# 20.380
# Biological Engineering Design
## Inflammation and Cardiovascular Disease

***John Essigmann***

**February 11, 2010**

# Human history at a glance …

Diagram showing evolution of man from ape, and a photo of a dog running have been removed due to copyright restrictions.

2.5 million years

We were strong and fast … even the wolves hung out with us

As Forest said last time, we consume more Calories than we burn off … and the Resultant obesity is associated with about 24 Million Americans developing diabetes

Photo by Glamhag on Flickr.

2.5 million years → ← 50 years →

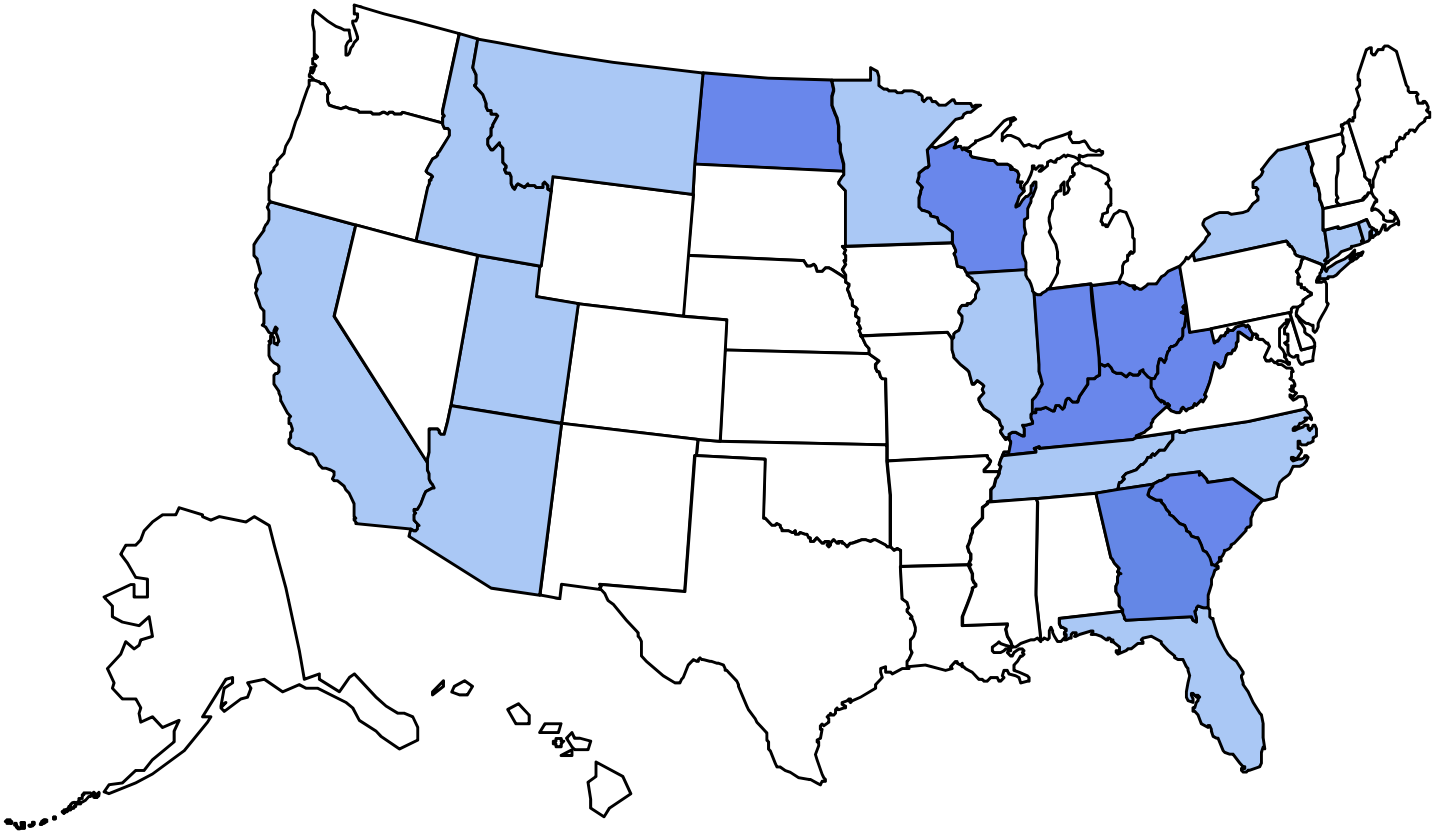
Ob/Ob Mouse

Obesity is also a risk factor for Coronary disease and stroke … and Inflammation plays a role in the disease

# Obesity Trends* Among U.S. Adults

## BRFSS, 1985

**(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” woman)**



No Data | <10% | 10%–14%

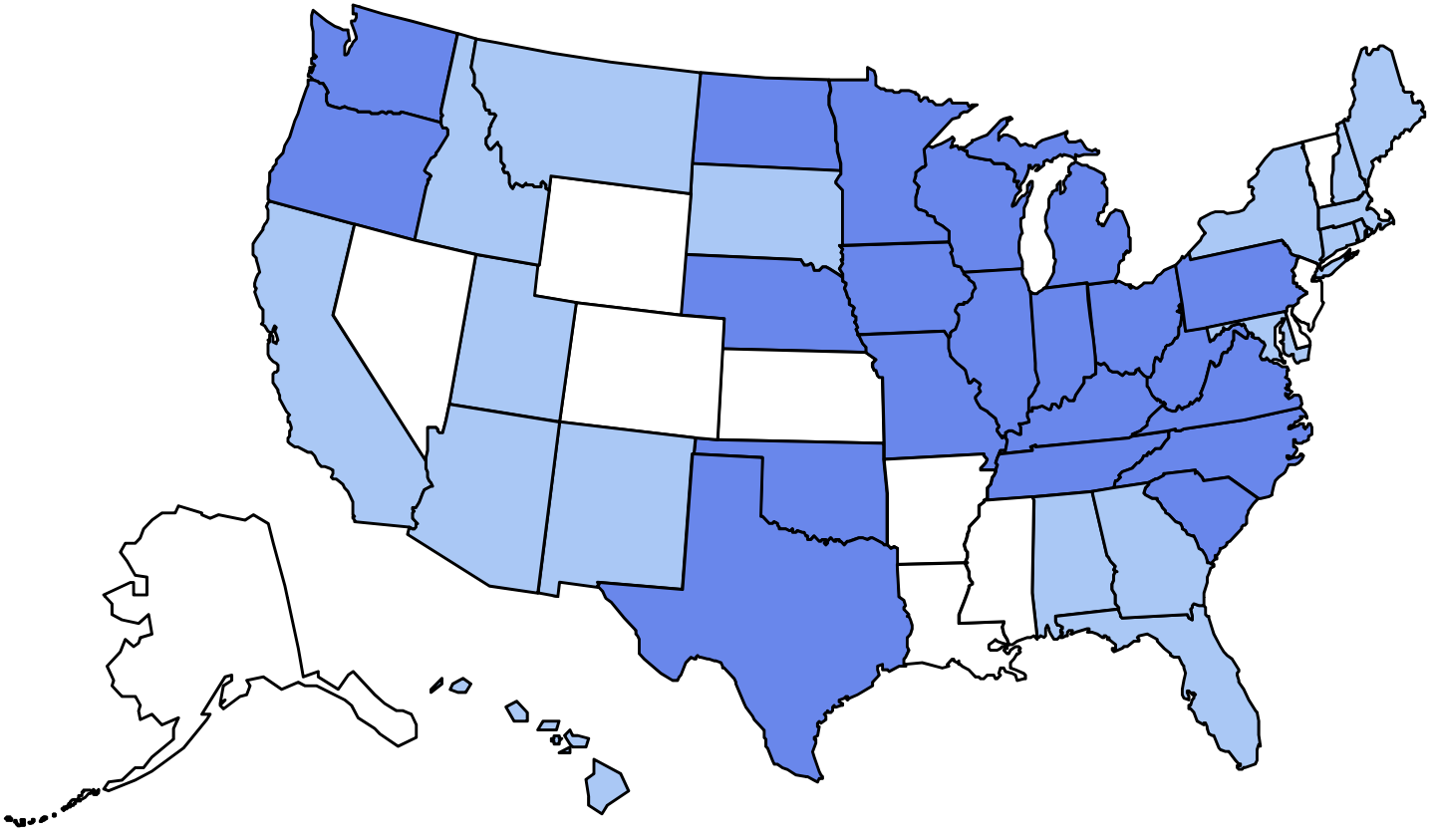
Public domain map created using data from www.cdc.gov/BRFSS.

**John Groopman, Johns Hopkins School of Public Health**

# Obesity Trends* Among U.S. Adults

## BRFSS, 1989

**(*BMI ≥30, or ~ 30 lbs overweight for 5’ 4” woman)**



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# Obesity Trends* Among U.S. Adults

## BRFSS, 1992

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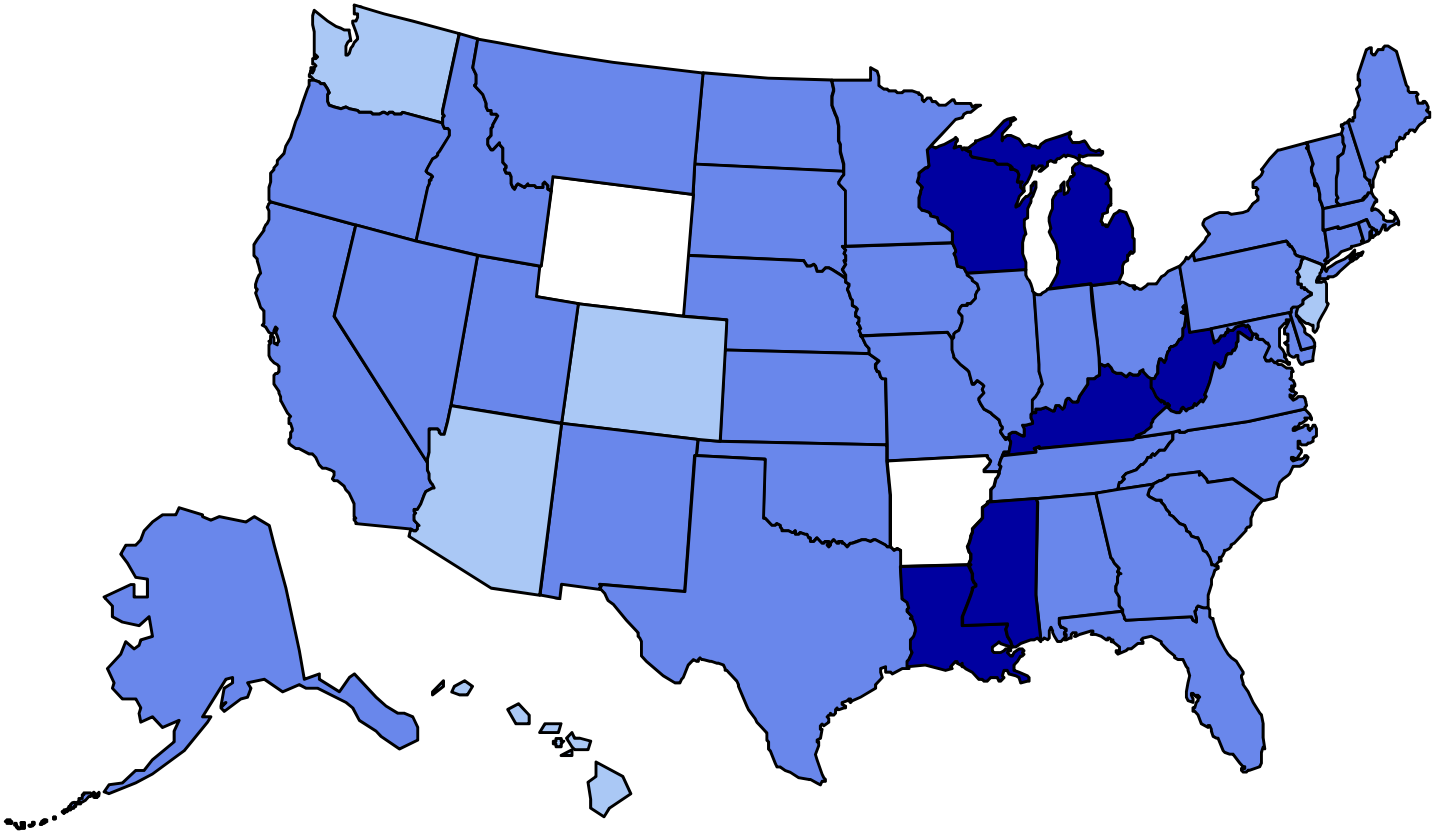


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# Obesity Trends* Among U.S. Adults

## BRFSS, 1994

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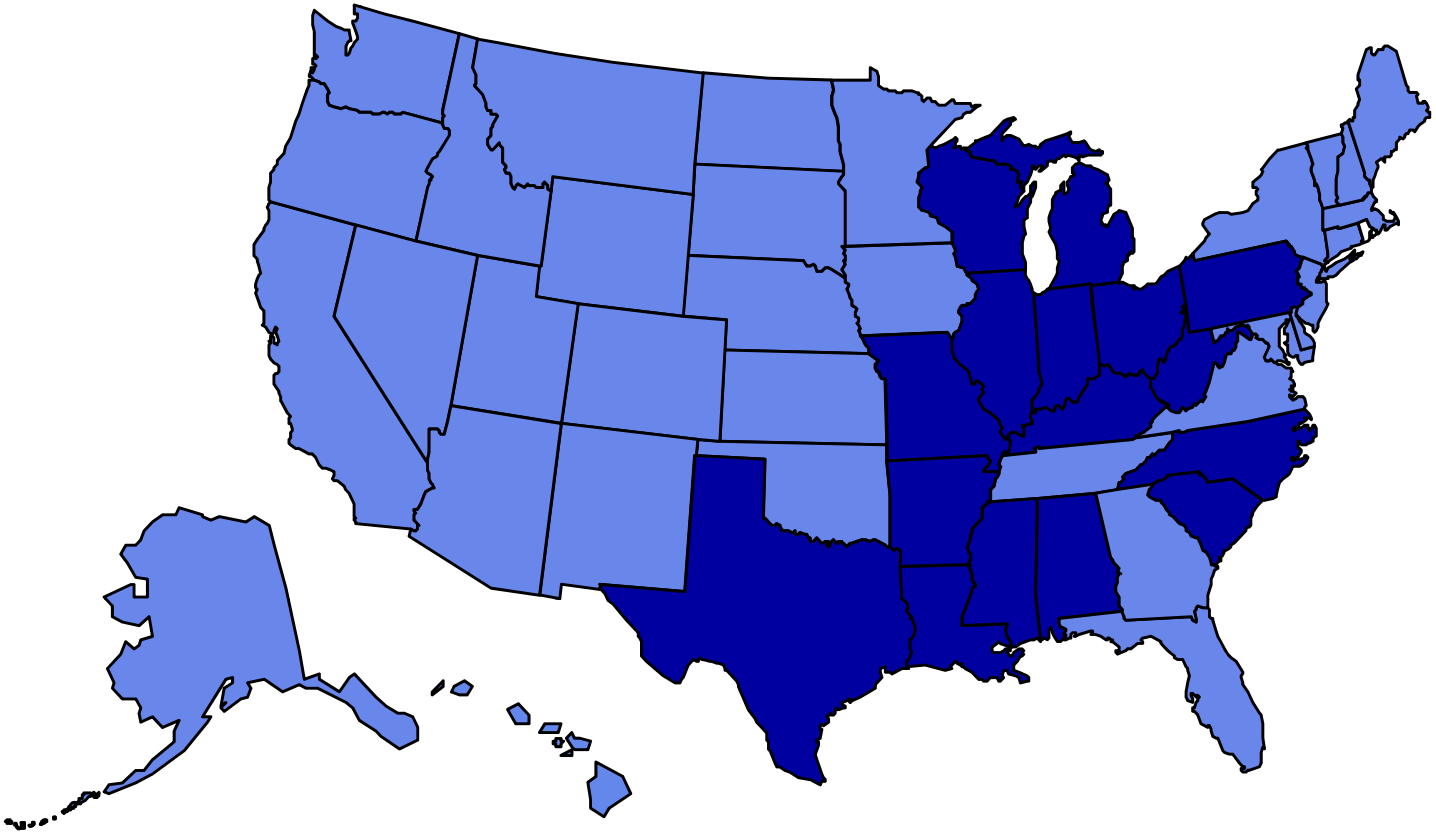


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# Obesity Trends* Among U.S. Adults

## BRFSS, 1997

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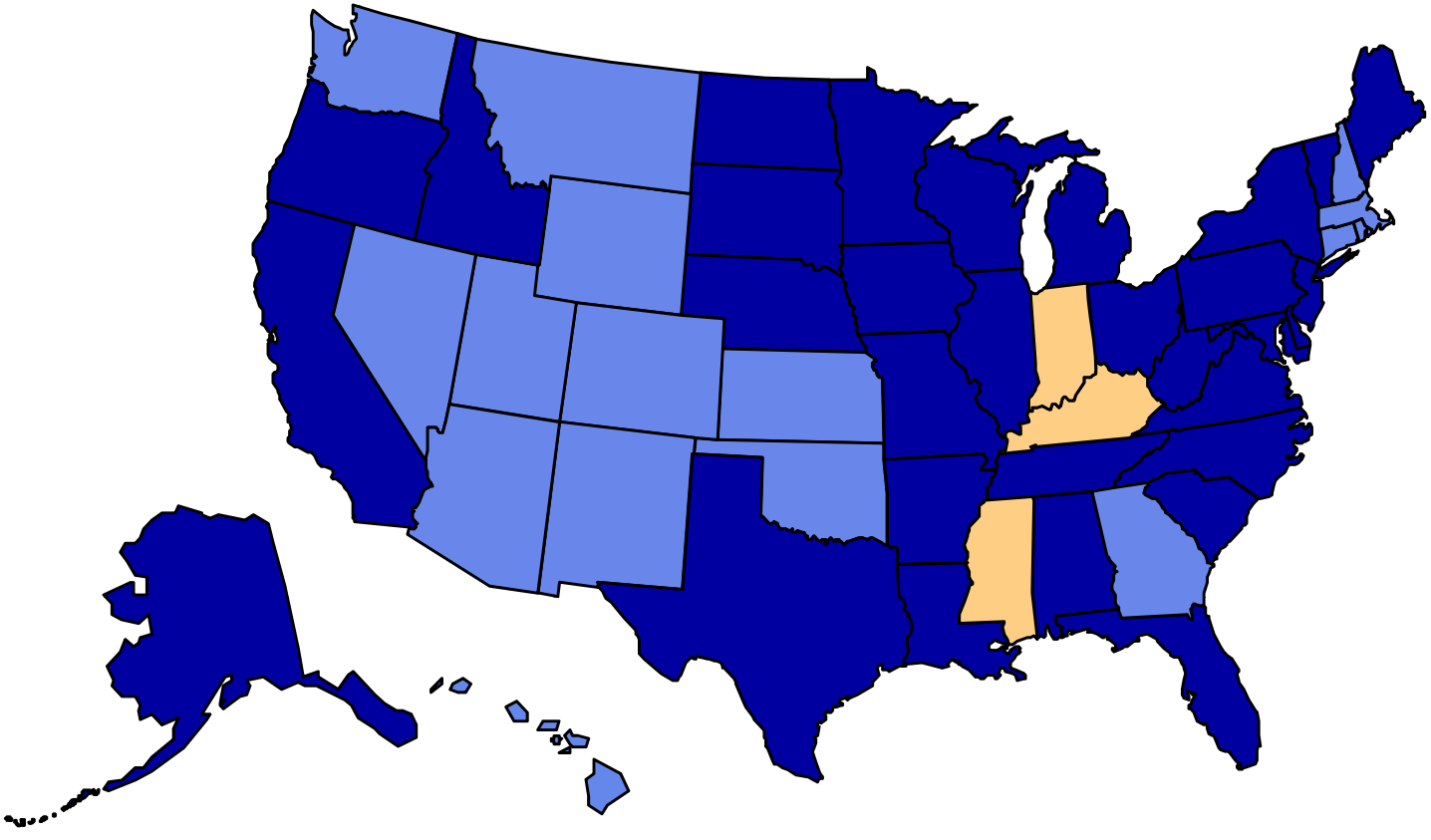


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# Obesity Trends* Among U.S. Adults

