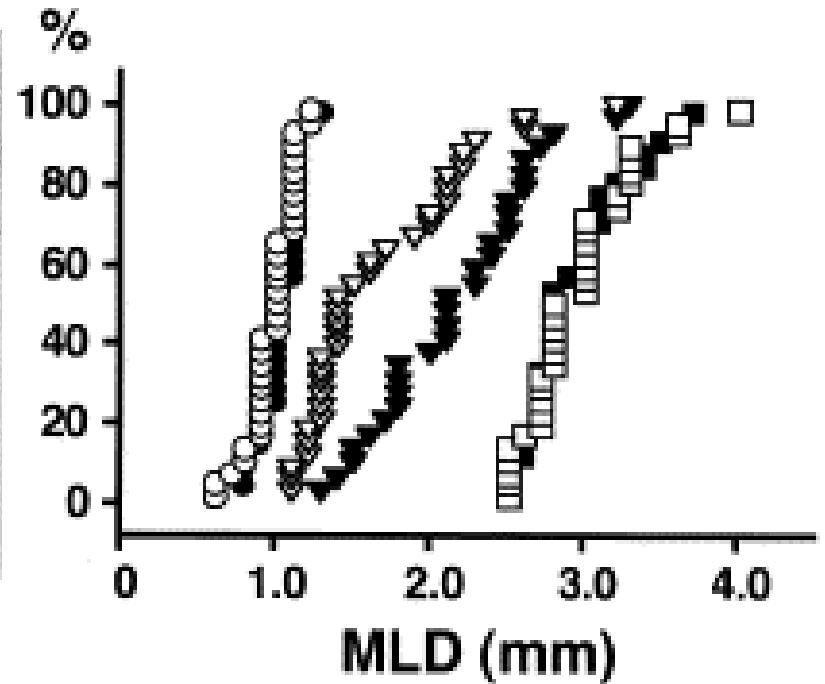
What is the underlying mechanism
of PPAR- γ to inhibit neointimal hyperplasia
other than VSMC-cycle arrest through p27 & Rb ?



control



rosiglitazone



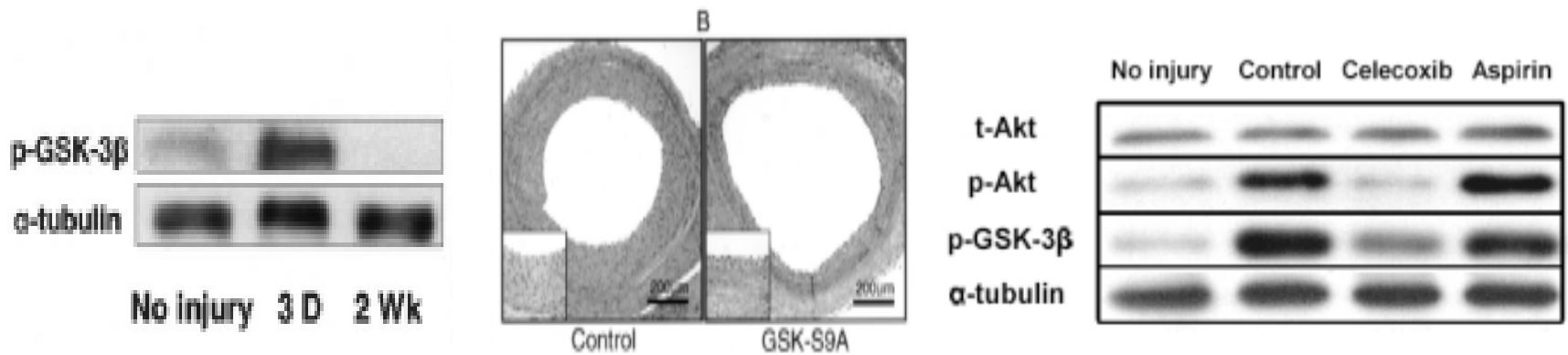
- in balloon injury model
- *Min et al, Metabolism 2002*

- after stenting, 6m F/U MLD
- *Takagi et al, JACC 2000*

GSK3 β is a key molecule in neointimal hyperplasia

[SNUH data]

- GSK is phosphorylated and inactivated in the vessel wall after balloon injury
- Its activation by celecoxib reduced neointimal hyperplasia.

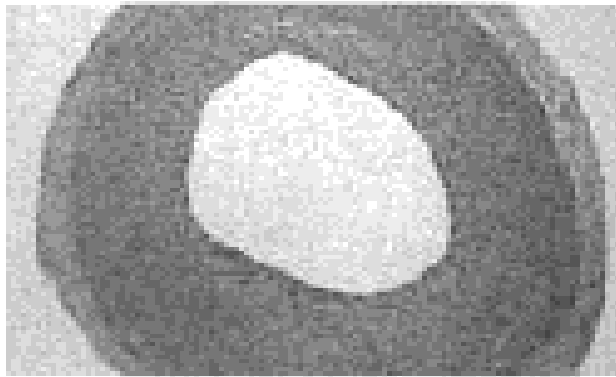


Park et al. *ATVB* 2003

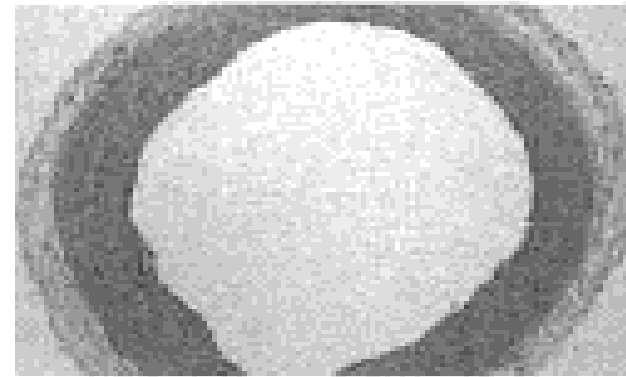
Yang et al. *Circulation* 2004

MMP-9 involves VSMC migration

MMP-9 & TIMP-1 are involved smooth muscle cell migration which is a key mechanism of restenosis after balloon injury.



Av1.LacZ4



Av1.TIMP1

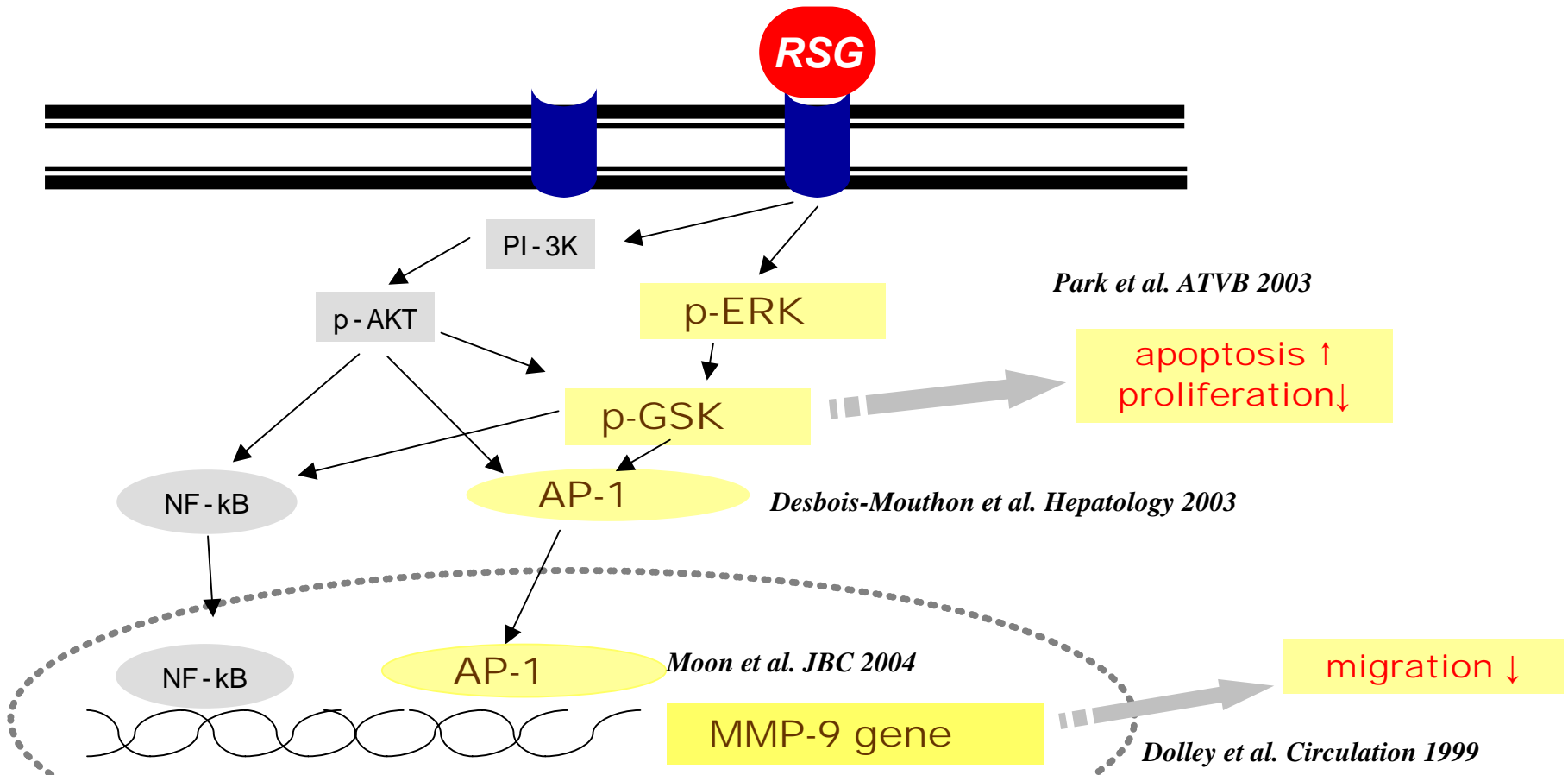
- in balloon injury model
- *Dolley et al. Circulation 1999*

