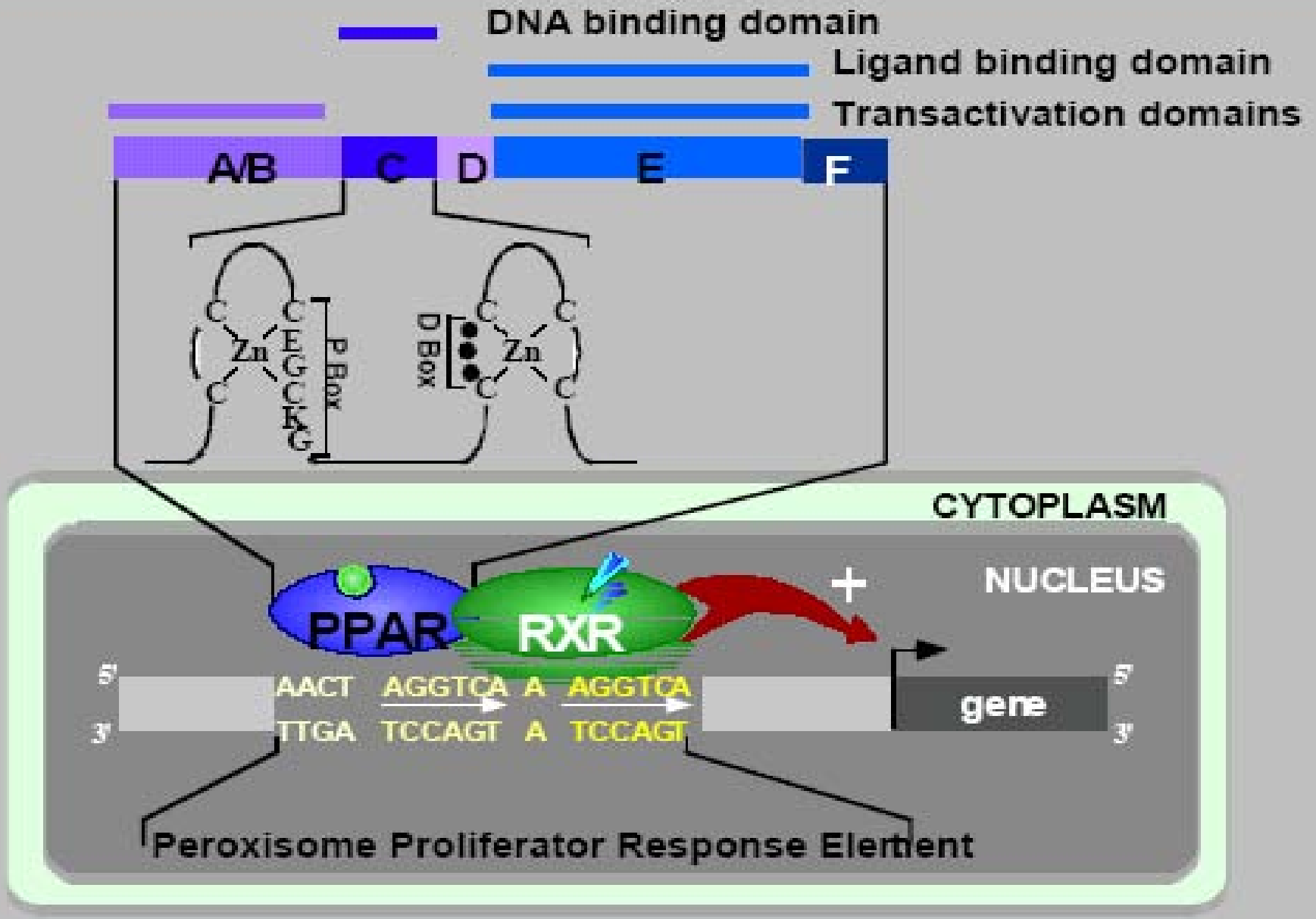
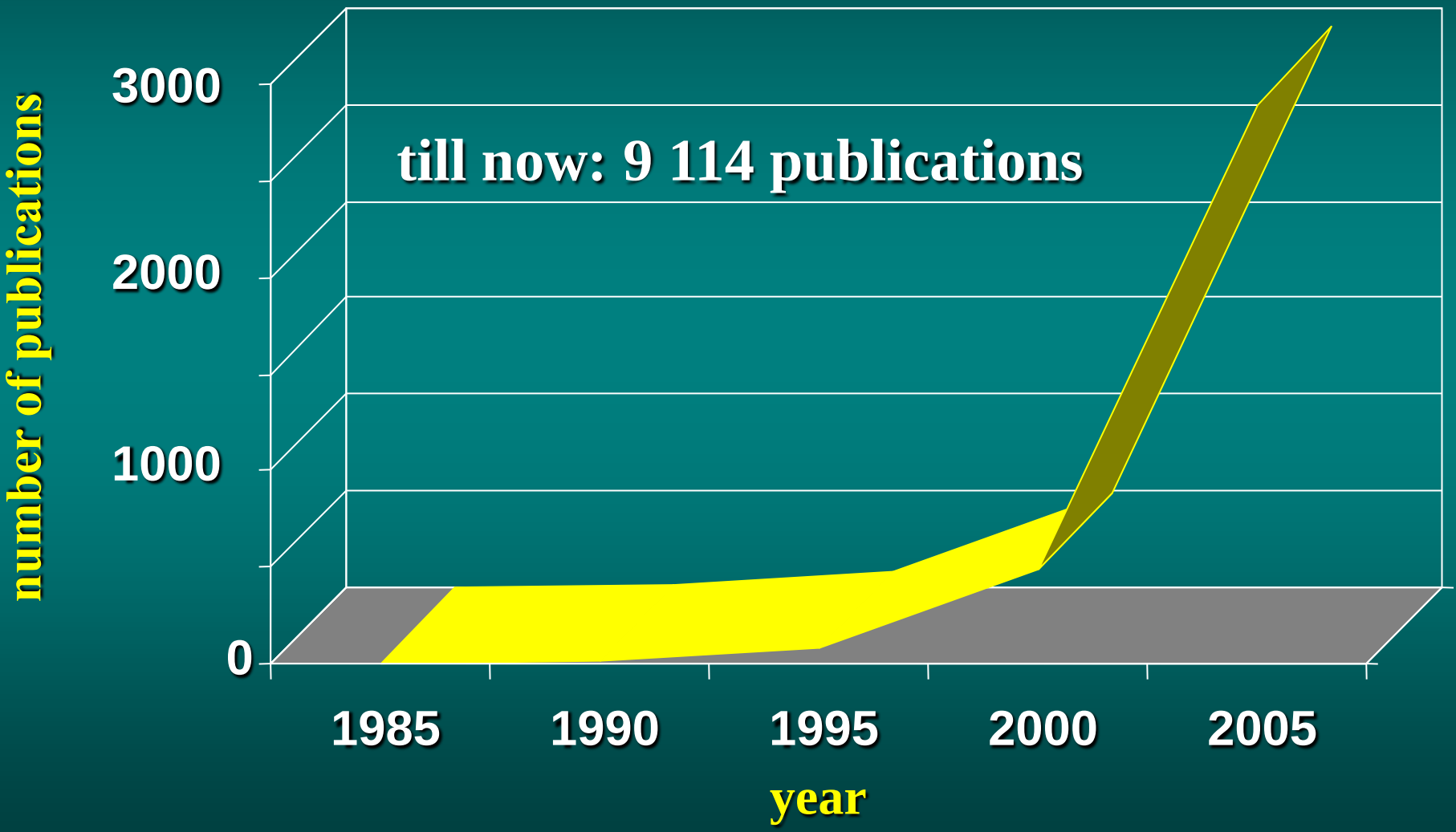
PPAR

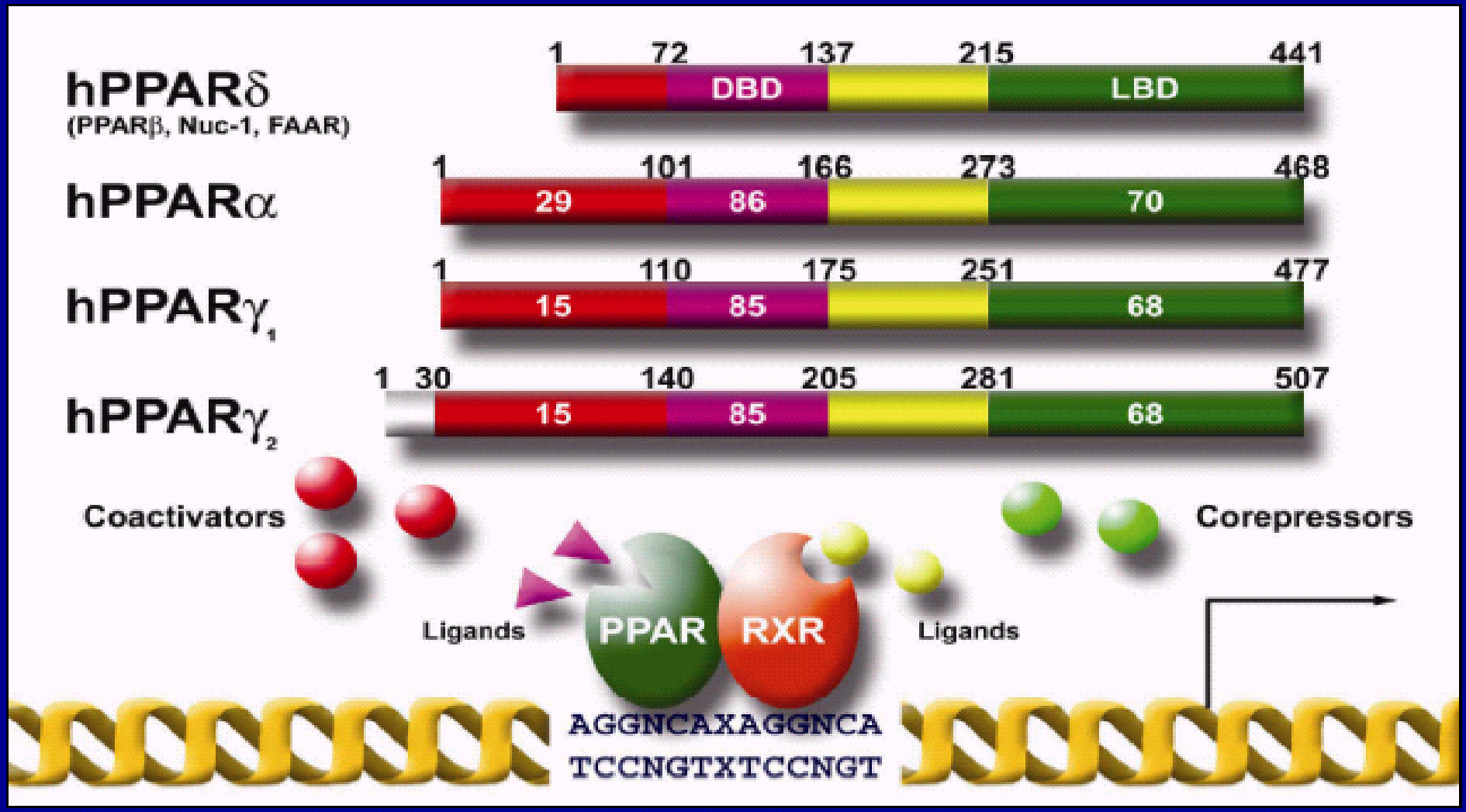
PPAR : RXR Is a Functional Unit



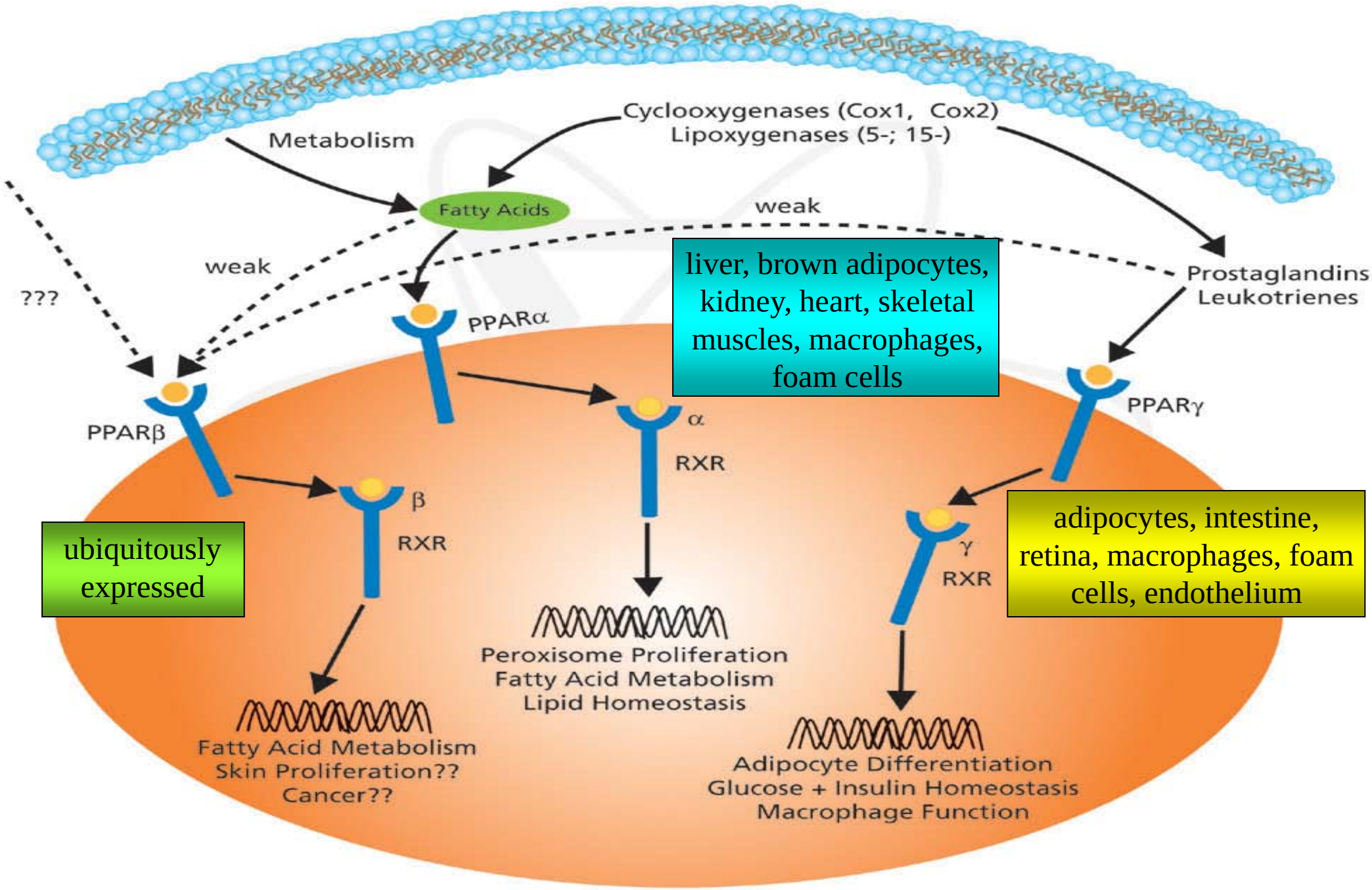
PPAR – history of research



PPARs: Isoforms of Receptors



Rosen ED, Spiegelman BM. *J Biol Chem* 2001; 276: 37731-37734


Metabolism
Cyclooxygenases (Cox1, Cox2)
Lipoxygenases (5-; 15-)
Fatty Acids
weak
weak
???
Prostaglandins
Leukotrienes
liver, brown adipocytes, kidney, heart, skeletal muscles, macrophages, foam cells
PPARα
PPARβ
PPARγ
α
RXR
β
RXR
γ
RXR
ubiquitously expressed
adipocytes, intestine, retina, macrophages, foam cells, endothelium
Peroxisome Proliferation
Fatty Acid Metabolism
Lipid Homeostasis
Fatty Acid Metabolism
Skin Proliferation??
Cancer??
Adipocyte Differentiation
Glucose + Insulin Homeostasis
Macrophage Function

PPARα, PPARγ, PPARδ

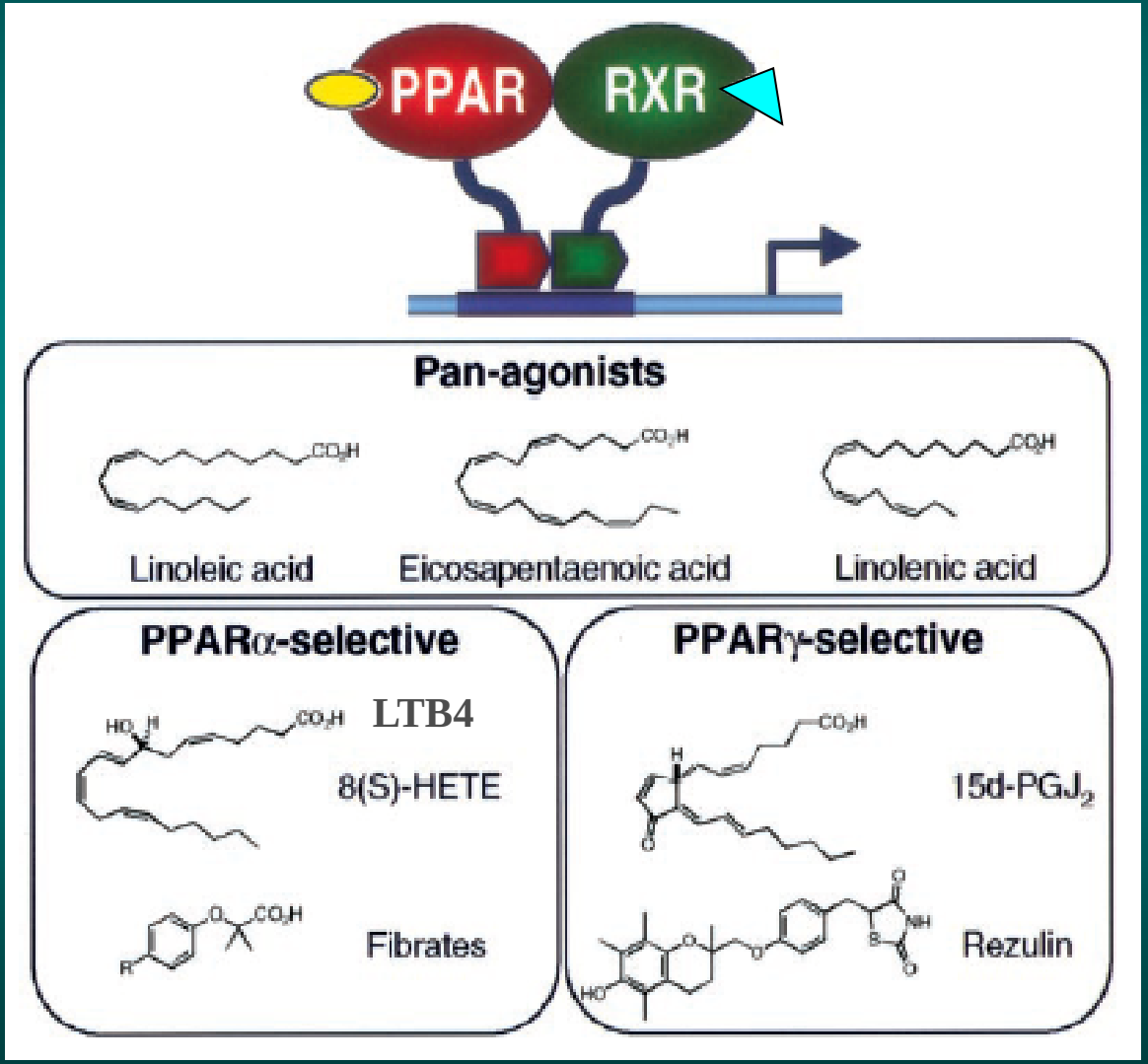
- Lipid-activated transcription factors

- Regulate:

