Cardiovascular Disease and Inflammation: What Can HDL Do About It?

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Cardiovascular and Pulmonary Branch
National Heart, Lung and Blood Institute
National Institutes of Health

Conflicts of Interests: Research grants with MedImmune, Shire, Corvidia, and Liposcience

Overview

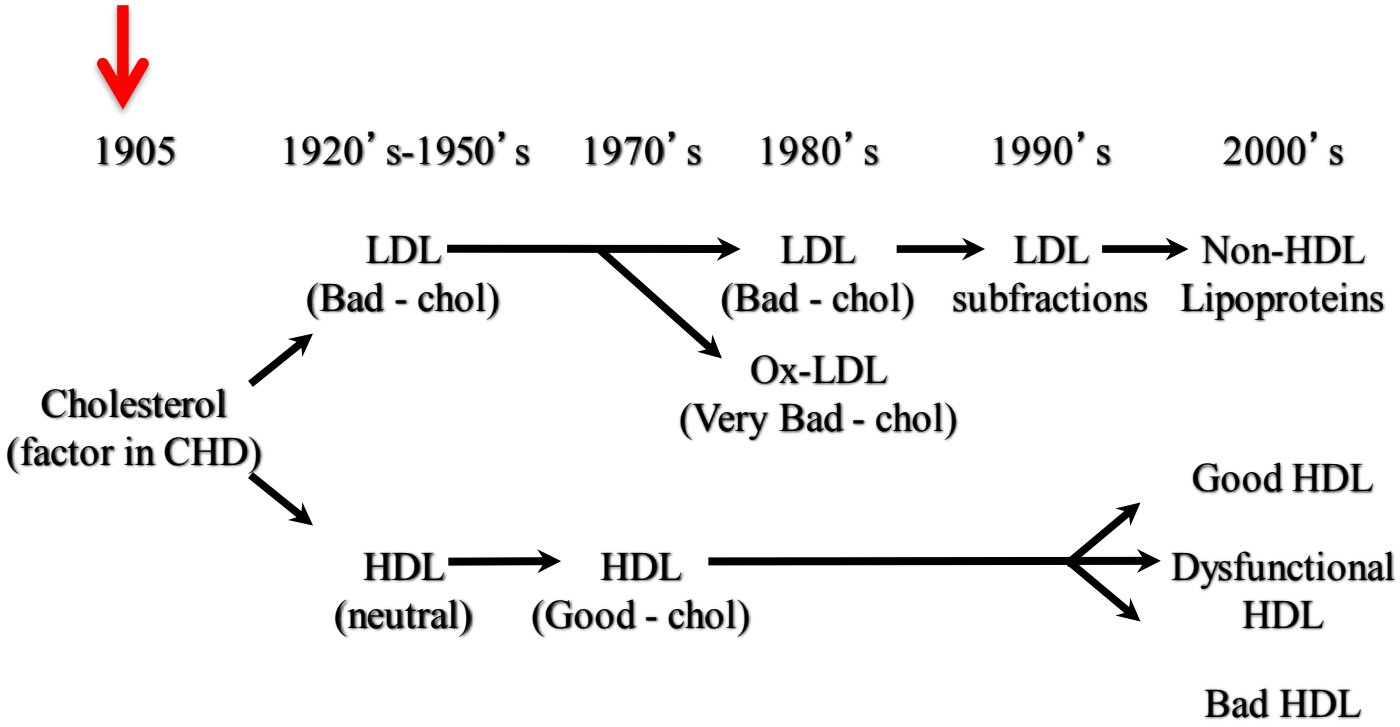
- History of Cholesterol Research
- Lipoprotein Metabolism
- Pathogenesis of Atherosclerosis
- HDL and Inflammation