Hypothesis

mechanism of PPAR- γ to inhibit neointimal hyperplasia

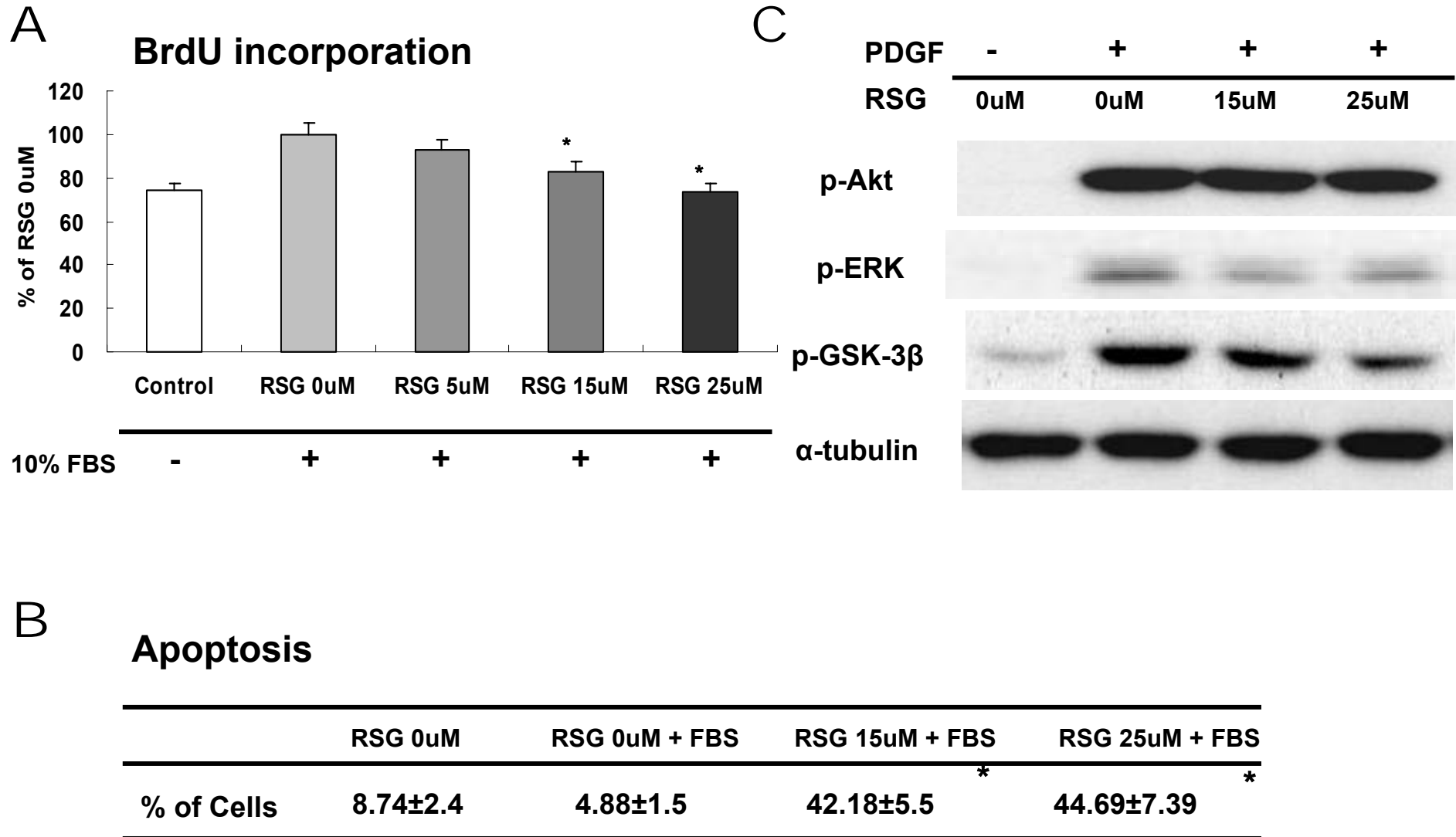
=

Rosiglitazone reduces NIH via GSK3 β and MMP-9 pathway ?



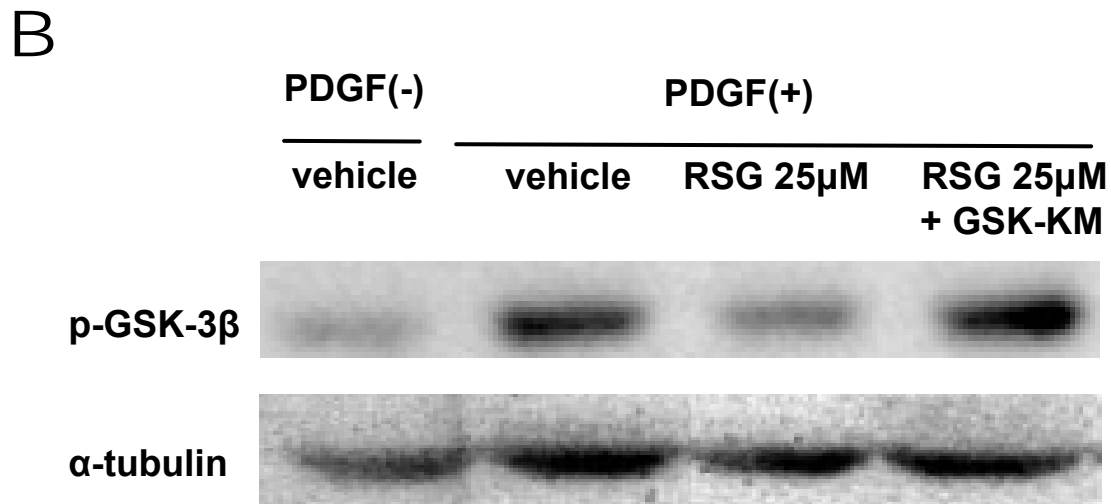
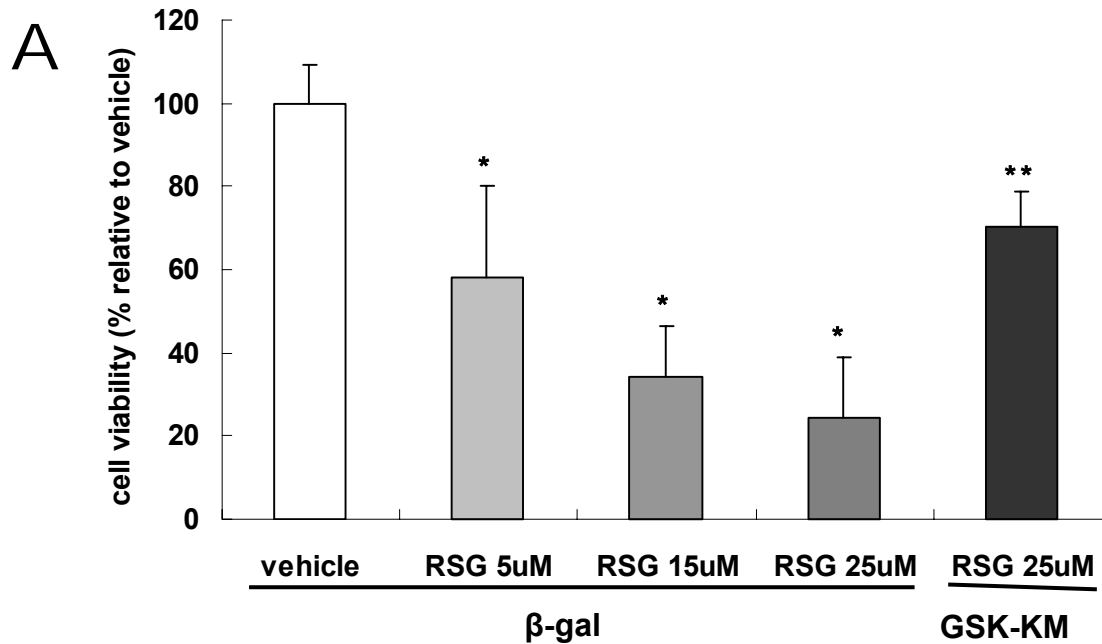
Rosiglitazone inhibits proliferation & induces apoptosis of rat VSMC

Figure 1



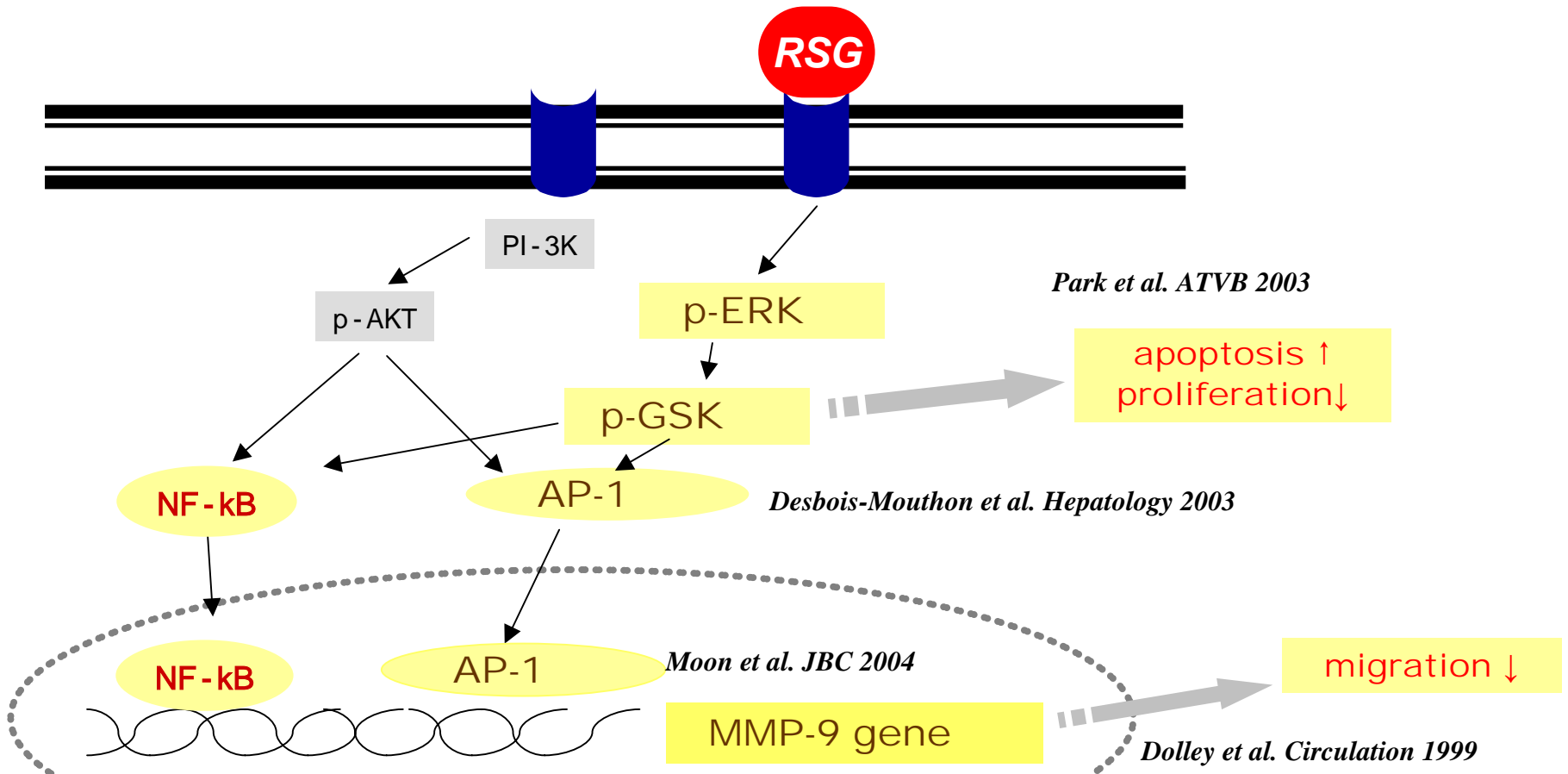
Rosiglitazone reduces **VSMC** viability by activation of GSK3 β