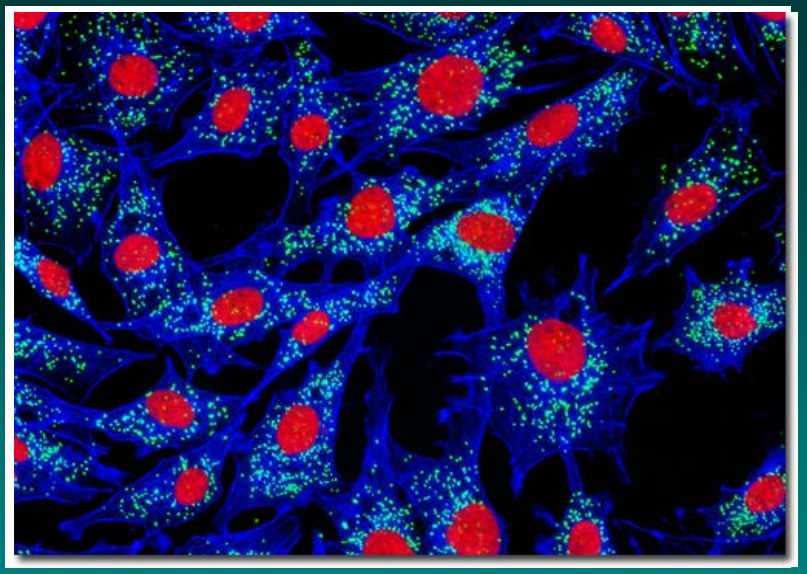
  * lipid metabolism

  * glucose homeostasis

- Impaired PPAR activity is believed to lead to dyslipidemia and insulin resistance

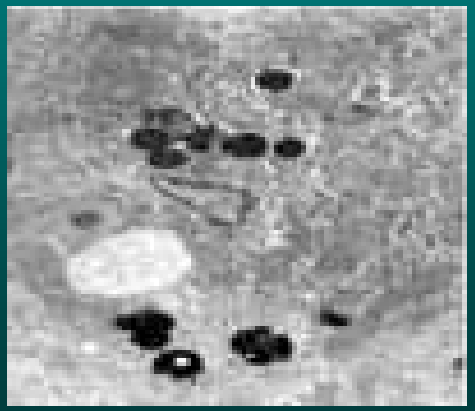


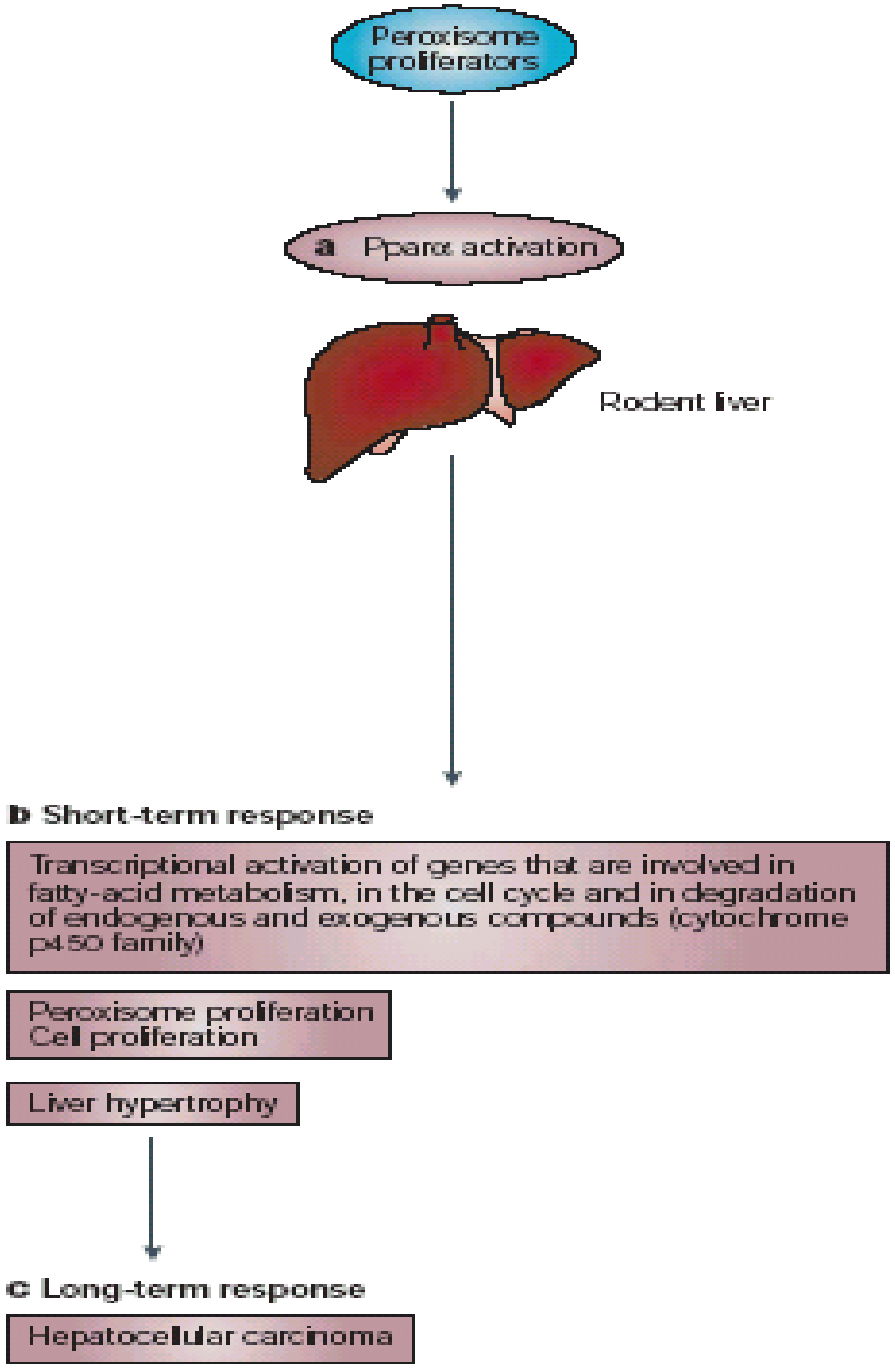
Peroxisomes



- The ubiquitous organelles, delineated by a single membrane, containing enzymes that utilize oxygen to subtract hydrogen atoms from organic substrates in an oxidative reaction that generates hydrogen peroxide.
- Peroxisomes typically contain catalase, an enzyme that uses H_2O_2 to oxidize formic acid, alcohols, phenols, and other substrates. Any remaining H_2O_2 present in the cell is broken down by catalase into water and free oxygen molecules.
- Peroxisomes are responsible for degradation of fatty acids and the catalysis of the initial steps in the synthesis of phospholipids, utilized in membrane formation.

peroxisome proliferators





Peroxisome proliferators

- Peroxisome proliferators are a diverse group of chemicals.

- Responses of rodent hepatocytes to peroxisome proliferators include proliferation of peroxisomes, increase in β-oxidation of fatty acids, resulting from induction of all enzymes in the peroxisomal β-oxidation cascade, and hepatomegaly.

- The peroxisome proliferator-response has gained considerable interest due to its association with metastatic hepatocellular carcinomas in rodents. The mechanism is not clear - these compounds do not bind directly and do not damage DNA.

PPARs (peroxisome proliferator-activated receptors)

- PPARs were cloned in 1990 as transcription factors that mediate the effects of synthetic peroxisome proliferators.

- Since this time PPARs have been described in a wide variety of species ranging from zebrafish and Xenopus to mouse and human.


Danio regio


Xenopus laevis

- The ligand binding pocket of PPARs is much larger than that of other NRs, with a volume of 1300 Å, of which the ligand occupies only about 30% to 40%.

- Overall, PPARs appear to have evolved as NRs adapted for binding to multiple natural ligands with relatively low affinity.

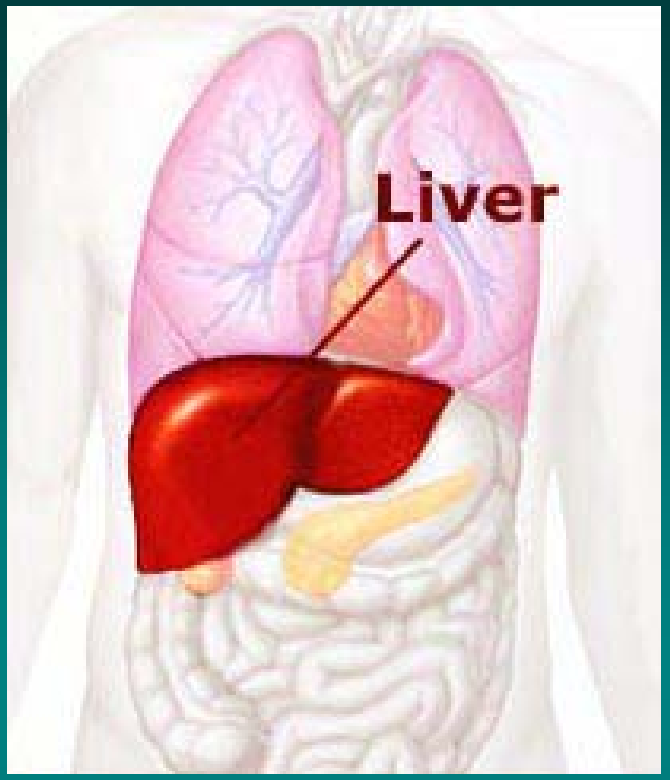
- The first PPRE was identified in the promoter of the acyl coenzyme A (acyl-CoA) oxidase gene, and then in a number of genes activated during adipocyte differentiation or associated with lipid metabolism.

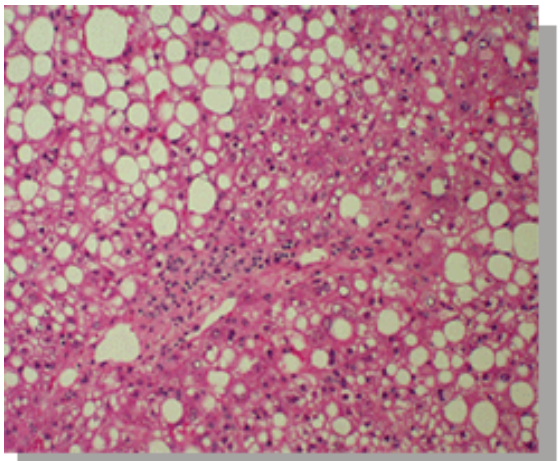
PPARα and lipid metabolism

In the liver, PPARα targets form a comprehensive ensemble of genes which participates in many if not all aspects of lipid catabolism. It includes:

* transport of fatty acids in the circulation,
* their uptake by the hepatocytes,
* intracellular binding by fatty acid binding proteins,
* activation by the acyl-CoA synthase,
* β-oxidation in the peroxisomes and mitochondria,
* ω-oxidation in the microsomes.

Ethanol inhibits PPARα activity – it may play a role in development of alcoholic fatty liver.





PPARα$^{-/-}$ mice

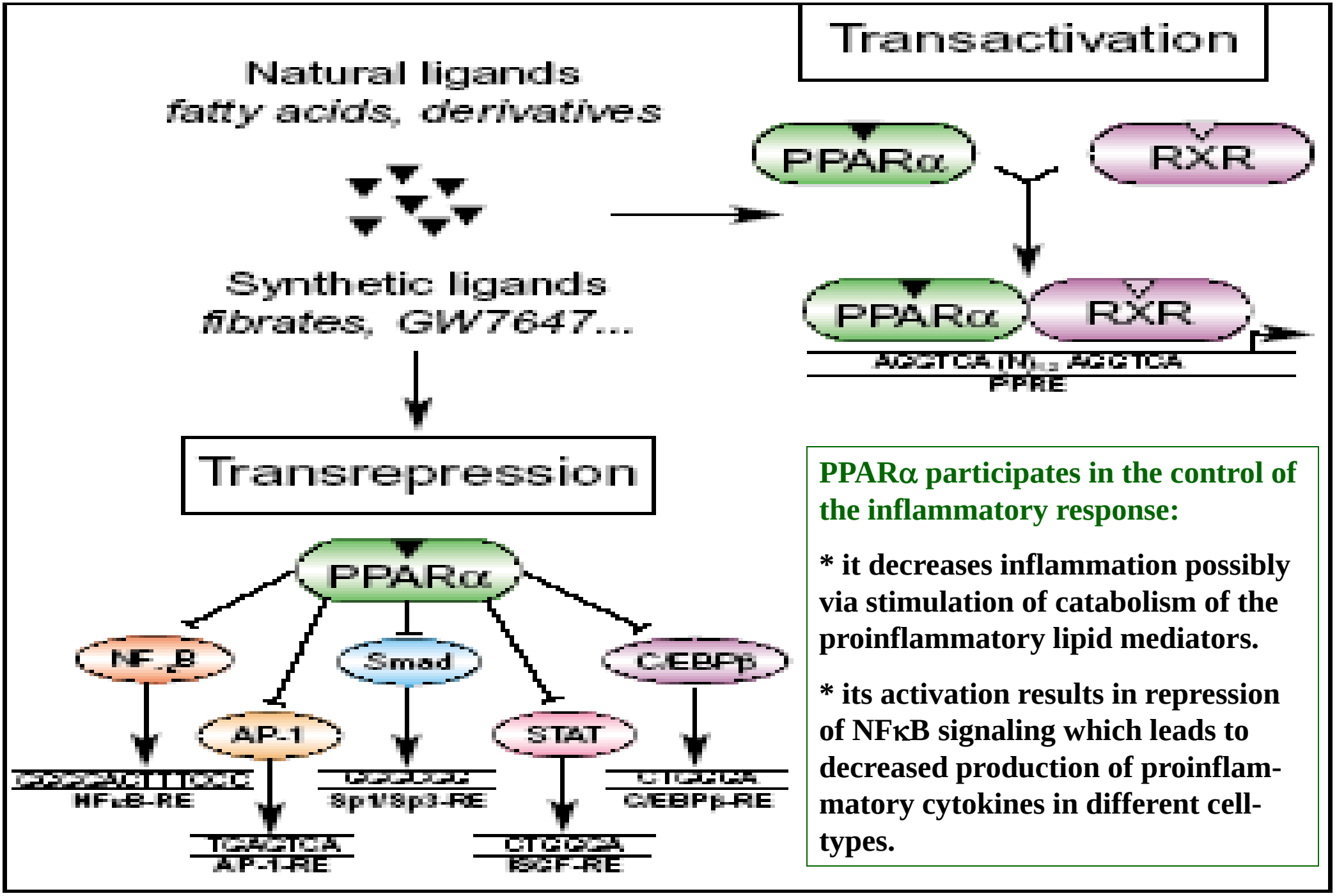
• Overall, PPARα acts as a global regulator of energy metabolism, which coordinates the rates of utilization of the various energy sources in relation to food availability.

• Accordingly, PPARα null mice which are viable and do not exhibit any obvious phenotype when kept under normal laboratory conditions and diet, experience serious difficulties during fasting, a situation that normally results in an enhanced fatty acid mobilization and increased β-oxidation in the liver as fatty acids represent the major energy source.

• Confronted to such a metabolic challenge, PPARα null mice are not capable of enhanced fatty oxidation and rapidly suffer from hypoglycemia and hypothermia.

• With age they develope obesity.

• Mice display increased inflammatory reactions and delayed wound healing.



PPARα participates in the control of the inflammatory response:

*** it decreases inflammation possibly via stimulation of catabolism of the proinflammatory lipid mediators.**

*** its activation results in repression of NFκB signaling which leads to decreased production of proinflammatory cytokines in different cell-types.**

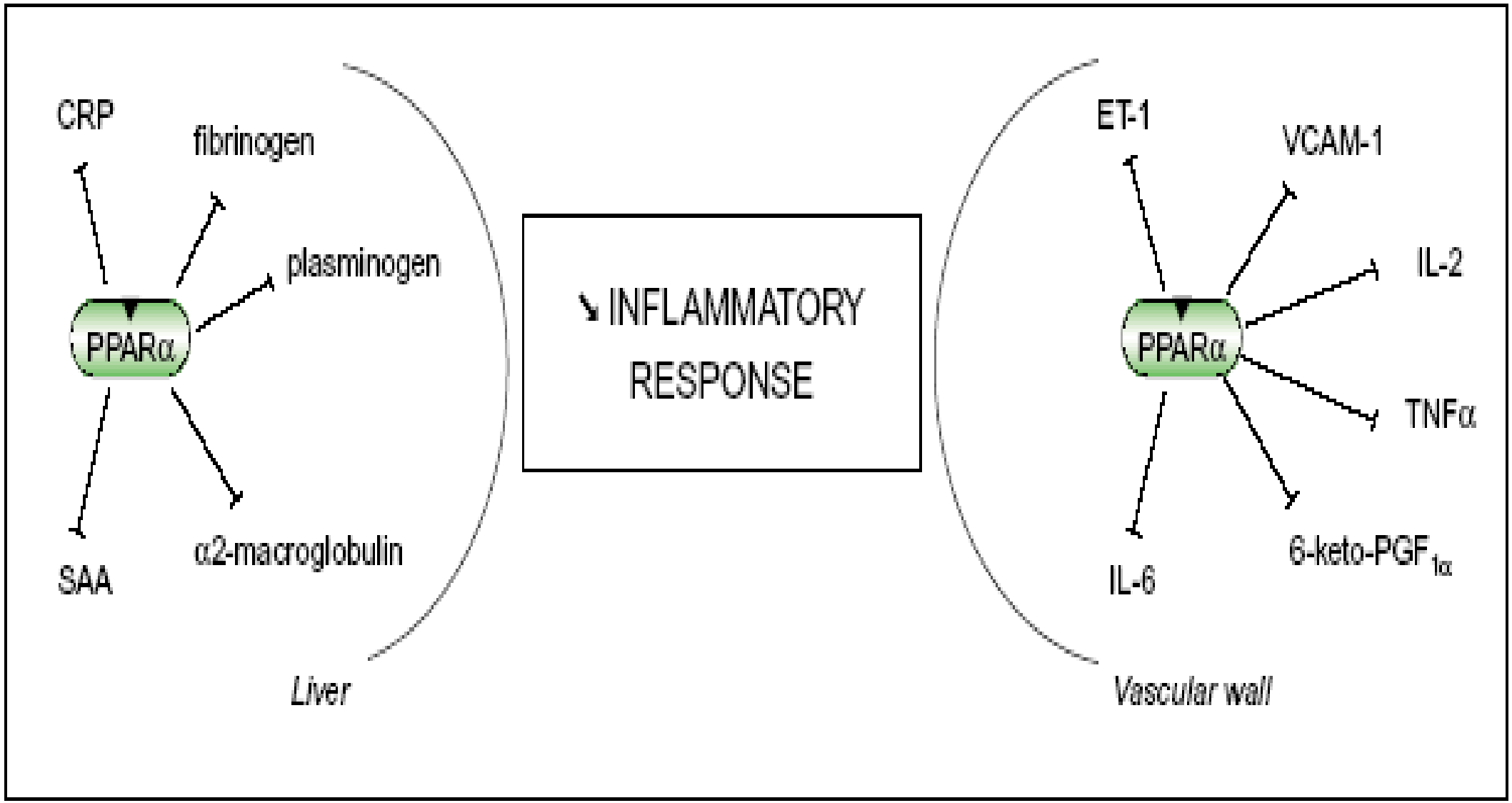
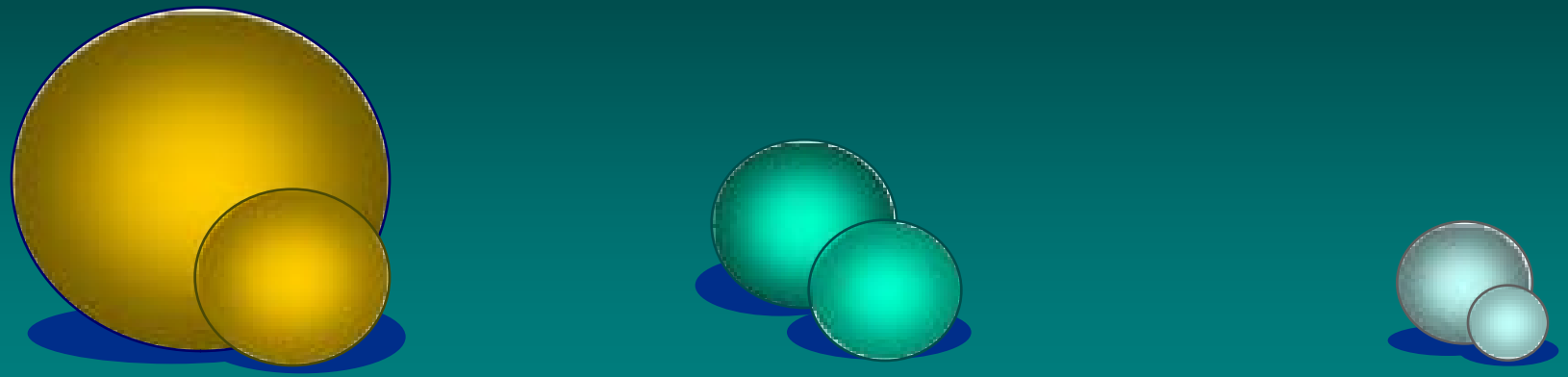


FIG. 3 – PPARα and the control of the inflammatory response. PPARα interferes with acute phase protein production in the liver and decreases the expression of several inflammatory mediators or cytokines directly in the vascular wall, thus exerting an overall effect on the systemic and local inflammatory response. CRP: C-reactive protein; SAA: serum amyloid A; ET-1: endothelin-1; VCAM-1: vascular cell-adhesion molecule-1; IL: interleukin; TNFα: tumor necrosis factor α.