## BRFSS, 2000

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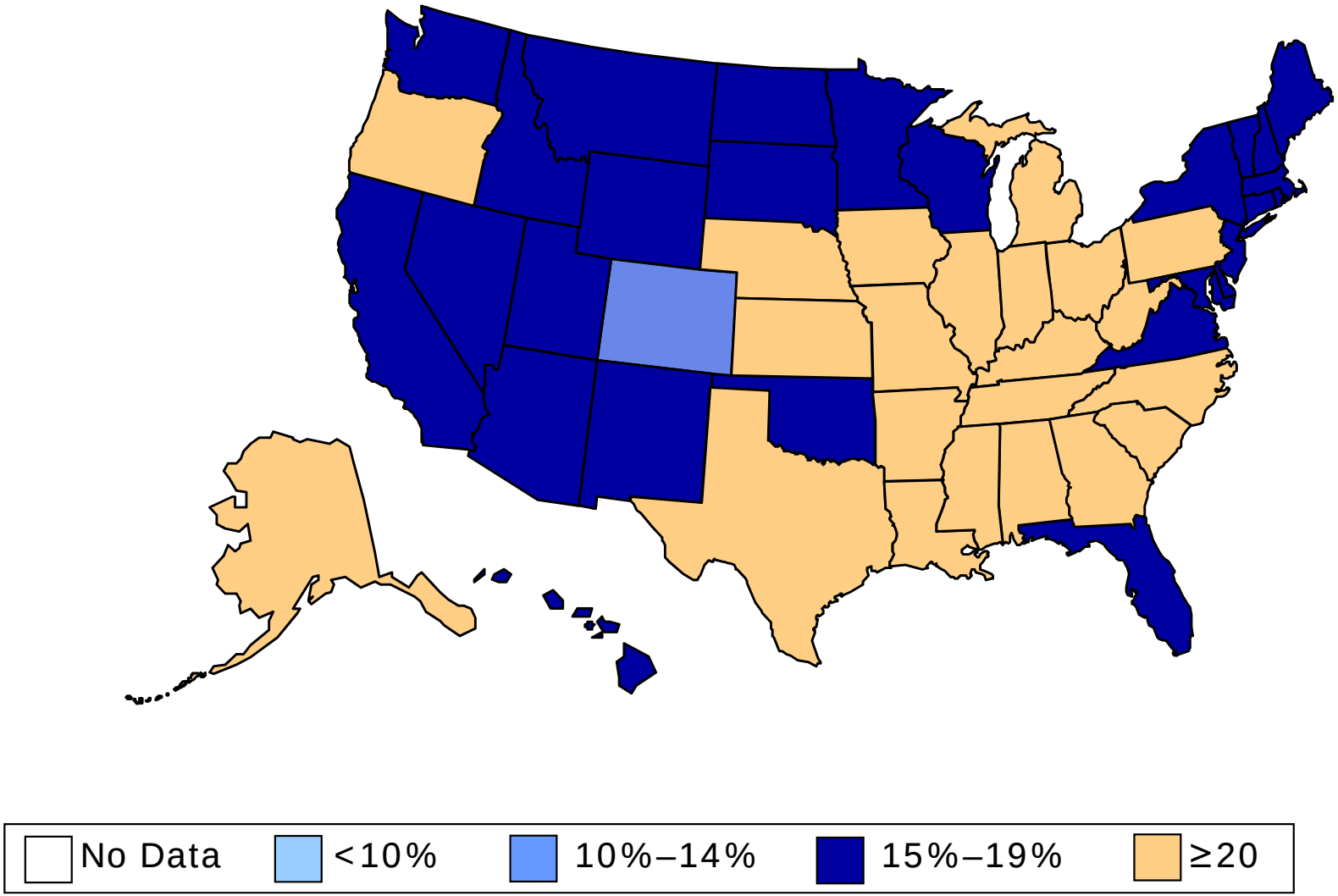


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# Obesity Trends* Among U.S. Adults

## BRFSS, 2003

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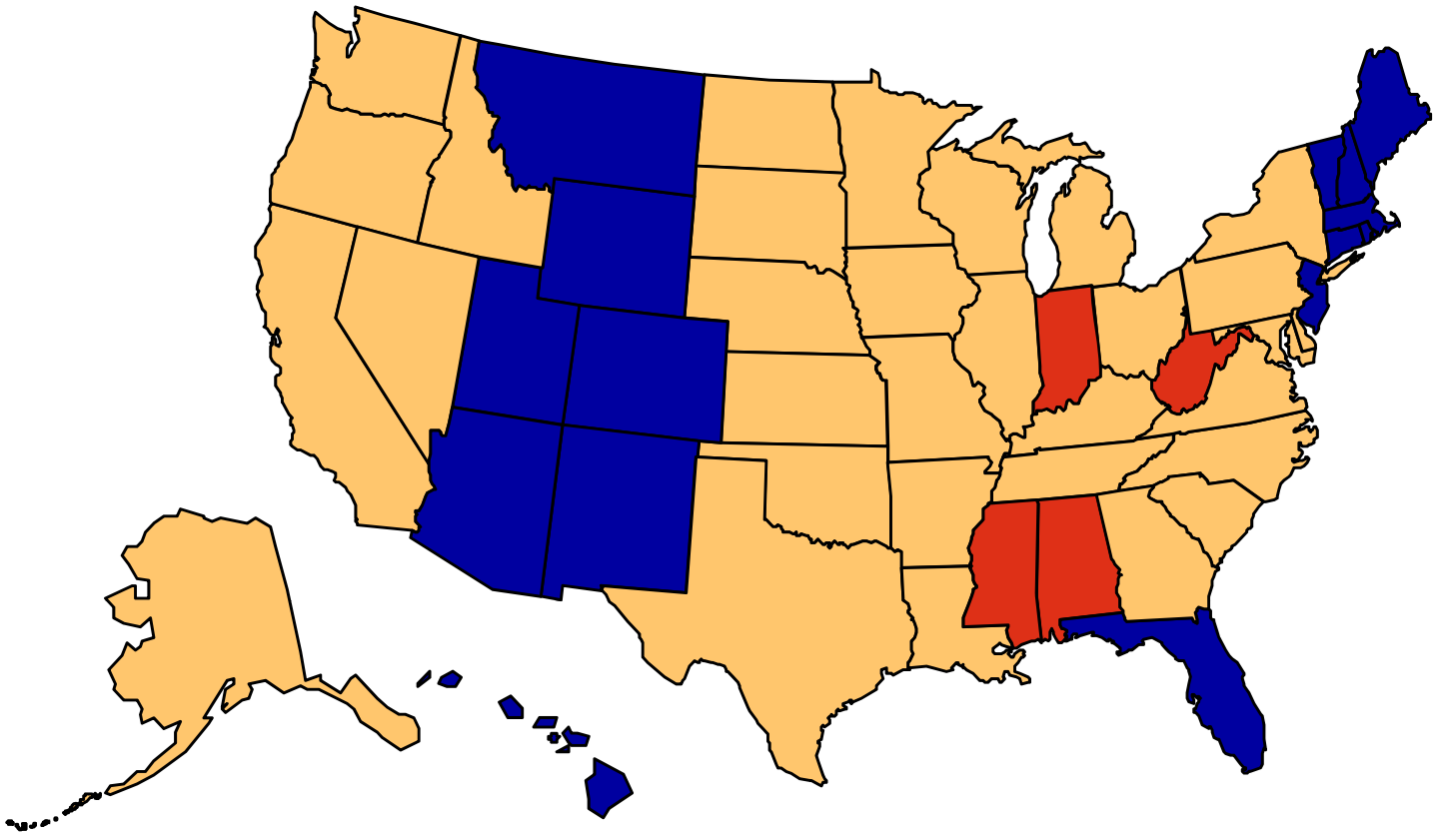


Public domain map created using data from www.cdc.gov/BRFSS.

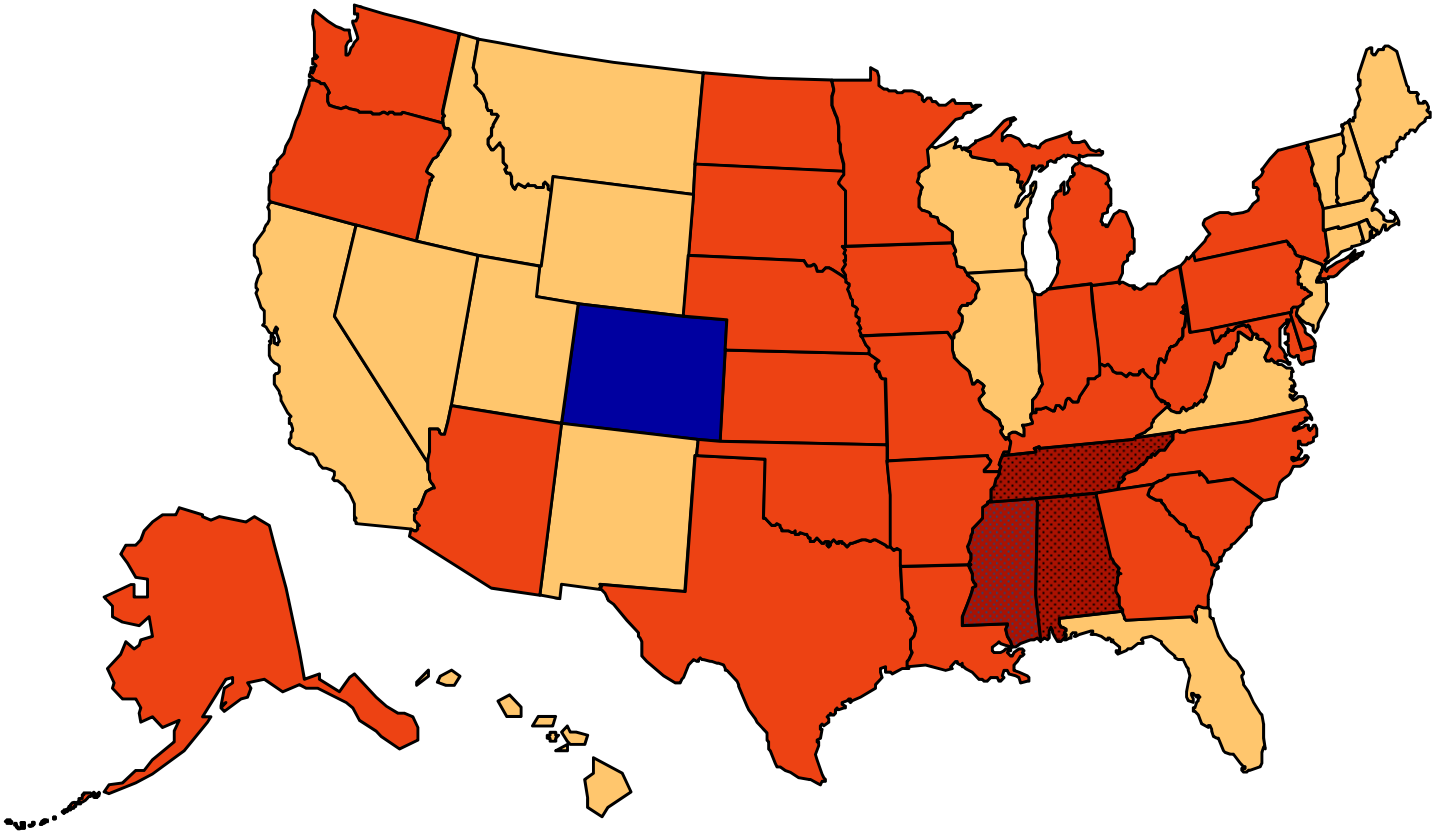
No Data | <10% | 10%–14% | 15%–19% | 20%–24% | ≥25%

Source: Behavioral Risk Factor Surveillance System, CDC

# Obesity Trends* Among U.S. Adults

## BRFSS, 2007

**(*BMI ≥30, or ~ 30 lbs. overweight for 5' 4" person)**



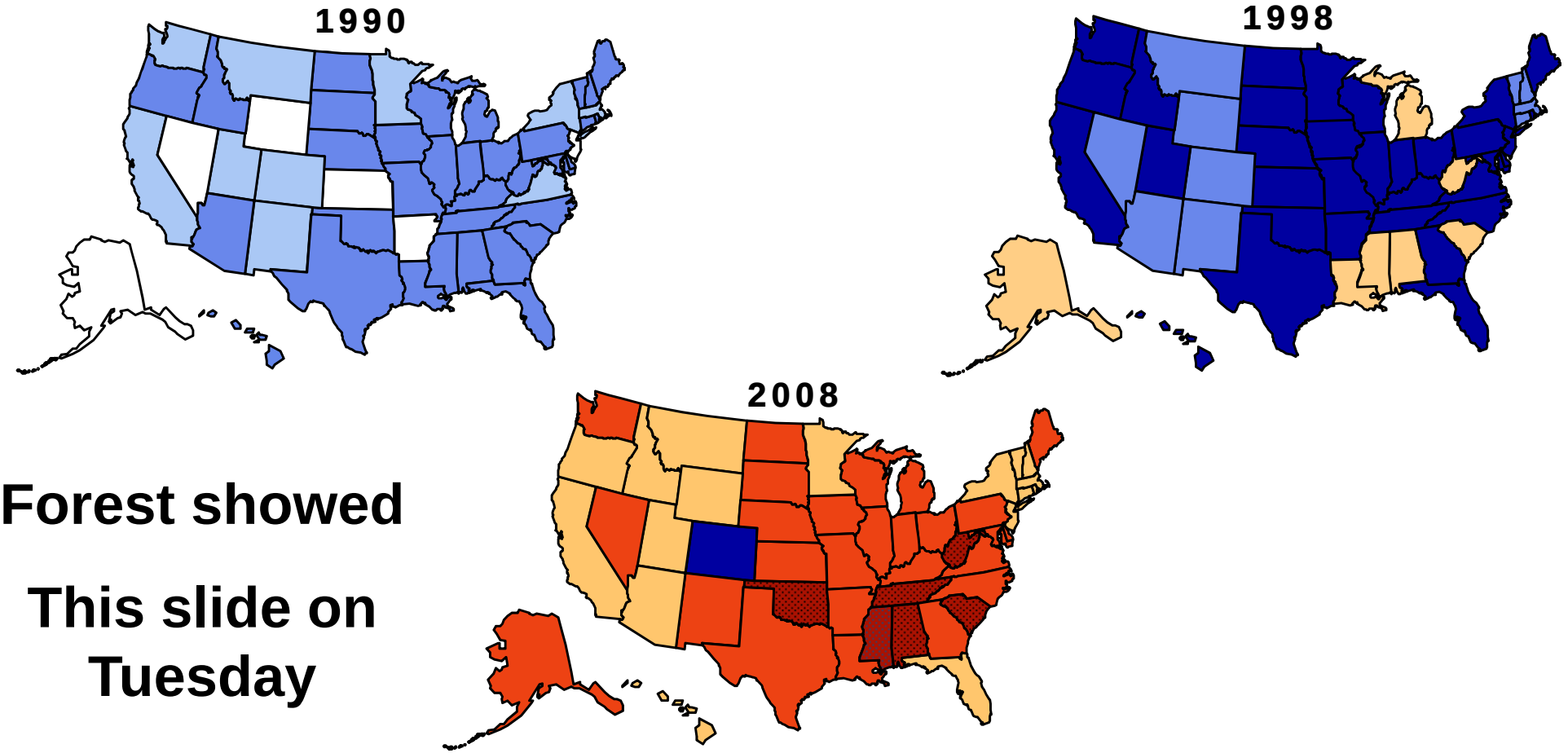
No Data | <10% | 10%–14% | 15%–19% | 20%–24% | 25%–29% | ≥30%

Public domain map created using data from www.cdc.gov/BRFSS.

# Obesity Trends* Among U.S. Adults

## BRFSS, 1990, 1998, 2008

(*BMI ≥30, or about 30 lbs. overweight for 5'4" person)




**Forest showed**

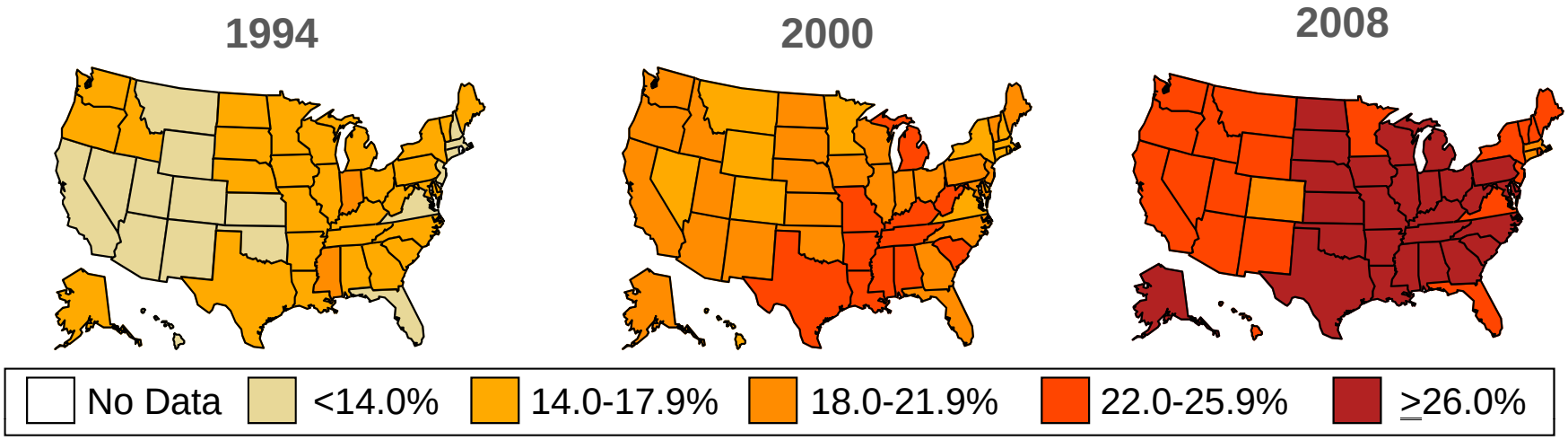
**This slide on Tuesday**

No Data | <10% | 10%–14% | 15%–19% | 20%–24% | 25%–29% | ≥30%

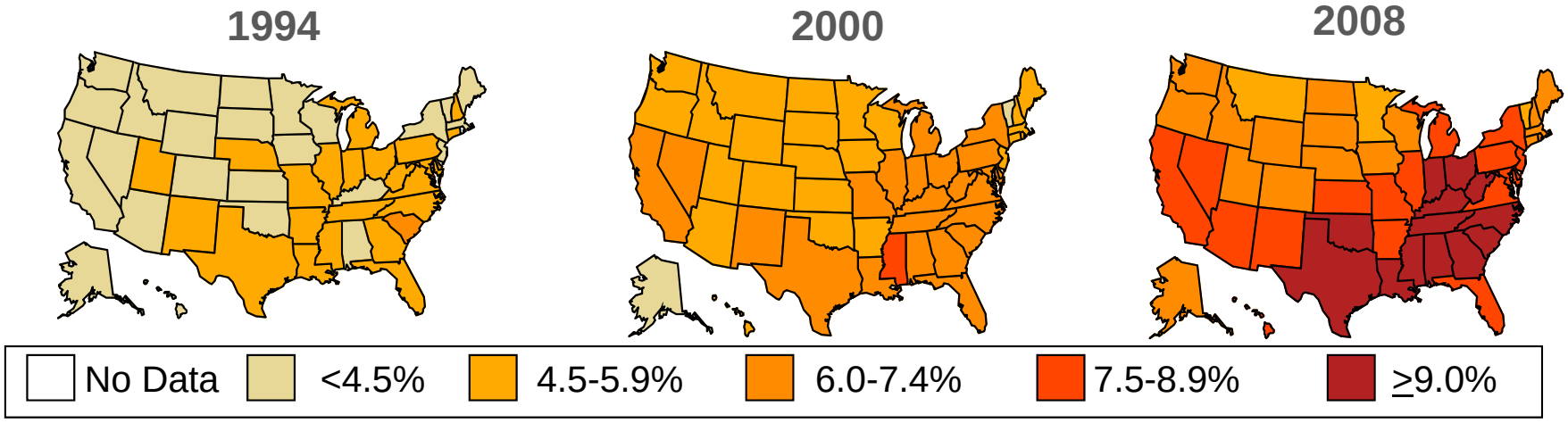
Public domain map created using data from www.cdc.gov/BRFSS.

# Age-adjusted Percentage of U.S. Adults Who Were Obese or Who Had Diagnosed Diabetes

## Obesity (BMI ≥30 kg/m$^2$)



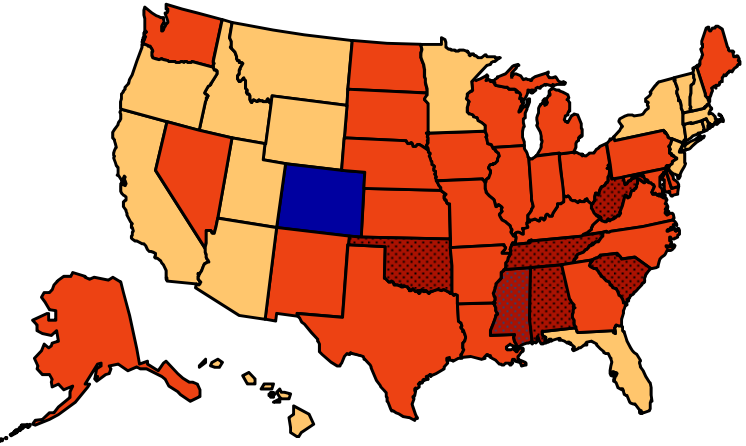

## Diabetes




CDC's Division of Diabetes Translation. National Diabetes Surveillance System available at http://www.cdc.gov/diabetes/statistics

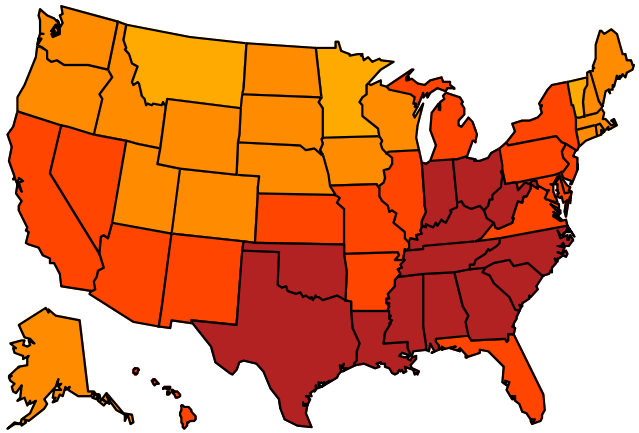
Public domain maps by the Centers for Disease Control.