History of Cardiovascular Disease Research

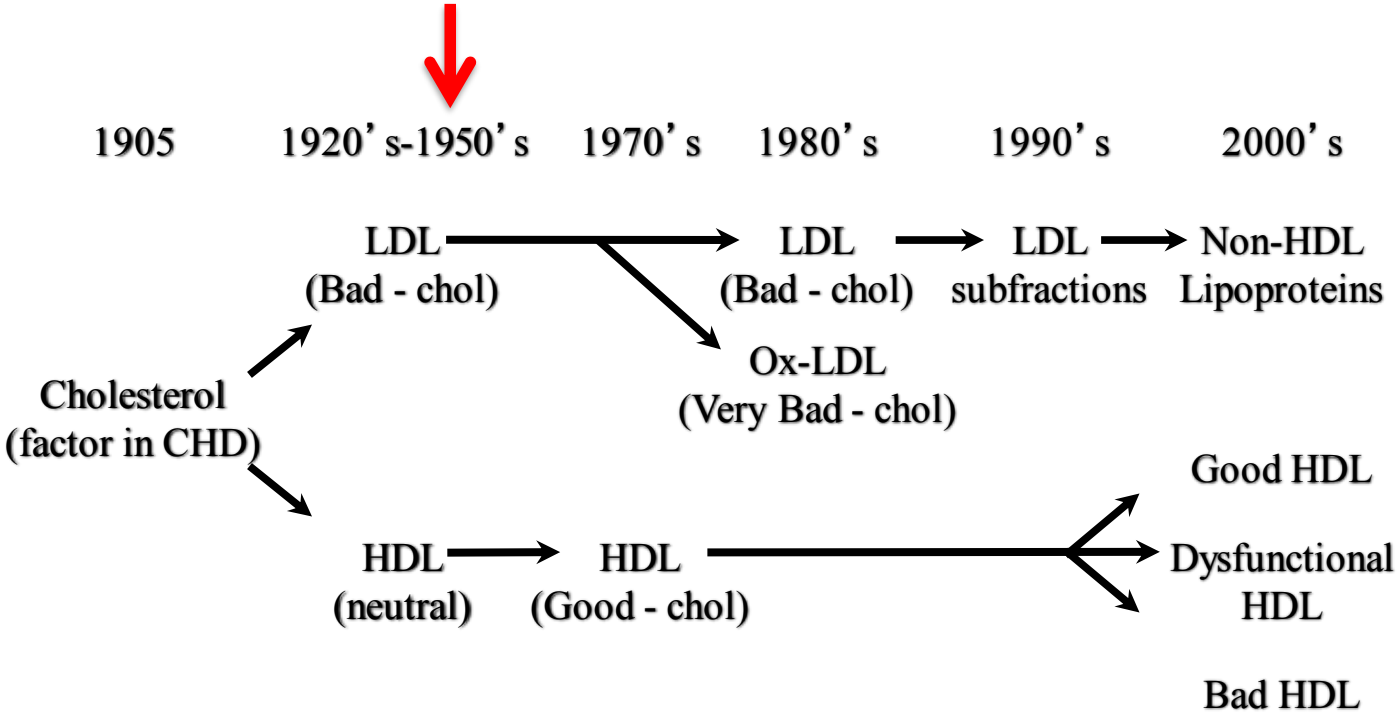
Rudolf Virchow
1821-1902

H. Anitchkov
1885-1964

Evolution of Cholesterol as a CHD Risk Biomarker



Evolution of Cholesterol as a CHD Risk Biomarker



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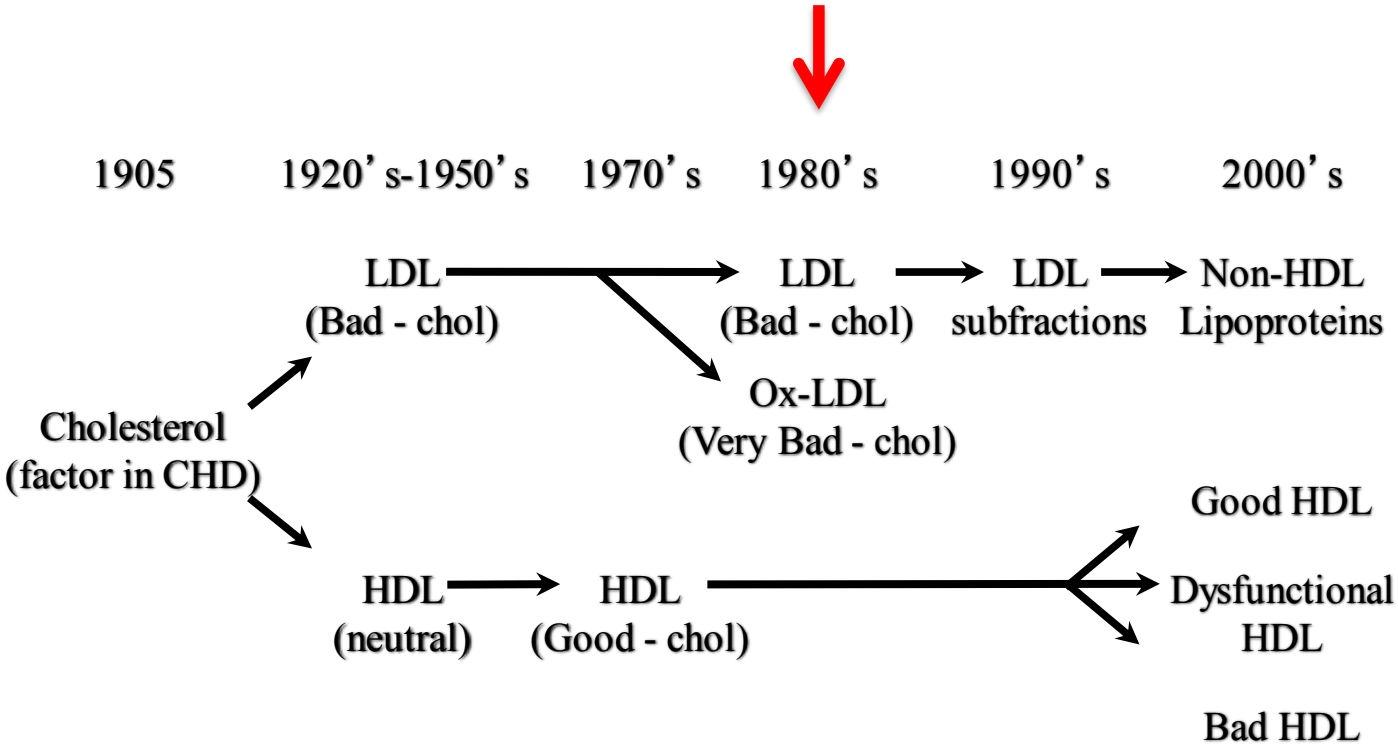
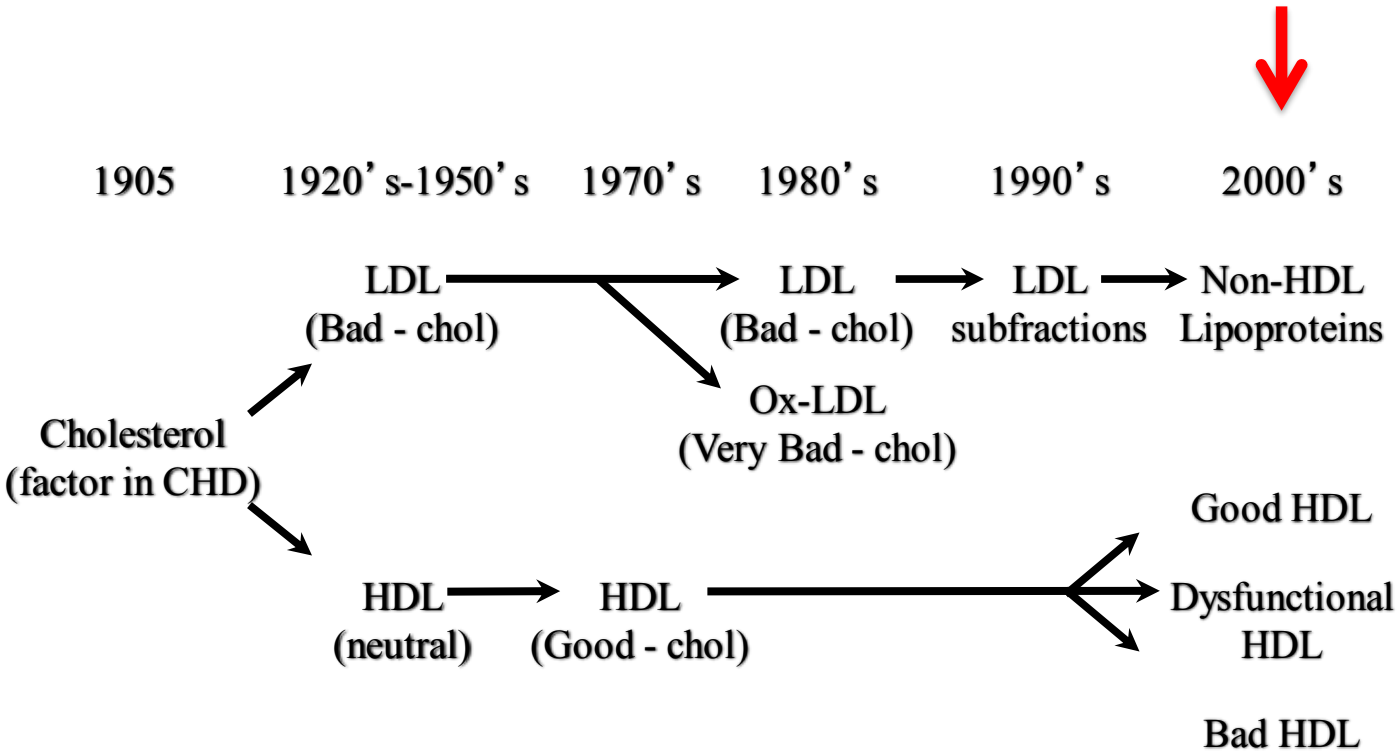