Lipoprotein Classes and Inflammation



Chylomicrons, VLDL, and their catabolic remnants

LDL

HDL

> 30 nm

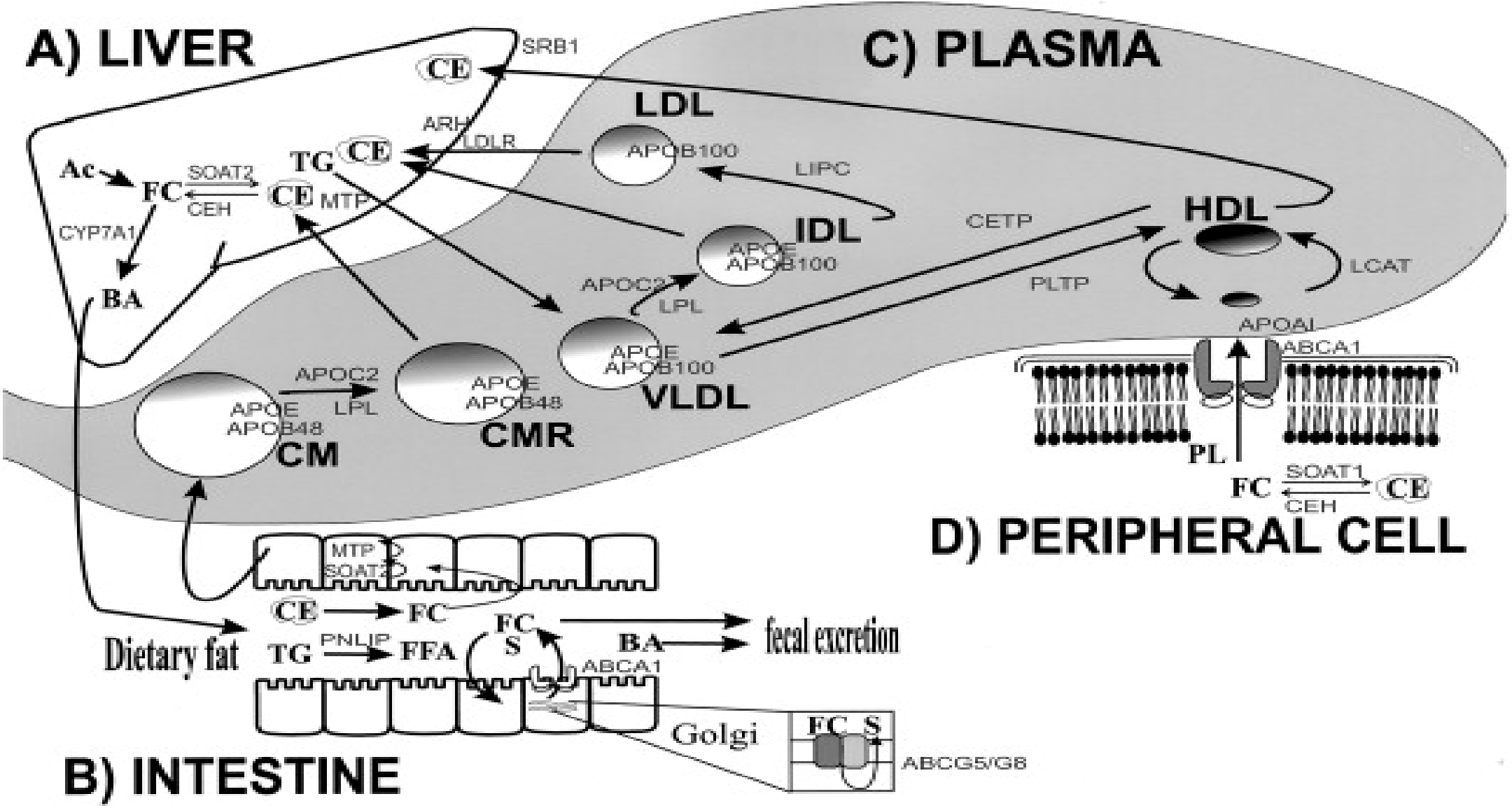
20–22 nm

9–15 nm

Potentially proinflammatory

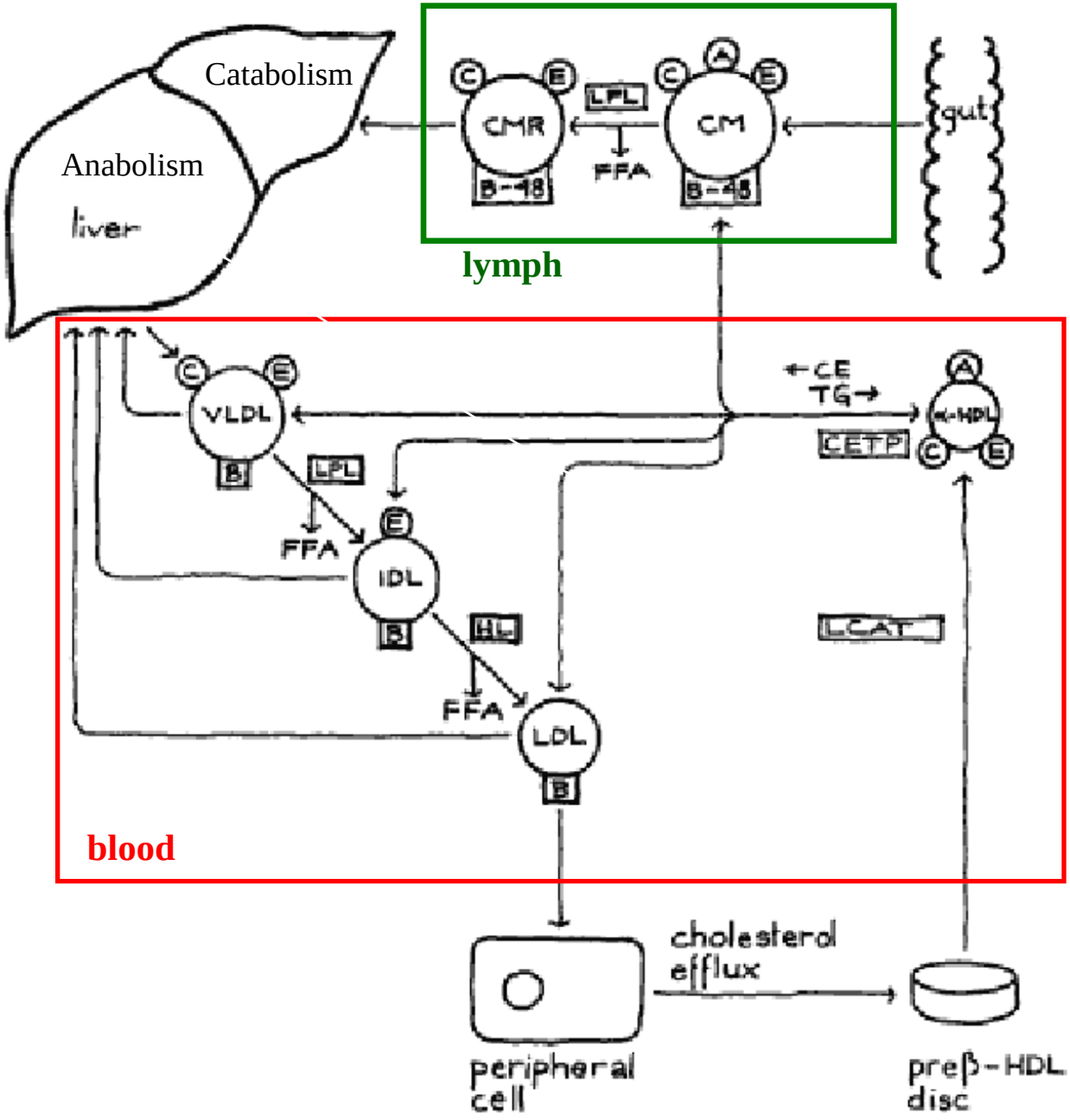
Potentially anti-inflammatory

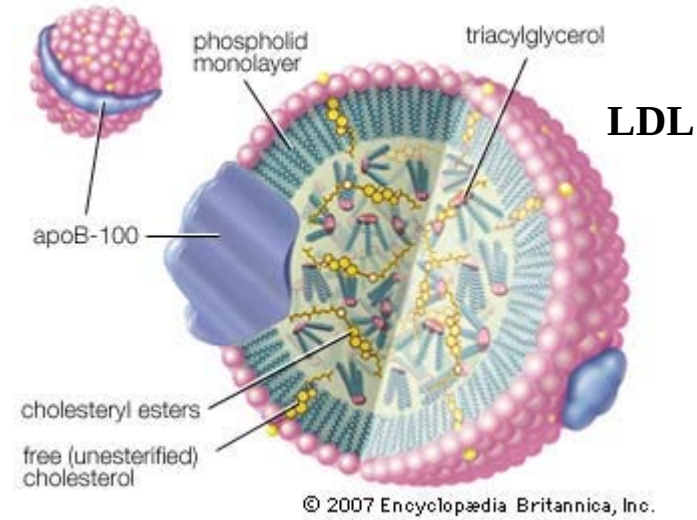
Doi H et al. *Circulation* 2000;102:670-676; Colome C et al. *Atherosclerosis* 2000;149:295-302; Cockerill GW et al. *Arterioscler Thromb Vasc Biol* 1995;15:1987-1994.



PPARα lowers trigliceride levels as a result of:

- **enhanced lipolysis, induction of FA uptake and catabolism**
- **reduced FA synthesis and VLDL production by the liver**
- **increased removal of LDL**



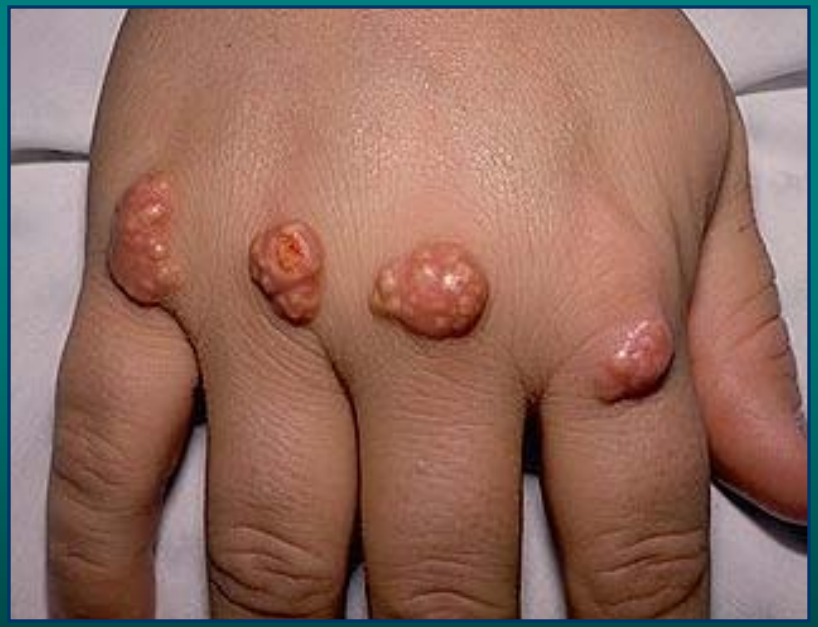


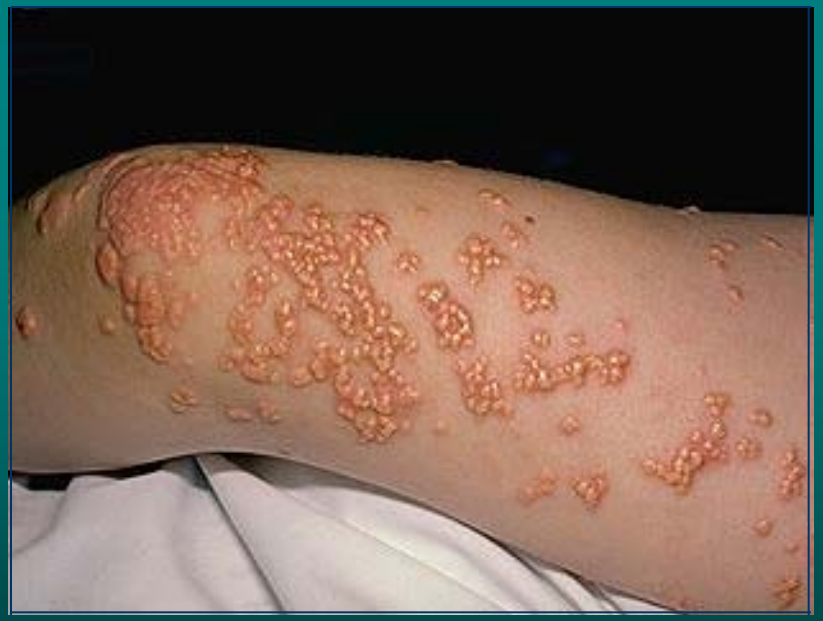
LDL

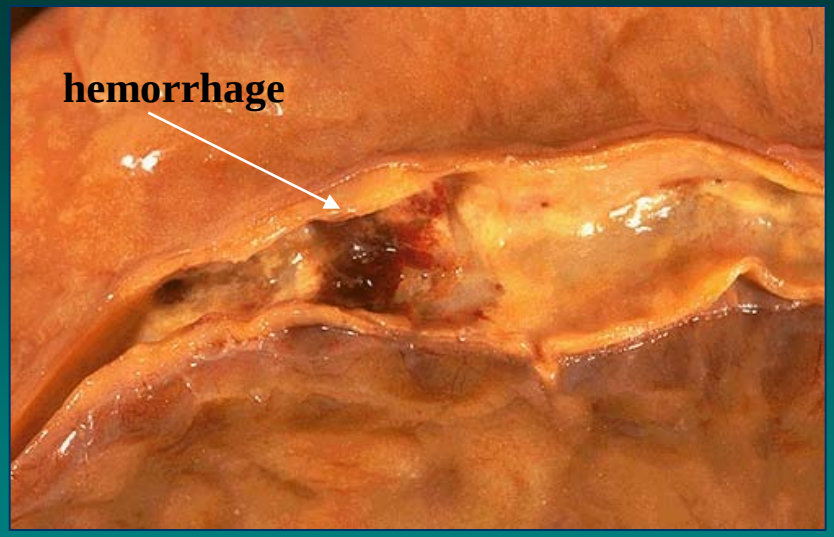
A-I, apo A-I;
B, apo B-100; B-48, apo B-48;
C, apo C;
CE, cholesterol ester;
CETP, cholesterol ester transfer protein;
CM, chylomicron;
CMR, chylomicron remnant;
E, apo E;
HL, hepatic lipase;
LCAT, lecithin:cholesterol acyltransferase
LPL, lipoprotein lipase;
TG, triglycerides;