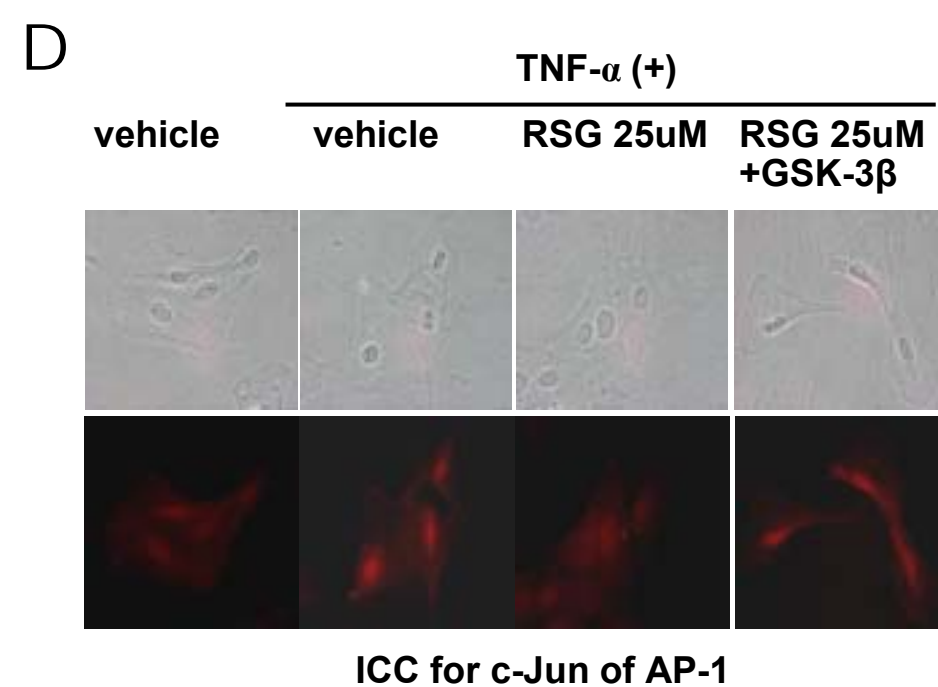
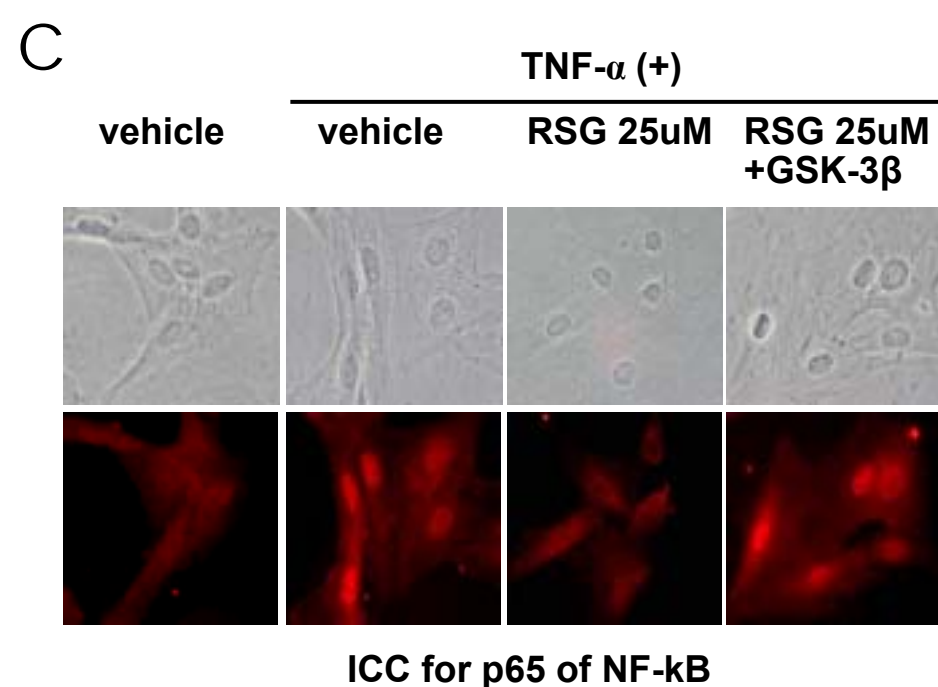
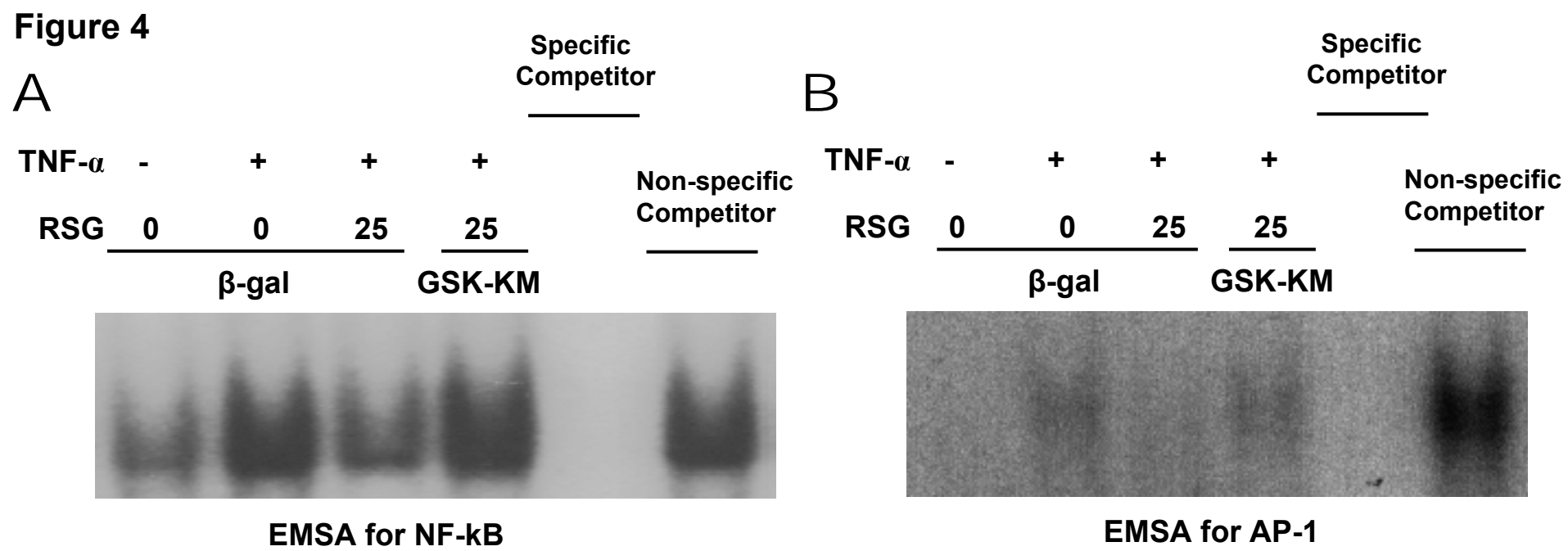
Figure 2



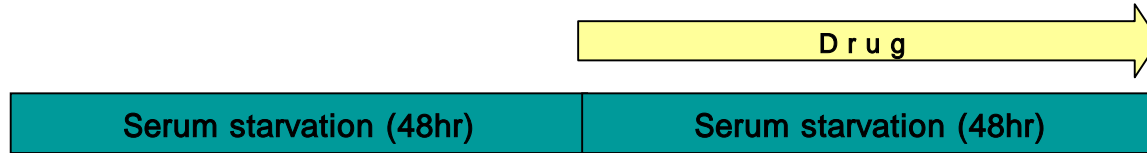
Hypothesis

Rosiglitazone reduces NIH via GSK3 β and MMP-9 pathway ?

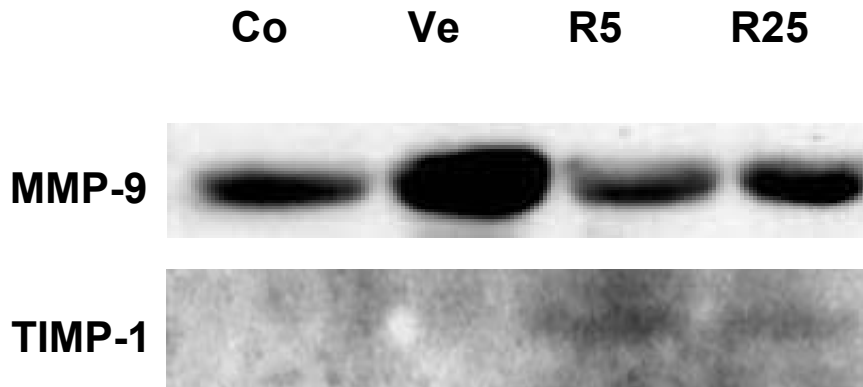




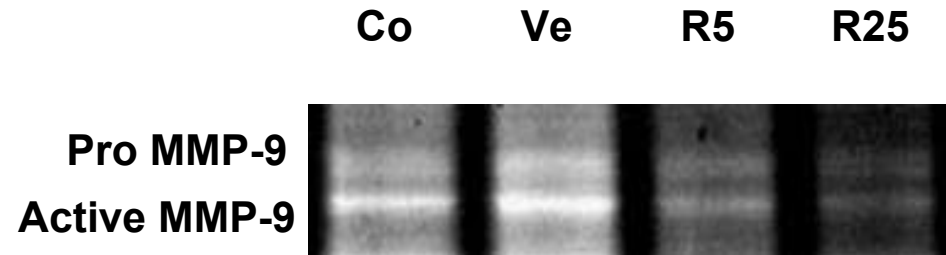
Rosiglitazone suppresses MMP-9 and induces TIMP-1