Evolution of Cholesterol as a CHD Risk Biomarker



Early Lipoprotein Research at the NIH

Circa mid 1960s

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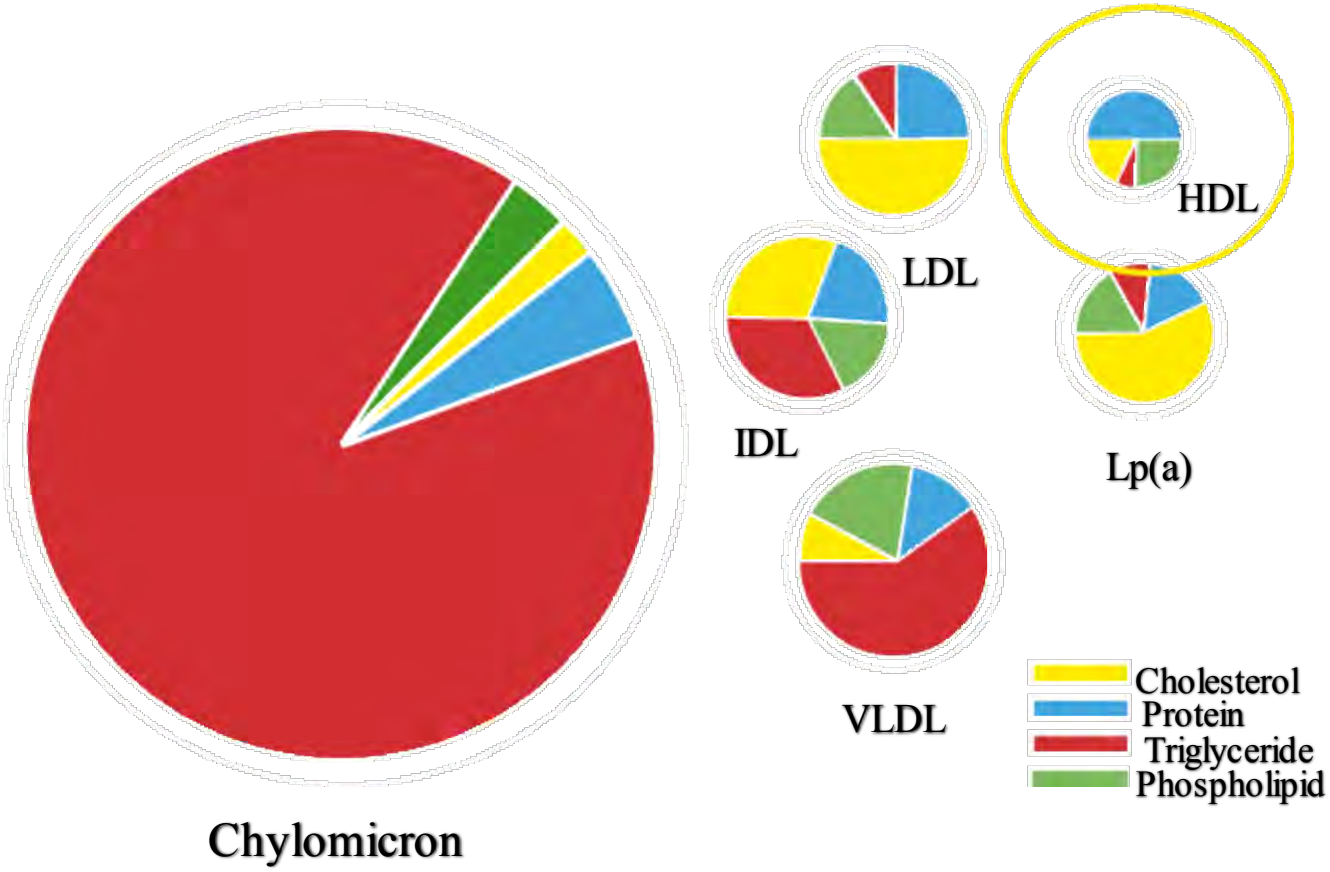
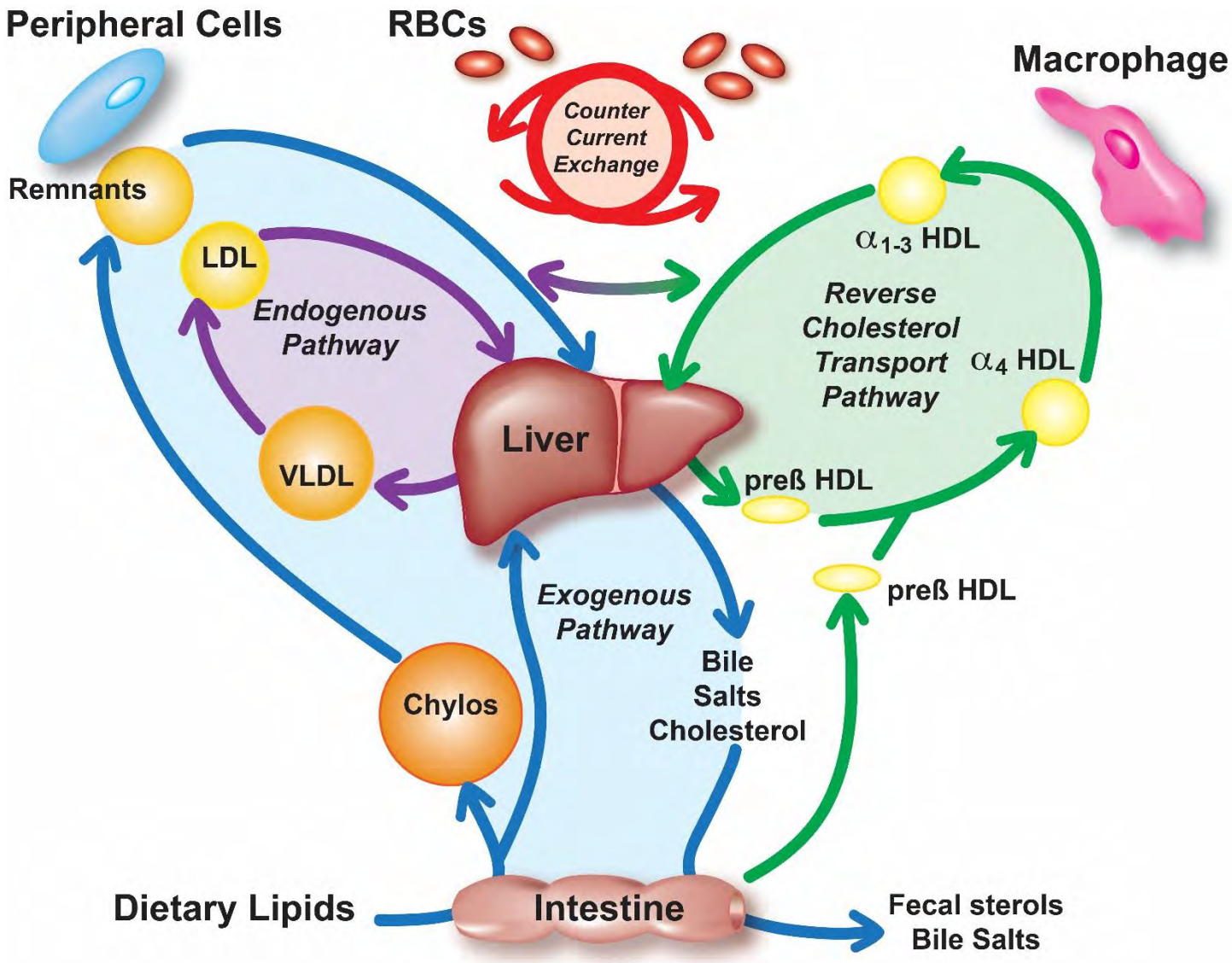
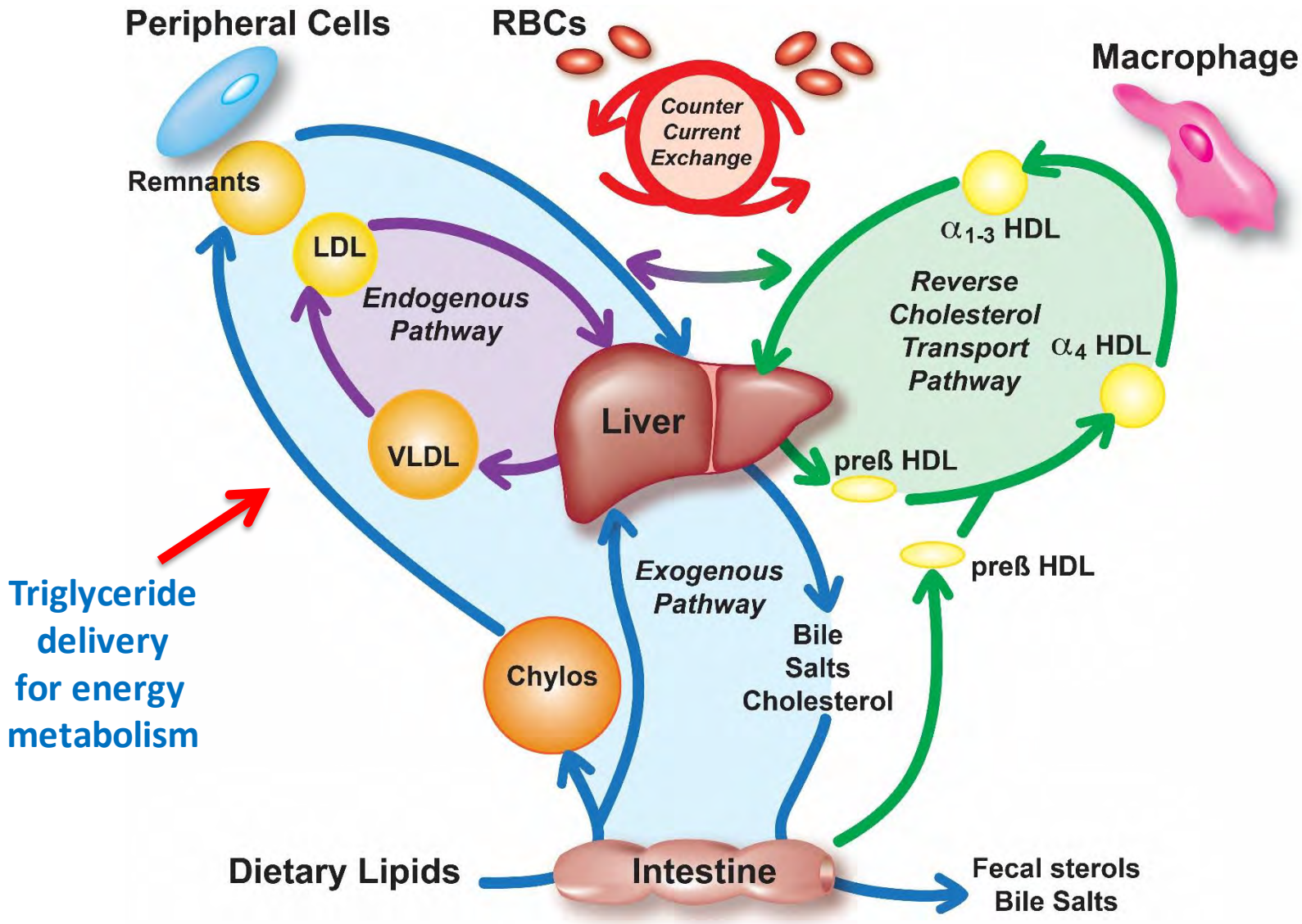
Major Classes of Lipoproteins