Eruptive xanthomas

Effect of dyslipidemia



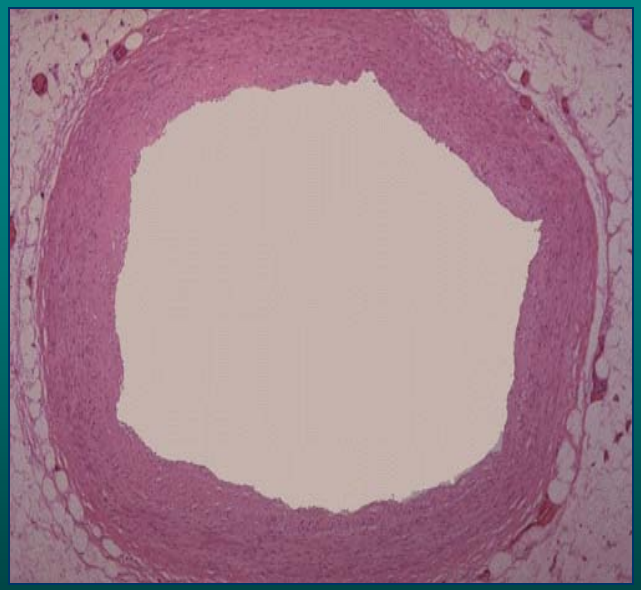




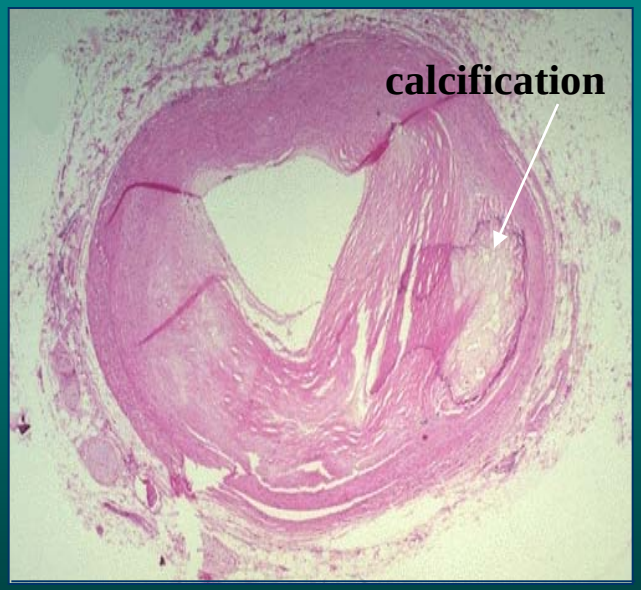
Atherosclerotic plaques

Effect of dyslipidemia

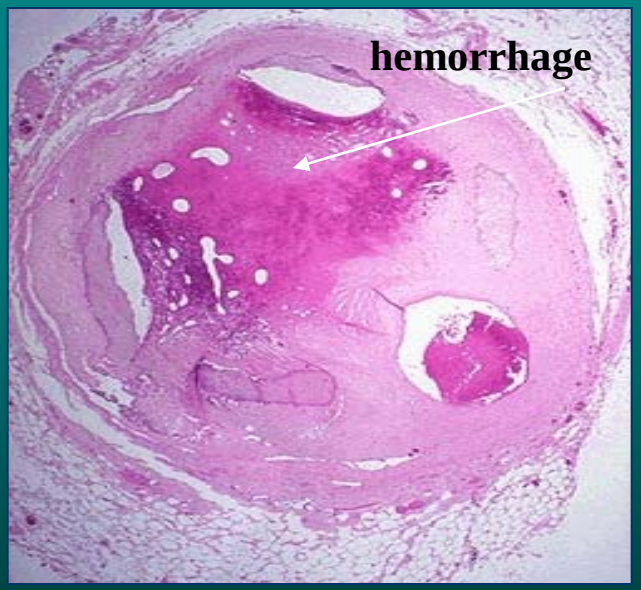
healthy vessel



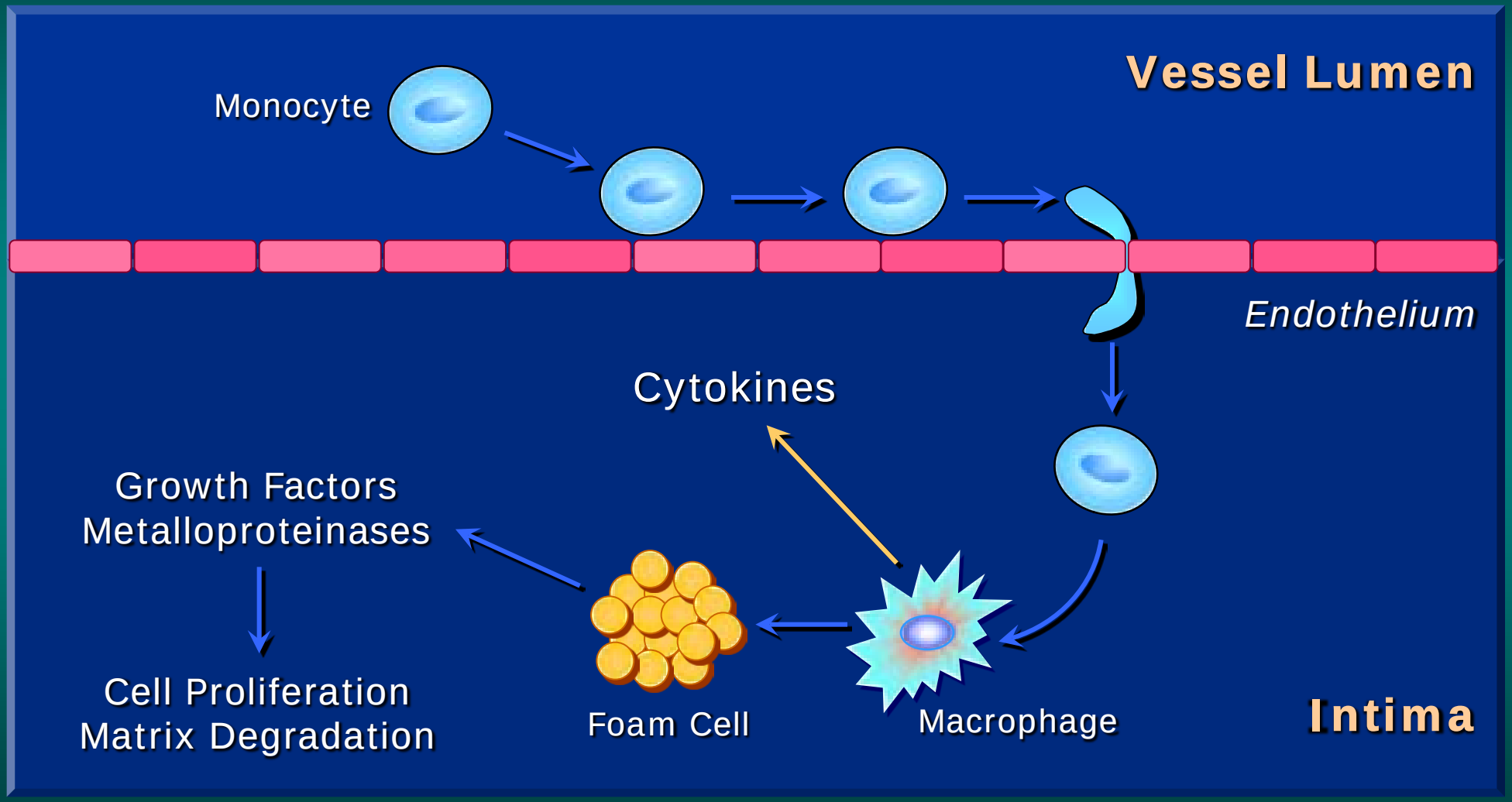
~70% lumen reduction



occlusive plaque

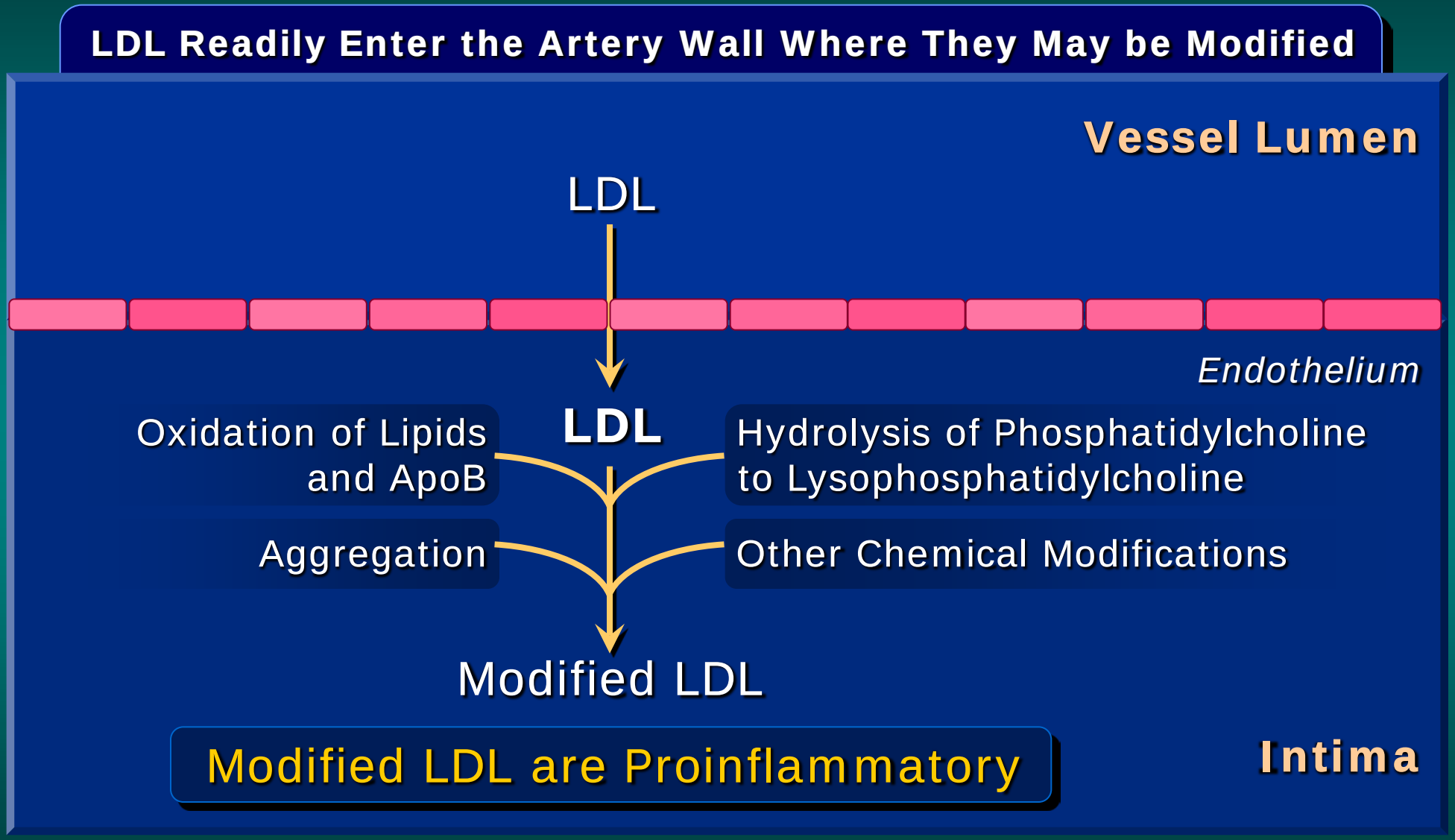


Atherosclerosis is an Inflammatory Disease



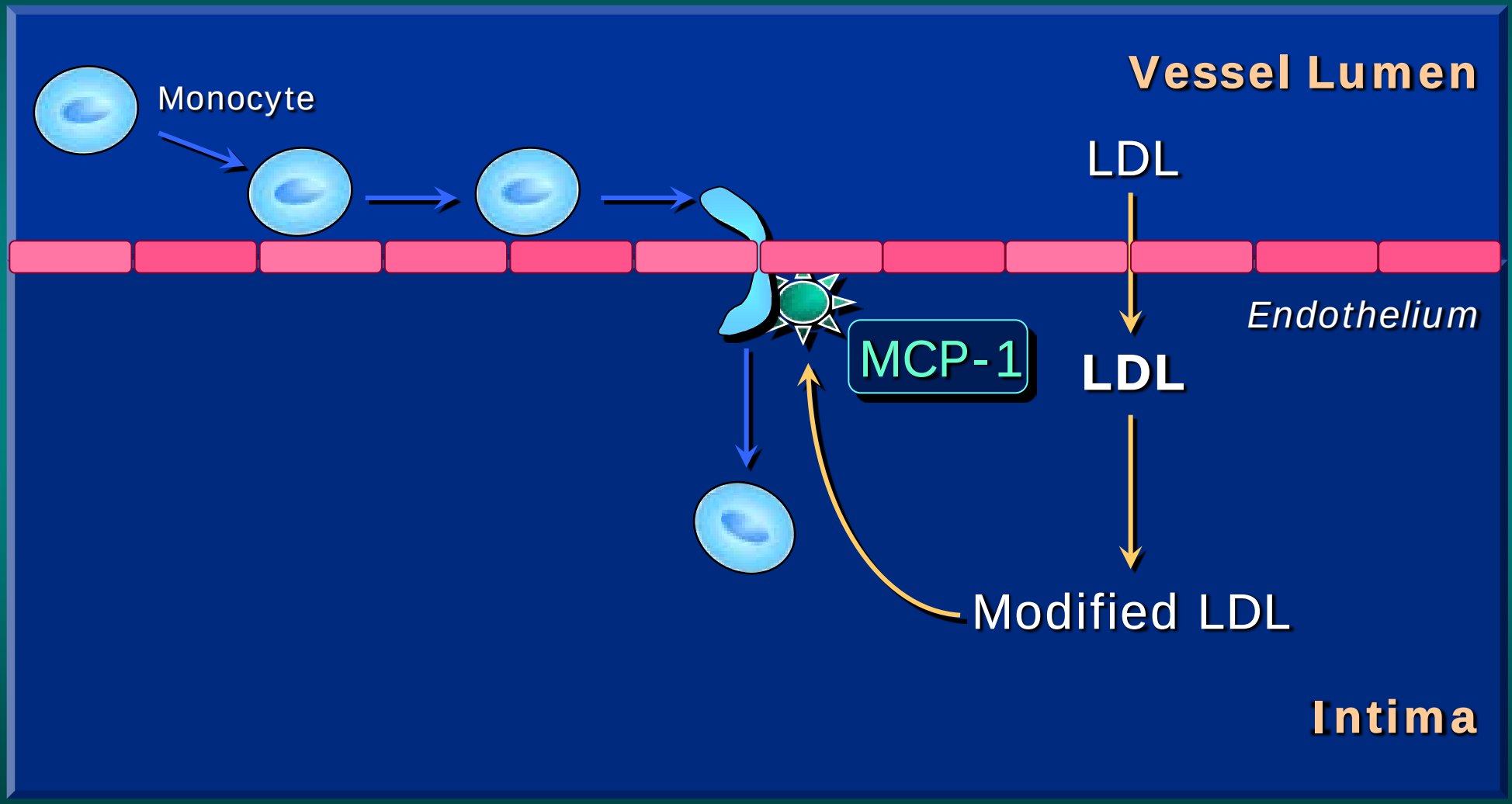
Ross R. *N Engl J Med* 1999;340:115-126.

Role of LDL in Inflammation



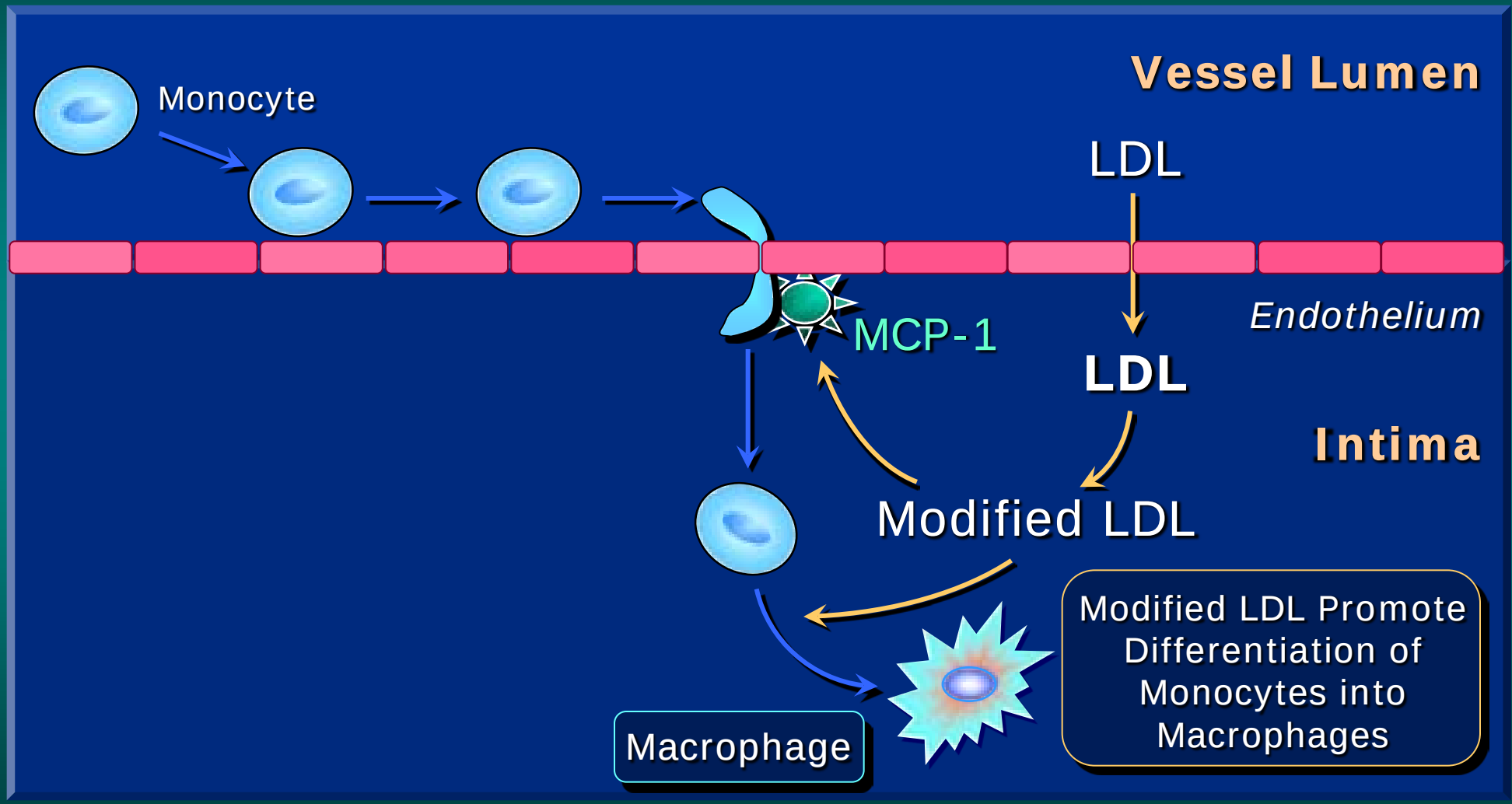
Steinberg D et al. *N Engl J Med* 1989;320:915-924.

Modified LDL stimulate expression of MCP-1 in endothelial cells



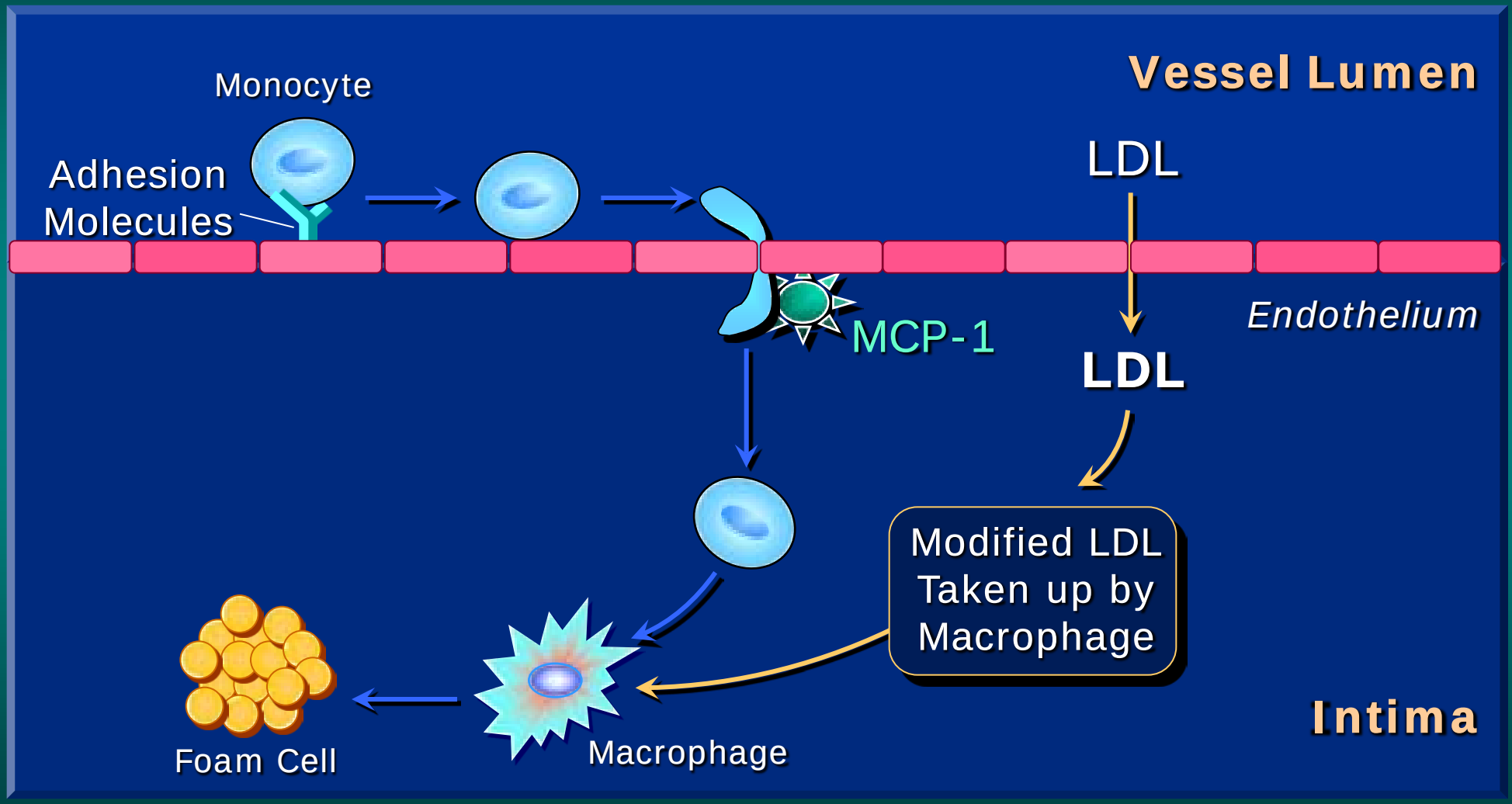
Navab M et al. *J Clin Invest* 1991;88:2039-2046.