# Cardiovascular Disease Follows Exactly the Same Pattern



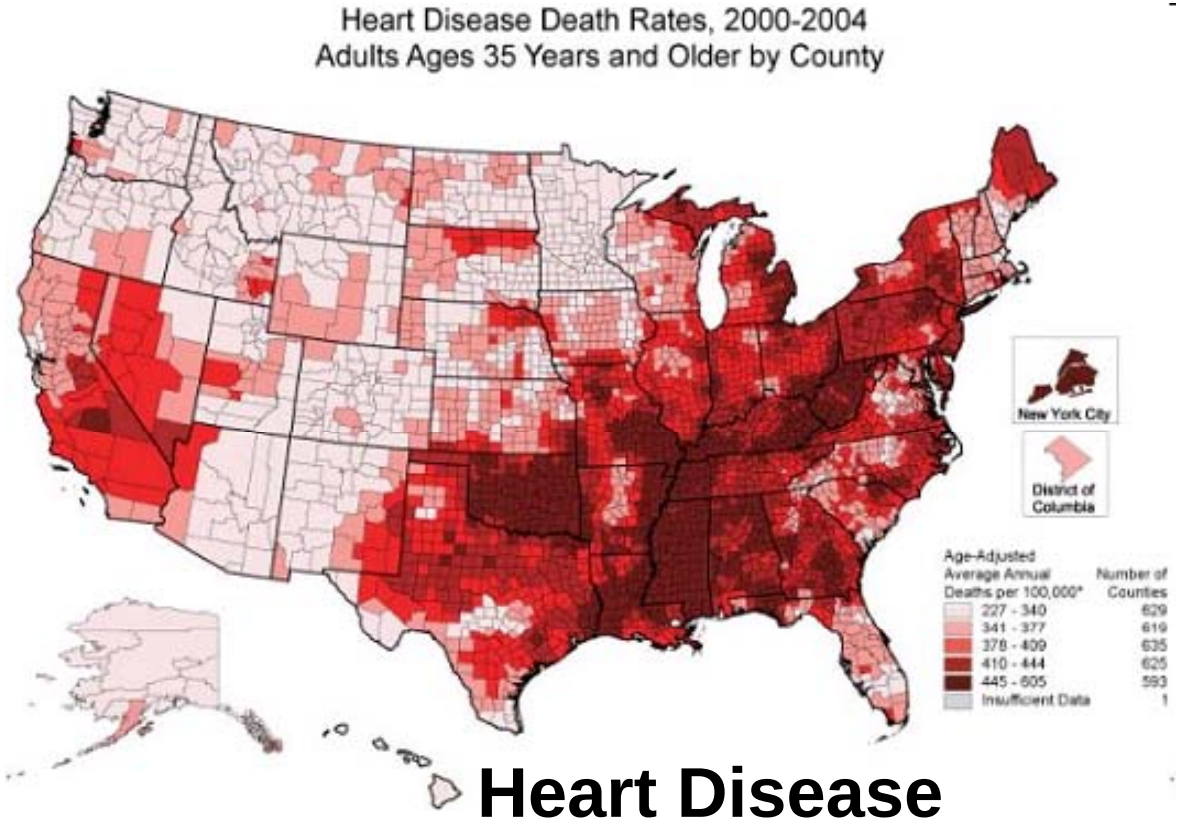
## Obesity (2008)

Public domain map created using data from www.cdc.gov/BRFSS.



## Diabetes (from Forest; 2008)

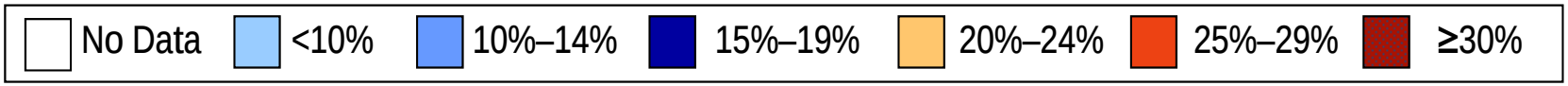
Public domain map by the U.S. Centers for Disease Control.




## Heart Disease (Death Rates) 2000-2004

Public domain map from the Centers for Disease Control. Data source: National Vital Statistics System and the U.S. Census Bureau.

**Legend for obesity data**



# What it Cardiovascular Disease?

- Starting at about your age … Gradual thickening of the arteries, usually at bends or bifurcation points
- LDL and cholesterol seep from blood through endothelial call layer into intima of artery (this is like an extracellular matrix)
- Possibly unfolded protein response is next (response to lipid)
- Eventually monocytes invade and differentiate into M1 and M2 macrophages
  - Role of Th2 (helper T-cells)

# What it Cardiovascular Disease? (Cont'd)

- Depending on the resolution of the inflammatory (M1) and anti-inflammatory (M2) arms of the pathway … an acute problem may develop in the form of a fragile fibrous cap (which is a point of weakness in the artery)
- Fibrous cap is under stress from above (shear pressure from blood flow)
- And below (macrophages turned into foam cells … which partially apoptose … and can result in regions of necrosis)
- Smooth muscle cells and fibroblasts try to fill in the chamber created by necrosis

# What it Cardiovascular Disease? (Cont'd)

- If this cell division and migration process succeeds, the cap is stabilized … you will die of something else
- If they fail, the cap is sheared off by blood flow, and tissue factor from intima mixes with blood
- Tissue factor activates blood platelets
- Thrombogenesis occurs (blood clot)
- A fibrous clot grows and can occlude the artery
- If it is a coronary artery, downstream necrosis will occur – Heart attack (Myocardial Infarction)
- Even if it is not a coronary artery, the clot can break free and cause distal damage (e.g., stroke)

# Video of Man Having a Heart Attack

http://www.youtube.com/watch?v=Qo3Nf_mJjAw

# First a Quick Overview

Sequence of Events Leading to Atheromatous Plaque

# Structure of a normal large artery

Figure removed due to copyright restrictions.
See Figure 1 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).

A large artery consists of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of SMCs. The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs.

# Initiation of atherosclerosis

Circulating leukocytes

The intima is composed of a single layer of endothelial cells overlying a subendothelial matrix

Figure removed due to copyright restrictions. See article citation below.
The figure shows a cross-section through an artery depicting circulating leukocytes adhering and migrating into the intima, where they divide.

**Outer wall of artery**

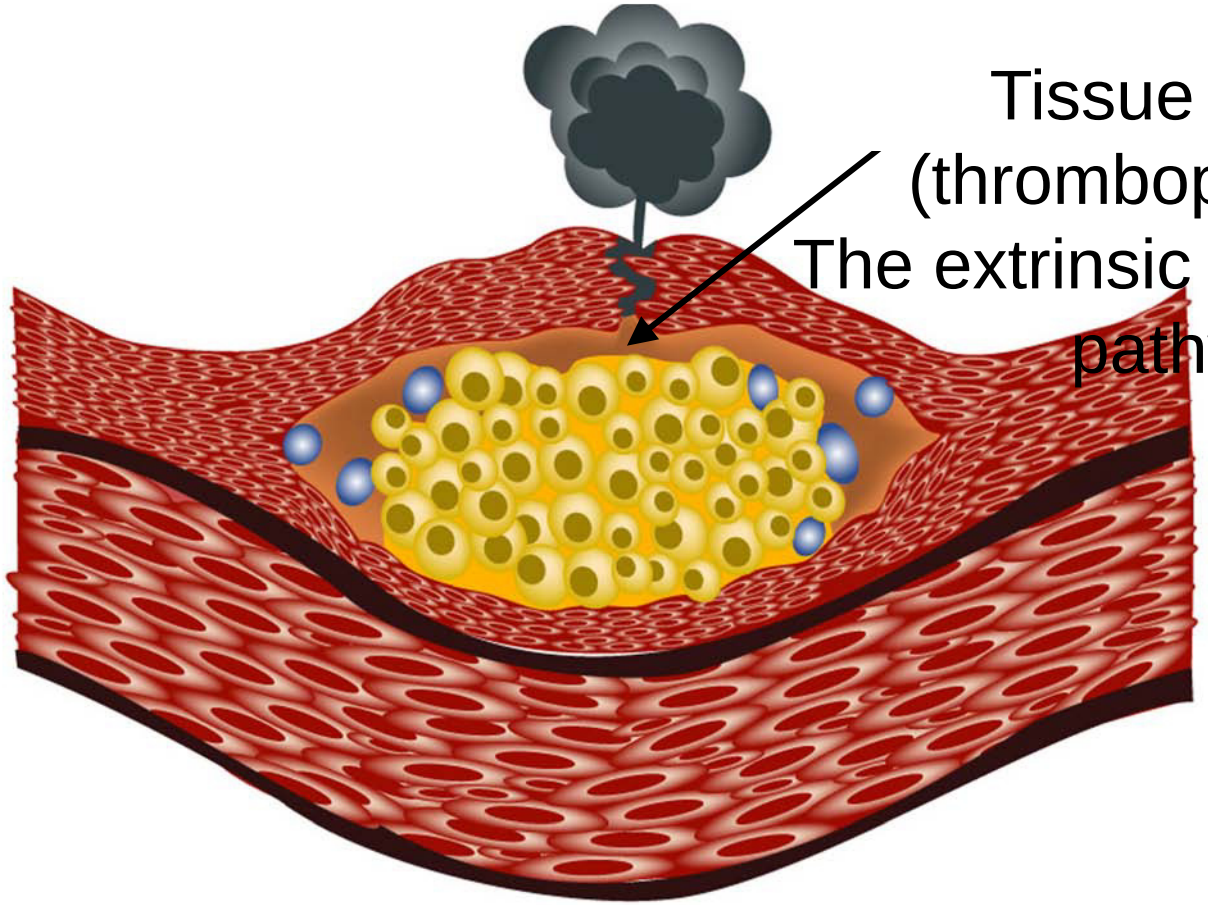
Packard and Libby, *Clinical Chemistry* 54: 24-38, 2008

# Progression of atherosclerosis

Figure removed due to copyright restrictions.
See Figure 2 from Packard, Rene R.S. and Peter Libby.
"Inflammation in Atherosclerosis: From Vascular Biology
to Biomarker Discovery and Risk Prediction." *Clinical Chemistry* 54 (2008).

# Thrombotic complication of atherosclerosis

Packard, Clin Chem 2008;54:24-38






Inflammatory mediators can inhibit collagen synthesis and evoke the expression of collagenases by macrophage foam cells within the intima. This imbalance diminishes the collagen content of the fibrous cap, rendering it weak and rupture-prone

# Now a More Detailed View

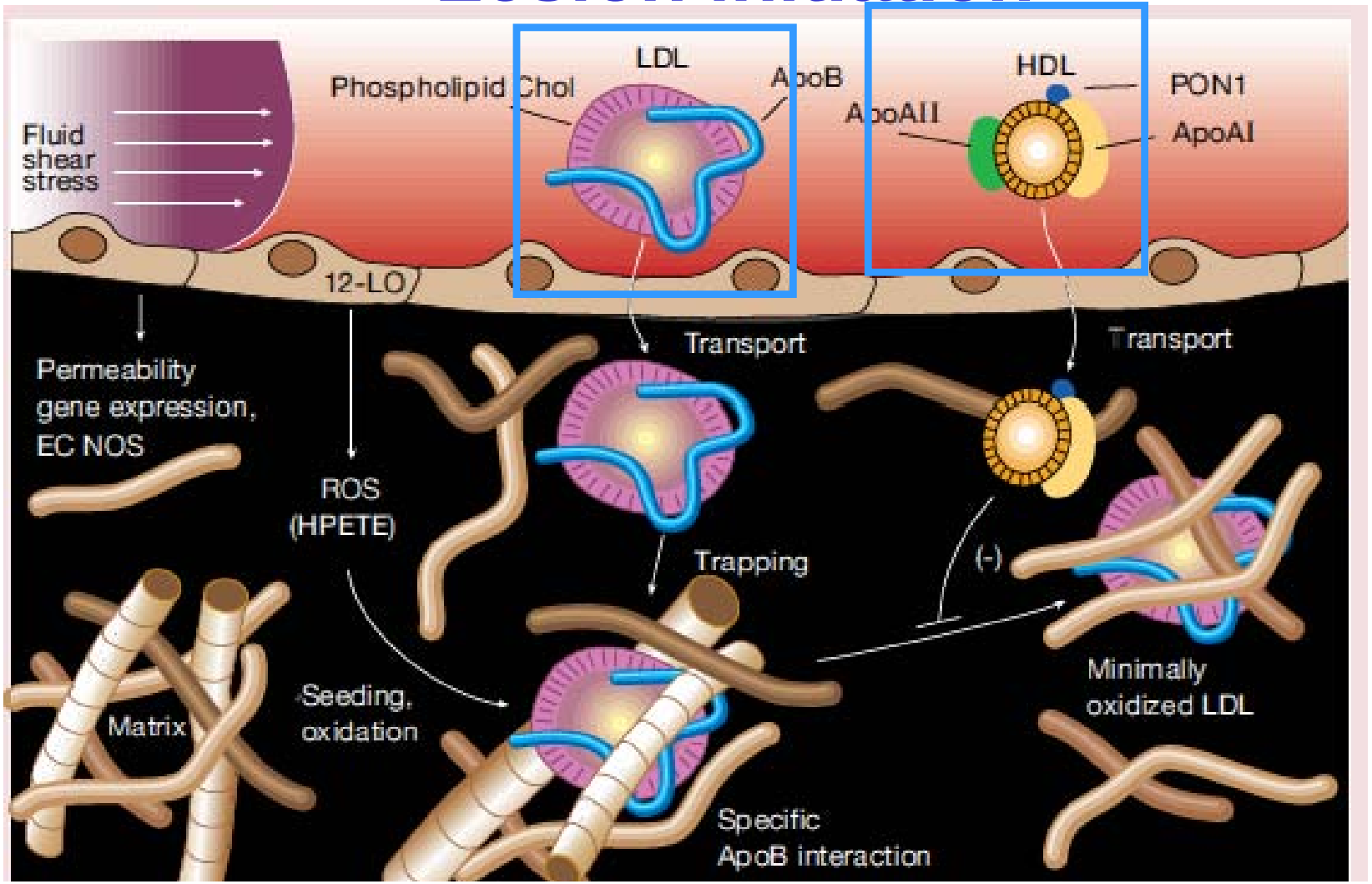
## Sequence of Events Leading to Atheromatous Plaque

**When systems biology was new …**

**Bob Tepper, founder, former president of Millennium Pharmaceuticals**

**Tried to take an integrated view of cancer, CVD and diabetes**

# Lesion initiation




Sites of lesion formation are determined in part by haemodynamic forces acting on endothelial cells. These influence the permeability of the endothelial barrier and expression of endothelial cell (EC) genes such as that for nitric oxide synthase (NOS). An important initiating event is the retention of LDL and other apolipoprotein B (apoB)-containing lipoproteins as a result of interaction with matrix components. The LDL undergoes oxidative modification as a result of interaction with reactive oxygen species (ROS) including products of 12/15 lipoxygenase (12-LO) such as HPETE. **Oxidation of LDL is inhibited by HDL**, which contains the antioxidant protein serum paraoxonase.

Figure showing lipoprotein transport pathways and fates removed due to copyright restrictions.

# Shuttling Lipids in Water … a Key Component of the Problem

Figure removed due to copyright restrictions. See Kaysen, G.A. "Dialysis removes apolipoprotein C-I, improving very low-density lipoprotein clearance Commentary." *Kidney International* 72 (2007).

Lipoproteins (liver) help create emulsions, but it is a great challenge to carry large volumes of a heterogeneous class of lipids

# Action of bile salts in emulsifying fats in the intestine (these micelles are in the intestine … those in the next slide are in the blood plasma)

Figure showing the chemical action of bile salts has been removed due to copyright restrictions.

**The lipids are then digested and transported through the intestinal mucosa … and end up in the blood stream**

# Generalized structure of a plasma lipoprotein

Figures of plasma lipoprotein structure and of a chylomicron binding to lipoprotein lipase have been removed due to copyright restrictions.

**Binding of a chylomicron to lipoprotein lipase on the inner surface of a capillary**

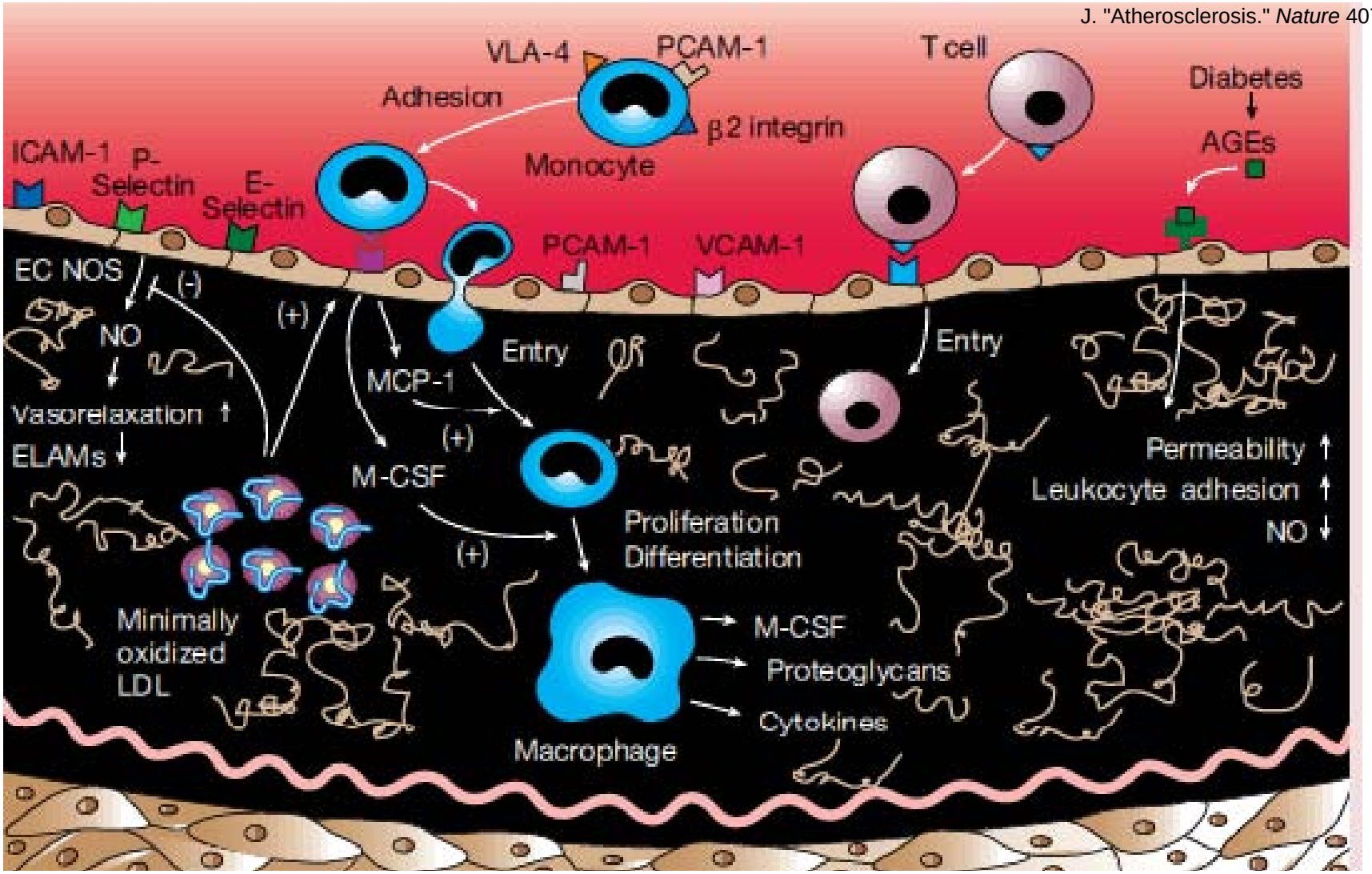
# Shuttling Lipids in Water … a Key Component of the Problem

Figure removed due to copyright restrictions. See Kaysen, G.A. "Dialysis removes apolipoprotein C-I, improving very low-density lipoprotein clearance Commentary." Kidney International 72 (2007).

Moreover … These lipid-glycoprotein conjugates need to be distinguished from sentinels of infections (e.g., LPS) … a hard task!

# Inflammation






Minimally oxidized LDL stimulates the overlying endothelial cells to produce adhesion molecules, chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1), and growth factors such as macrophage colony-stimulating fa\ctor (M-CSF), resulting in the recruitment of monocytes to the vessel wall. Oxidized LDL has other effects, such as inhibiting the production of NO, an important mediator of vasodilation and expression of endothelial leukocyte adhesion molecules (ELAMs). Among endothelial cell adhesion molecules likely to be important in the recruitment of leukocytes are ICAM-1, P-selectin, E-selectin, PCAM-1 and VCAM-1. Important adhesion molecules on monocytes include b2 integrin, VLA-4, and PCAM-1. Advanced glycosylation endproducts (AGEs) are formed in diabetes and these promote inflammation via specific receptors on endothelial cells.