Lipoprotein Metabolism Pathways



Lipoprotein Metabolism Pathways



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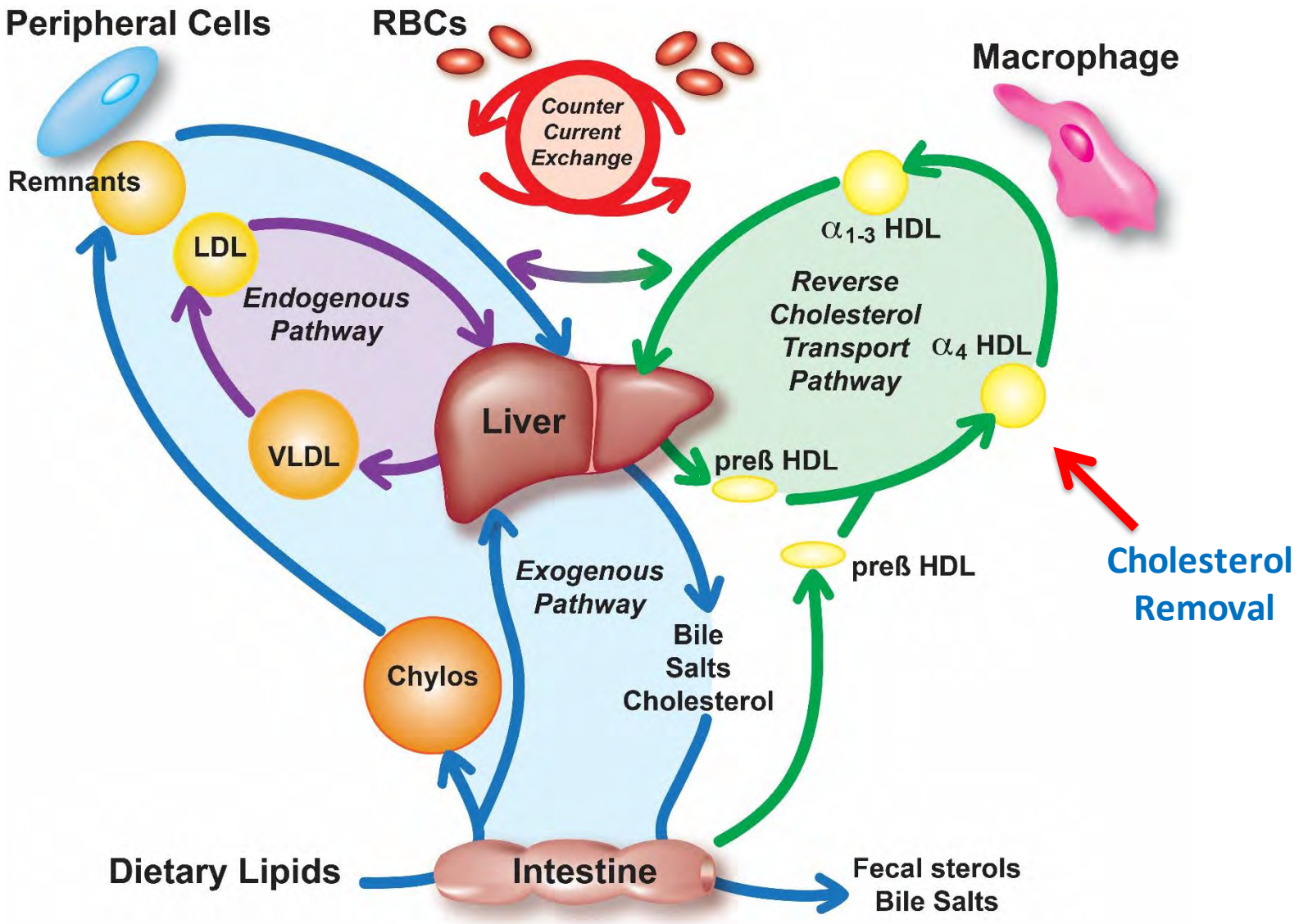
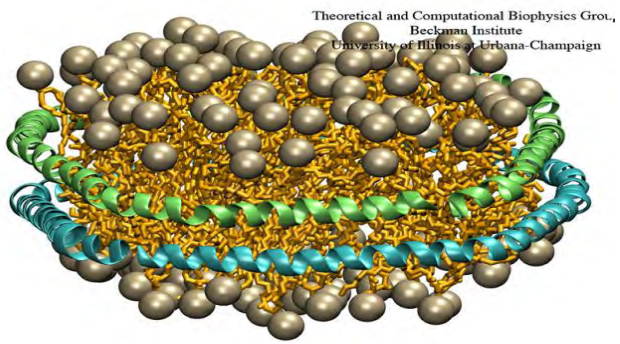
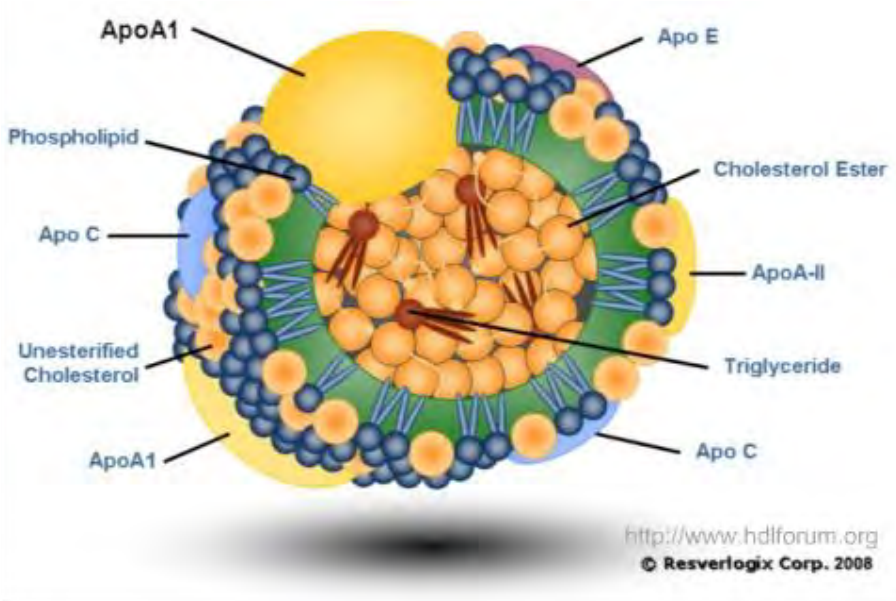