Differentiation of monocytes into macrophages



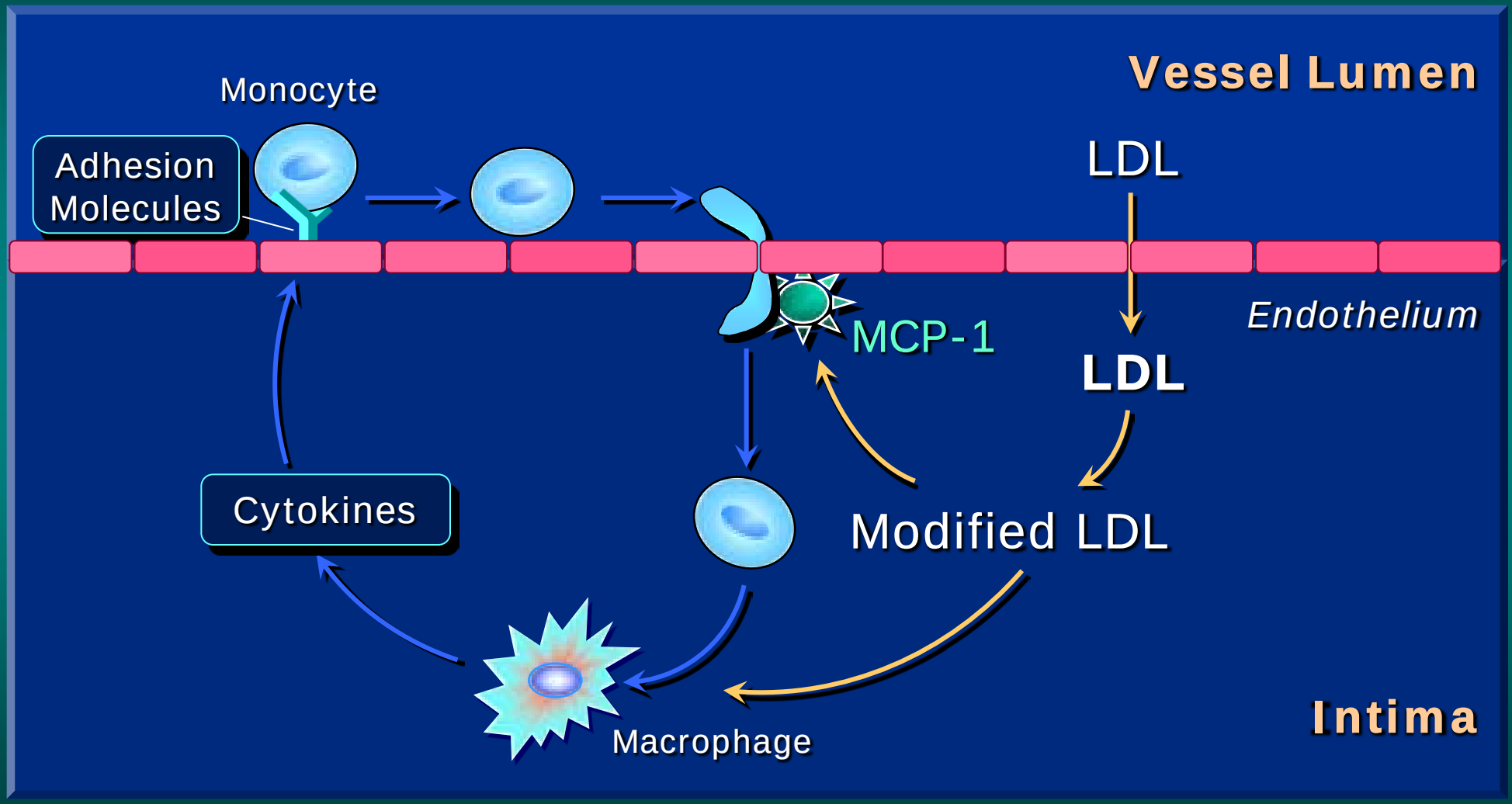
Steinberg D et al. *N Engl J Med* 1989;320:915-924.

Macrophages express receptors that take up modified LDL



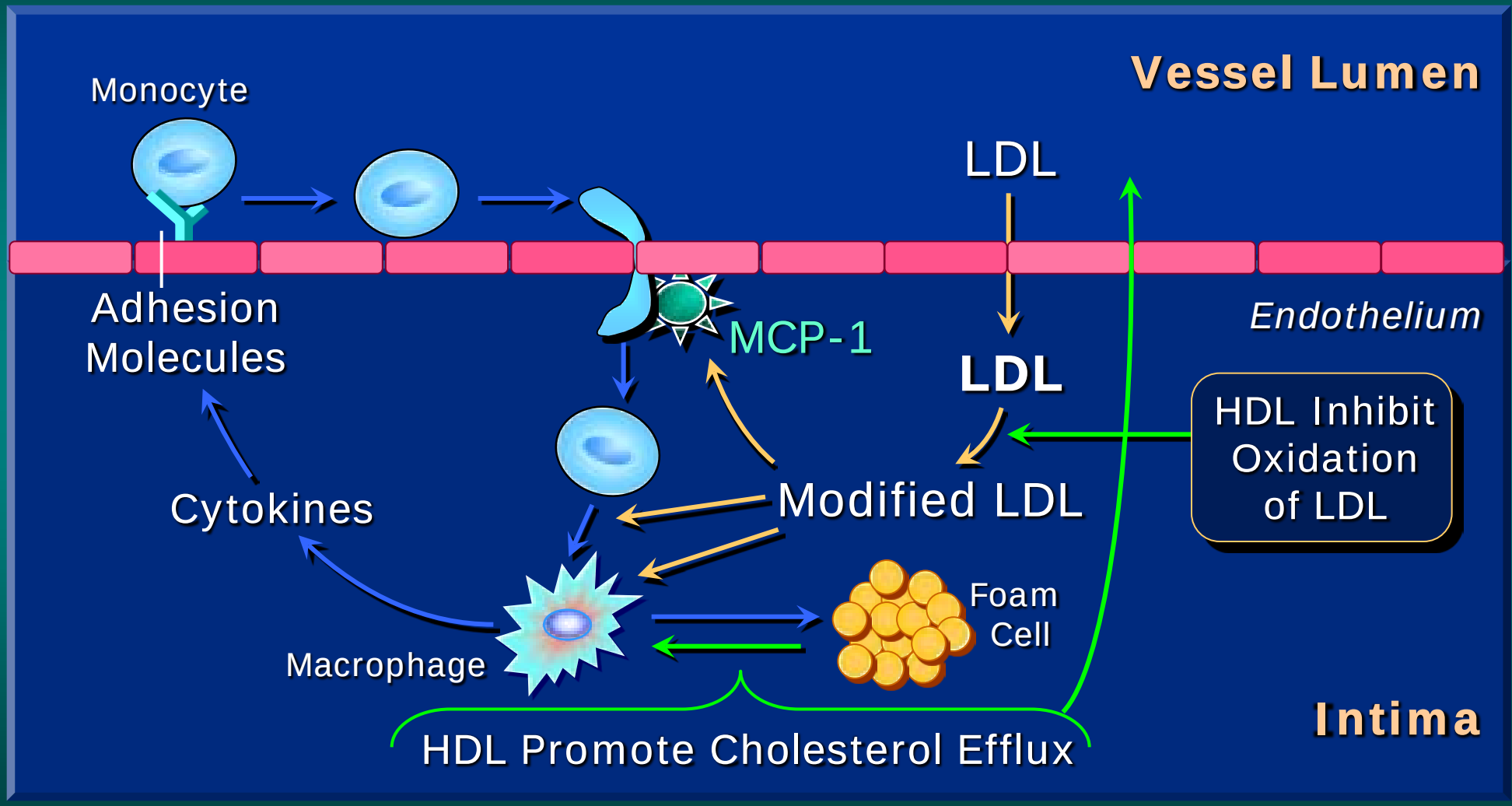
Steinberg D et al. *N Engl J Med* 1989;320:915-924.

Modified LDL induces macrophages to release cytokines that stimulate adhesion molecule expression in endothelial cells



Nathan CF. *J Clin Invest* 1987;79:319-326.

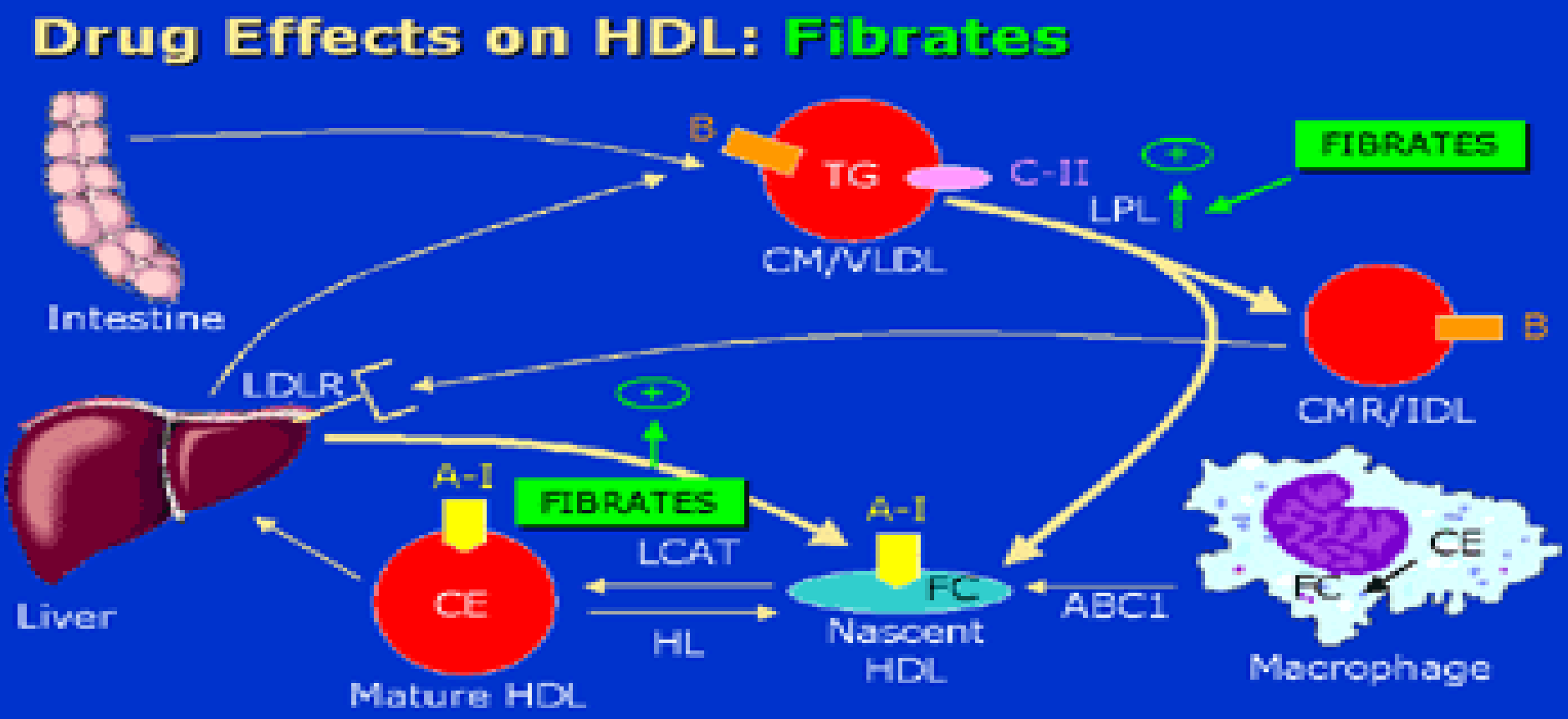
HDL prevent formation of foam cells, reduced adhesion molecules and inhibit the oxidative modification of LDL

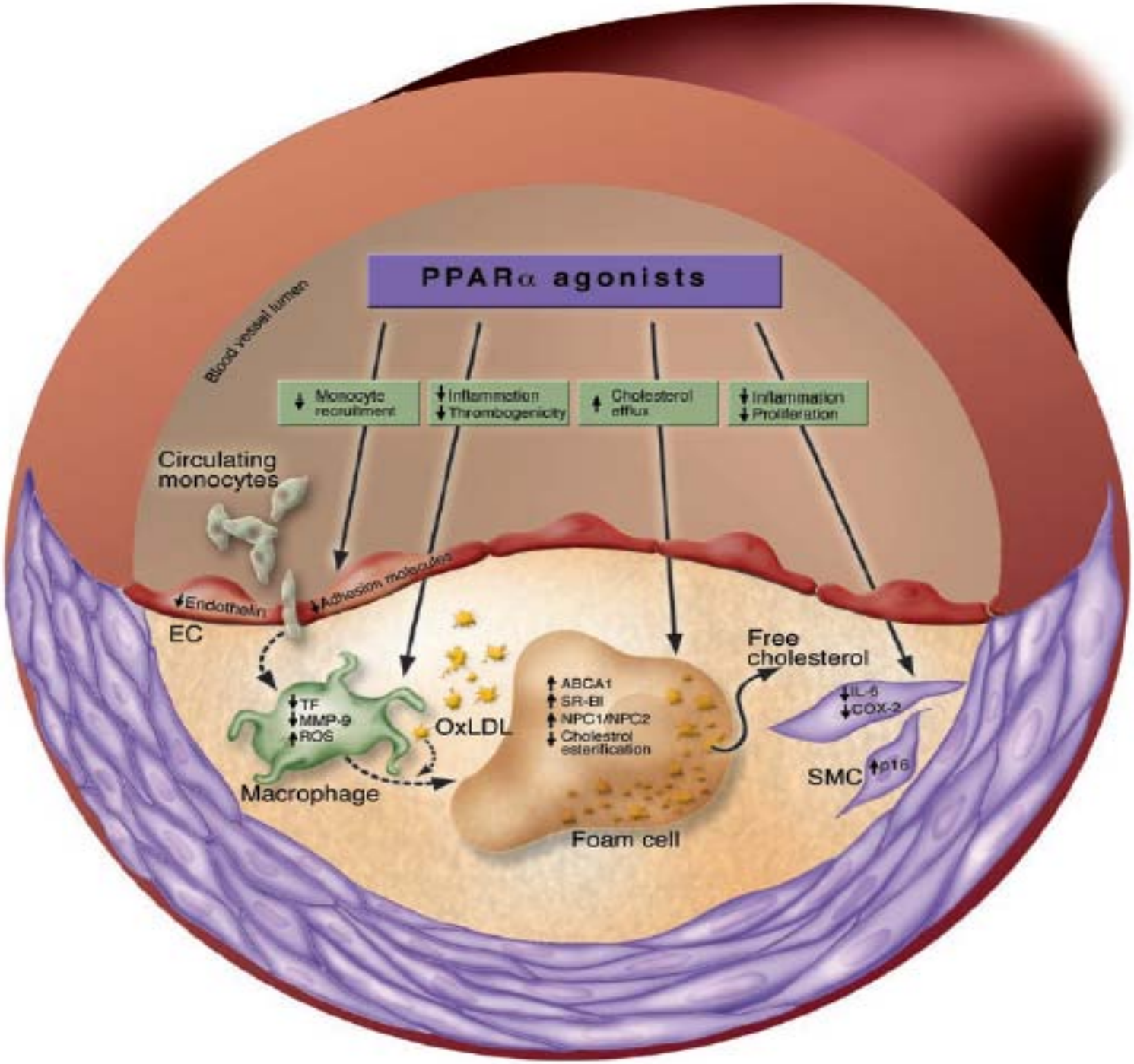


Mackness MI et al. *Biochem J* 1993;294:829-834.

Mechanisms by which fibrates correct dyslipidemia:

- transcriptional induction of synthesis of the major HDL apolipoproteins (apoA-I and apoA-II, lecithin:cholesterol acyltransferase) as well as lipoprotein lipase.
- decrease in hepatic apoC-III transcription and enhanced clearance of triglyceride-rich lipoproteins.