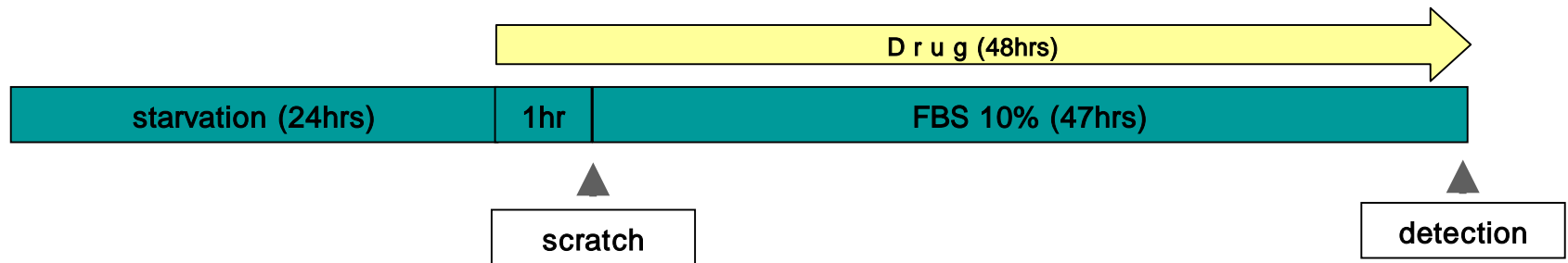
western blot



zymography



Rosiglitazone suppresses MMP-9 and induces TIMP-1, which results in **inhibition of VSMC migration**



scratch assay

Vehicle

RSG 5uM

RSG 15uM

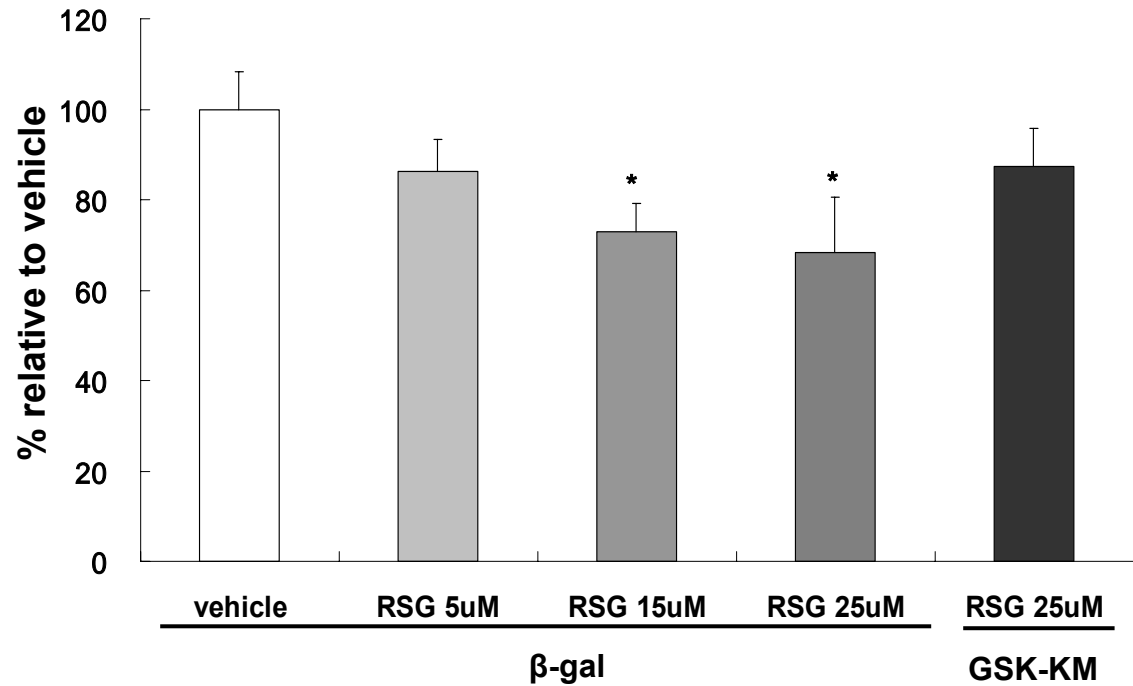
RSG 25uM



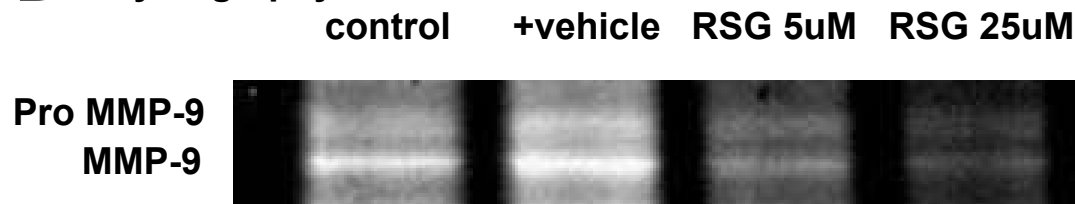
Rosiglitazone inhibits VSMC migration by activation of GSK3 β

Figure 3

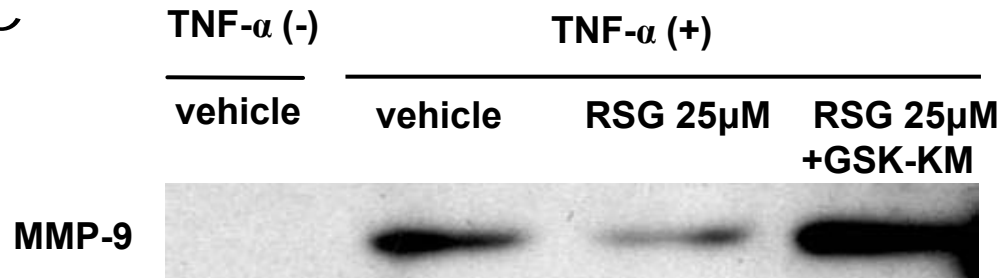
A Migration



B Zymography

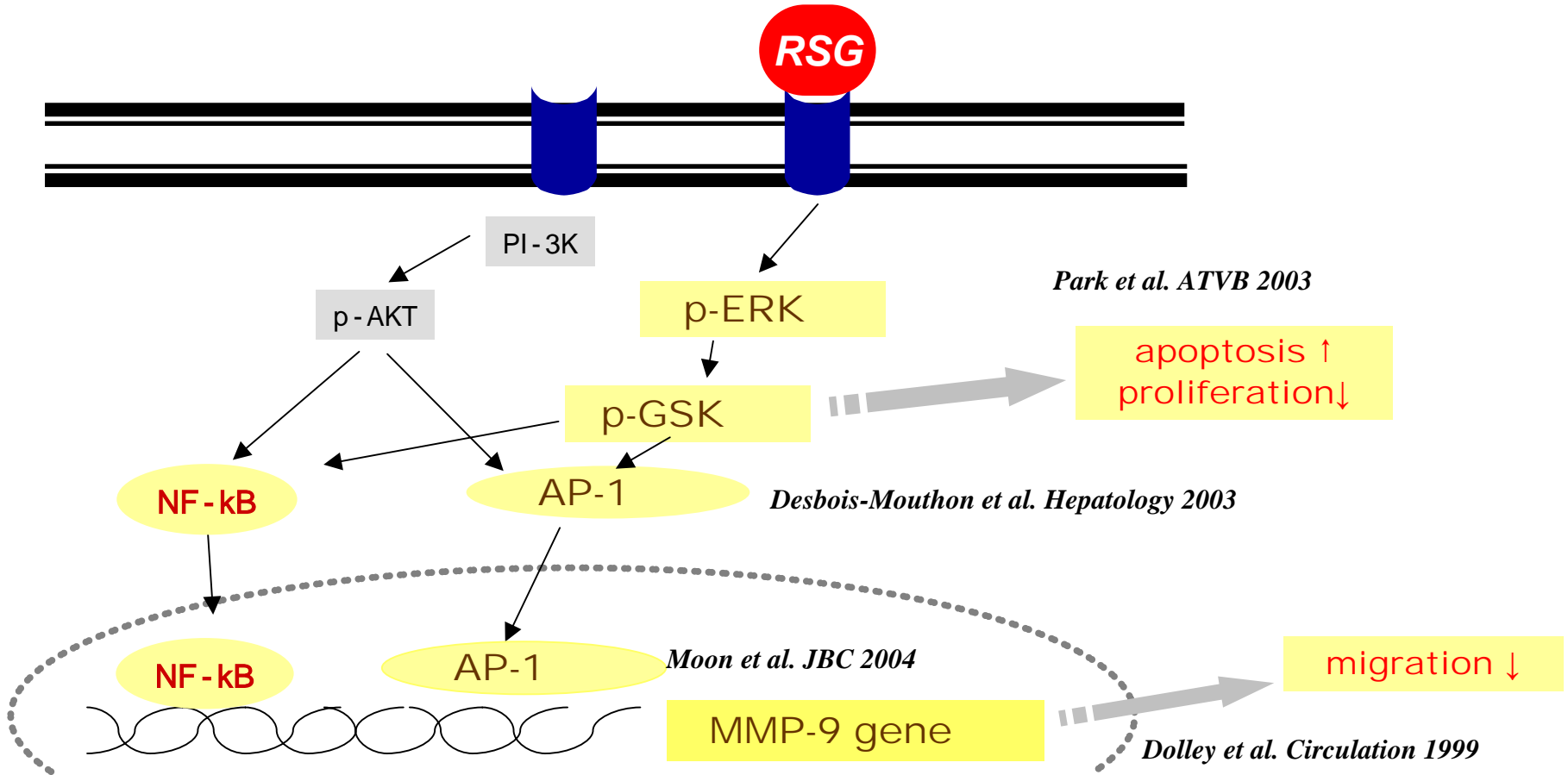


C



Hypothesis

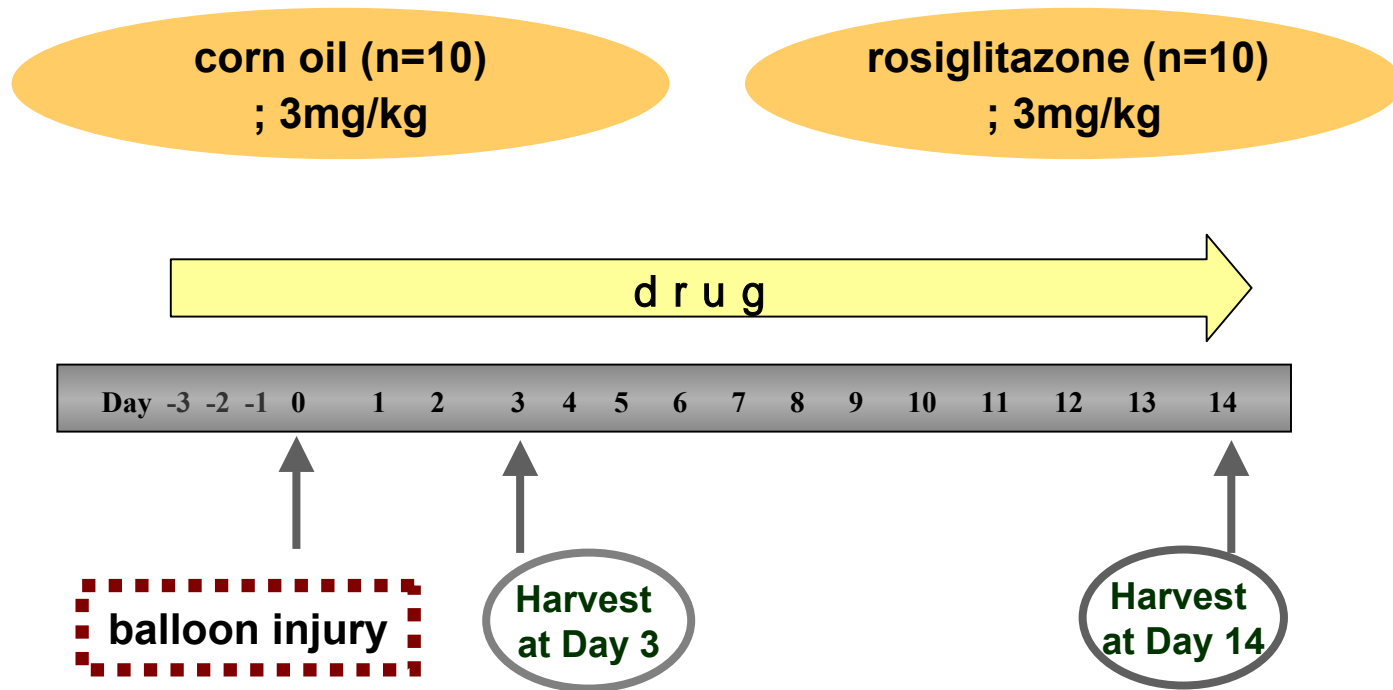
Rosiglitazone reduces NIH via GSK3 β and MMP-9 pathway ?