HDL Structure and Composition

Pre-beta Discoidal Shaped HDL



Alpha Spherical Shaped HDL

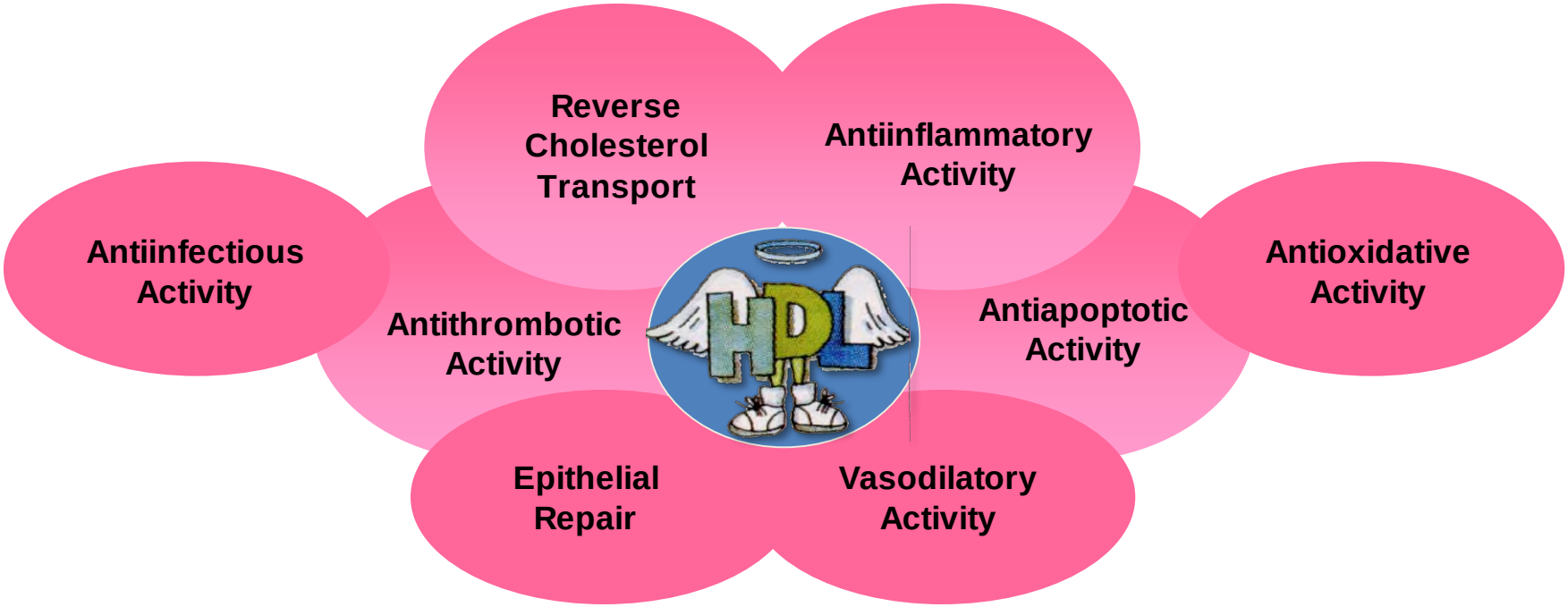


Initial Cholesterol Acceptor

Delivers Cholesterol to Liver

HDL transports over 80 different proteins and over 200 species of lipids.

Pleiotropic Anti-Atherogenic Effects of HDL



Curr Med Res Opin 2004:20:1253-1268
Annu Rev Med 2003:53:321-341

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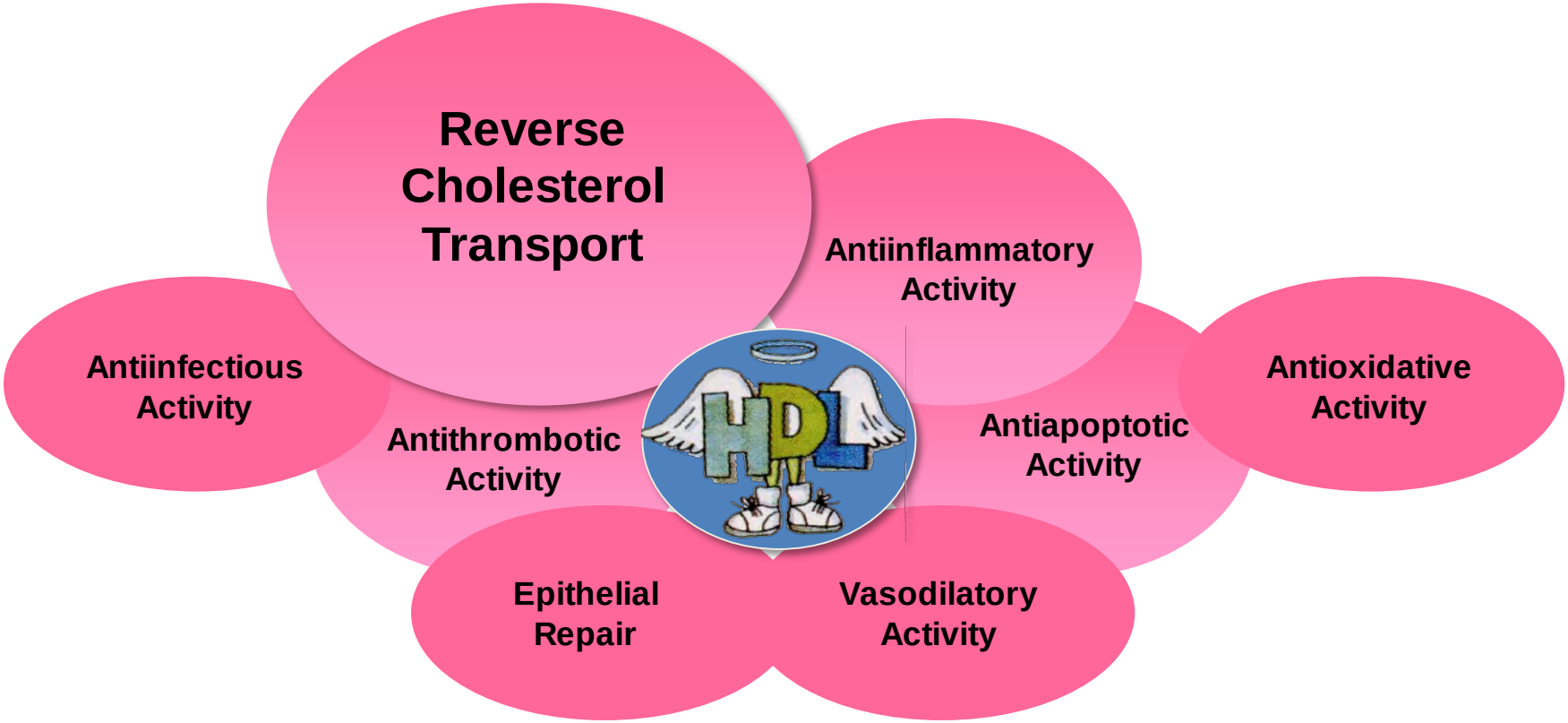

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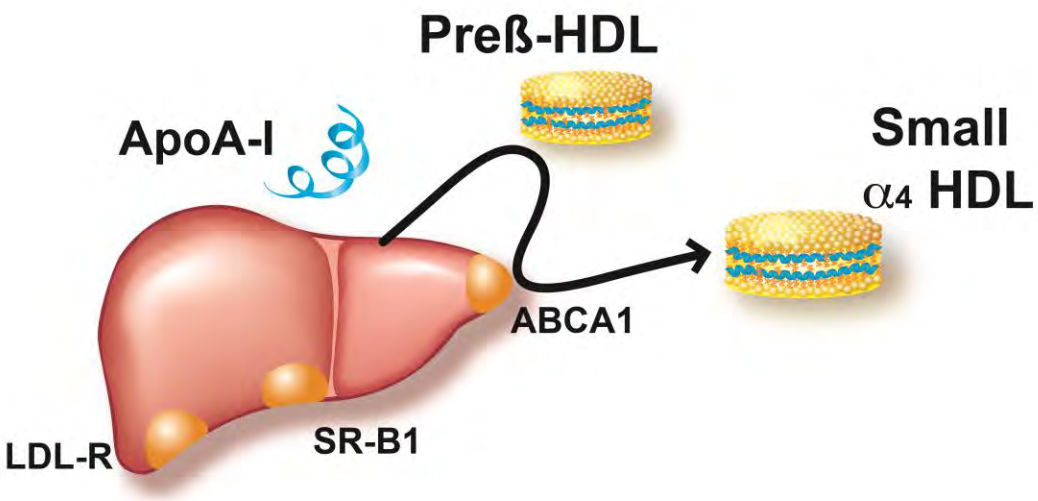
Reverse Cholesterol Transport Pathway