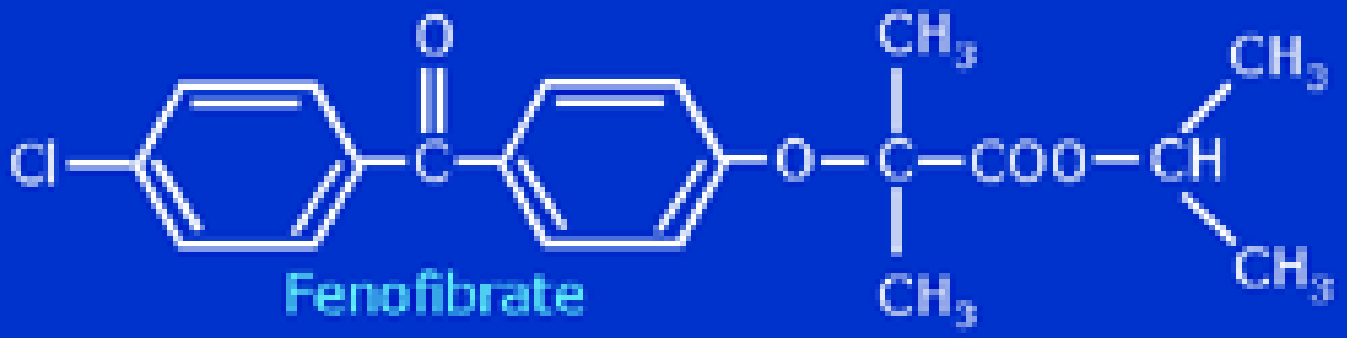




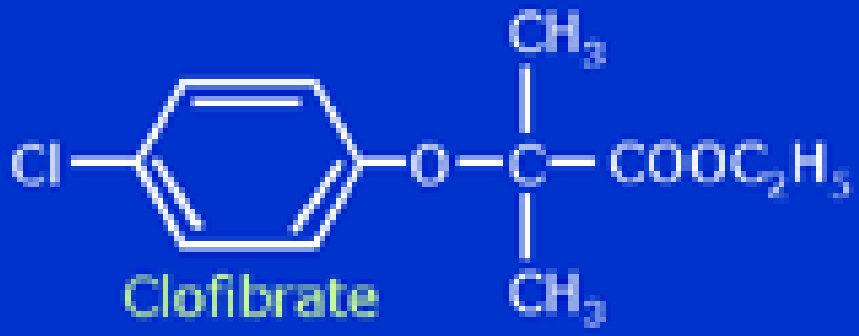
PPARα and atherosclerosis

Levebvre et al. 2006

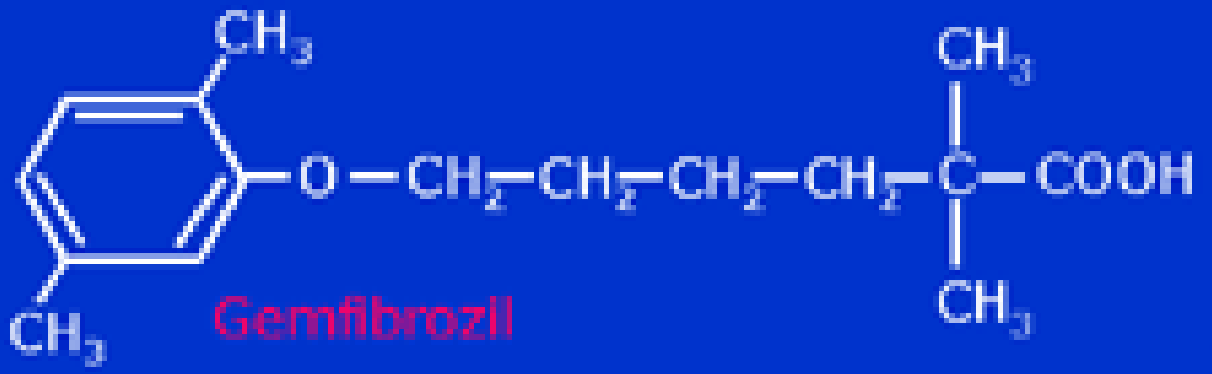
Fibrate Chemical Structures



Fenofibrate

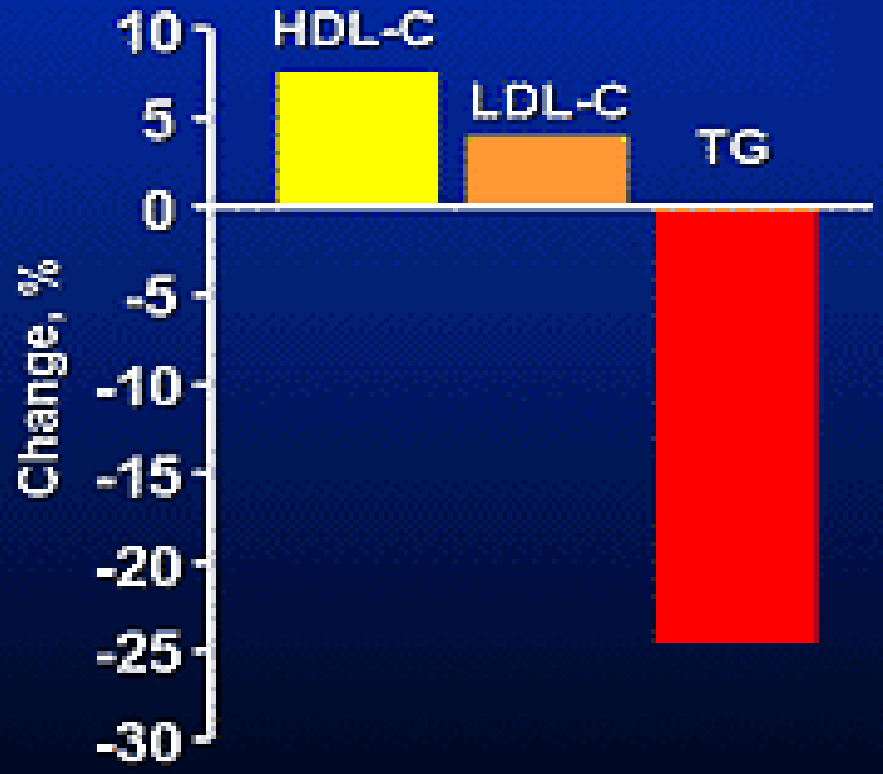


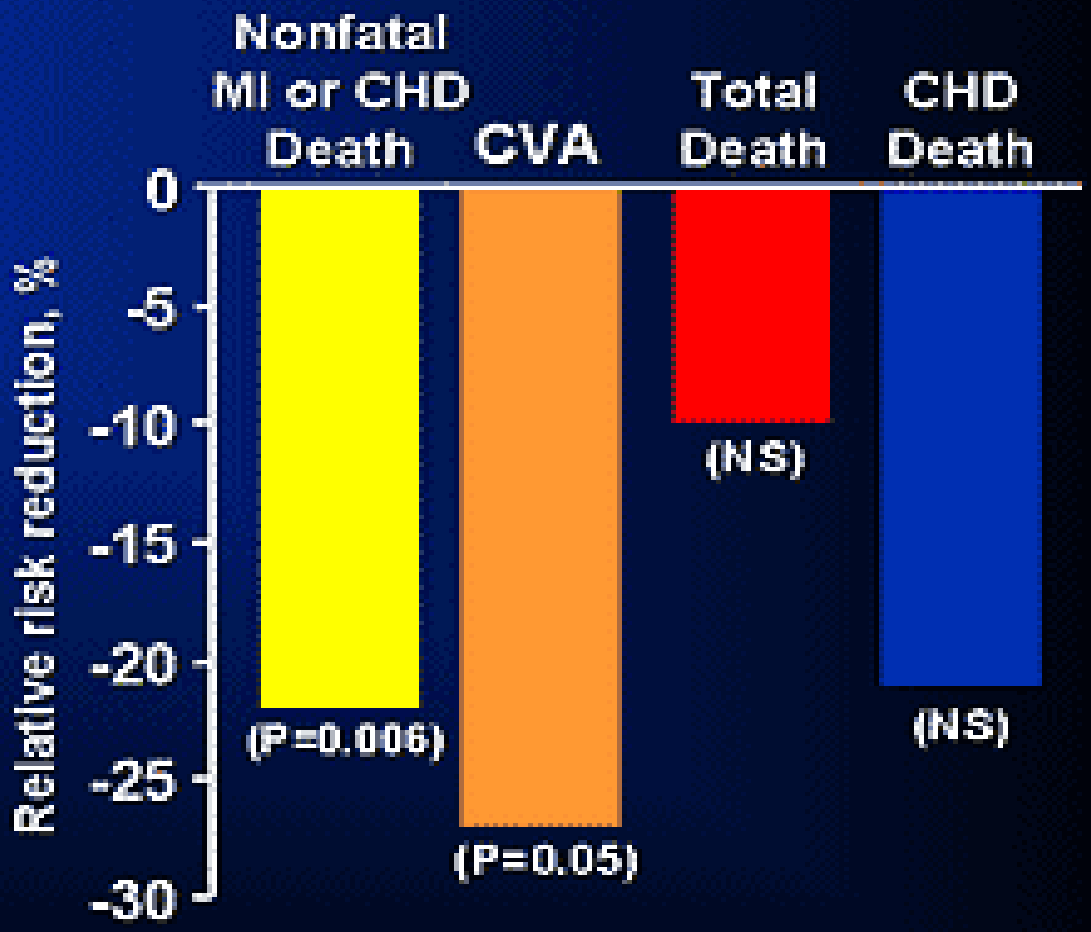
Clofibrate



Gemfibrozil

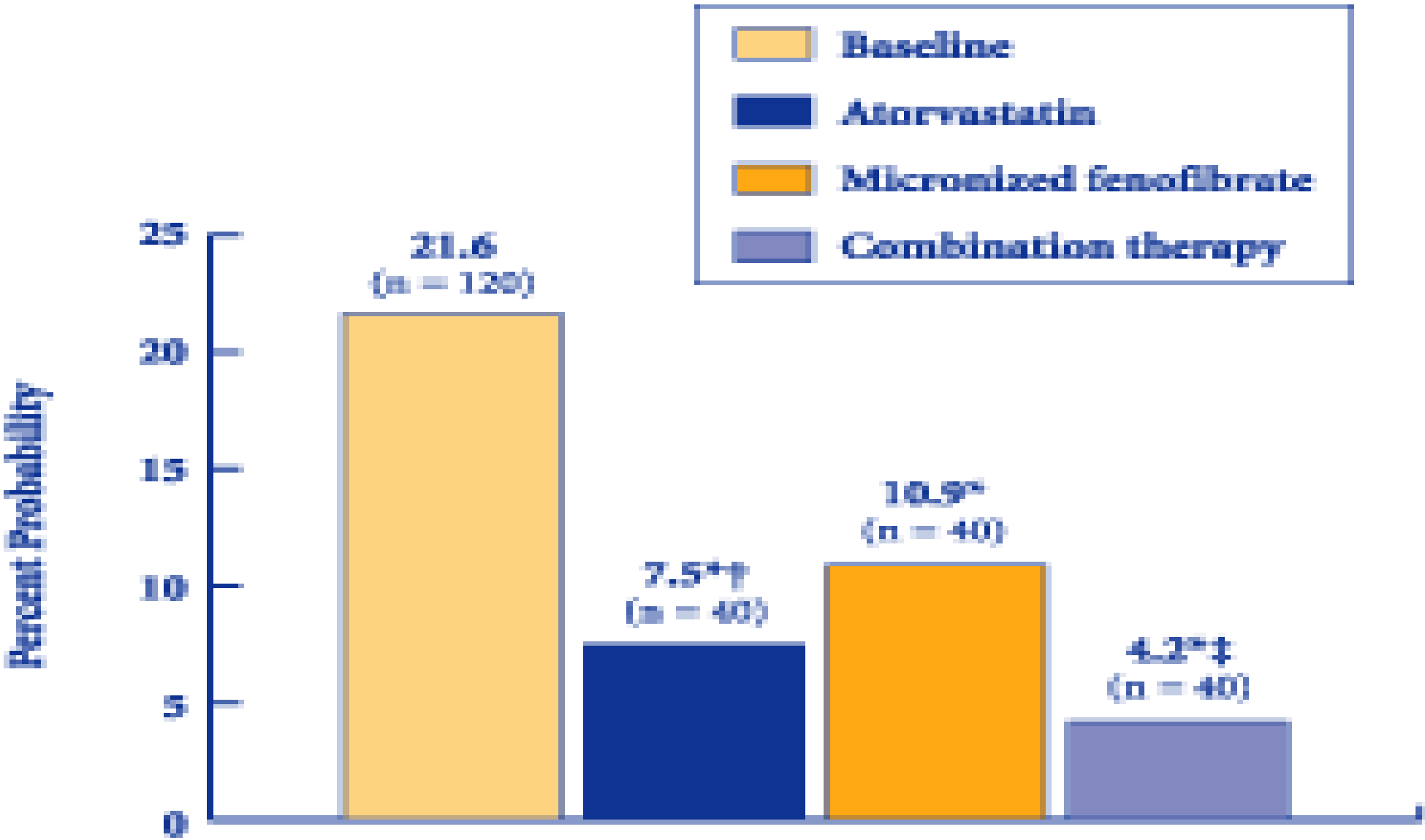
VA-HIT Results: Gemfibrozil vs Placebo





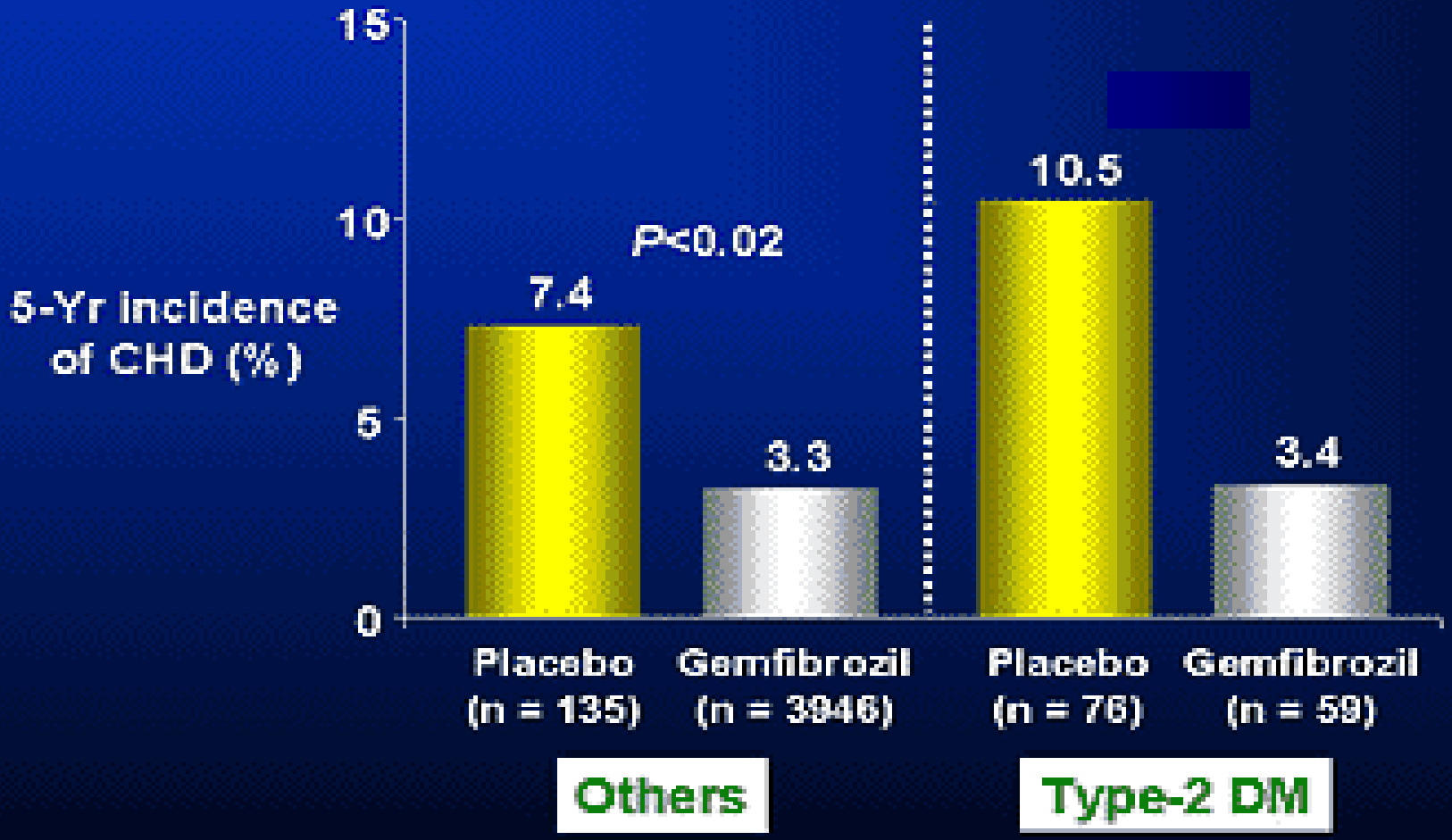
MI – myocardial infarction, CVA - cerebrovascular accident (stroke), CHD – coronary heart disease

Probability of myocardial infarction during 10 years



Data are %. *P <.0001 vs. baseline;
†P <.05 vs. fenofibrate; ‡P <.05 vs. both monotherapies.

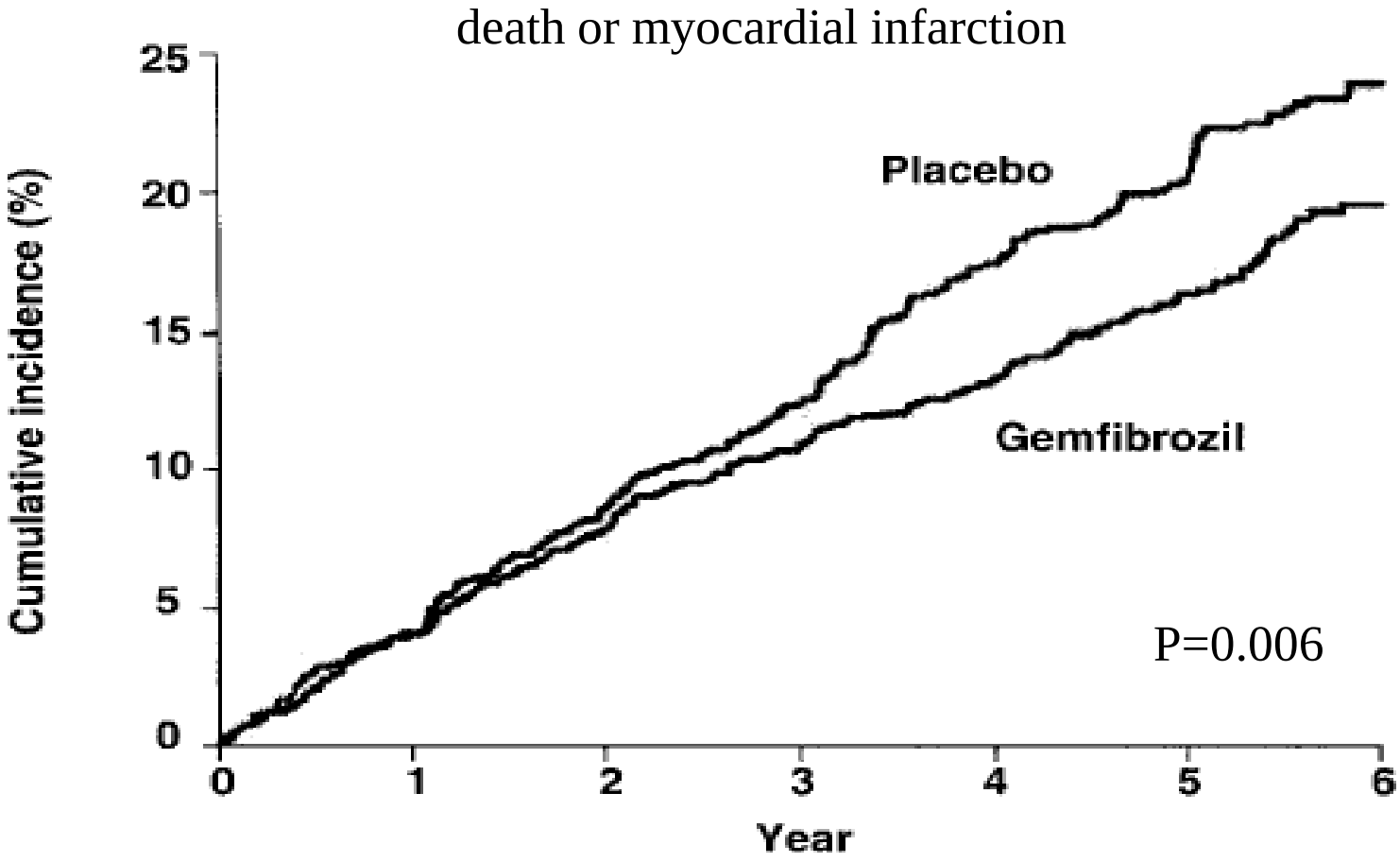
Primary CHD Prevention in Patients With Type 2 Diabetes: The Helsinki Heart Study



*Myocardial infarction or cardiac death.
NS=not significant.