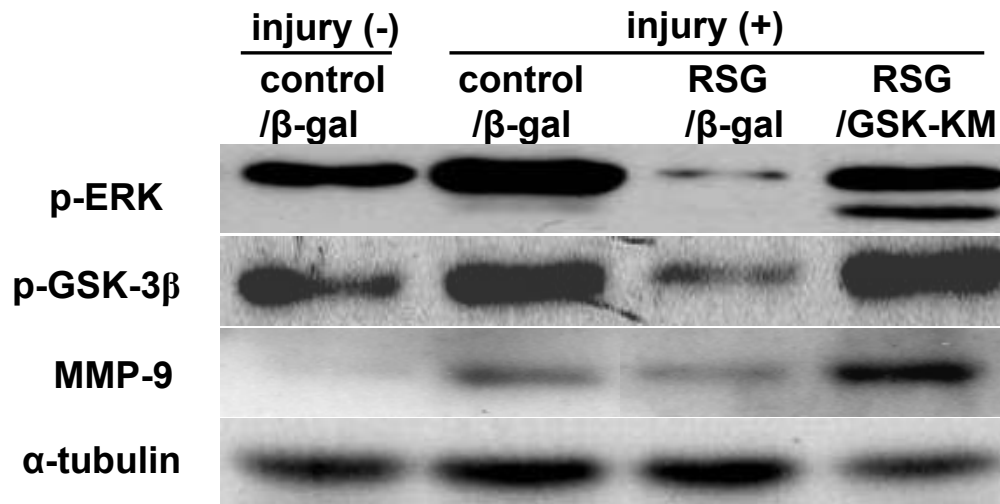
True in in-vivo situation ?

Animal experiment to confirm that rosiglitazone inhibits neointimal hyperplasia

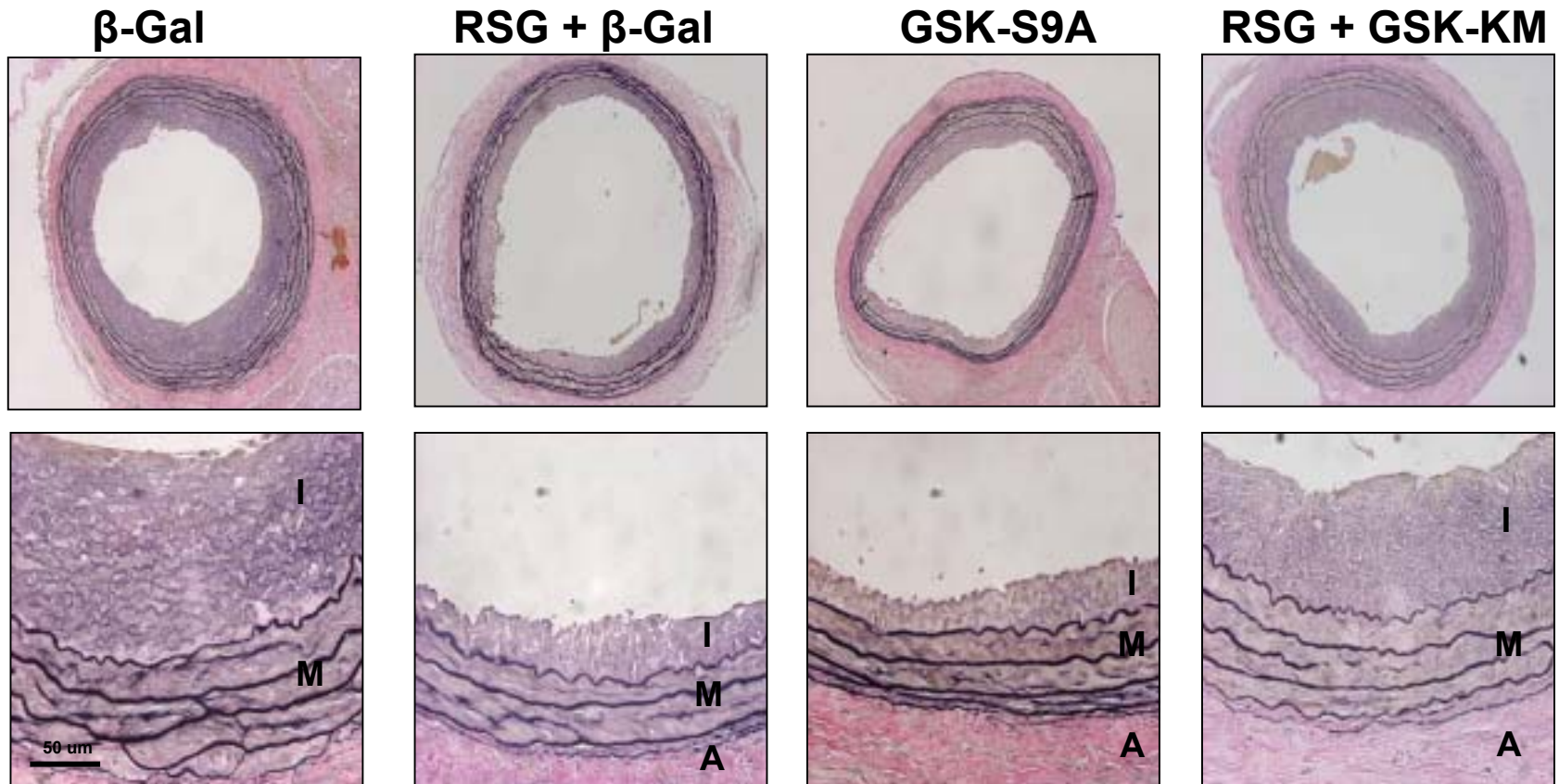
- in rat carotid injury model



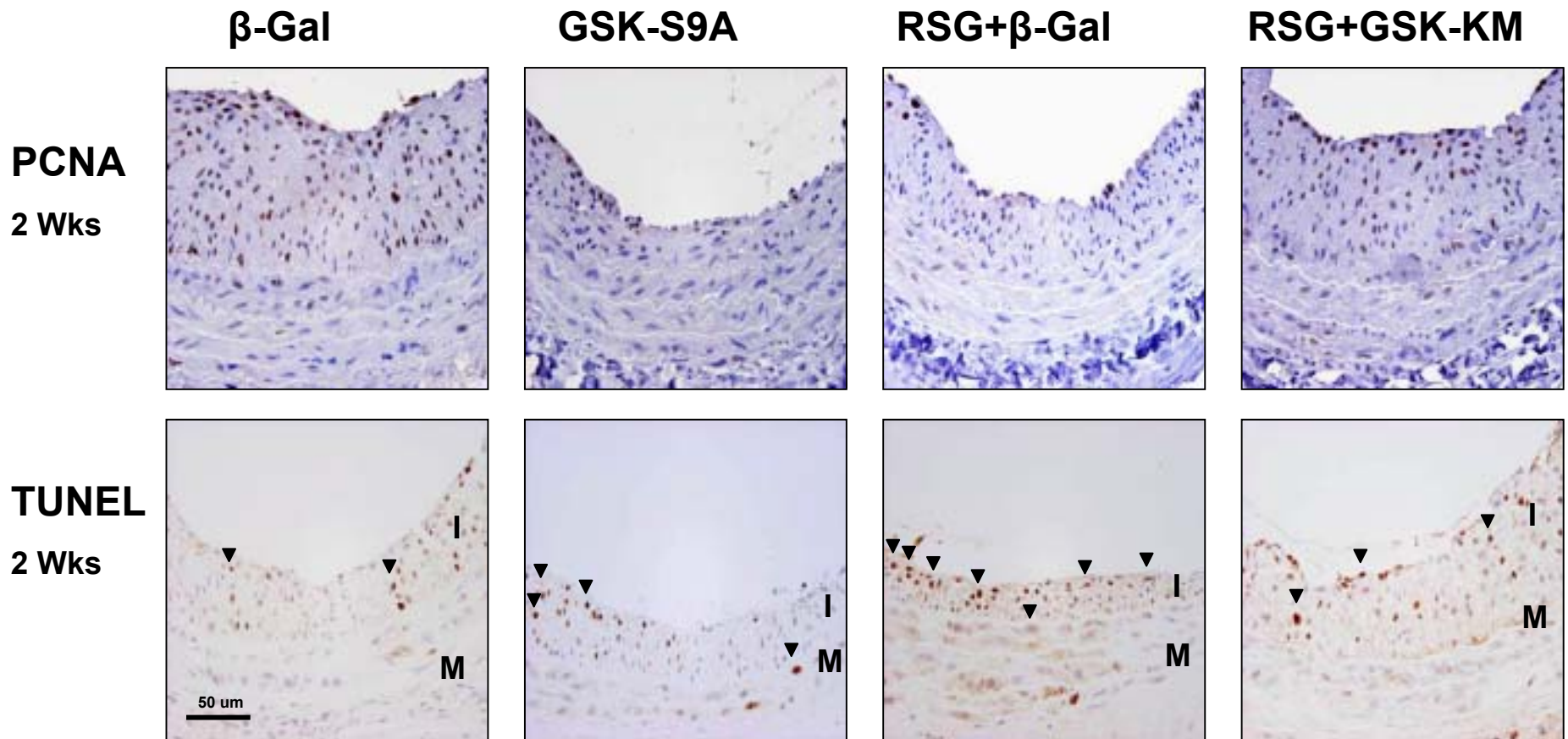
Balloon injury activate ERK and inactive GSK3 β
resulting in MMP-9 induction,
which was suppressed by Rosiglitazone
through GSK3 β activation