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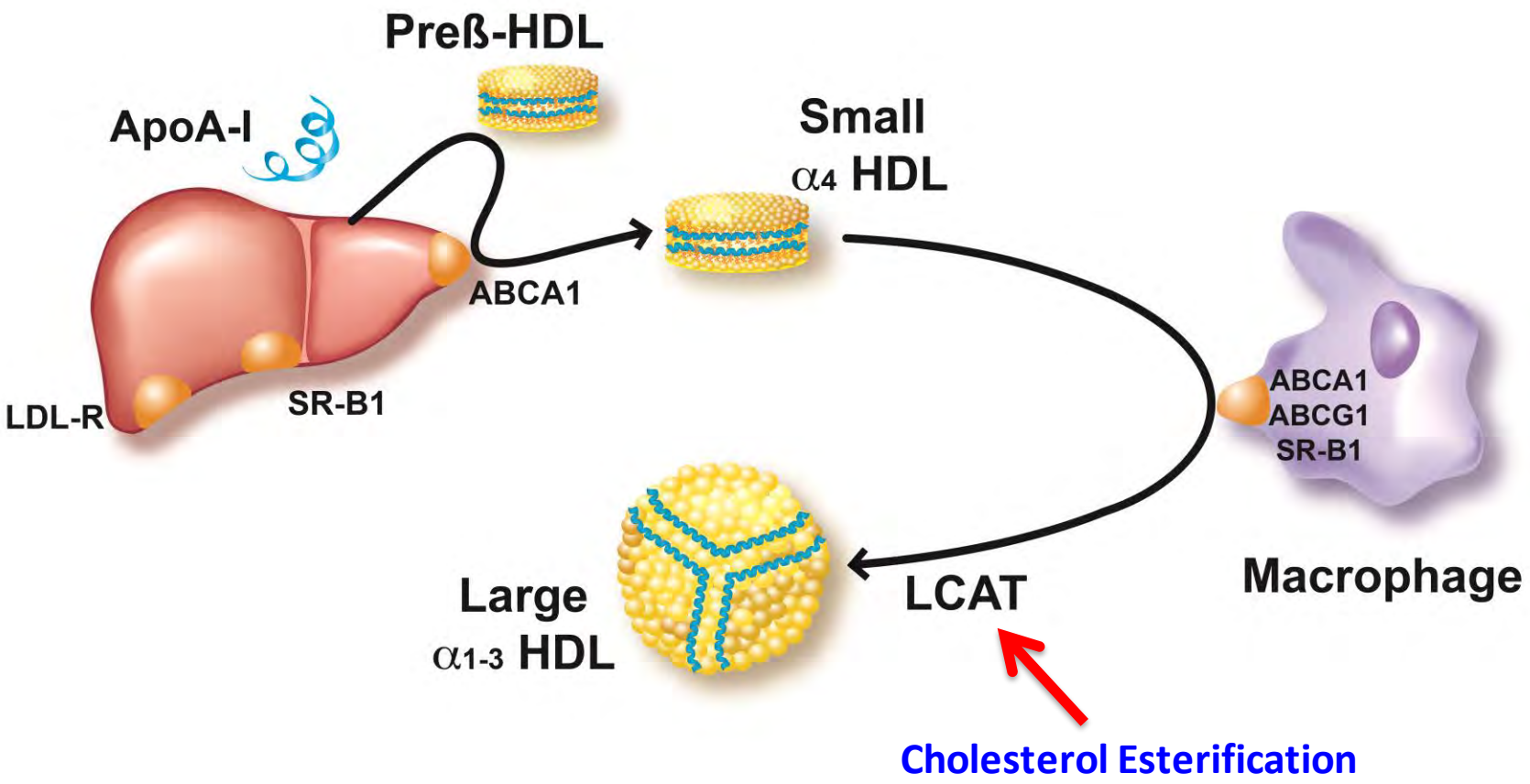

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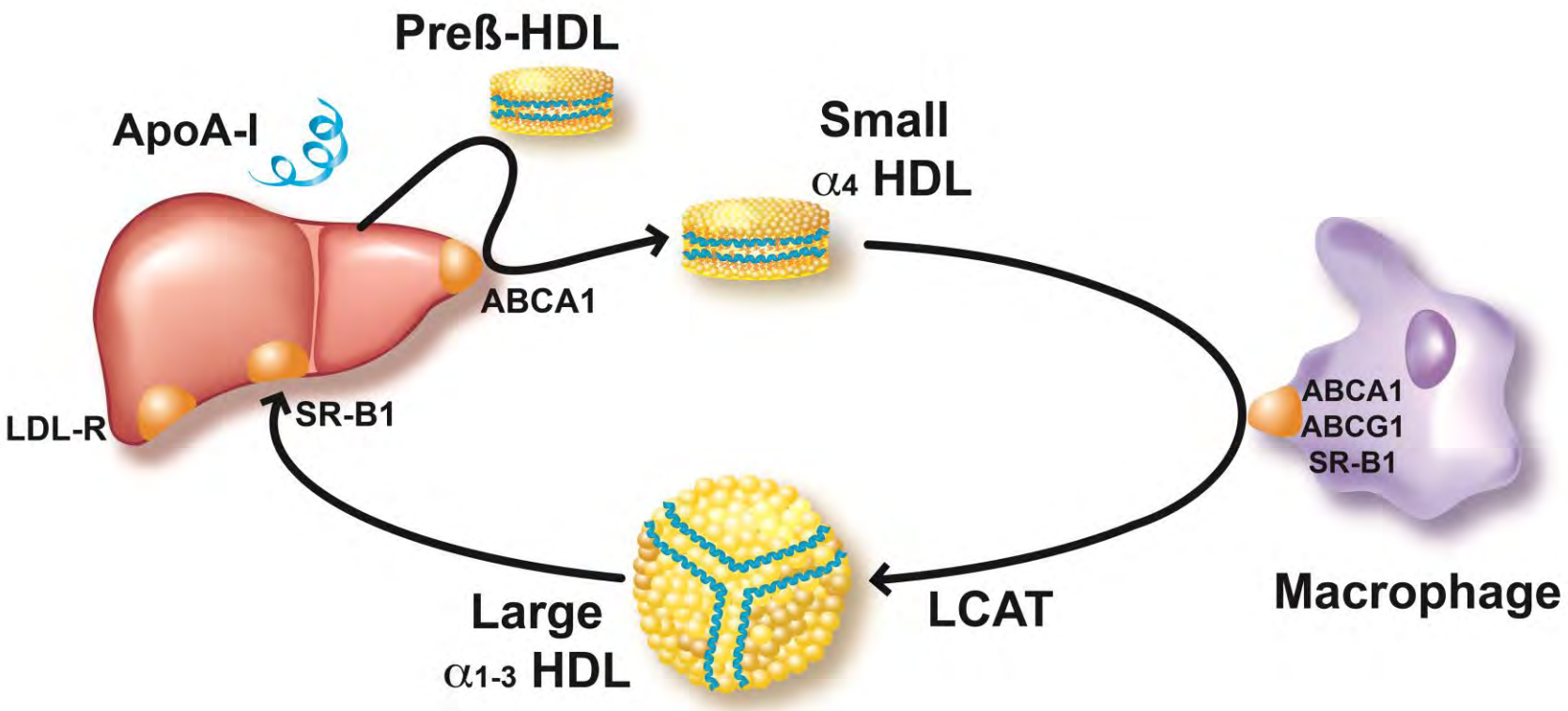

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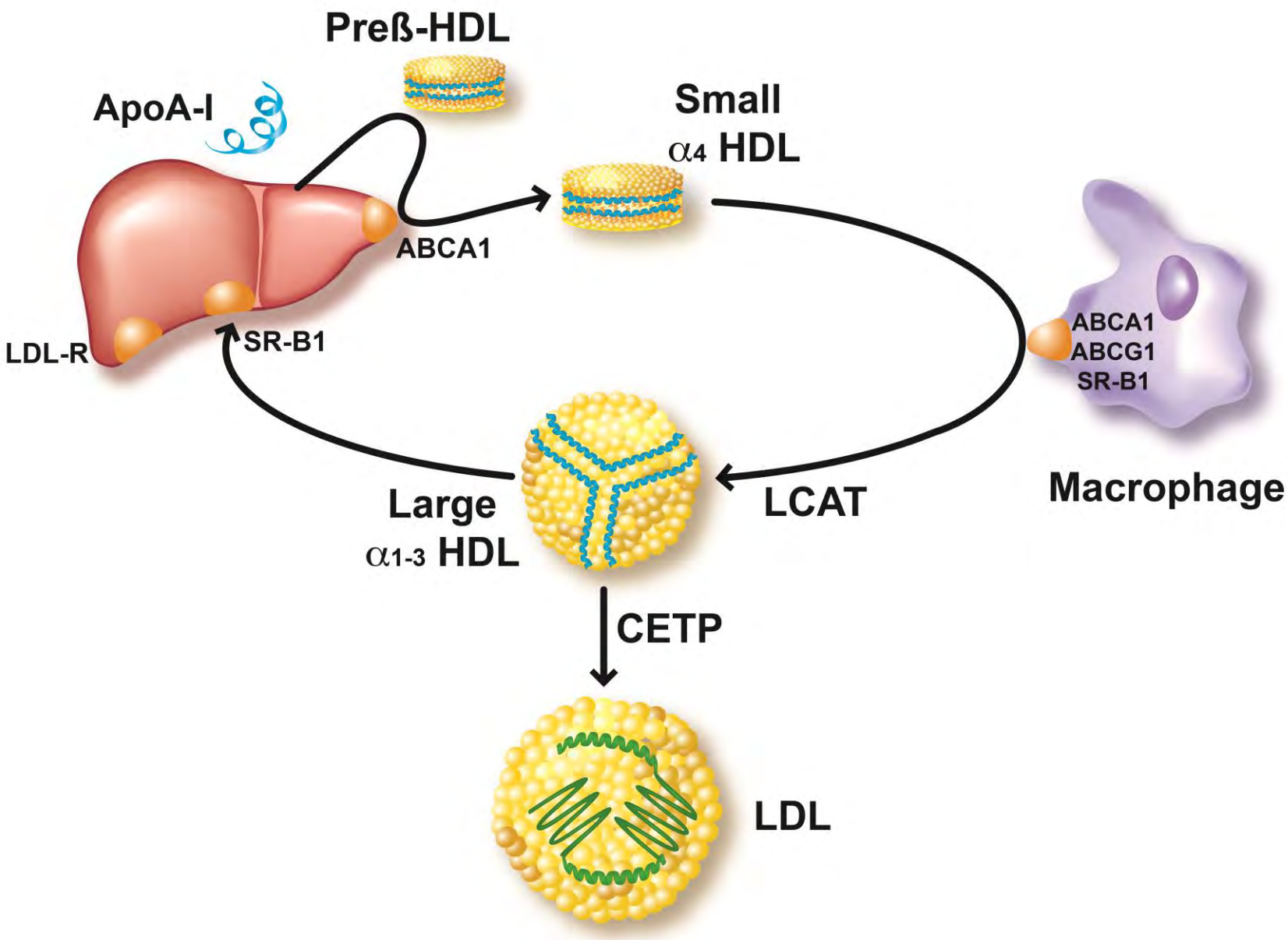