# Foam-cell formation

Figure removed due to copyright restrictions.
See Figure 5 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).

Highly oxidized aggregated LDL is formed in the vessel as a result of the action of reactive oxygen species (ROS) and the enzymes sphingomyelinase (SMase), secretory phospholipase 2 (sPLA 2 ), other lipases, and myeloperoxidase (MPO). The oxidized aggregated LDL is recognized by macrophage scavenger receptors such as SR-A, CD36 and CD68. Scavenger receptor expression is mediated by cytokines such as tumour necrosis factor-a (TNF-a) and interferon-g (IFN-g). Foam cells secrete apolipoprotein E (apoE), which may facilitate removal of excess cellular cholesterol via HDLs. The death of foam cells leaves behind a growing mass of extracellular lipids and other cell debris. – Probably contributes to Unfolded Protein Response

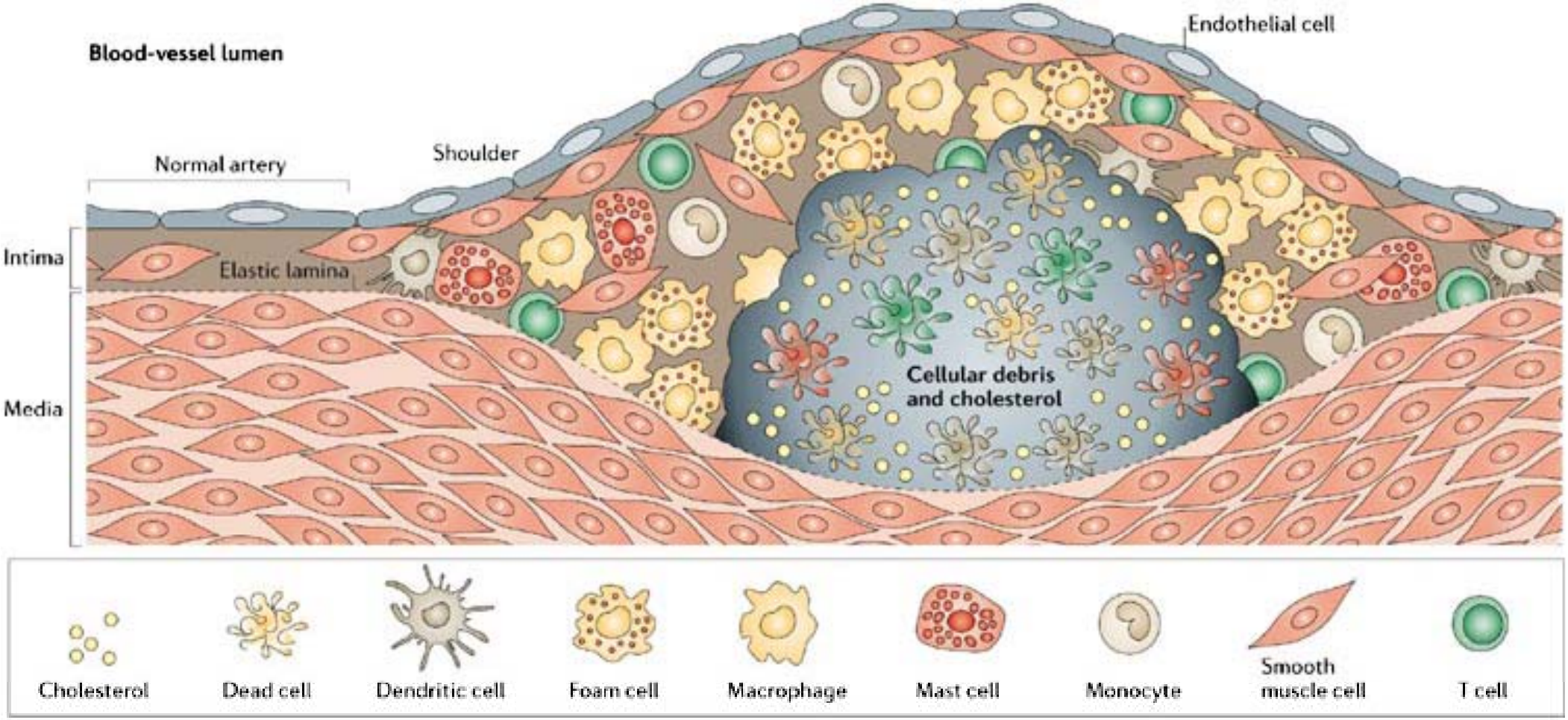
# Formation of fibrous plaques

Figure removed due to copyright restrictions.
See Figure 6 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).

# Complex lesions and thrombosis

Figure removed due to copyright restrictions.
See Figure 7 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).

# Complex lesions and thrombosis



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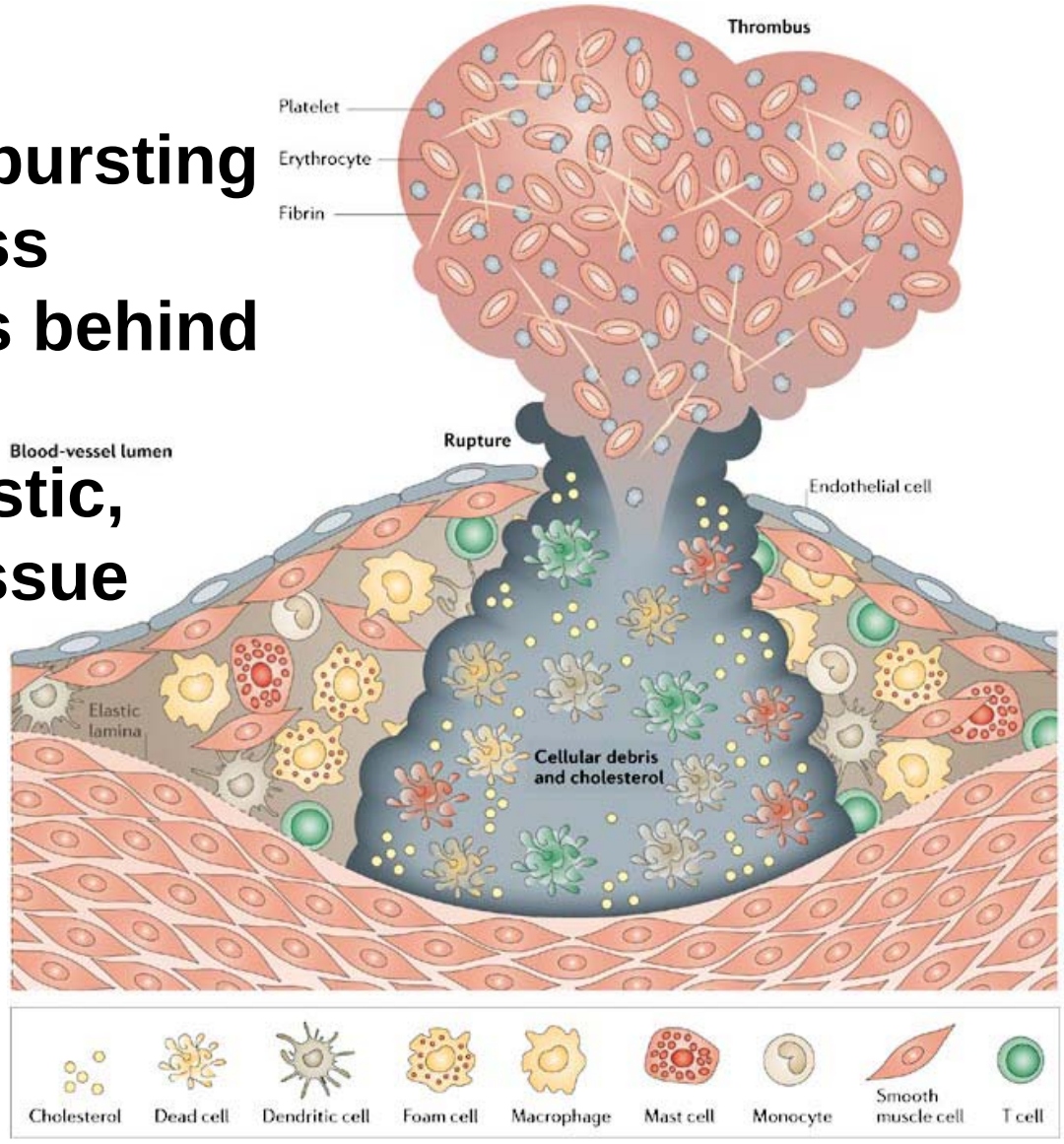
Source: Hansson, Goran K. and Peter Libby. "The immune response in atherosclerosis: a double-edged sword." *Nature Reviews Immunology* 6 (2006). © 2006.

nature REVIEWS IMMUNOLOGY

# Complex lesions and thrombosis

- **It is like the bursting of an abscess**
- **And it leaves behind scarring**
- **And non-elastic, thickened tissue**



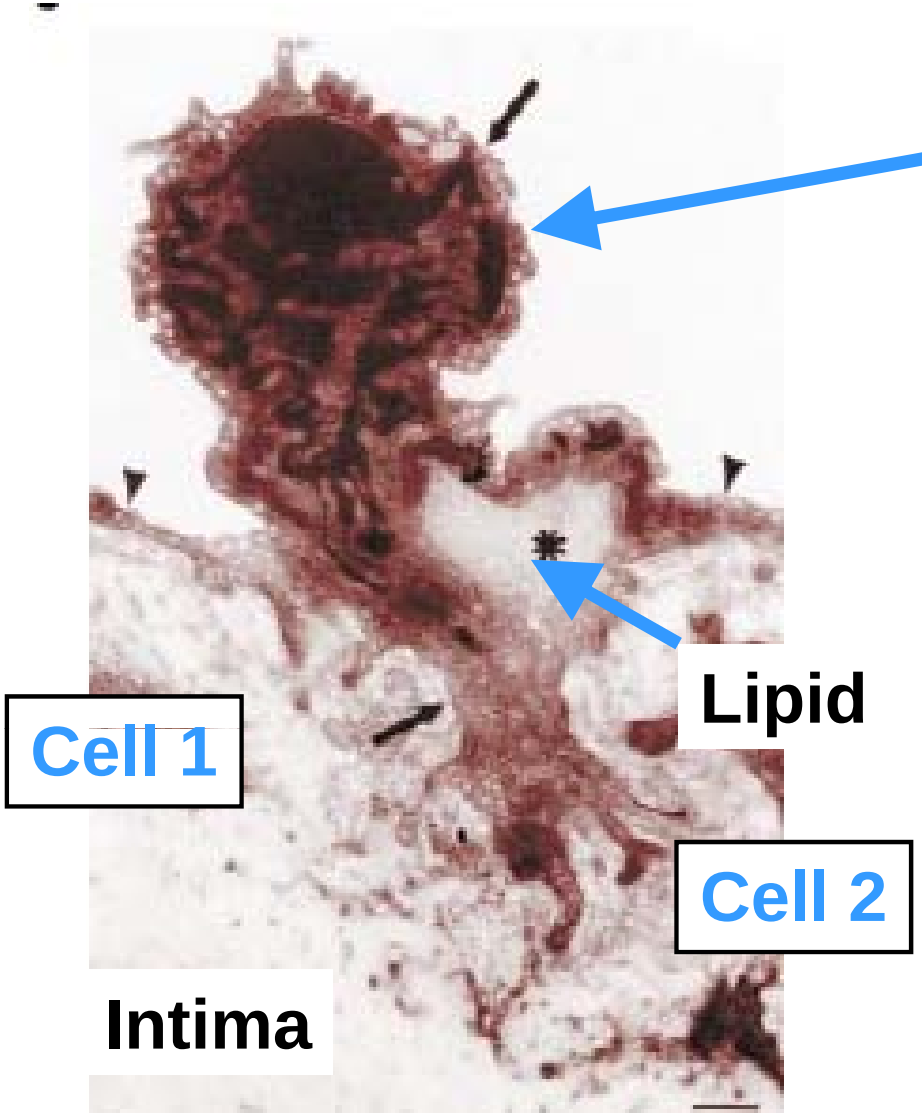



Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006) | doi:10.1038/nri1882

# Stages in the development of atherosclerotic plaques

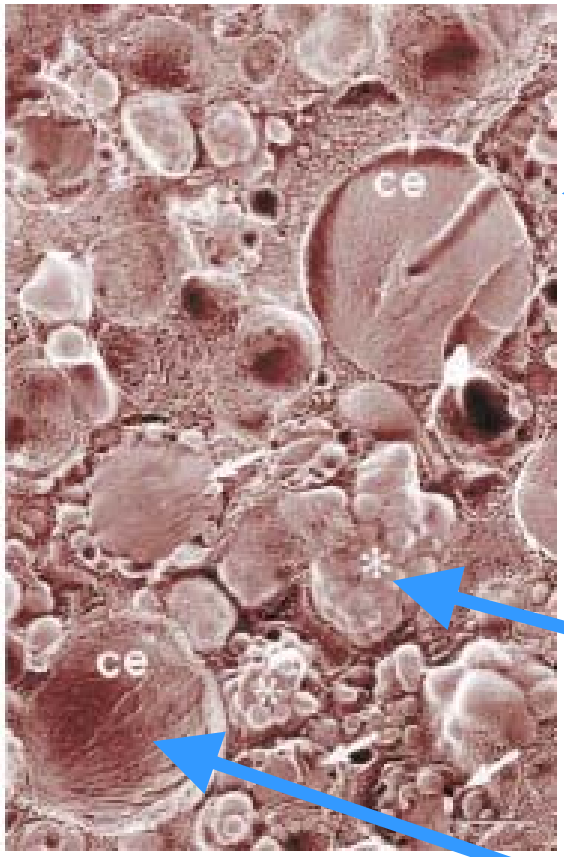
Figure removed due to copyright restrictions.
See Figure 2 from Lusis, Aldons J. "Atherosclerosis." *Nature* 407 (2000).

# Stages in the development of atherosclerotic plaques




**Monocyte transmigration**.
The thin-section electron micrograph of a cross-section of the aorta of a 9-weekold apoE-deficient mouse shows a monocyte (arrow) moving between two endothelial cells (arrowheads) to enter the intima (int). The asterisk denotes a cluster of lipid underneath the endothelial cell.

# Stages in the development of atherosclerotic plaques



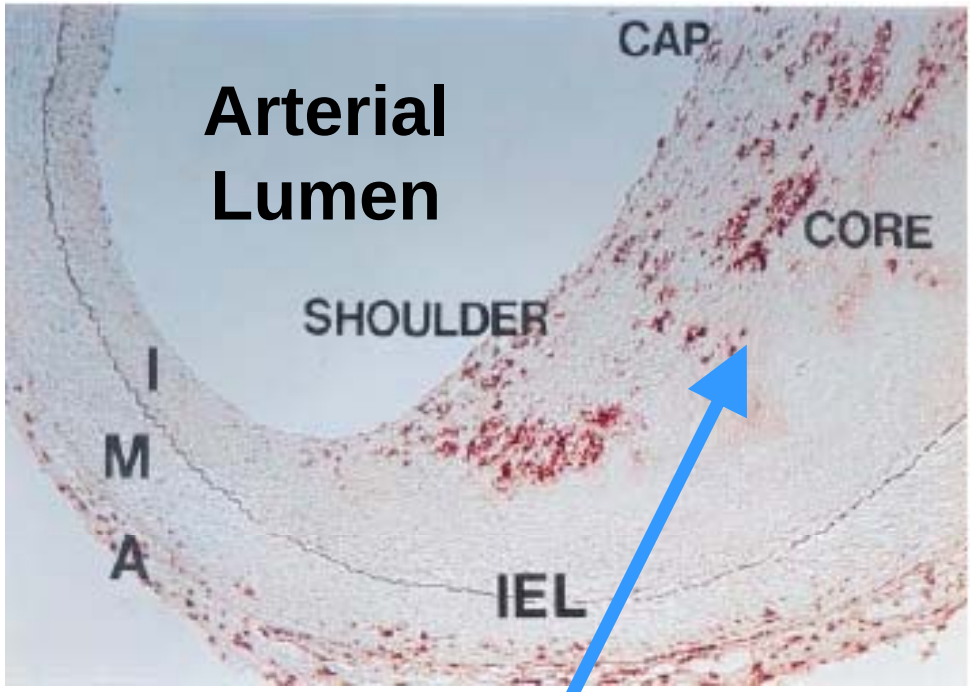

**LDL**

**Cytoplasm of a foam cell**

**CE = Cholesterol esters**

**Foam-cell Formation**. Freezeetch electron micrograph of the cytoplasm of a macrophage foam cell in the intima of a rabbit fed a high-fat diet for two weeks. Large lipid droplets with the onion skin configuration typical of cholesterol esters (ce) as well as other lipid-filled compartments (arrows) can be recognized. Some compartments contain large aggregated LDL particles (asterisk) resembling those in previous figure.

# Stages in the development of atherosclerotic plaques

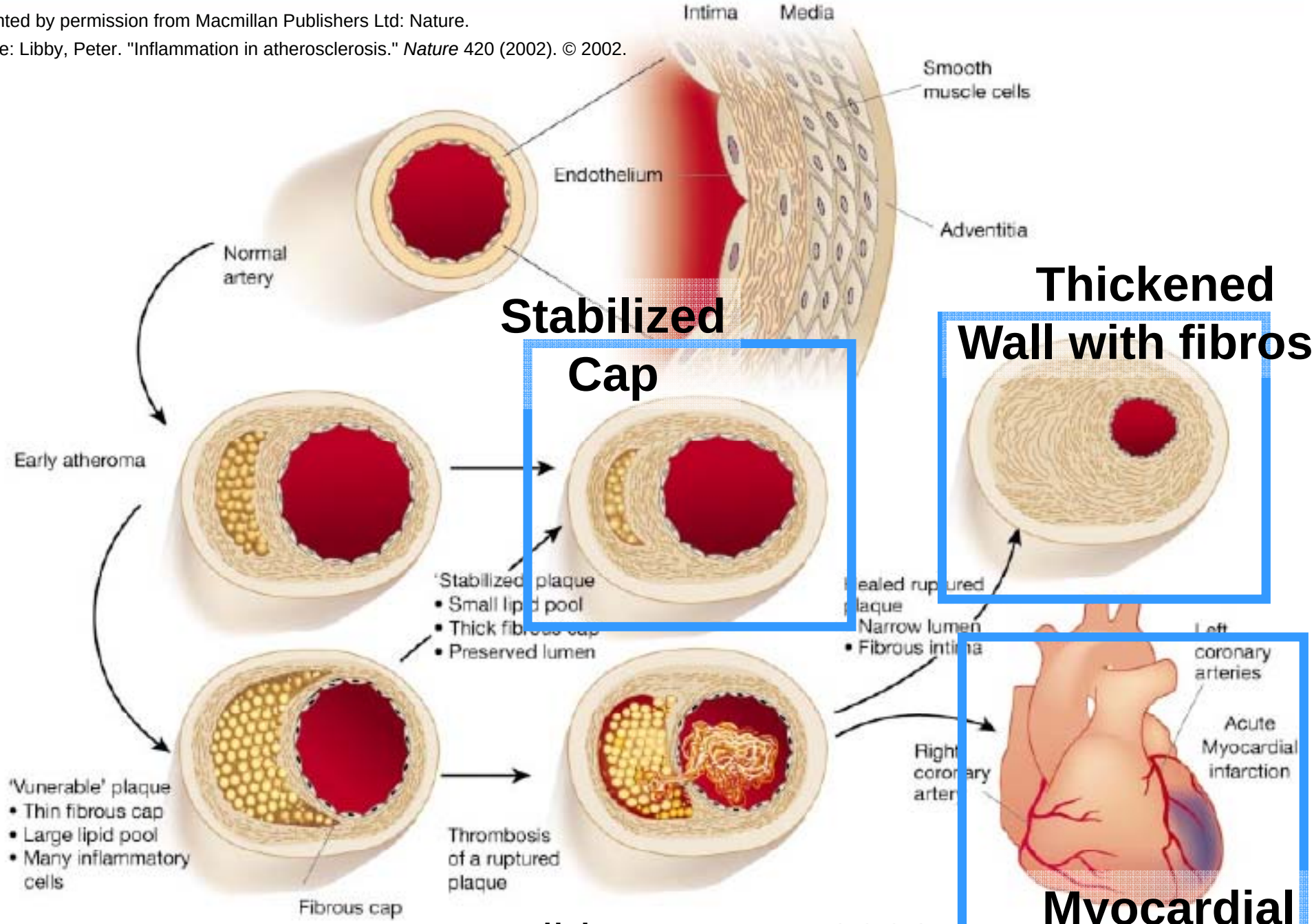



Fibrous lesion. Light micrograph (2400x) of a section of an advanced human coronary atherosclerotic lesion that has been immunostained for the acrophage specific antigen EMB-11 (red). A, adventitia; I, intima; IEL, internal elastic lamina; M, media