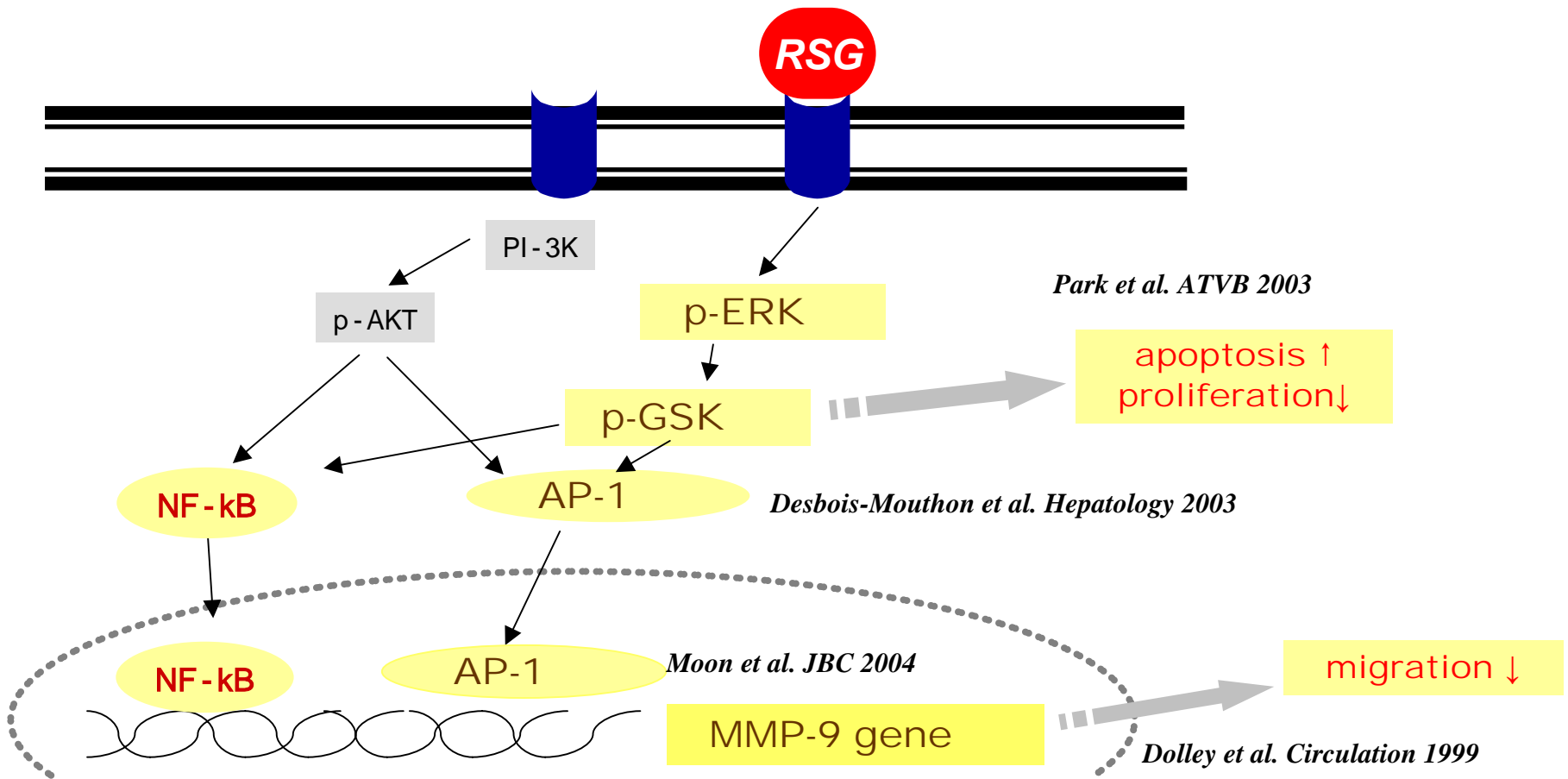
Rosiglitazone inhibits neointimal hyperplasia, which is partially reversed by GSK3 β inactivation



Rosiglitazone inhibits **proliferation** & induces **apoptosis** of VSMC
after vascular injury,
which is partially reversed by GSK3 β inactivation



Rosiglitazone reduces neointimal formation via activating GSK3 β and inhibiting MMP-9



PPAR

PPAR polymorphism influence CAD progression

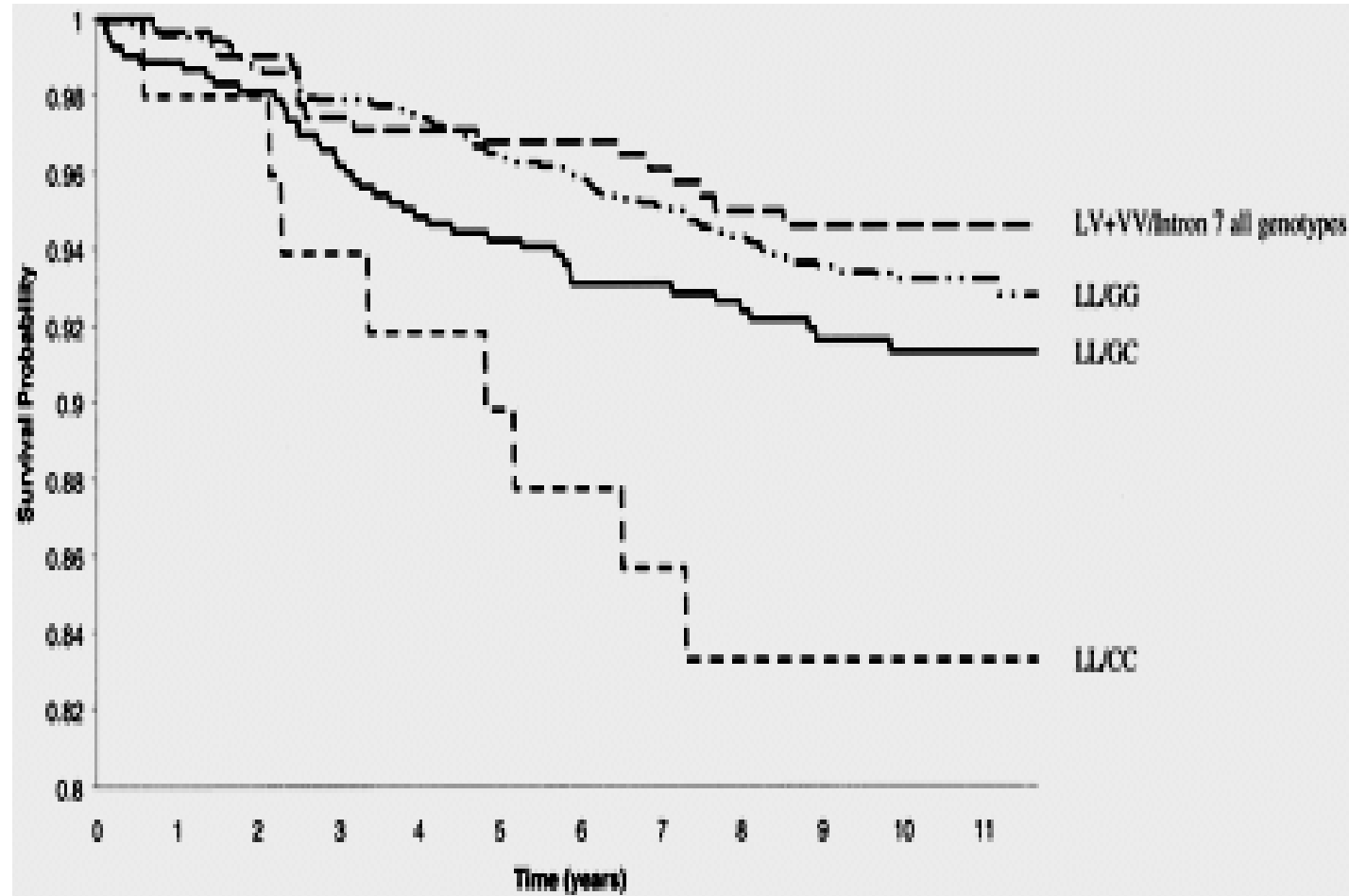
L162V polymorphism & G to C transversion in intron 7 of PPAR-alpha gene

IHD patients

N= 3309

11yr F/U
(retrospective)

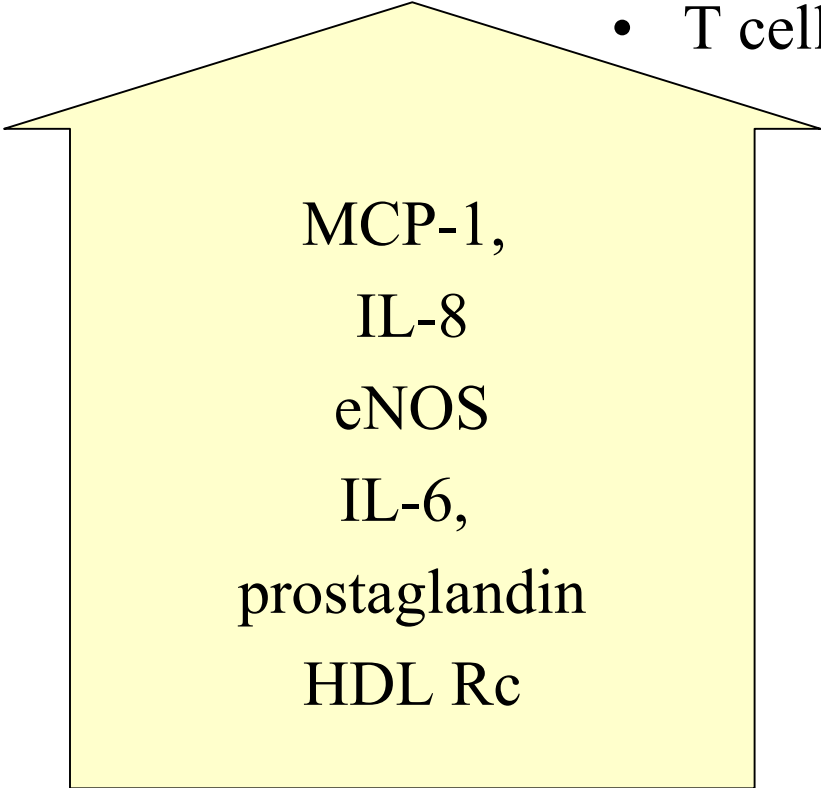
survival



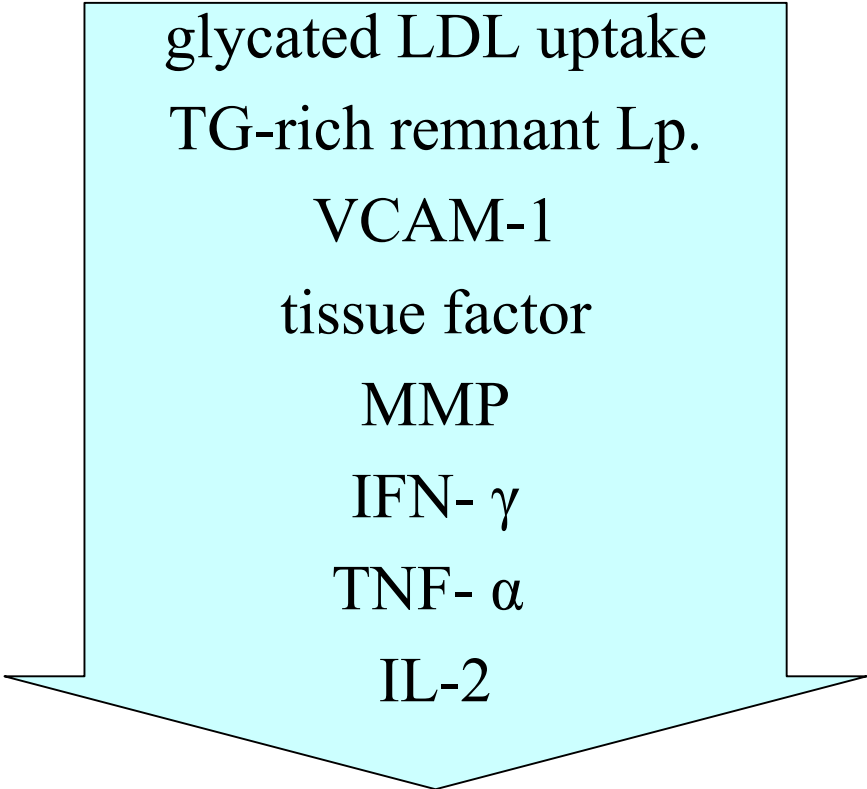
David et al. Circulation. 2002

PPAR - cellular actions

- endothelial cell,
- VSMC,
- macrophage
- T cell.