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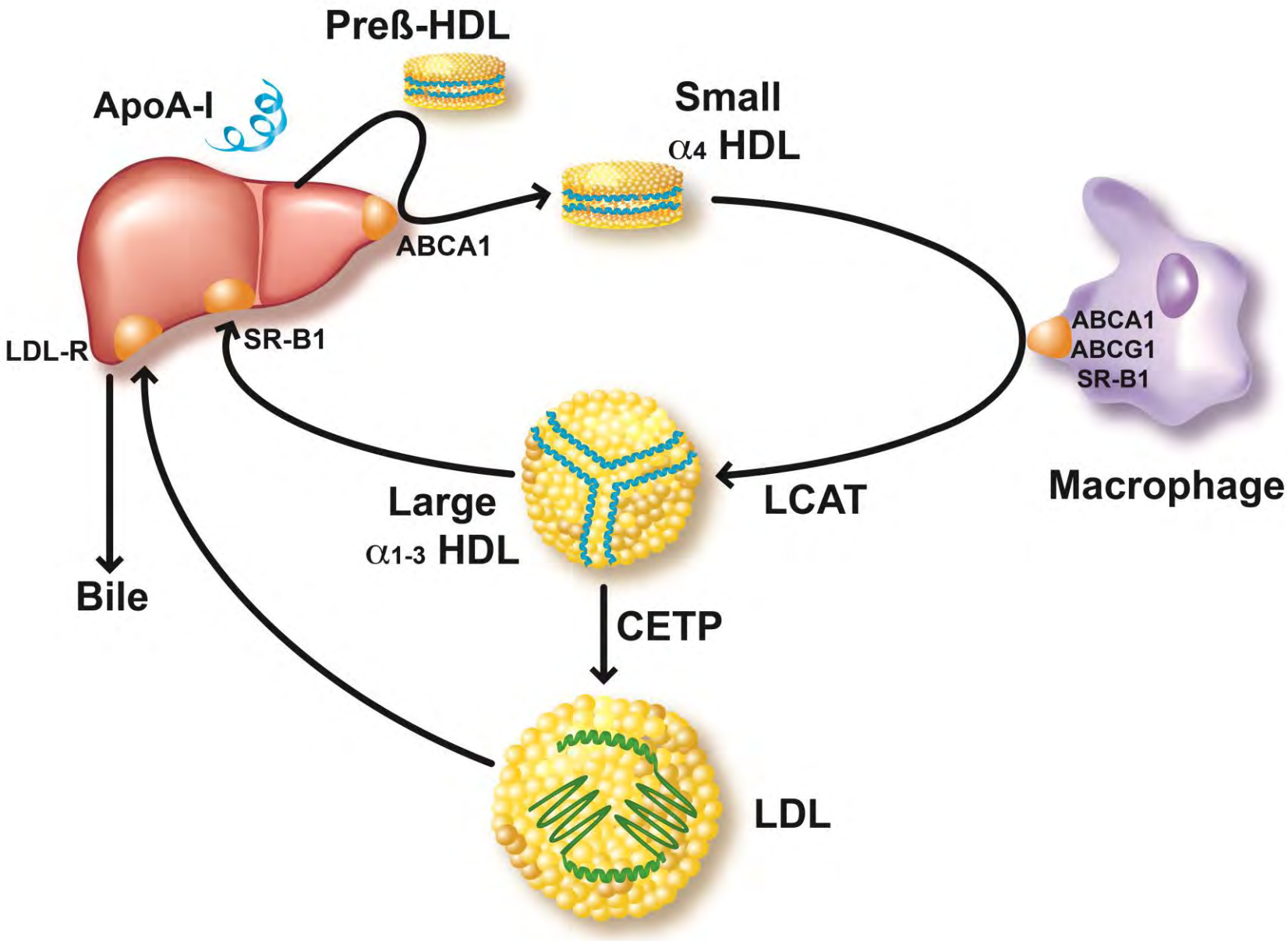
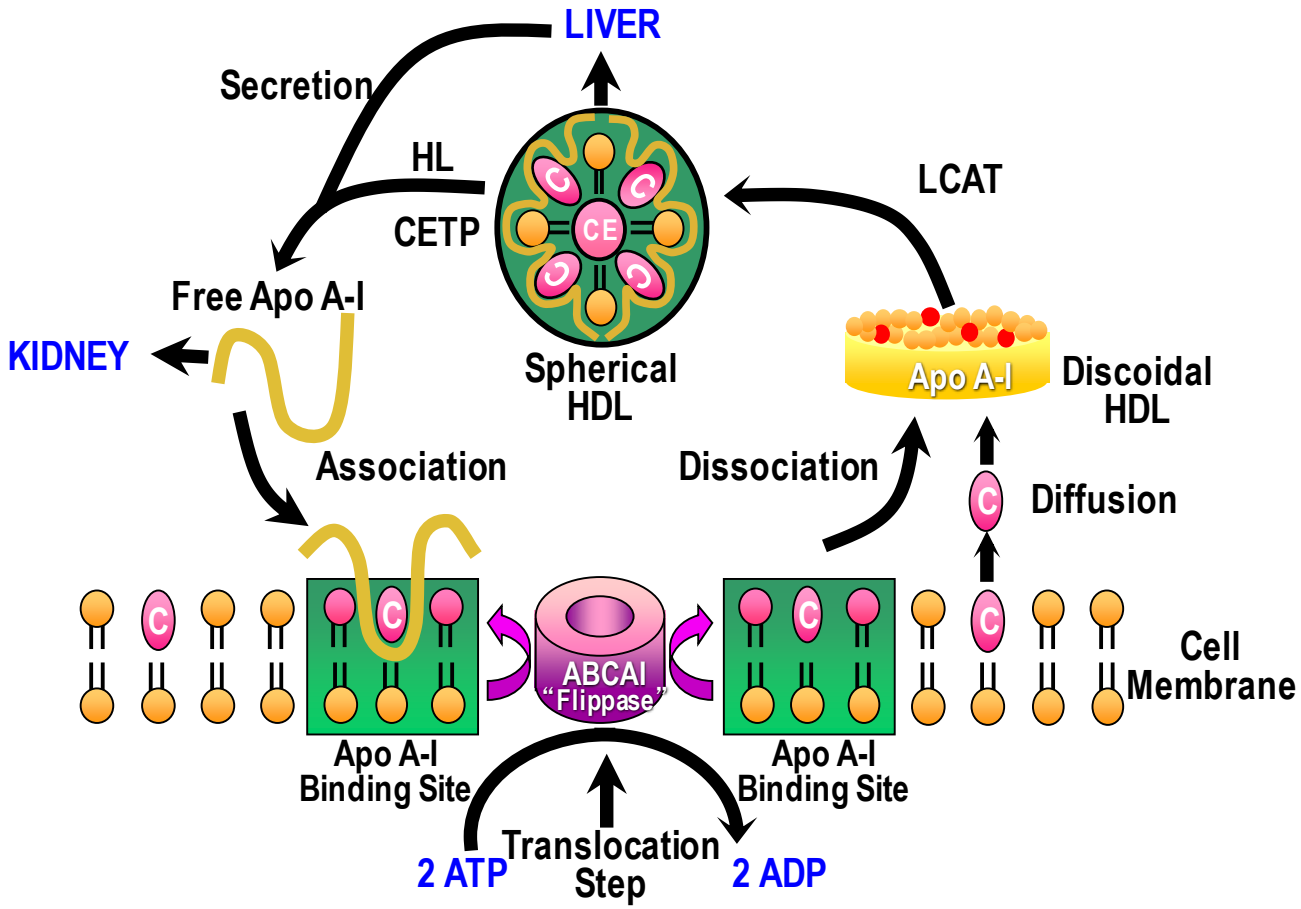