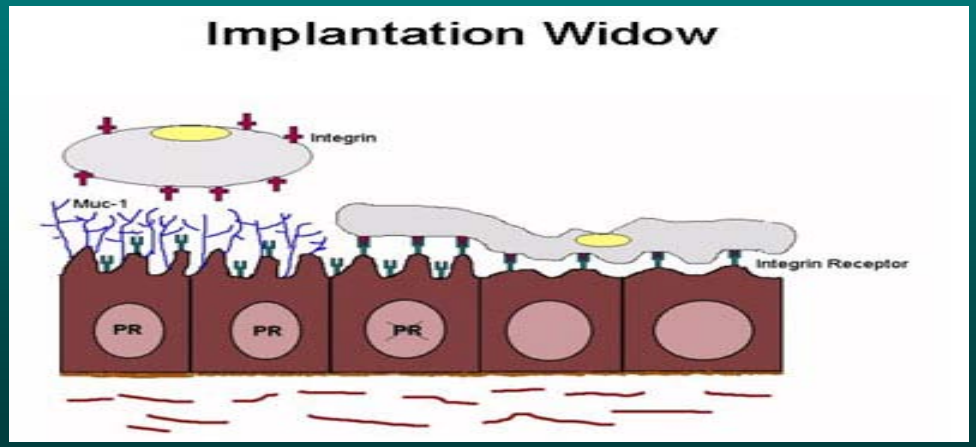
Koskinen P et al. *Diabetes Care*. 1992;15:820-825

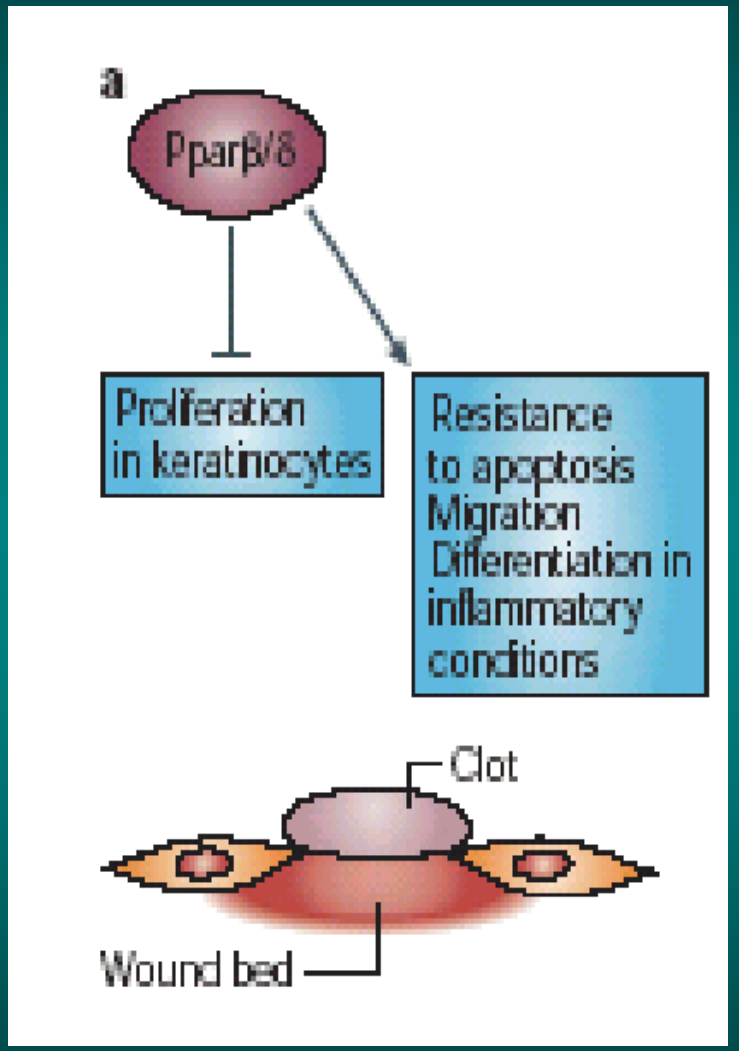
Treatment with fibrates reduces cardiovascular risk



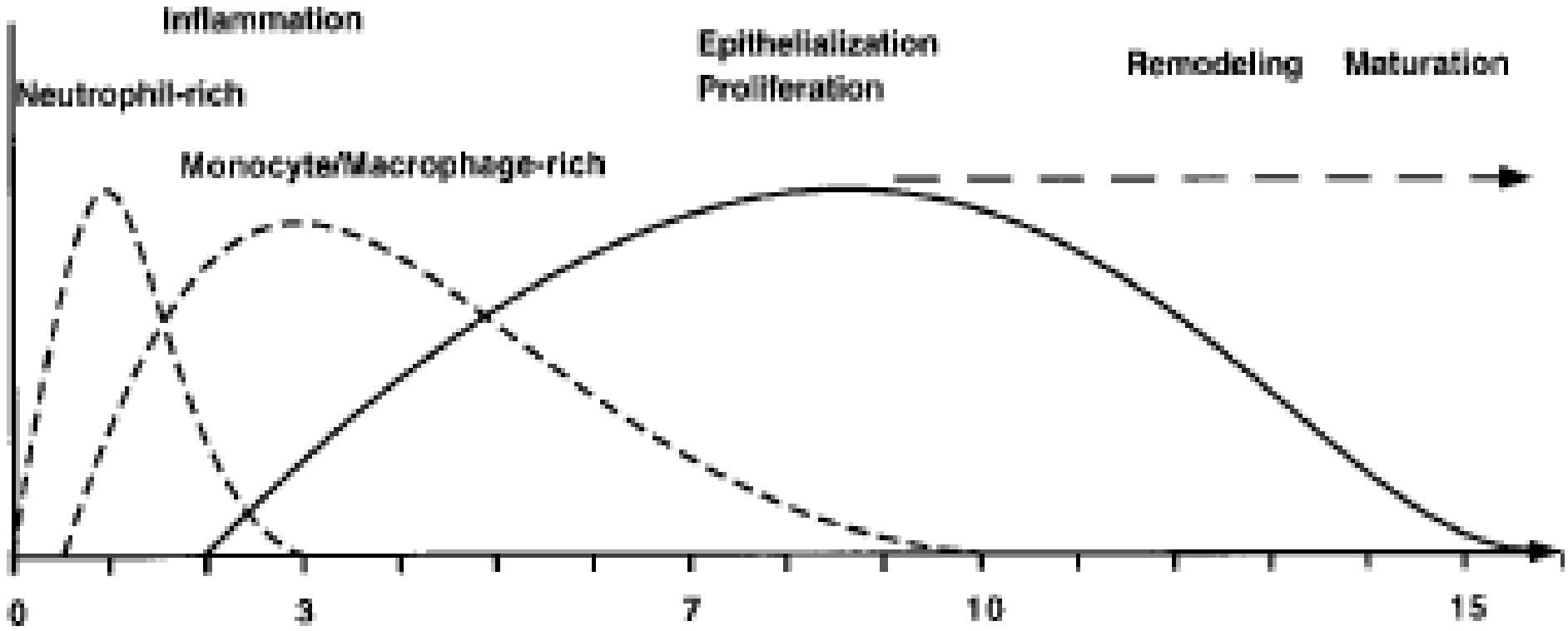
PPARβ (PPARδ)

- PPARβ is relatively poorly characterized.
- Its expression is rather ubiquitous, with varying levels in different organs.
- Endogenous ligand for PPARδ is prostacyclin (PGI2).
- PPARδ seems to play a very important role in implantation of embryo.
- It was also implicated in oligodendrocyte maturation.





PPAR expression in epidermis during wound healing



PPARβ – wound healing

- **Recent studies have demonstrated an involvement of PPARδ in regulation of wound healing. Its activation:**

  * contributes to lipid biosynthesis in sebocytes and keratinocytes

  * ameliorates inflammatory responses in the skin.

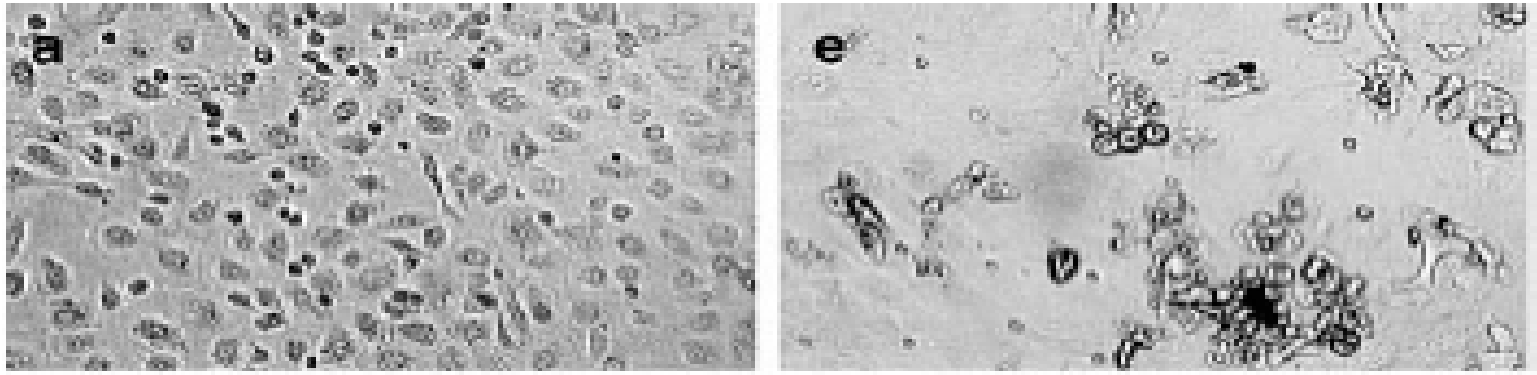
  * diminishes proliferation and accelerates differentiation of keratinocytes

  * enhances keratinocyte resistance to apoptotic signals.

- **Increased proliferation and death of keratinocytes at the edges of epidermal wounds in PPARδ mutant mice most likely participate in the healing delay observed in these animals.**

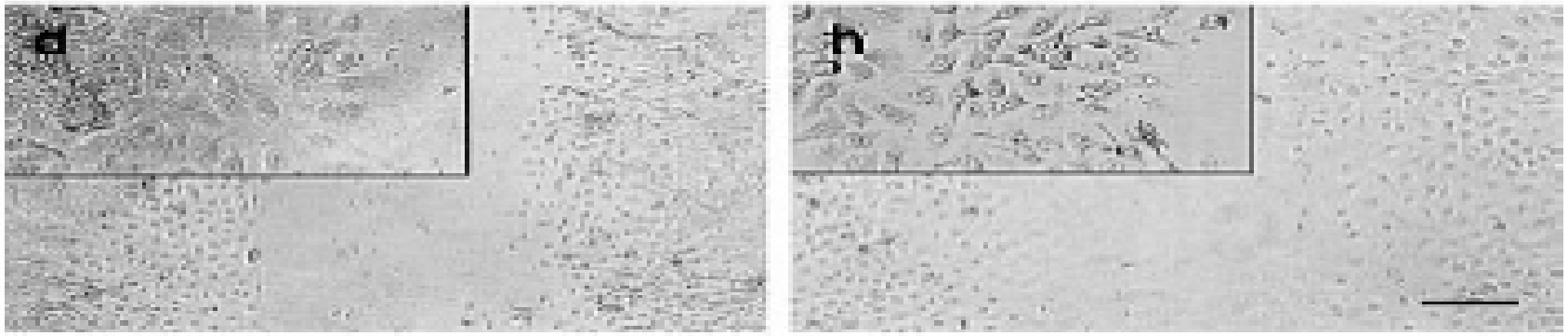
Effect of PPARβ deficiency on keratinocyte adhesion...

Plating



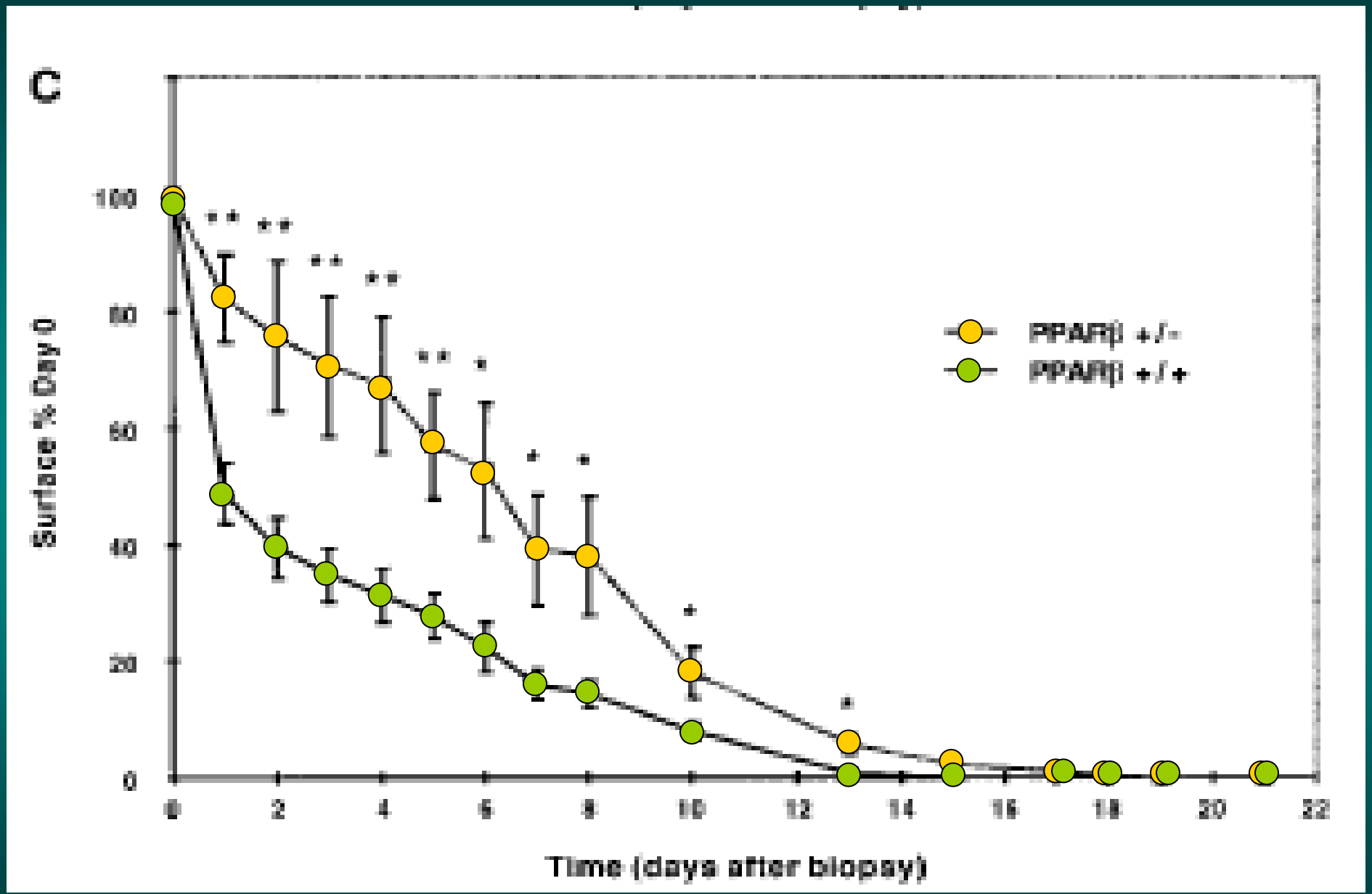
...and in vitro wound healing.

Day 4



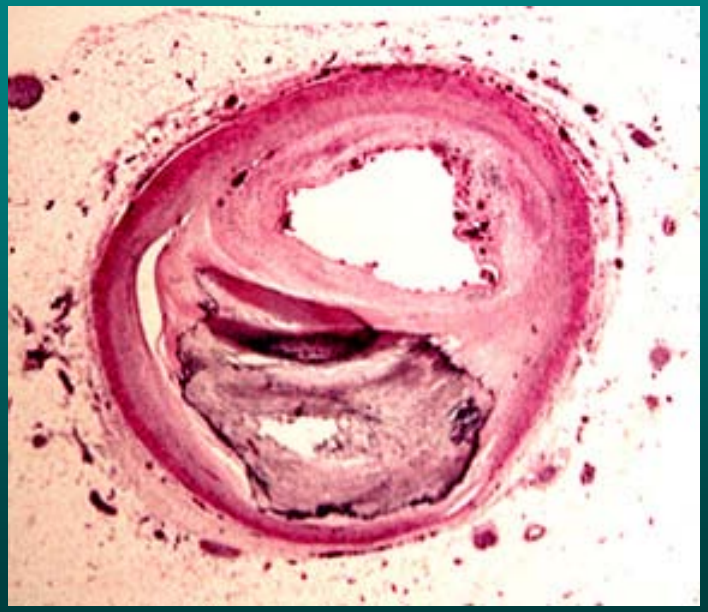
PPARβ +/+

PPARβ +/-


C
Surface % Day 0
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
Time (days after biopsy)
PPARβ +/-
PPARβ +/+

PPARβ – atherosclerosis

- Some studies indicate, that activation of PPARδ may influence atherogenesis, although the final output of its action is not known yet.
- Artificial, selective ligand of PPARδ was reported to cause a dramatic rise in HDL cholesterol, while lowering the levels of LDL small dense lipoprotein, fasting triglicerides, and insulin.
- On the other hand, PPARδ, whose expression is increased during differentiation of macrophages, increases the expression of genes involved in lipid uptake and storage, what may promote the macrophage lipid accumulation and foam cell formation.



PPARβ overexpressing mice

- Overexpression of PPARβ in adipose tissue specifically induces expression of genes required for fatty acid oxidation and energy dissipation, which then leads to improved lipid profiles and reduced adiposity.

- Importantly, these animals are completely resistant to obesity that is induced by a high-fat diet and by genetic predisposition.

- As predicted, treatment of obese mice with a synthetic PPARβ agonist depletes lipid accumulation. In parallel, PPARβ-deficient mice challenged with a high-fat diet show reduced energy expenditure and are prone to obesity. Maybe PPARβ serves as a widespread regulator of fat burning and is a potential target in the treatment of obesity.

- The Marathon Mice are capable of continuous running of up to twice the distance of a wild-type littermate. This is achieved by targeted expression of an activated form of PPARβ in skeletal muscle, which resulted in a dramatic increase in "nonfatiguing, red" type I muscle fibers.


marathon mouse

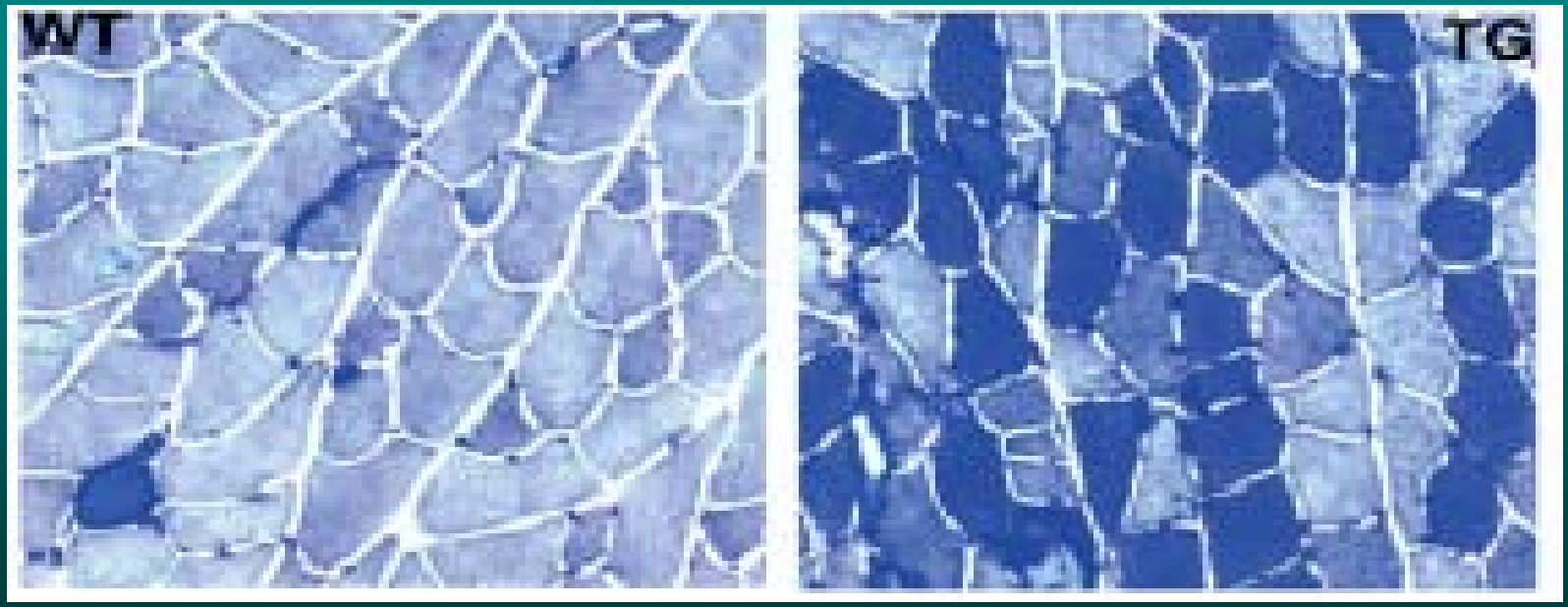
Increased Oxidative Type I Fibers in the PPARδ Transgenic Mice

Skeletal muscle fibers are generally classified as type I or type II fibers.