MCP-1,
IL-8
eNOS
IL-6,
prostaglandin
HDL Rc



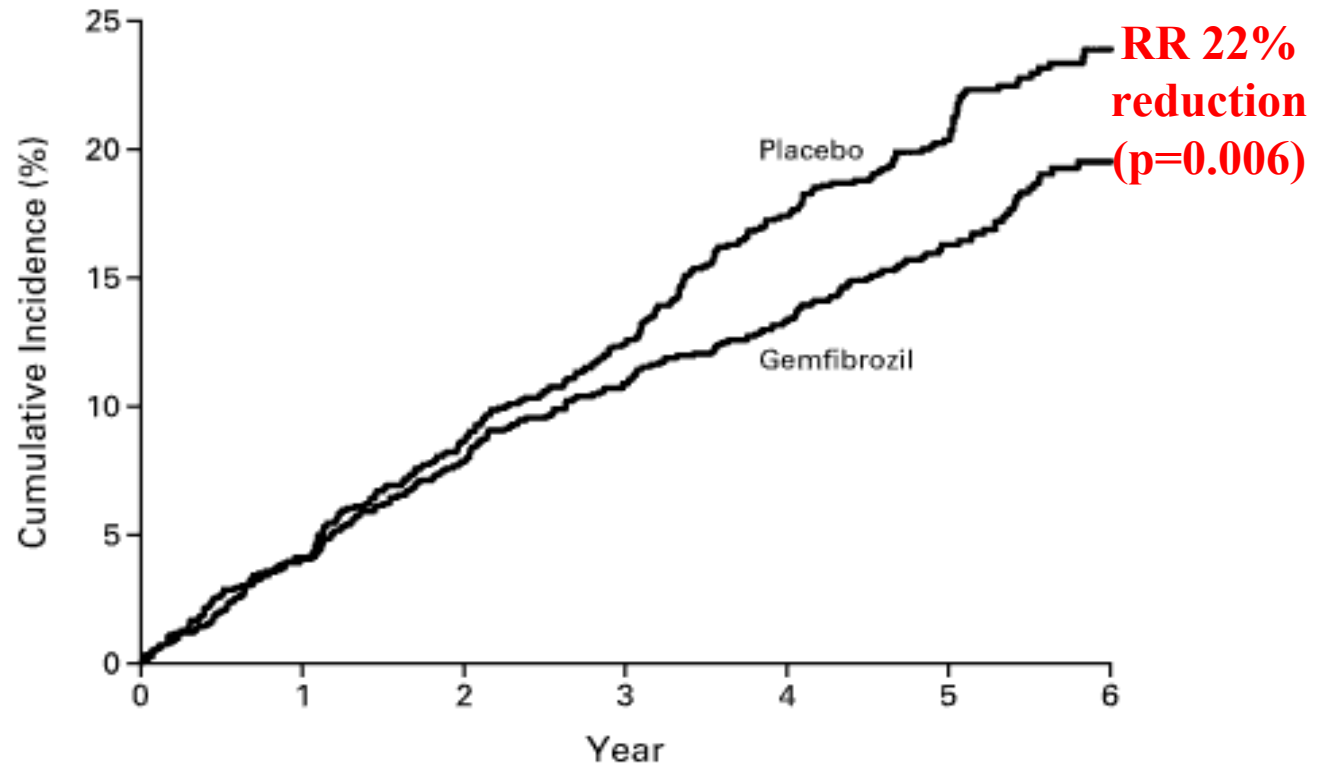
glycated LDL uptake
TG-rich remnant Lp.
VCAM-1
tissue factor
MMP
IFN- γ
TNF- α
IL-2

Fibrate reduces CV event

- Effect of **bezafibrate** treatment over five years on **coronary plaques** causing 20% to 50% diameter narrowing
 - *Ericsson et al. Am J Cardiol. 1997*
- Effect of **fenofibrate** on **progression of coronary-artery disease** in type 2 diabetes.
 - *DAIS Investigators. Lancet. 2001*
- Prevention of the **angiographic progression** of coronary and vein-graft atherosclerosis by **gemfibrozil** after coronary bypass surgery in men with low levels of HDL cholesterol
 - *Frick et al. Circulation. 1997.*
- **Primary-prevention trial** with **gemfibrozil** in middle-aged men with dyslipidemia: safety of treatment, changes in risk factors, and incidence of coronary heart disease.
 - *Frick et al. N Engl J Med. 1987*
- **Gemfibrozil** for the **secondary prevention** of coronary heart disease in men with low levels of high-density lipoprotein cholesterol
 - *Rubins et al. N Engl J Med. 1999*

Fibrate reduces CV event

male CHD patients
with low HDL
N= 2531
6yr F/U
CV event



No. AT RISK

Placebo	1267	1200	1118	1040	962	666	466
Gemfibrozil	1264	1201	1128	1067	1005	706	498

Rubins et al. N Engl J Med 1999

Fibrate improves endothelial function

- **Ciprofibrate** therapy improves **endothelial function** and reduces **postprandial lipemia and oxidative stress** in type 2 diabetes mellitus
 - Evans *et al. Circulation*. 2000; 101: 1773–1779.
- **Gemfibrozil** improves **insulin sensitivity and FMD** in type 2 diabetic patients.
 - Avogaro *et al. Eur J Clin Invest*. 2001; 31: 603–609
- Effect of **fenofibrate** on brachial artery **FMD** in type 2 diabetes mellitus.
 - Playford *et al. Am J Cardiol*. 2002; 90: 1254–1257.
- Short-term TG lowering with **fenofibrate** improves **vasodilator function** in subjects with hypertriglyceridemia.
 - Capell *et al. Arterioscler Thromb Vasc Biol*. 2003; 23: 307–313.
- **Exercise-induced vasomotion** of angiographically normal and stenotic coronary arteries **improves** after cholesterol-lowering drug therapy with **bezafibrate**.
 - Seiler *et al. J Am Coll Cardiol*. 1995; 26: 1615–1622.

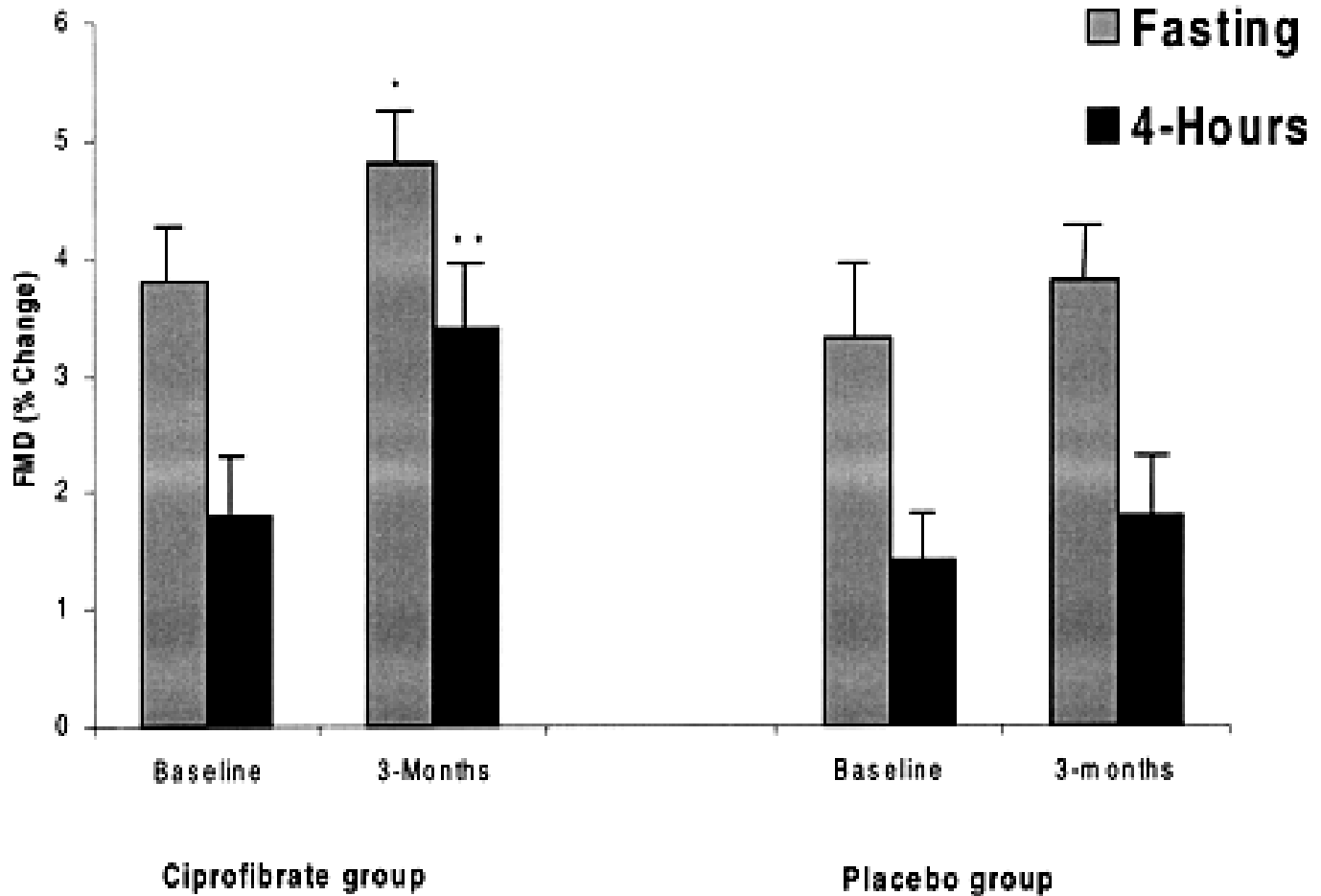
Fibrate improves endothelial function

type 2 DM

N= 21

3m F/U

FMD



PPAR – **clinical** data summary

- PPAR polymorphism \Leftrightarrow CAD progression
- fibrate intervention
 - reduce atherosclerosis and CAD event
 - improve endothelial function