**Section of a human coronary artery**

# Three Possible Resolutions






Libby, *Nature*, 2002

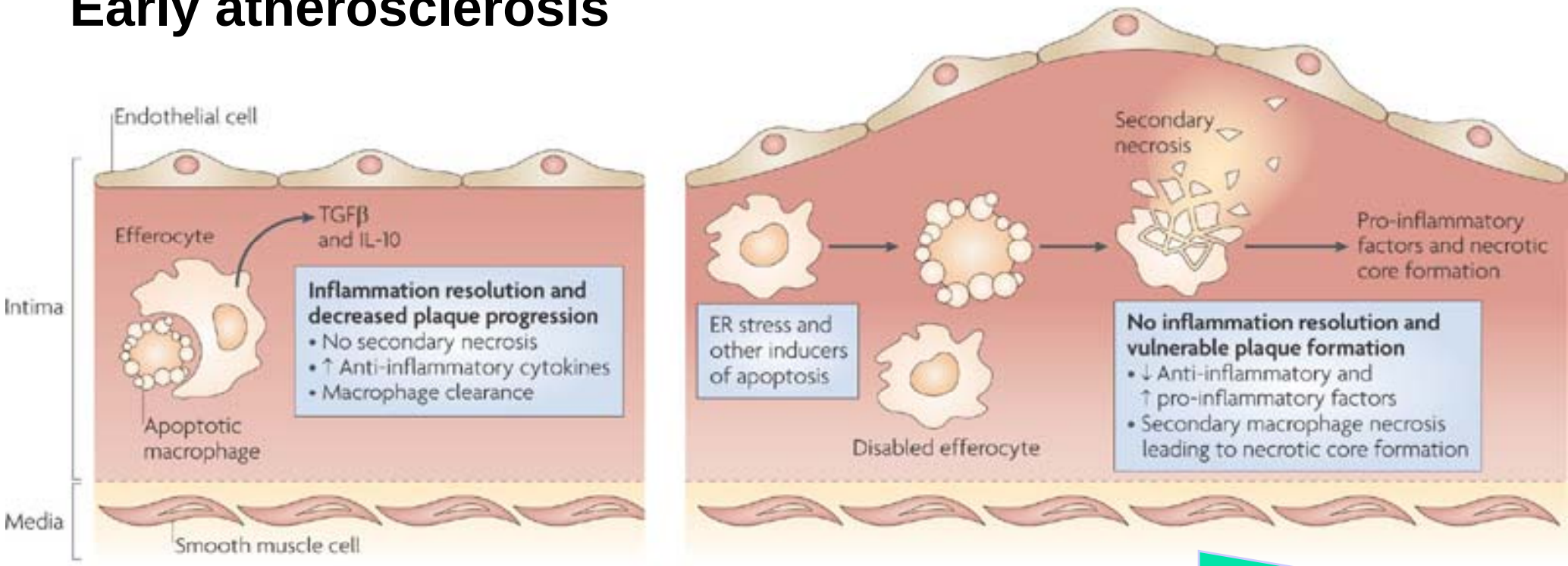
# Immune Cell Involvement

In Atherosclerosis

# Immune Cell Infiltration and Balance

**Advanced atherosclerosis**

**Early atherosclerosis**

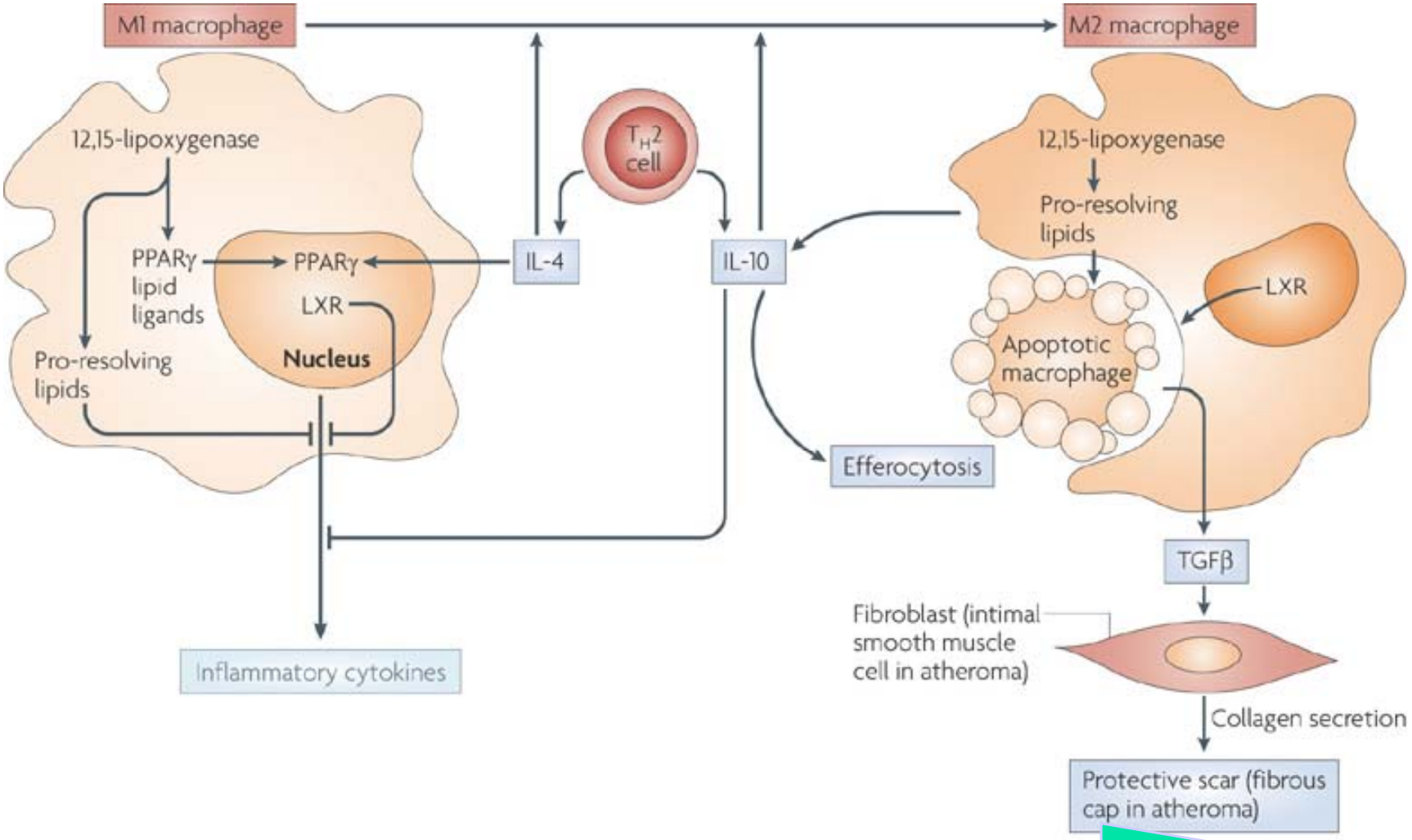



**Good → Bad**

**Efferocytosis = Successful clearage of apoptotic macrophages = good; when this fails, the system tips toward fibrous cap formation, and necrosis – necrosis draws in more inflammatory cells**

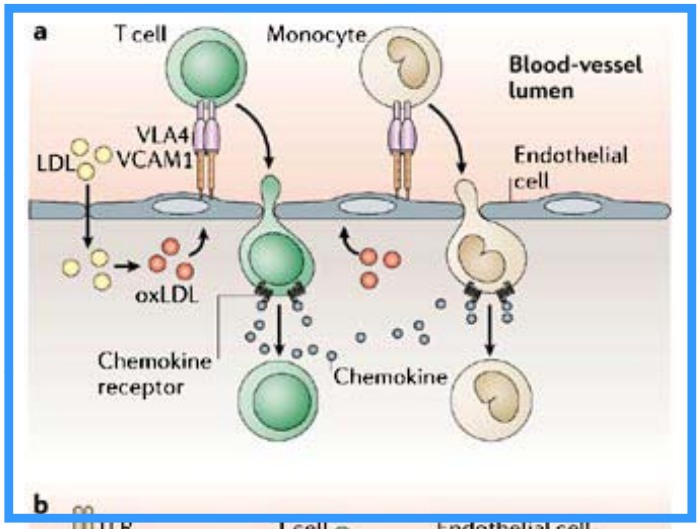
Ira Tabas, Nature Reviews Immunology 10, 36-46 (January 2010)

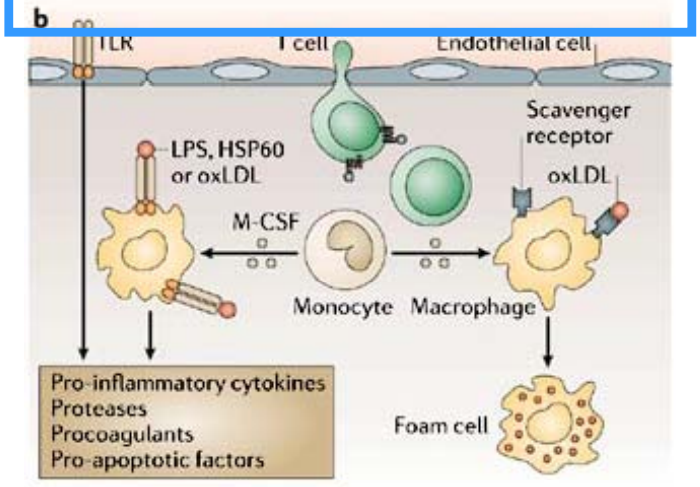
# Healthy Response to CVD Would Shift from Left to Right

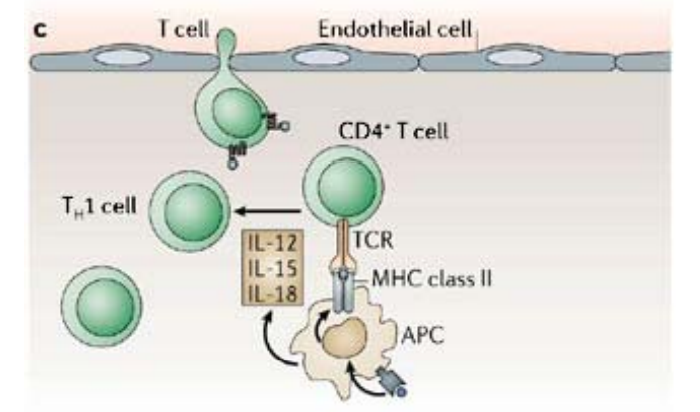


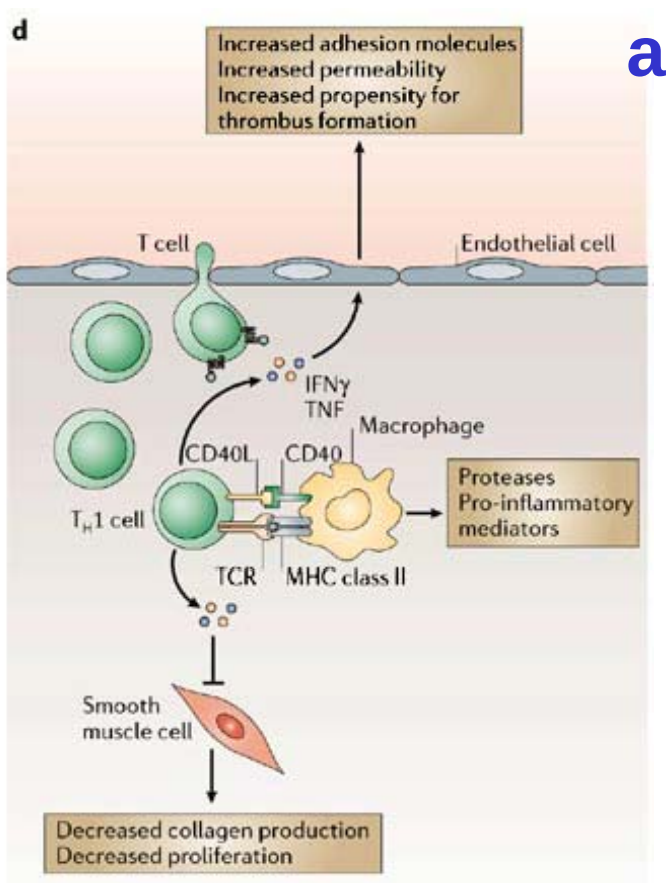

**Bad → Good**

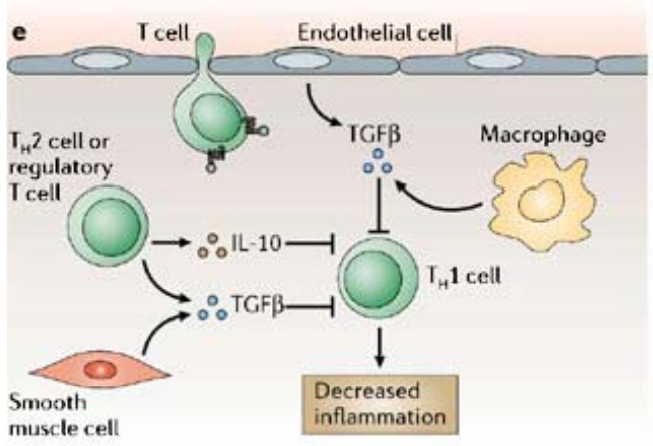
Ira Tabas, Nature Reviews Immunology 10, 36-46 (January 2010)



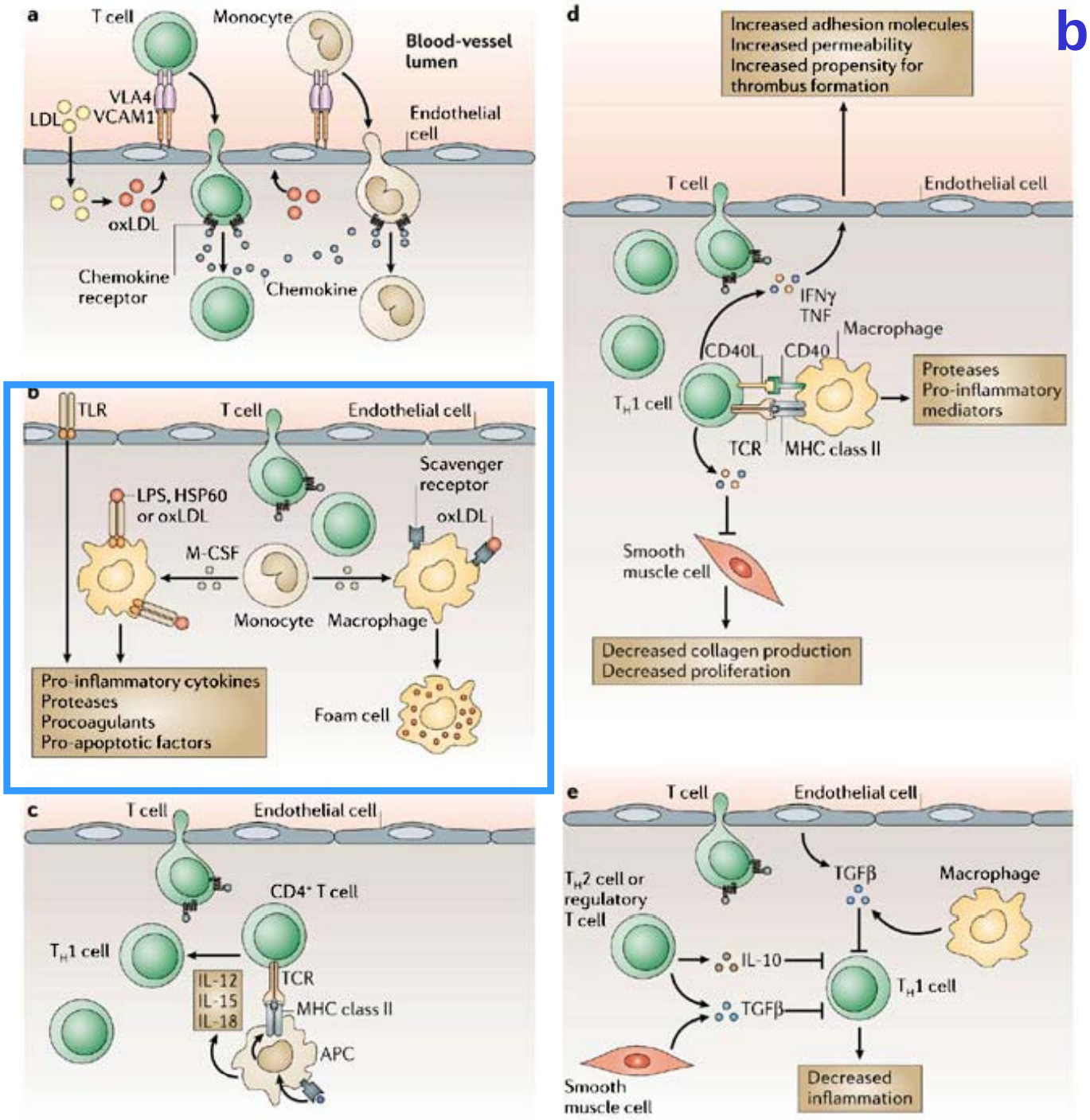




**a.**

- LDL diffuses from the blood
- LDL particles associate with proteoglycans of the extracellular matrix
- LDL modified by enzymes and oxygen radicals → oxLDL
- Biologically active lipids are released and induce endothelial cells to express leukocyte adhesion molecules
- Monocytes and T cells bind to VCAM1-expressing endothelial cells through very late antigen 4 (VLA4)
- Monoc. and T cells respond to locally produced chemokines by migrating into the arterial tissue

Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006)



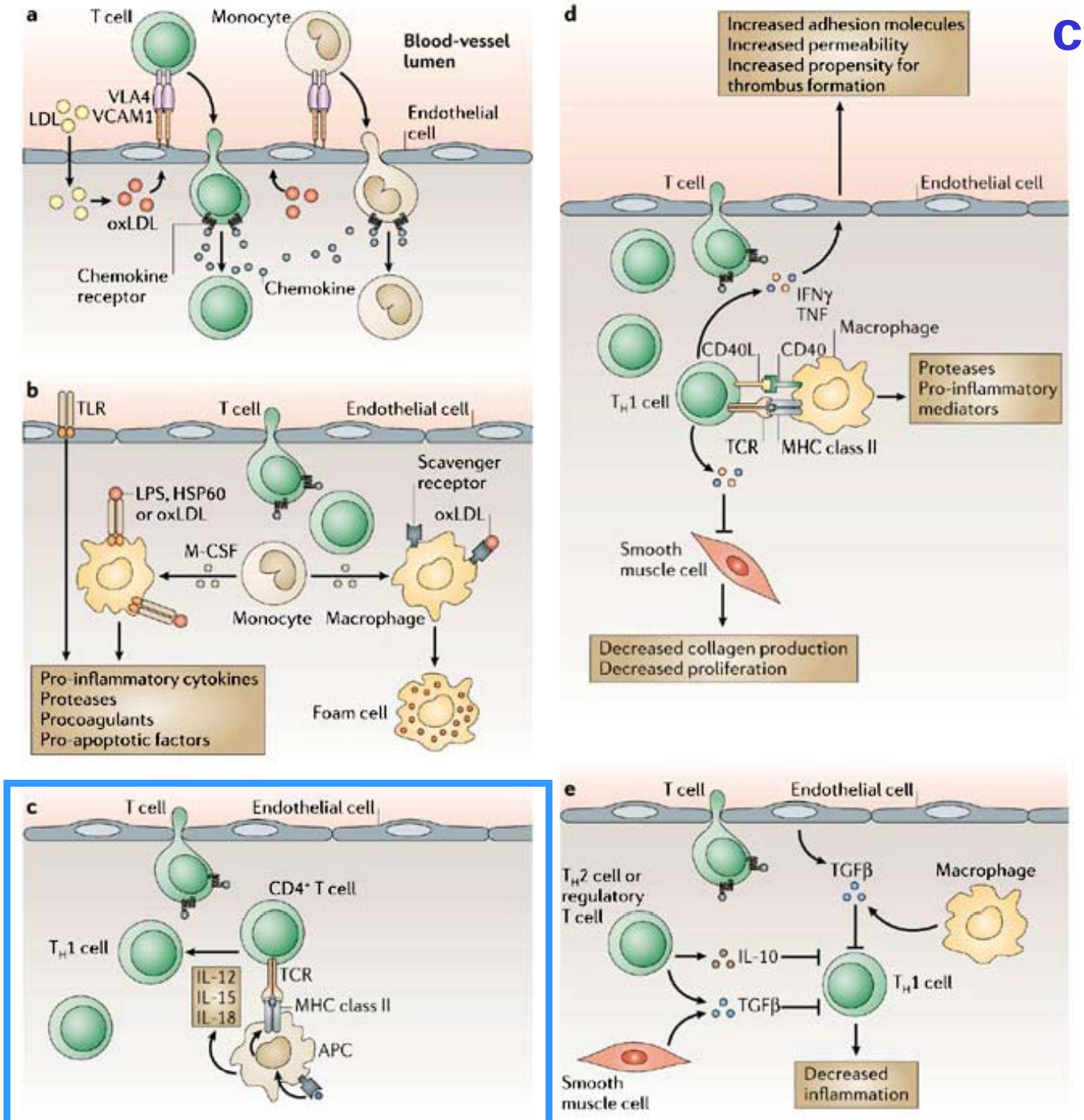

**b.** • Monocytes differentiate into macrophages in response to local macrophage colony-stimulating factor (M-CSF)

• Expression of many pattern-recognition receptors increases, including scavenger receptors and Toll-like receptors (TLRs)