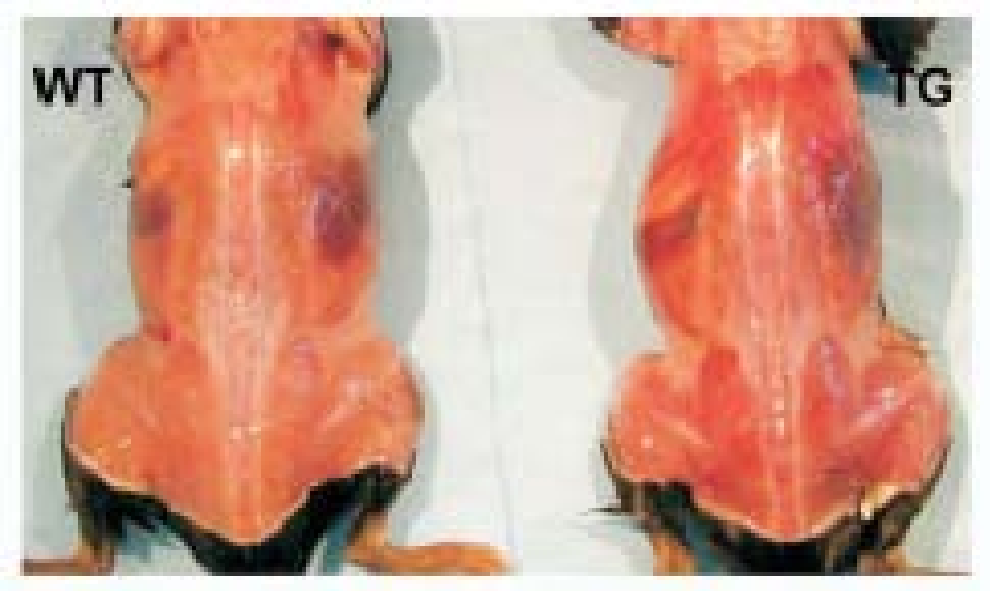
Type I fibers (oxidative/slow) are mitochondria rich and mainly use oxidative metabolism for energy production, which provides a stable and long-lasting supply of ATP, and thus are fatigue-resistant.

Type II (glycolytic/fast) fibers comprise three subtypes, IIa, IIx, and IIb. Type IIb fibers have the lowest levels of mitochondrial content and oxidative enzymes, rely on glycolytic metabolism as a major energy source, and are susceptible to fatigue, while the oxidative and contraction functions of type IIa and IIx lie between type I and IIb

Metachromatic staining of the plantaris muscle. Type I fibers are stained dark blue.

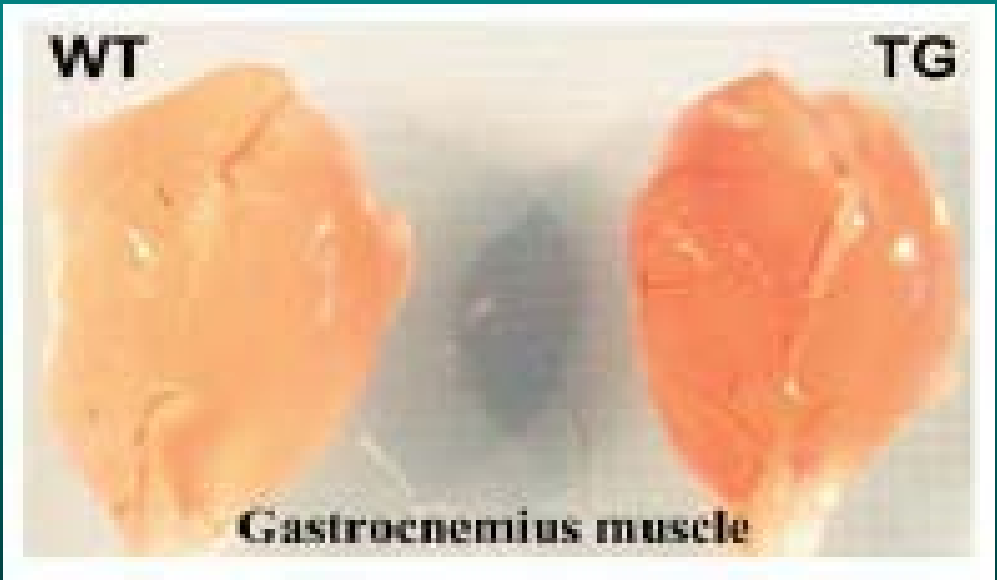


Wang et al. 2004



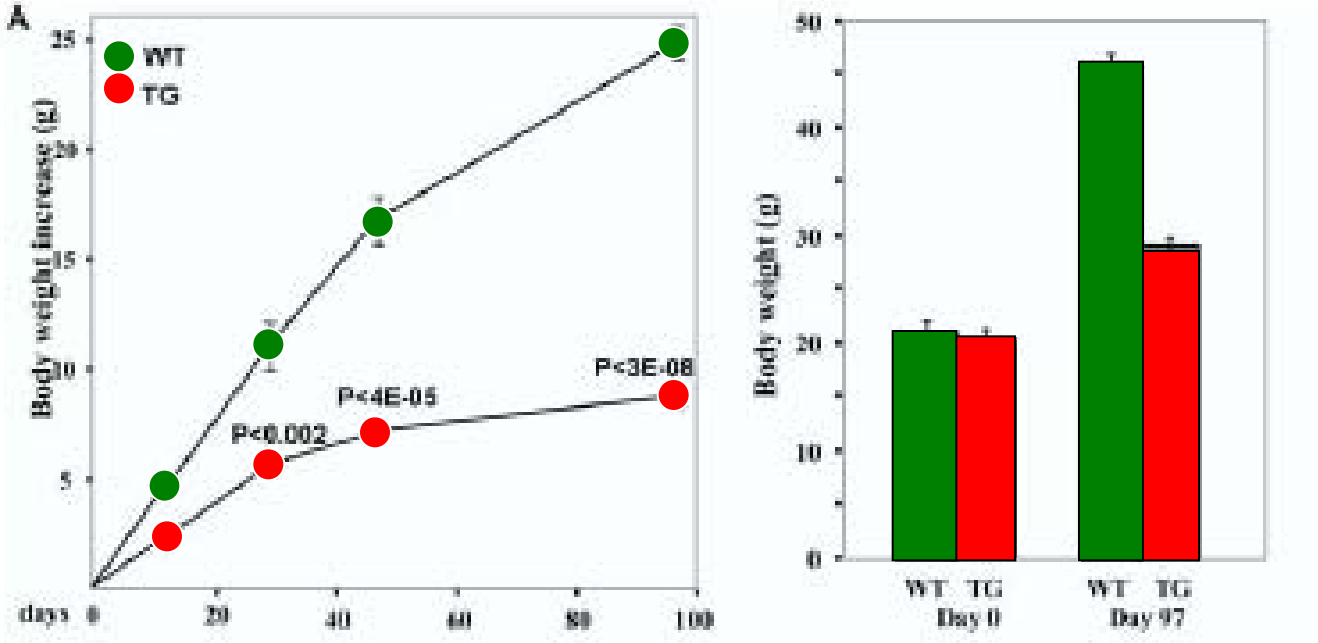
Skeletal muscles in the PPARδ Transgenic Mice

Muscles in transgenic mice (TG)
are redder than those in wild-type mice (WT)

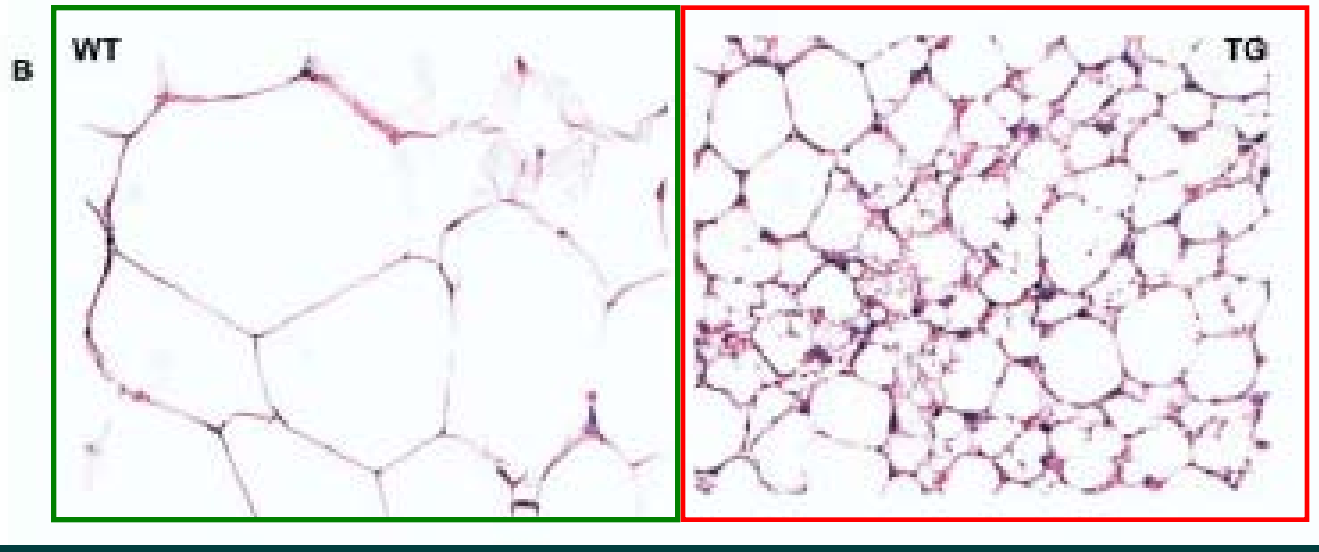


Wang et al. 2004

Effect of PPARδ overexpression on obesity



Body weight

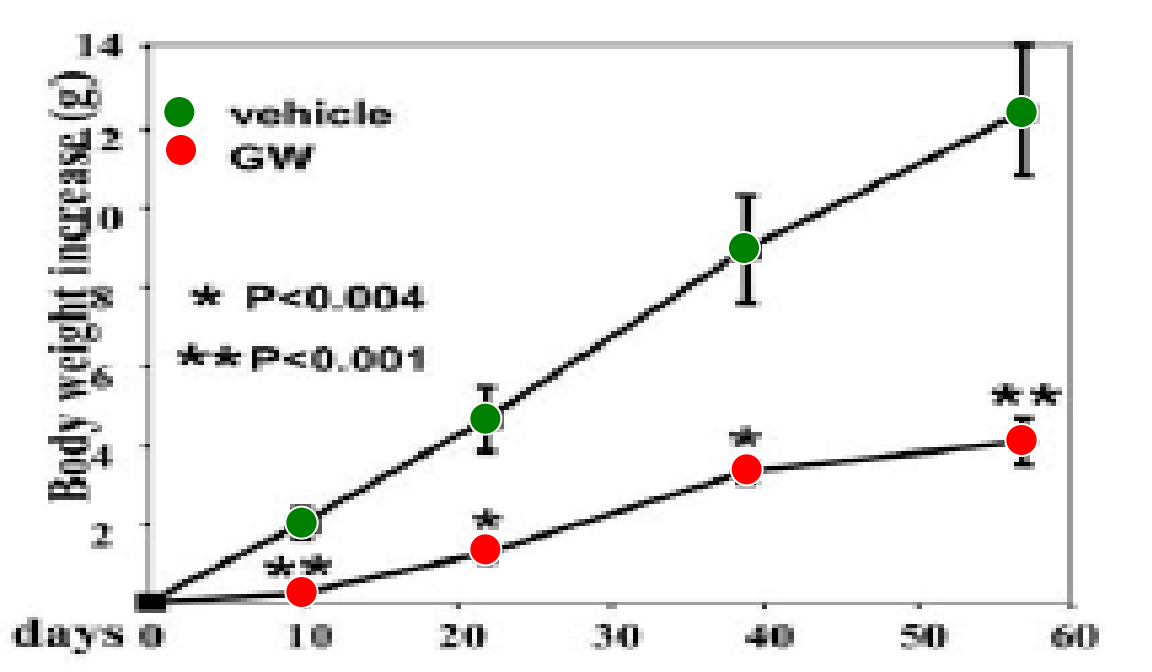


Adipose tissue morphology

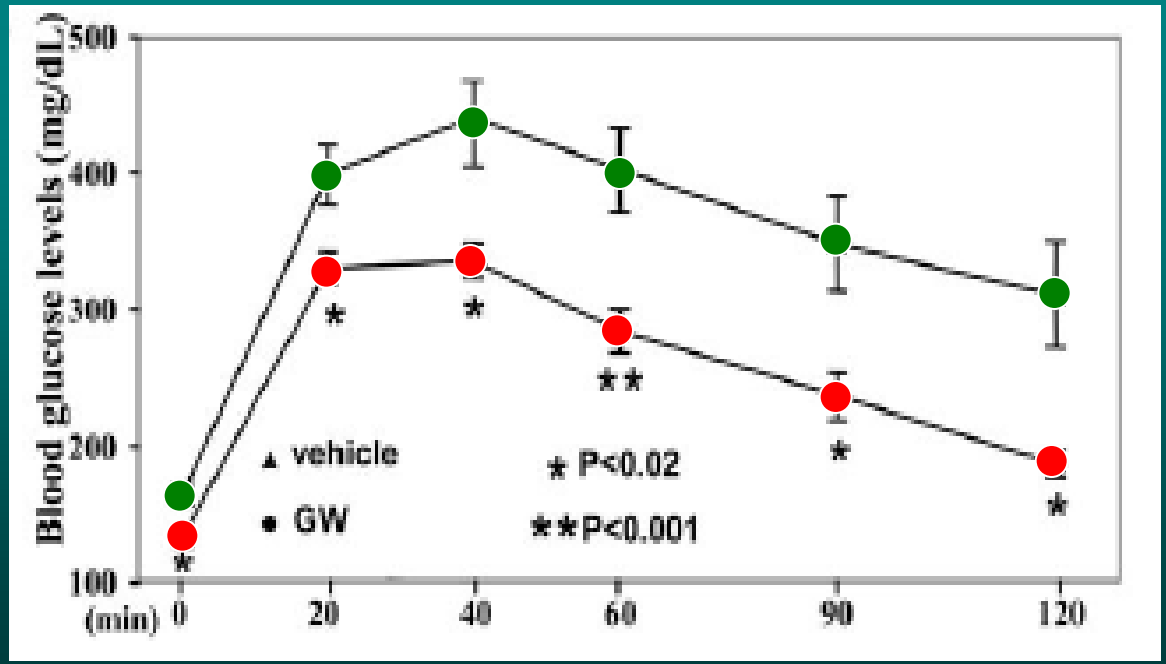
Wang et al. 2004

Effect of pharmacological activation of PPARδ on obesity

Old mice fed a high-fat diet



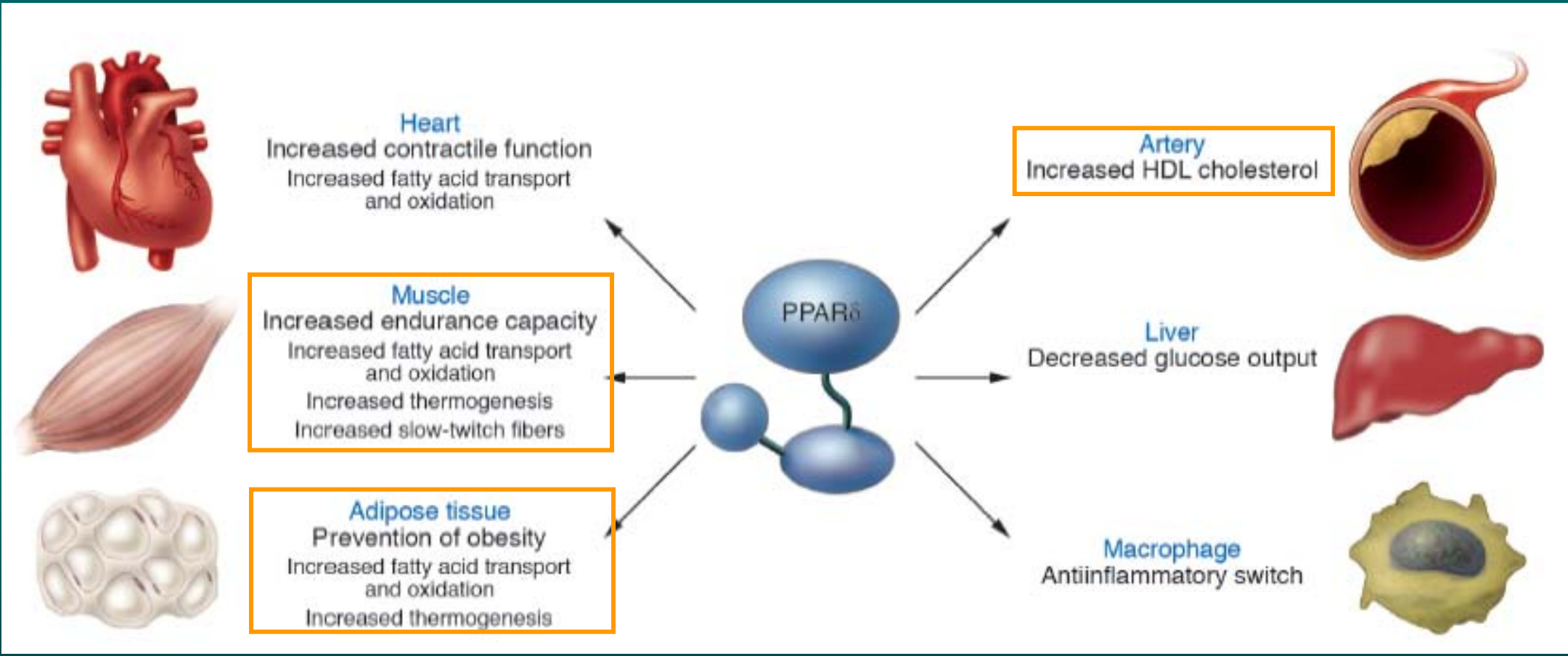
Body weight



Blood glucose level after glucose injection

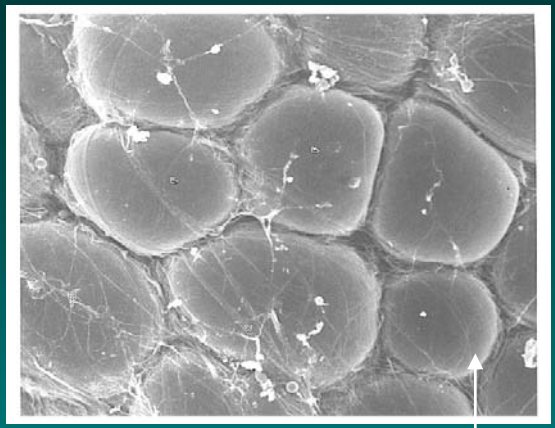
Wang et al. 2004

Therapeutic targets of PPARδ



Barish et al. 2006

Thank you and see you next week...



What would be profitable to remember in June:

- Expression pattern and physiological role of PPARs
- Current and potential usage of PPARα ligand in the clinic
- Potential clinical significance of PPARβ

Slides can be found in the library and at the Heme Oxygenase Fan Club page:

https://biotka.mol.uj.edu.pl/~hemeoxygenase