.. well correlated with **animal studies**

PPAR- α

improves lipid metabolism

reduces vascular inflammation

improves vascular tone

PPAR
&
Cardiovascular disease

Metabolic to Vascular

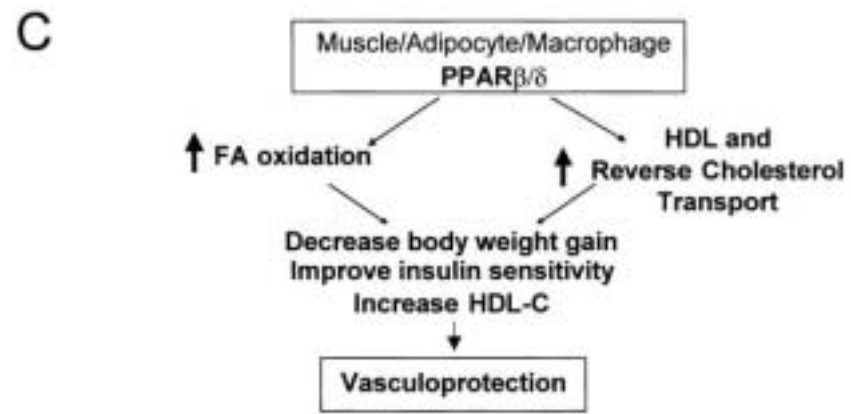
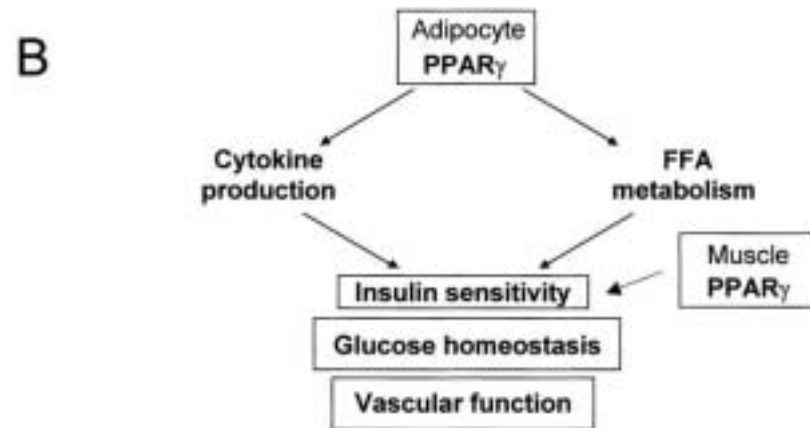
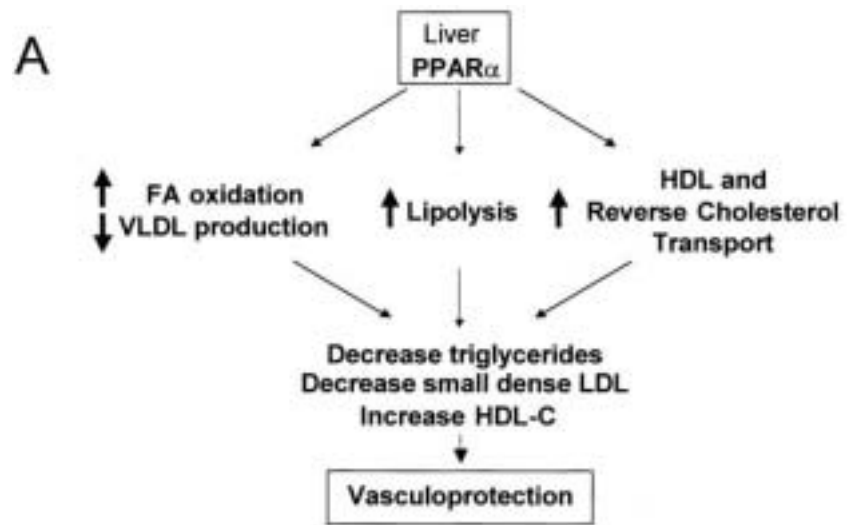
actions

of

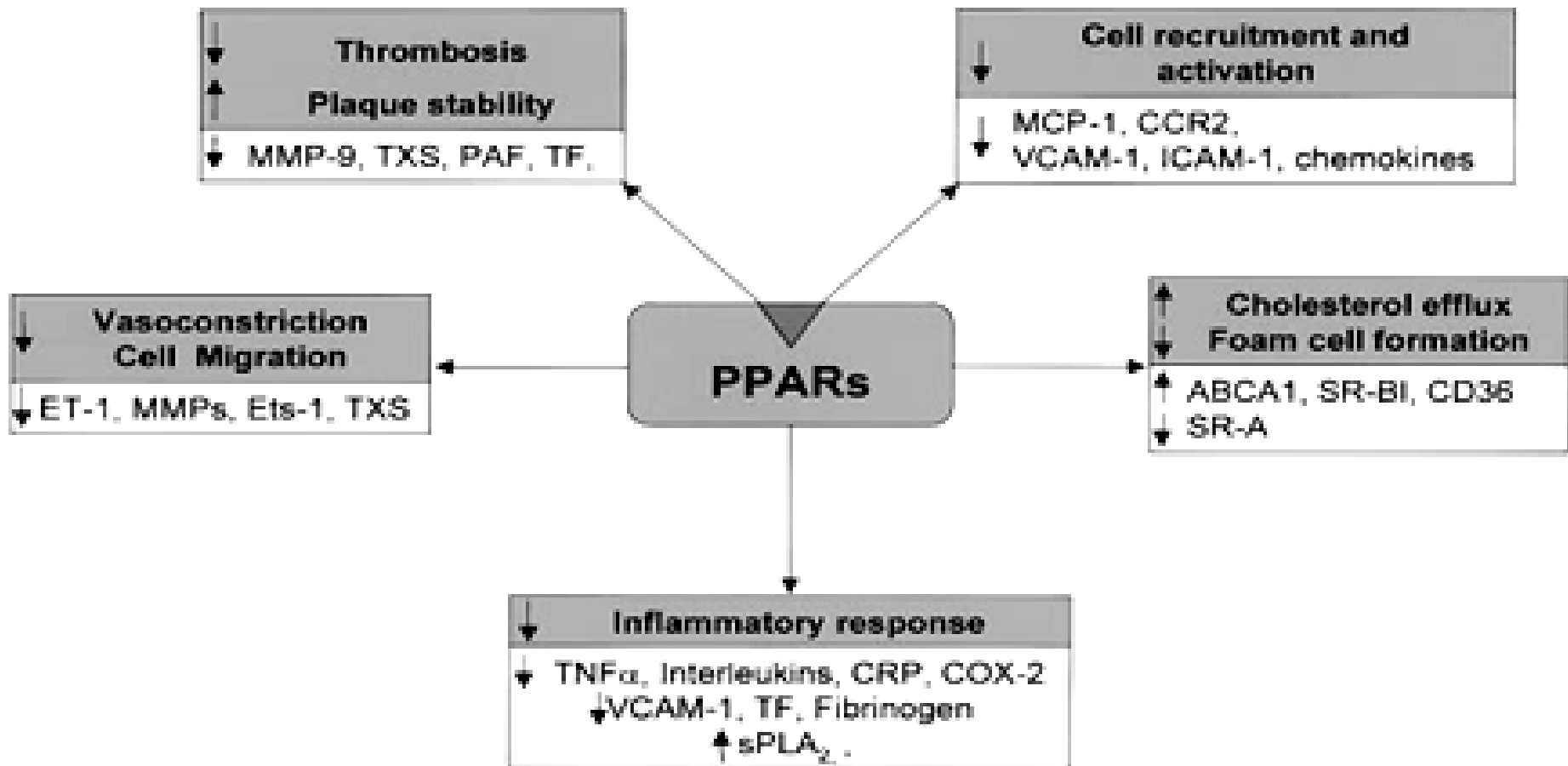
PPAR α ,

PPAR γ ,

PPAR β/δ .



Overall actions of PPARs



Ongoing cardiovascular endpoint trials with PPAR and agonists

- FIELD : **Fenofibrate** Intervention and **Event** Lowering in **Diabetes**
- RECORD : **Rosiglitazone** Evaluated for **Cardiac** Outcomes and for Regulation of glycaemia in **Diabetes**
- PROACTIVE : PROspective **pioglitazone** Clinical Trial In **macroVascular** Events
- DREAM : **Diabetes** REduction Assessment with ramipril and **rosiglitazone** Medication
- CHICAGO : A Study Evaluating **Carotid** Intima-Media Thickness in Atherosclerosis Using **Pioglitazone**

Ongoing cardiovascular endpoint trials with PPAR and agonists

- PERISCOPE : **Pioglitazone** Effect on Regression of **Intravascular Sonographic Coronary** Obstruction Prospective Evaluation
- STARR : Study of **Atherosclerosis** with Ramipril and **Rosiglitazone**
- BARI-2D: Bypass Angioplasty Revascularization Investigation – Type 2 diabetes
- RECORD: **Rosiglitazone** Evaluated for **Cardiac** Outcomes and Regulation of Glycemia in **Diabetes**
- ACCORD: Action to Control Cardiovascular Risk in diabetes
- ACT NOW: Act Now for the Prevention of Type 2 Diabetes

Acknowledgement

-
- PPAR project members
 - ; PPAR- γ , α
 - ; PPAR- γ
 - ; PPAR- γ
 - ; PPAR- δ
 - ; PPAR- δ

Therapeutic Perspectives in PPAR

- selective PPAR modulator (SPPARM)

different modification of 3-dimensional conformation of ligand
Rc => different interaction with cofactors

ex.) effects on glucose metabolism without stimulating adipocyte differentiation

- combined PPAR α /PPAR γ
 - ragaglitazar, tesaglitazar

Summary ; mechanism of PPAR- γ to inhibit NIH [SNUH data]

- Rosiglitazone
 - Induction of VSMC apoptosis via ERK/GSK pathway (WST-1, FACS, western blot)
 - Attenuation of AP-1 activity (EMSA)
 - Inhibition of VSMC migration via suppression of MMP-9 and induction of TIMP-1 (western blot, zymography, scratch assay)
 - Reduction of VSMC proliferation (PCNA)
 - Induction of VSMC apoptosis (TUNEL)
 - Inhibition of neointimal hyperplasia (in rat)

Rosiglitazone inhibits neointimal hyperplasia, which is partially reversed by GSK3 β inactivation