• Scavenger receptors mediate macrophage uptake of oxLDL particles, which leads to intracellular cholesterol accumulation and the formation of foam cells

• TLRs bind LPS, heat-shock protein 60 (HSP60), oxLDL and other ligands, which instigates production of many pro-inflammatory molecules by macrophages

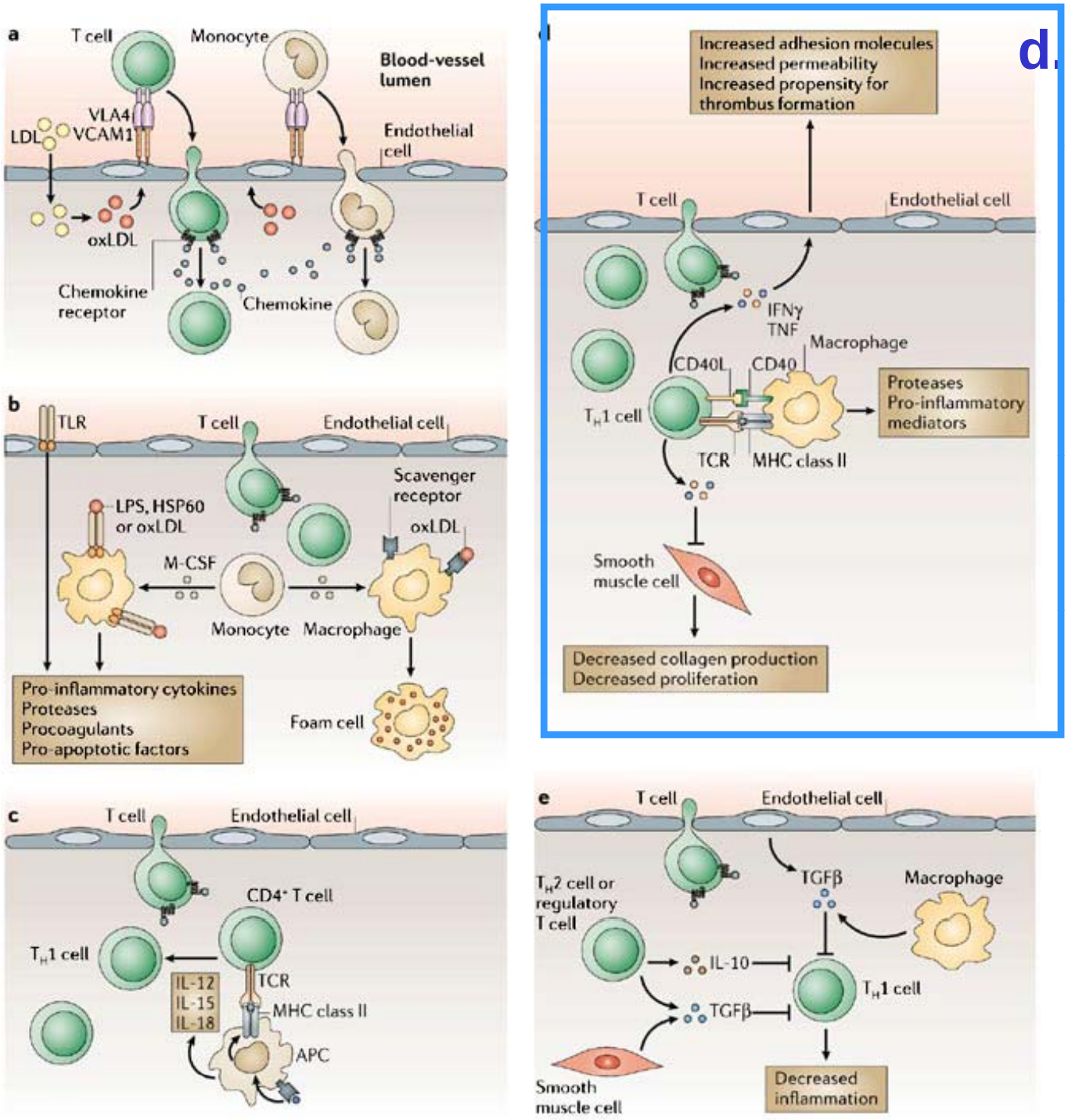
Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006)




Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006)

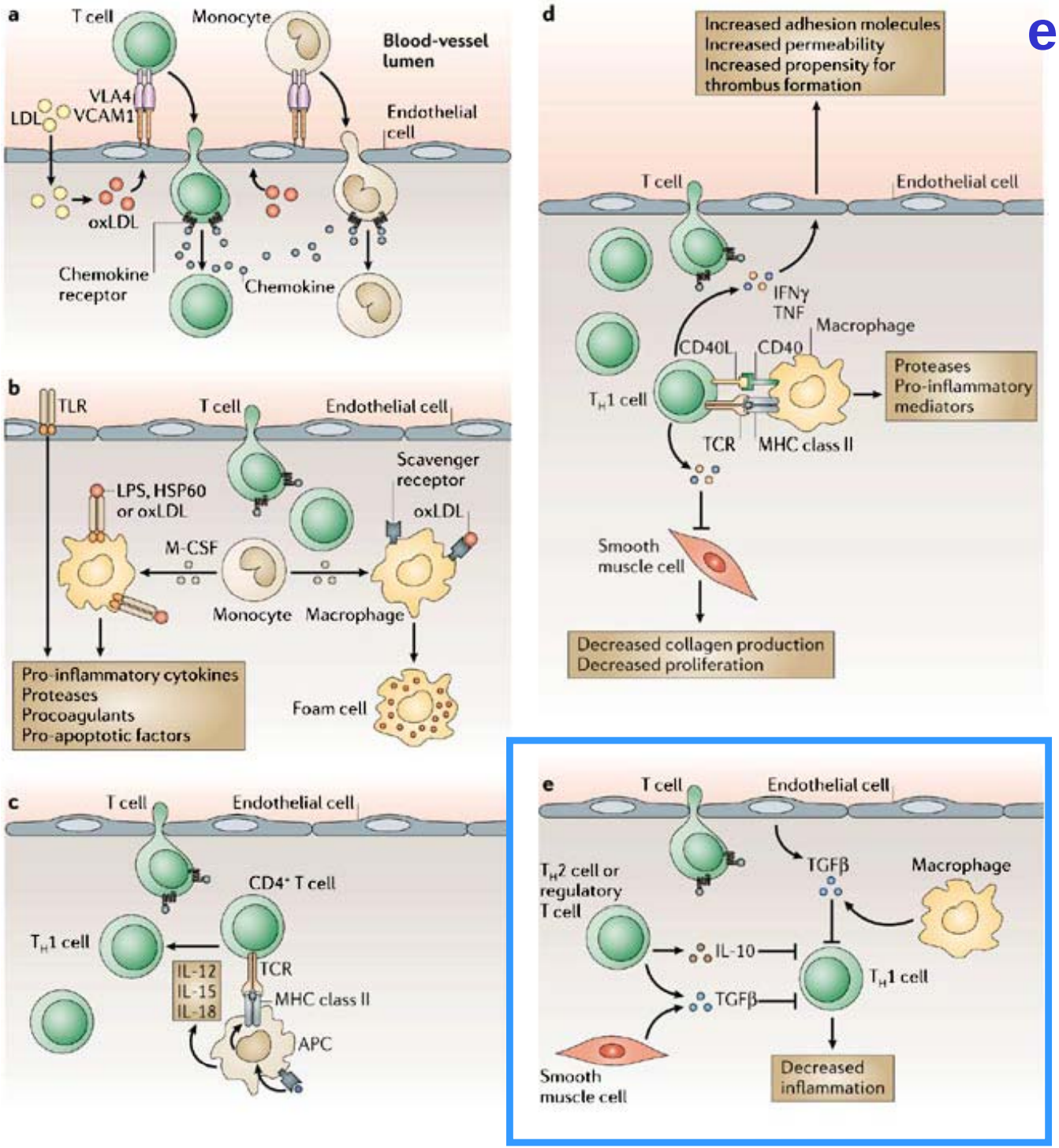
**C.**

- T cells undergo activation after interacting with APCs, such as macrophages or dendritic cells
- APCs process and present local antigens including oxLDL, HSP60 and possibly components of local microorganisms
- A T helper 1 (Th1)-cell-dominated response ensues, possibly owing to the local production of interleukin-12 (IL-12), IL-18 and other cytokines
- Antigen presentation and TH1-cell differentiation might also occur in regional lymph nodes




- Th1 cells produce inflammatory cytokines including IFN-g and TNF and express CD40 ligand (CD40L)
- These messengers prompt macrophage activation, production of proteases and other pro-inflammatory mediators, activate endothelial cells, increase adhesion-molecule expression and the propensity for thrombus formation, and inhibit smooth-muscle-cell proliferation and collagen production

Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006)




**e.**

- Response is shut down – thanks to Th2 cells
- Plaque inflammation attenuated in response to the anti-inflammatory cytokines IL-10 and TGF-b)
- These are produced by several cell types including regulatory T cells, macrophages and, for TGF-b, also vascular cells and platelets. TCR, T-cell receptor

Hansson *et al. Nature Reviews Immunology* **6**, 508-519 (July 2006)

Figure removed due to copyright restrictions.

See Figure 1 from Libby, Peter, et al. "Inflammation in Atherosclerosis: From Pathophysiology to Practice." *Journal of the American College of Cardiology* 54 (2009).

# Libby, 2009

# Cells Involved in Atherosclerosis Express Pattern-Recognition Receptors Involved in Innate Immunity

Figure removed due to copyright restrictions.
See Figure 2 from Libby, Peter, et al. "Inflammation in Atherosclerosis: From Pathophysiology to Practice." *Journal of the American College of Cardiology* 54 (2009).

While we usually think of these “receptors” in the context of response to a bacterial infection -- in the arterial wall they respond to LDL, Apolipop. and other agents to trigger inflammagion

**Libby, 2009**

# Cells Involved in Adaptive Immunity and Their Effect on Arterial Lesions

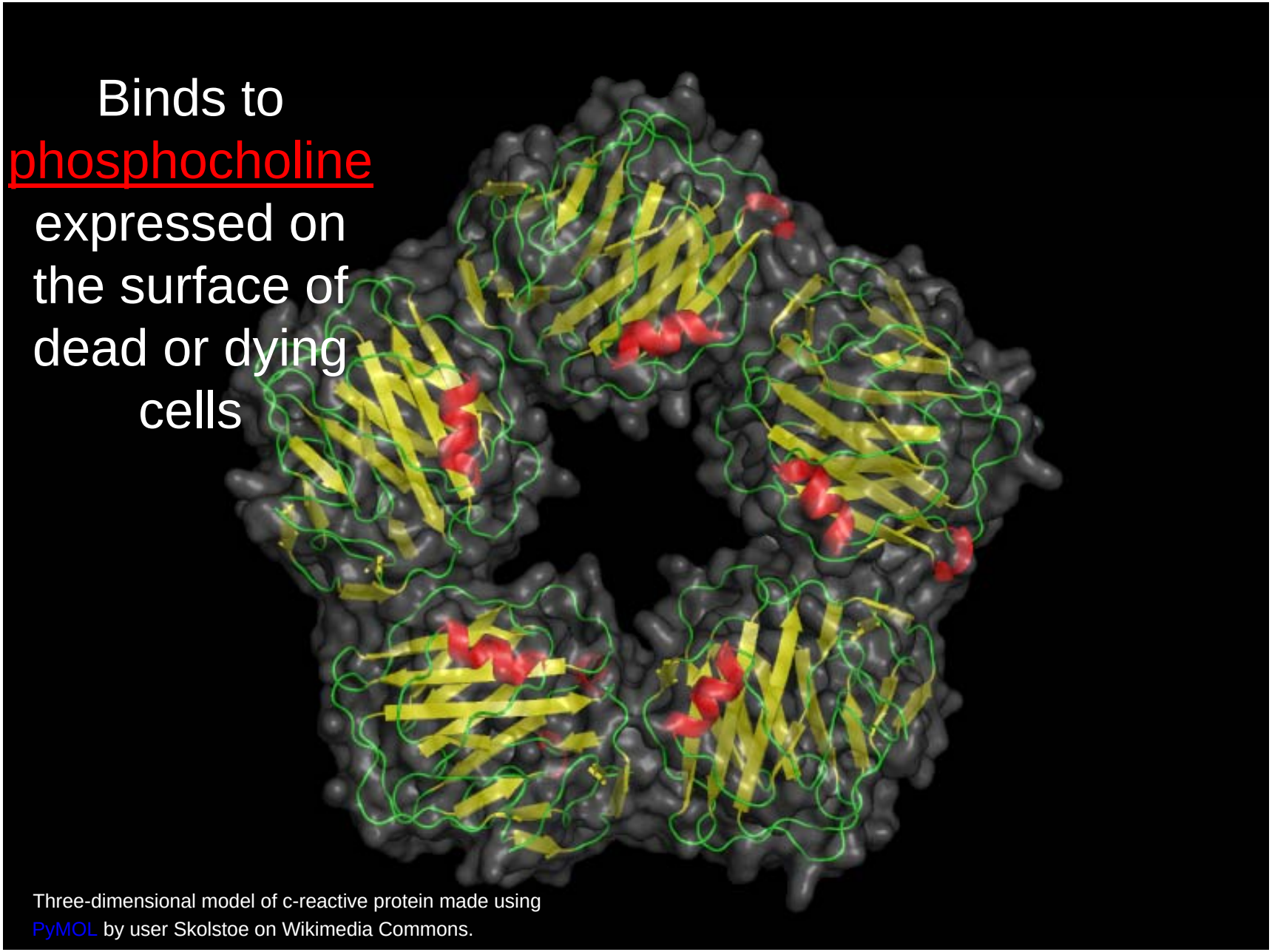
**Five classes of lympho-cytes**

Figure removed due to copyright restrictions.
See Figure 3 from Libby, Peter, et al. "Inflammation in Atherosclerosis: From Pathophysiology to Practice." *Journal of the American College of Cardiology* 54 (2009).

**Libby, 2009**

# C-Reactive Protein
# A new diagnostic marker of inflammation

Binds to phosphocholine expressed on the surface of dead or dying cells

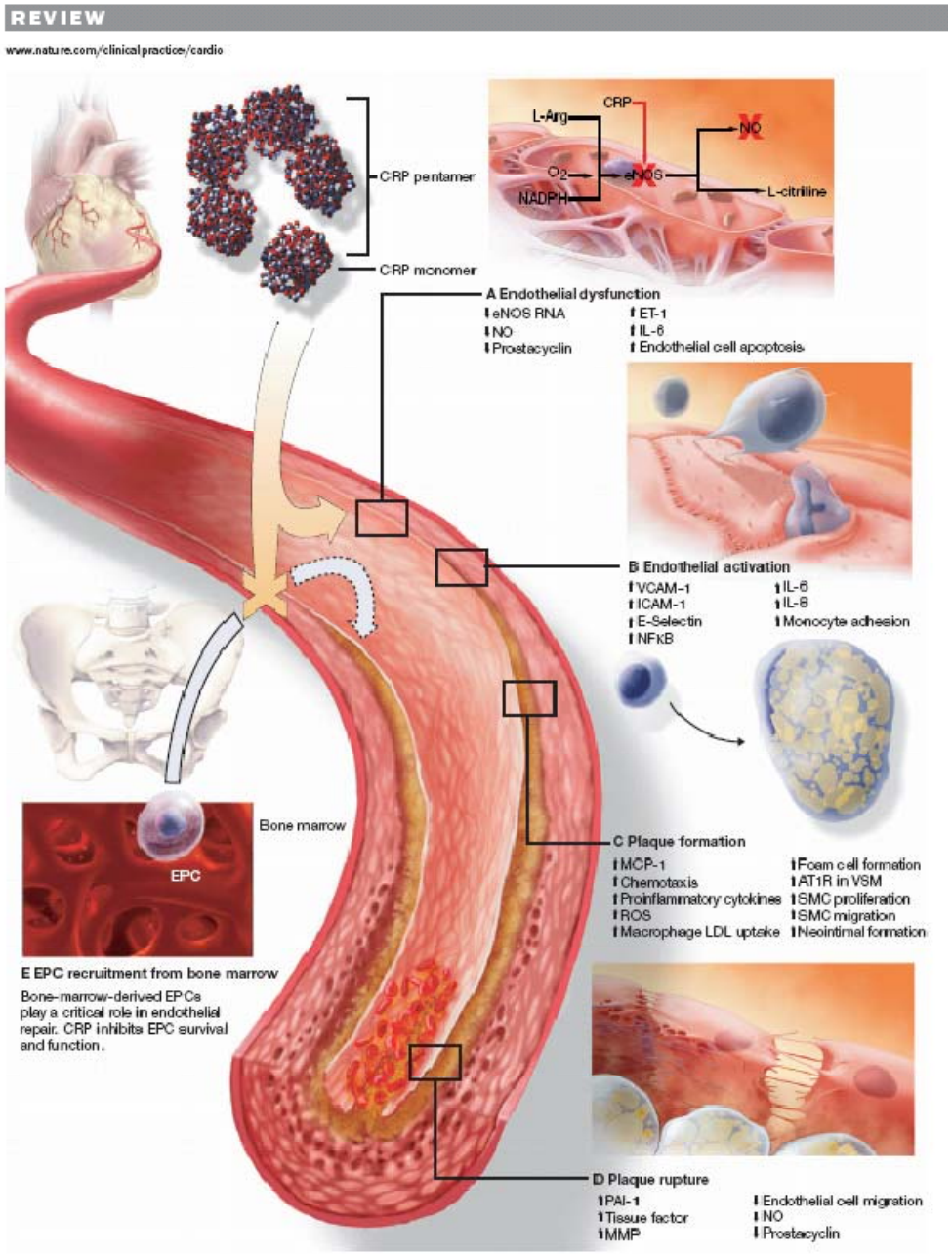


Three-dimensional model of c-reactive protein made using PyMOL by user Skolstoe on Wikimedia Commons.

# Inflammation is Sensed in Many Organs
# That information is transmitted to the liver

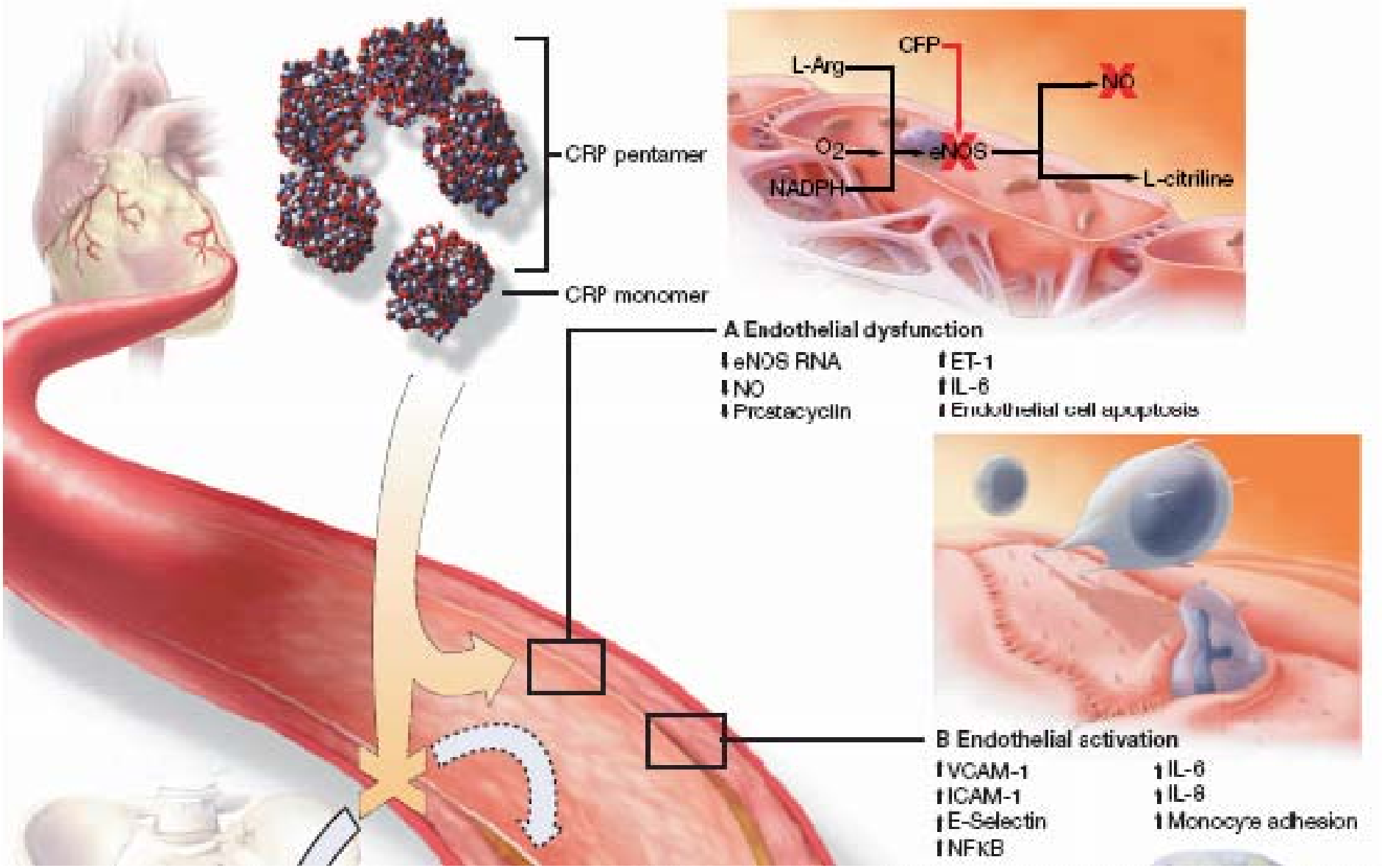
Figure removed due to copyright restrictions.
Inflammation sensed by the heart, blood vessel wall, macrophages, and adipose tissue leads to the release of cytokines that transmit this information to the liver. See Figure 1 from Rader, Daniel, J. "Inflammatory Markers of Coronary Risk." *New England Journal of Medicine* 343 (2000).

# Role of C-Reactive Protein in CVD

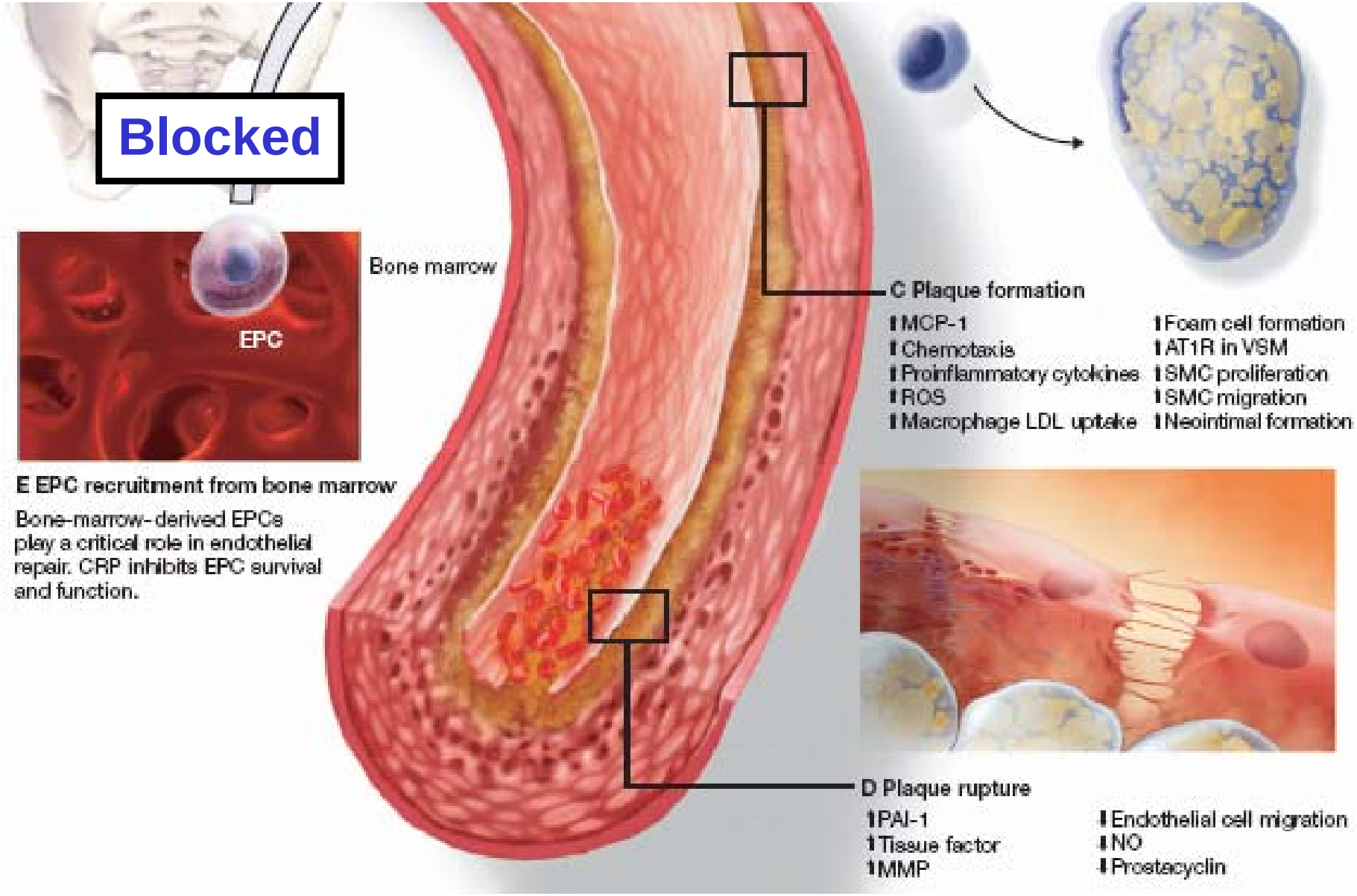



**Verma et al., 2005**

# Role of C-Reactive Protein in CVD



# Role of C-Reactive Protein in CVD



# Treatment of CVD

Treatments that address the immune mediated component of the disease

# Addressing the disease

- Diet, exercise … still the best (stop smoking = inflammatory)
- Statins, anti-hypertensives, platlet-directed anti-inflammatory and anticoagulative agents, and anything that reduces insulin resistance
- Omega-3 FAs are precursors of protectins … effective
- Future
  - Agents that shift the macrophage M1 – M2 balance (Omega-3 FA and drug S1P lipid) might do this by binding to MPhage)
  - Activators of PPARs
  - Inducers of IL-10 and TGF-beta (if local) retard plaque progression (note = Protein drugs present delivery obstacles)
  - Immunize high risk people with apoptotic cells → increase IgM to apoptotic cell surface proteins

# A Few Project Ideas

Thoughts I had while preparing this lecture

# Project Ideas

- Tetrathiomolybdate has been tried (successfully) to combat CVD – Tom Maciag paper
- Use a phage display library to find plaques.  Deliver a payload (drug) or image the lesion
  - Renata Pasqualini paper
- Blood vessels become “leaky” in the vicinity of an infection. Design a nanoparticle to squeze through the space to deliver a drug – Shiladit Sengupta
- National Geographic approach to ID novel therapeutic targets
  - Search for genes at intersection of the CVD, Diabetes and Cancer ven diagrams

# Do we have a chance to conquer complex diseases?

Scan of article from National Geographic (January 2010) removed due to copyright restrictions.
Read the article from National Geographic.

# Or We Could Throw in the Towel and Do Something Useful With Tools Available Today

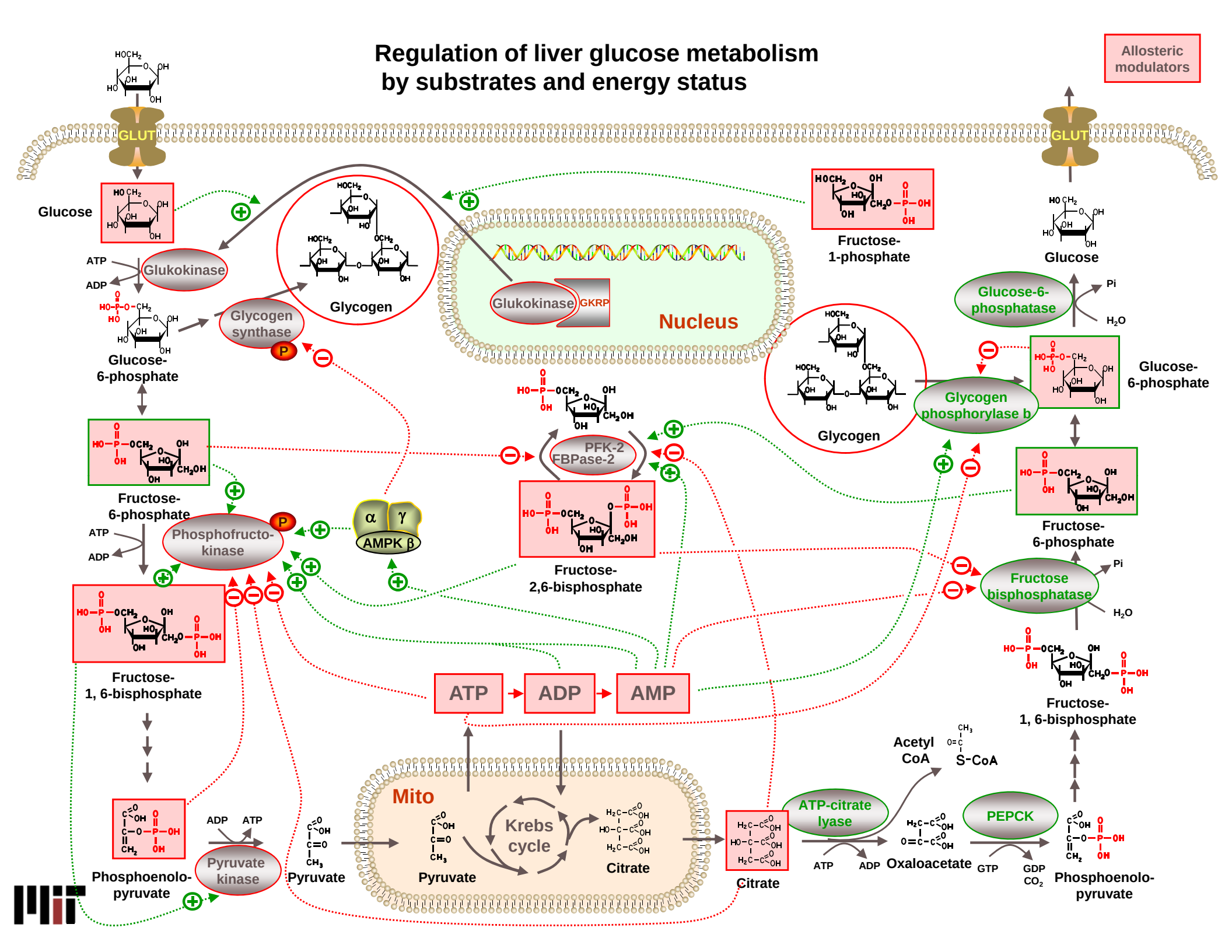
Photo from Flickr by feastoffun.com.

# Adiponectin and an Ob/Ob Background

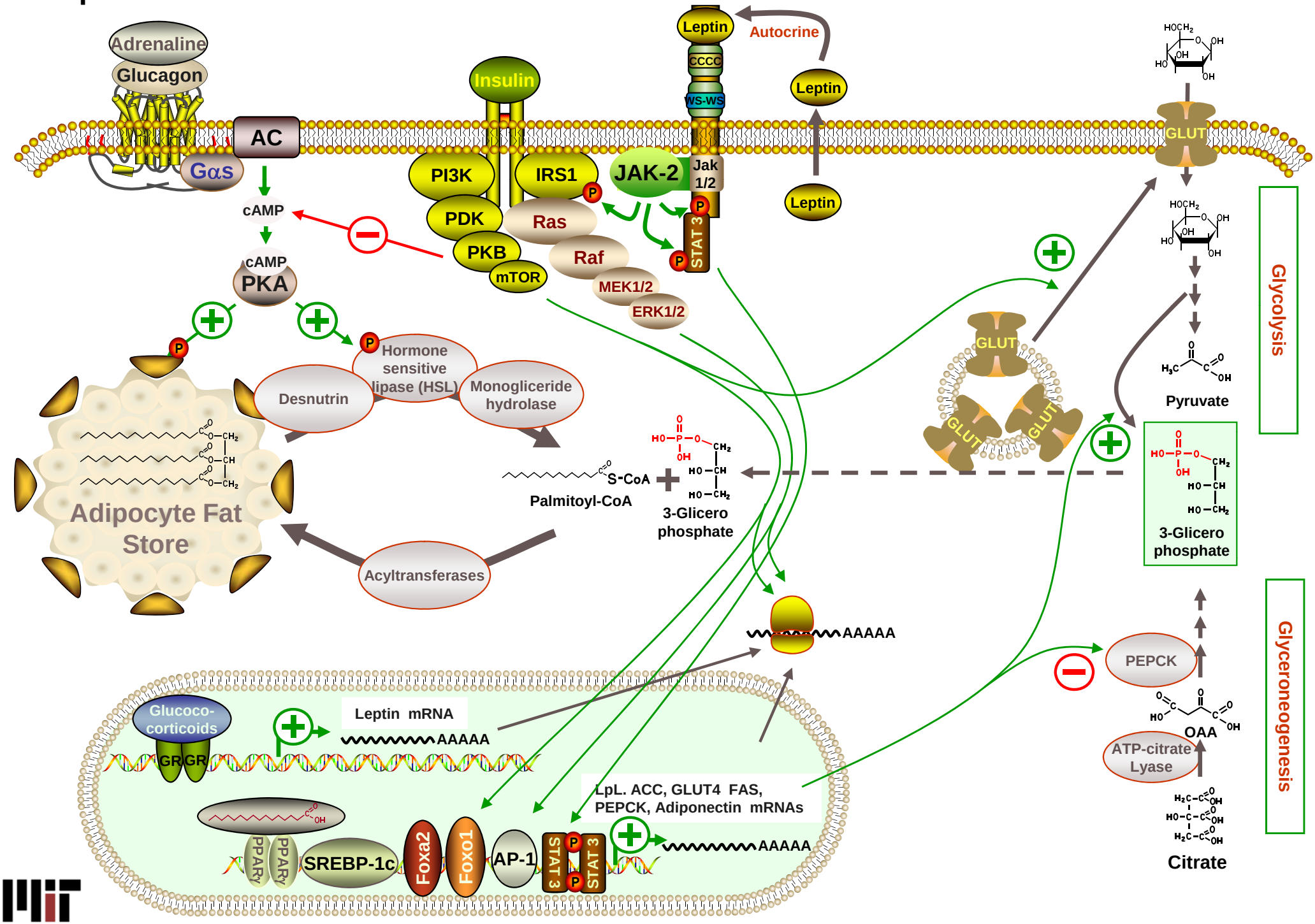
**BE Design: We could grow <u>healthy</u> sumo wrestlers**

# Extra Slides Dealing With Pathways

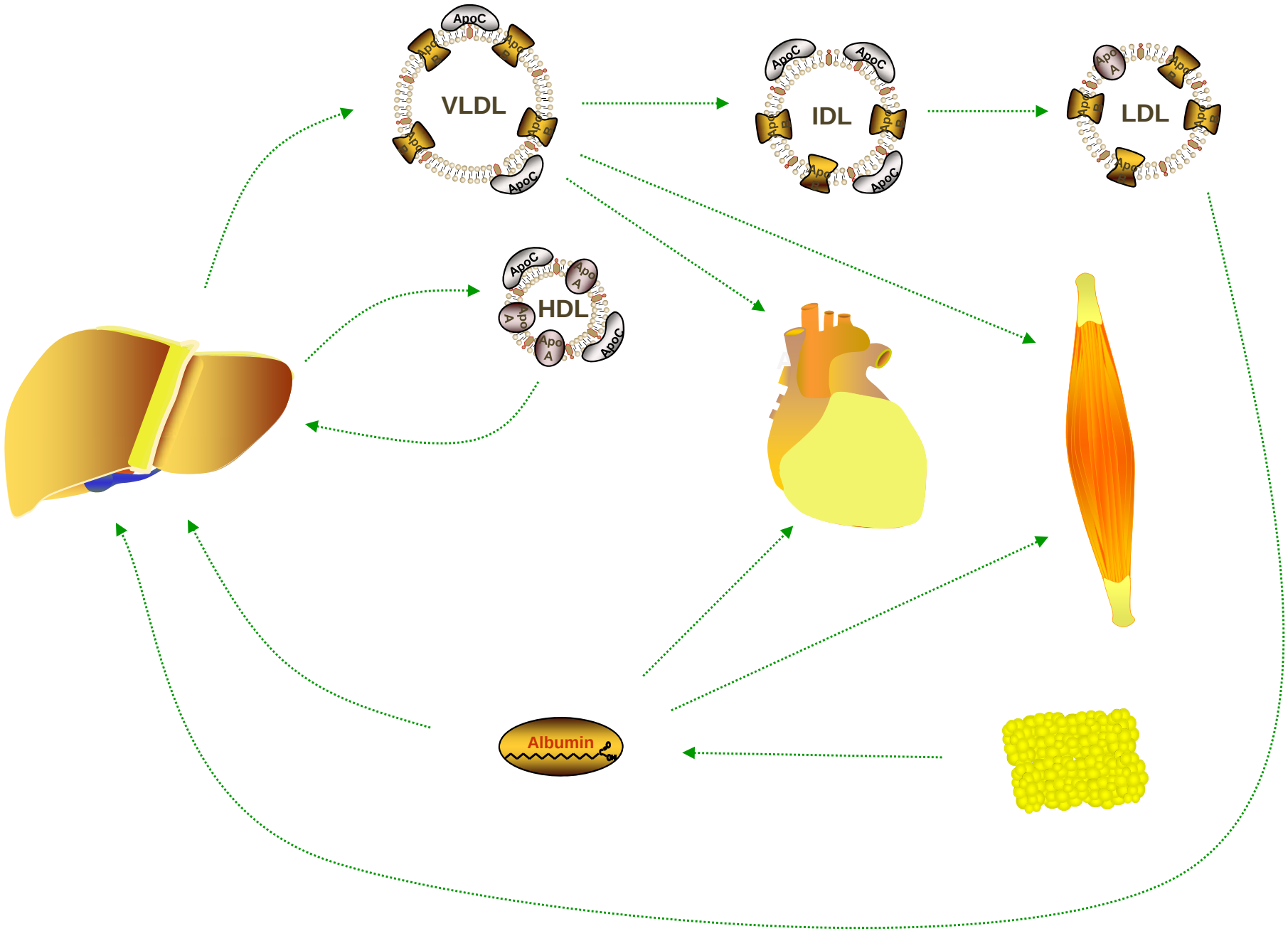
May be useful for your presentations


Regulation of liver glucose metabolism
by substrates and energy status
Allosteric modulators
GLUT
GLUT
Glucose
Glukokinase
ATP
ADP
Glucose-6-phosphate
Glycogen synthase
P
Glycogen
Glukokinase
GKRP
Nucleus
Fructose-1-phosphate
Glucose
Glucose-6-phosphatase
Pi
H2O
Glucose-6-phosphate
Glycogen
Glycogen phosphorylase b
Fructose-6-phosphate
PFK-2
FBPase-2
Fructose-2,6-bisphosphate
Fructose-6-phosphate
ATP
ADP
Phosphofructo-kinase
P
α
γ
AMPK β
Fructose bisphosphatase
Pi
H2O
Fructose-1, 6-bisphosphate
Fructose-1, 6-bisphosphate
ATP
ADP
AMP
Mito
Krebs cycle
Pyruvate
Citrate
Acetyl CoA
S-CoA
ATP-citrate lyase
ATP
ADP
Oxaloacetate
PEPCK
GTP
GDP
CO2
Phosphoenolo-pyruvate
ADP
ATP
Pyruvate kinase
Phosphoenolo-pyruvate
Pyruvate
Citrate

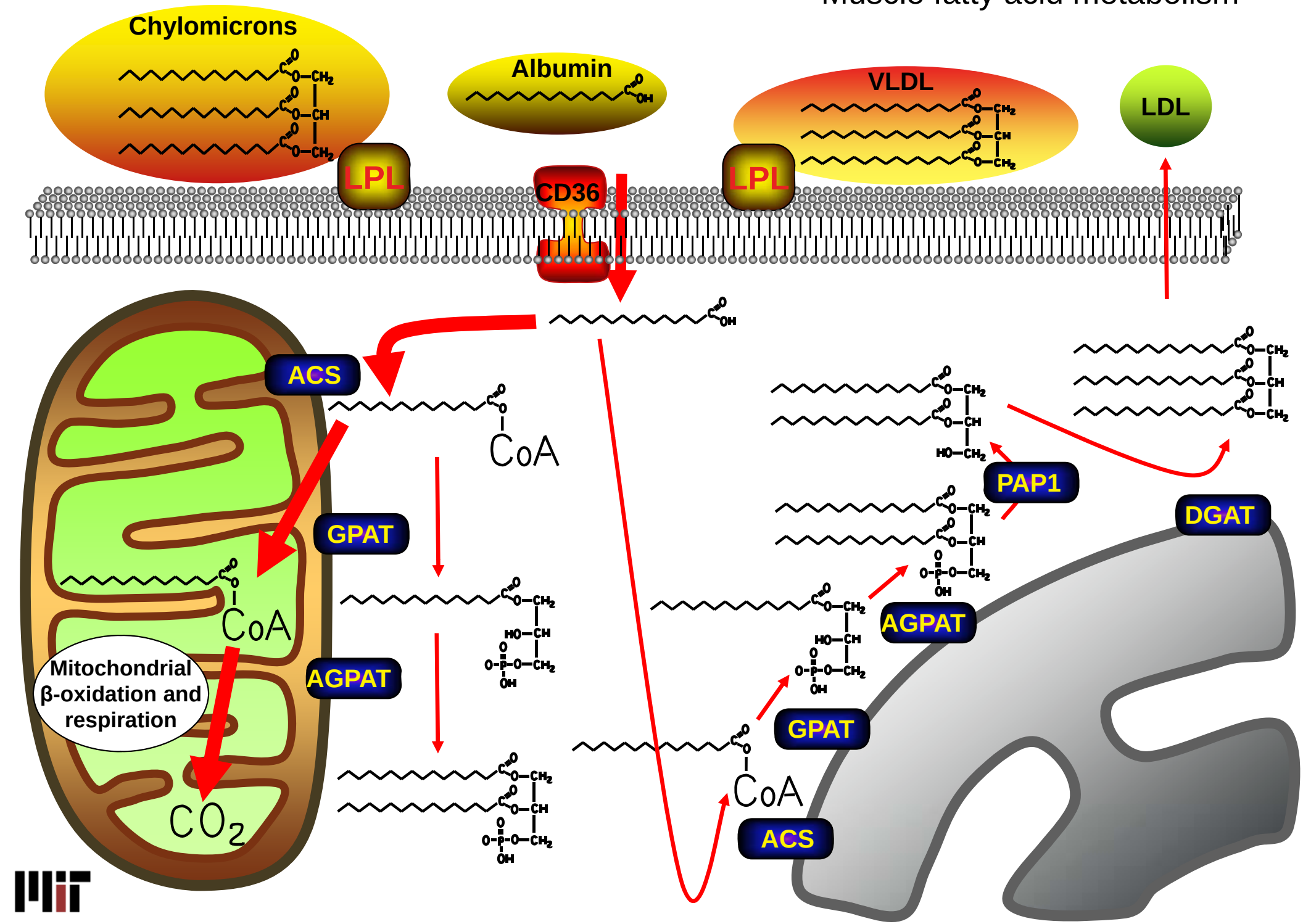
# Regulation of glucose, FA and TG metabolism in adipose tissue



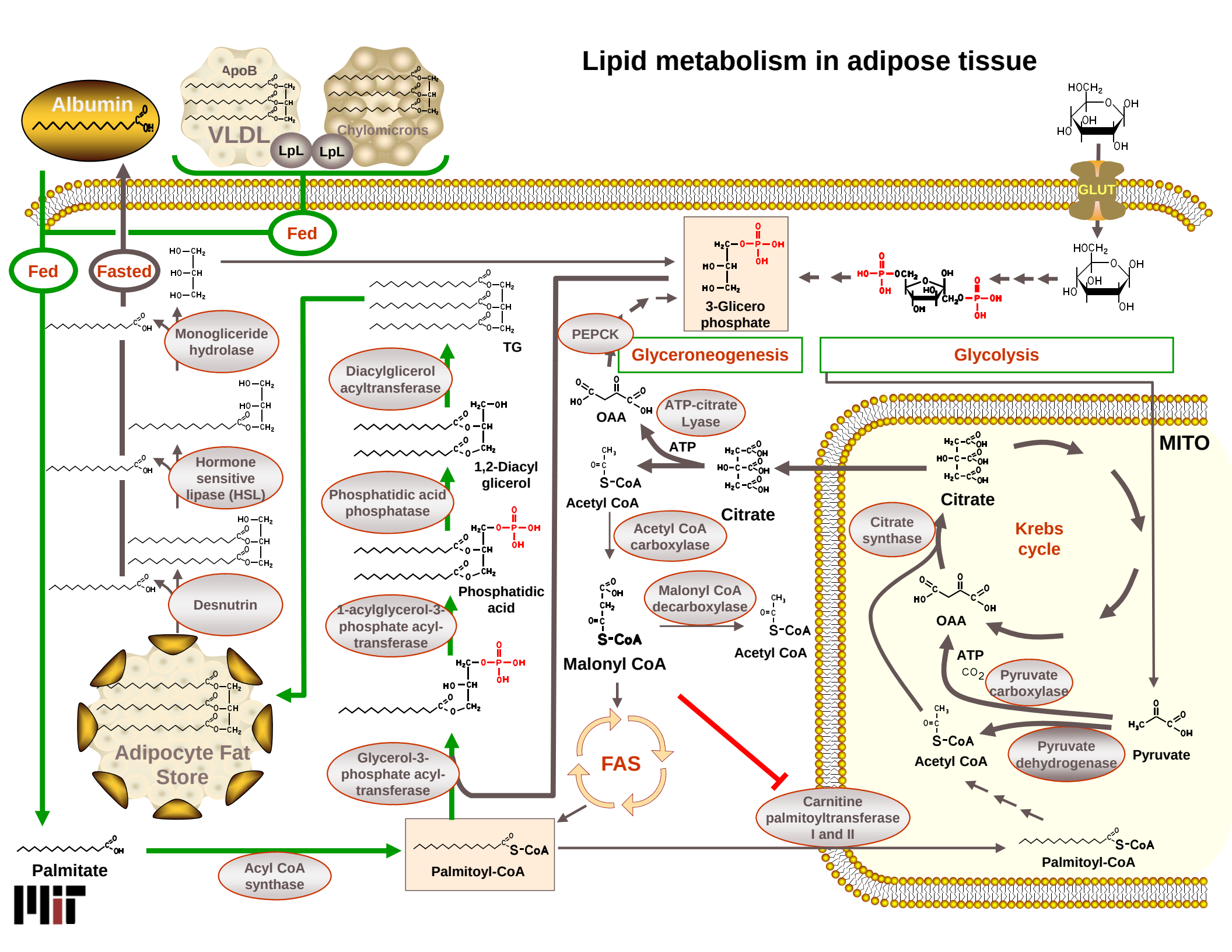
# Lipids circulation and usage in fasted state



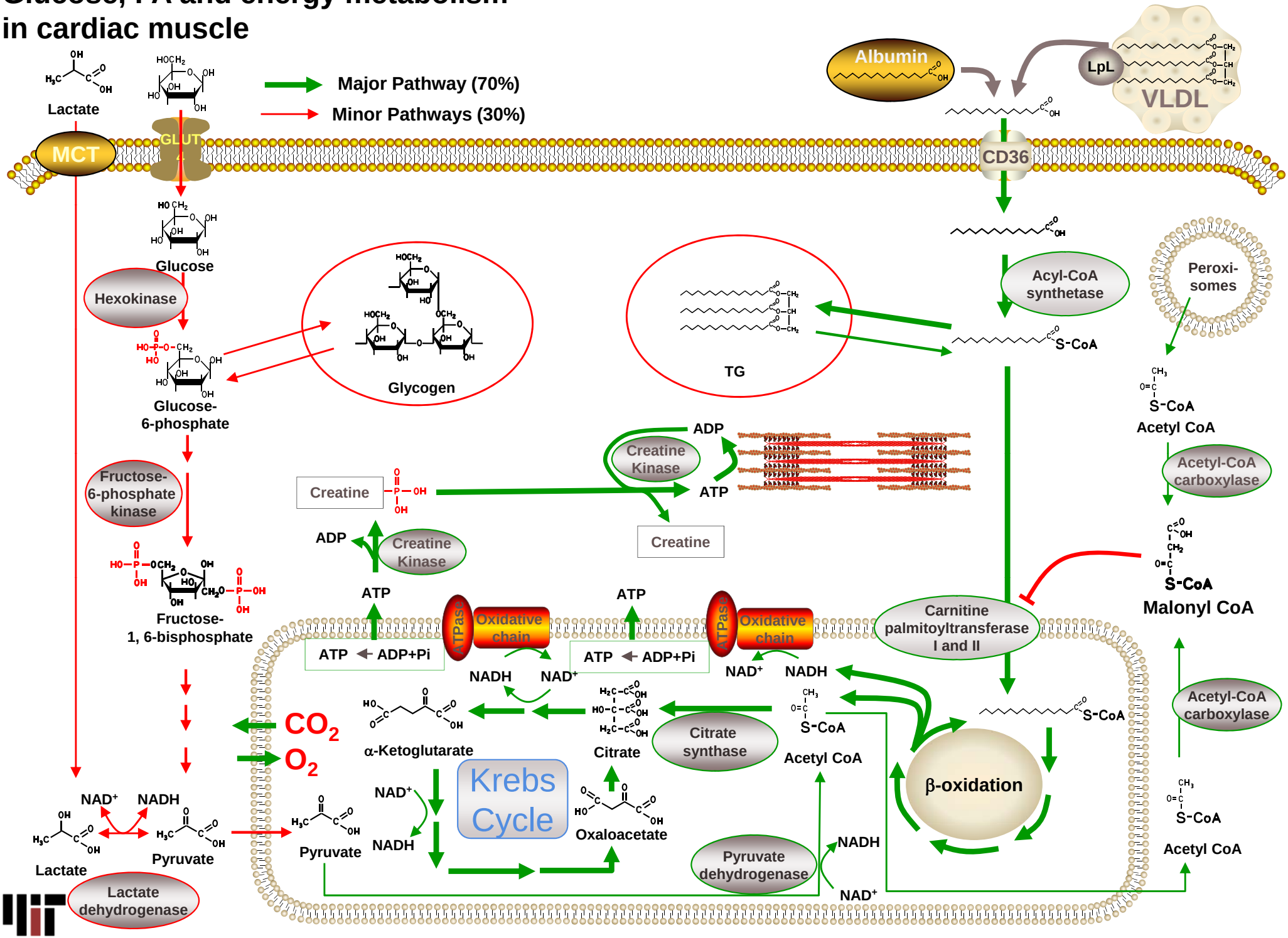
# Muscle fatty acid metabolism



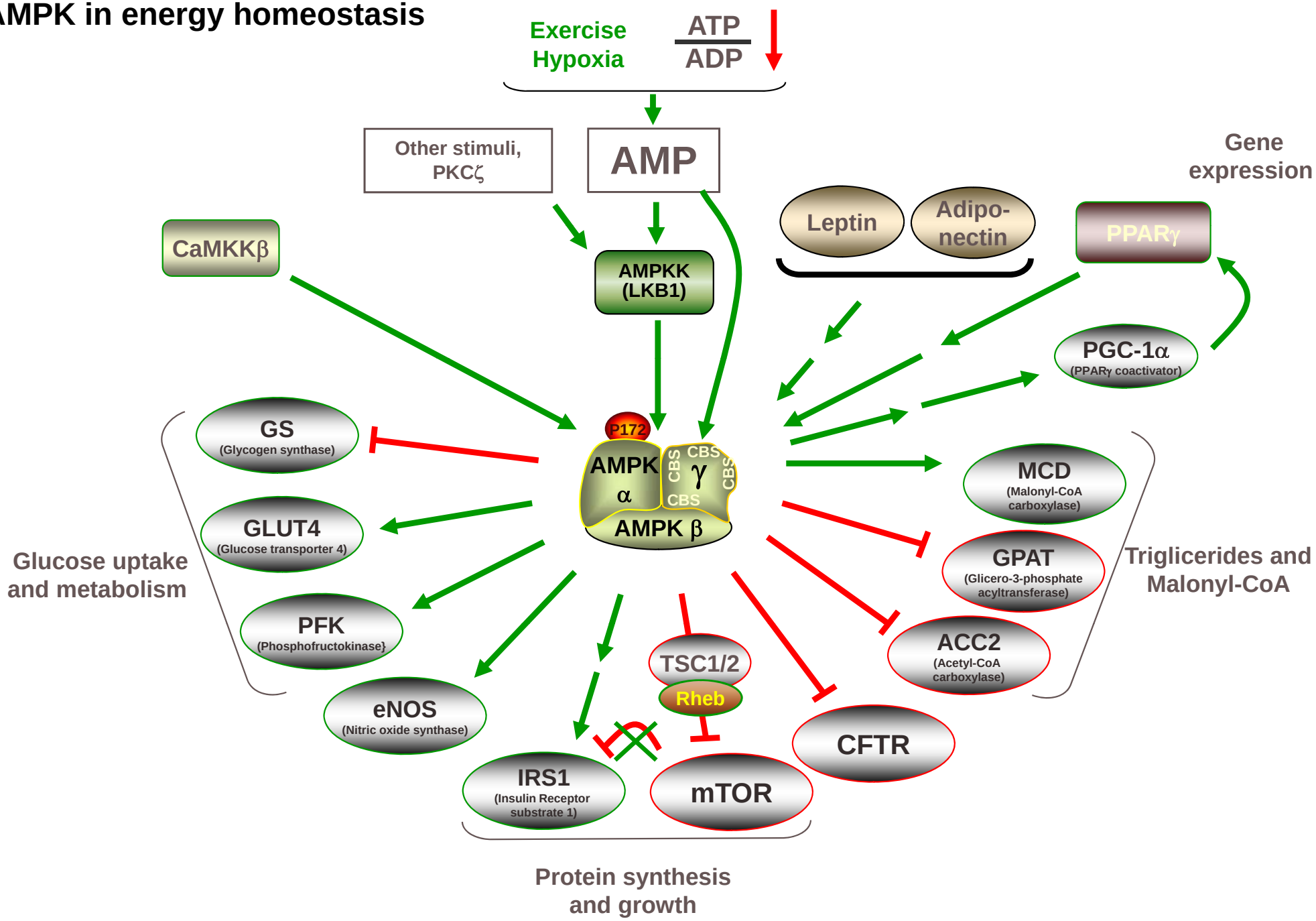
# Lipid metabolism in adipose tissue



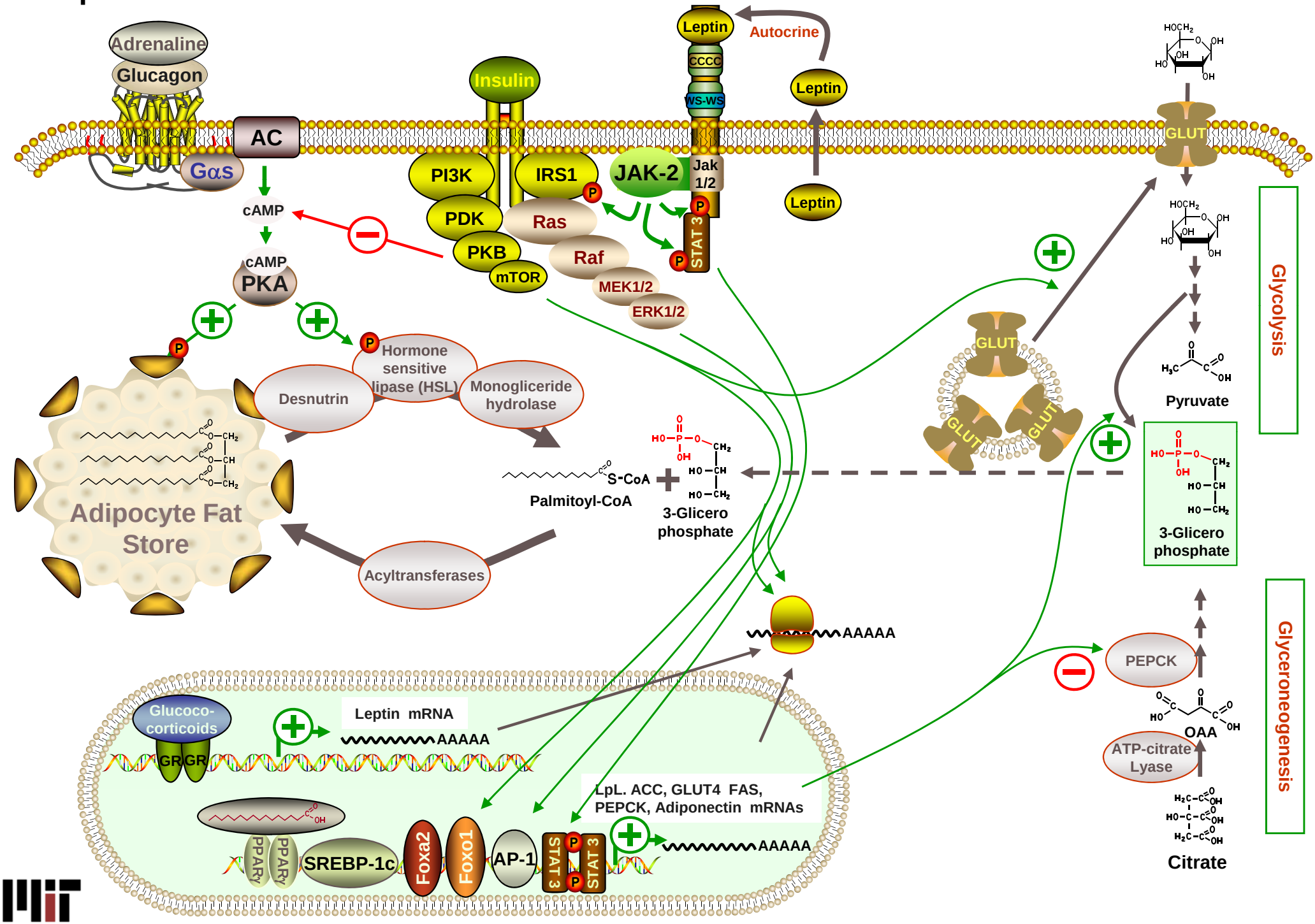
# Glucose, FA and energy metabolism in cardiac muscle



# AMPK in energy homeostasis

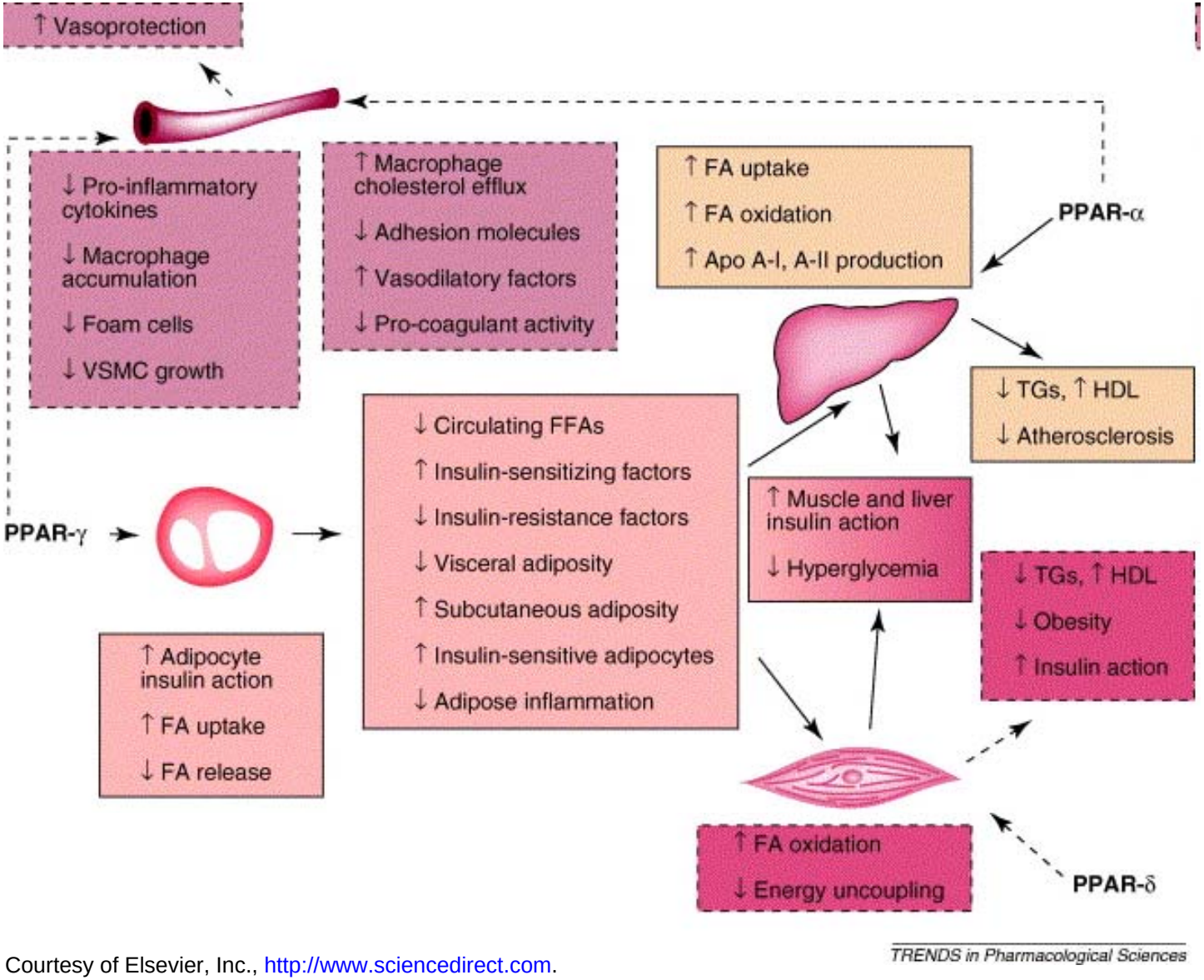


# Regulation of glucose, FA and TG metabolism in adipose tissue



# Inflammation links classic risk factors to altered cellular behavior within the arterial wall and secretion of inflammatory markers in the circulation

Figure removed due to copyright restrictions.
See Figure 4 from Packard, Rene R.S. and Peter Libby. "Inflammation in Atherosclerosis: From Vascular Biology to Biomarker Discovery and Risk Prediction." *Clinical Chemistry* 54 (2008).



20.380J / 5.22J Biological Engineering Design

Spring 2010