Model of Cholesterol Efflux to HDL



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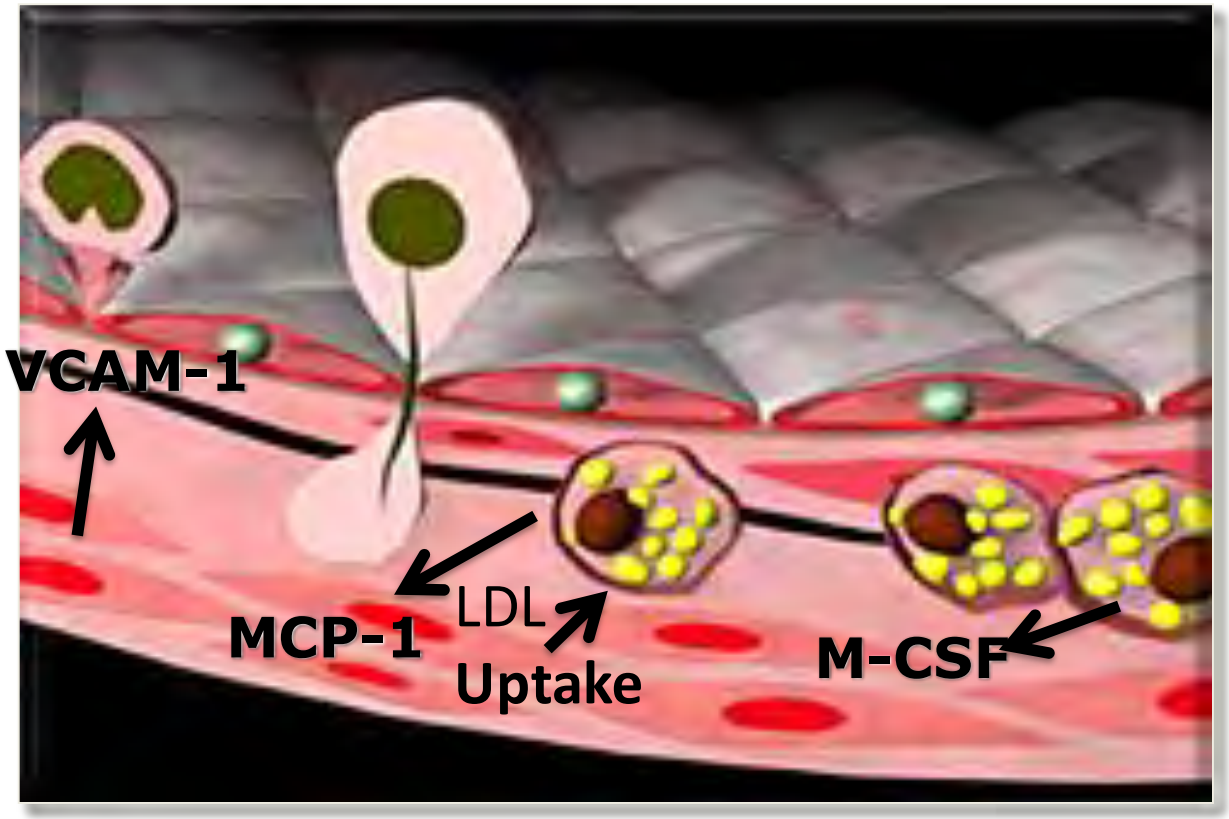
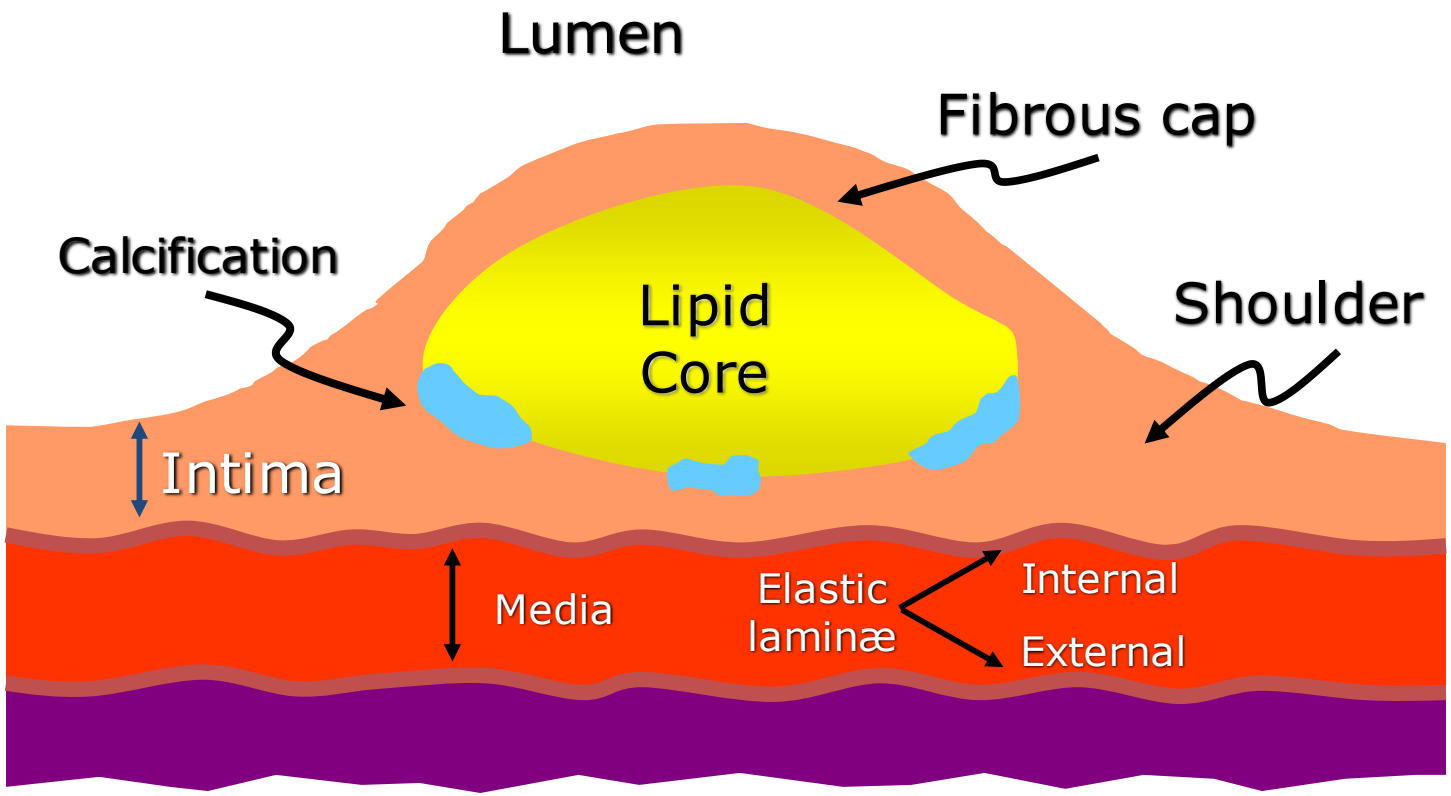
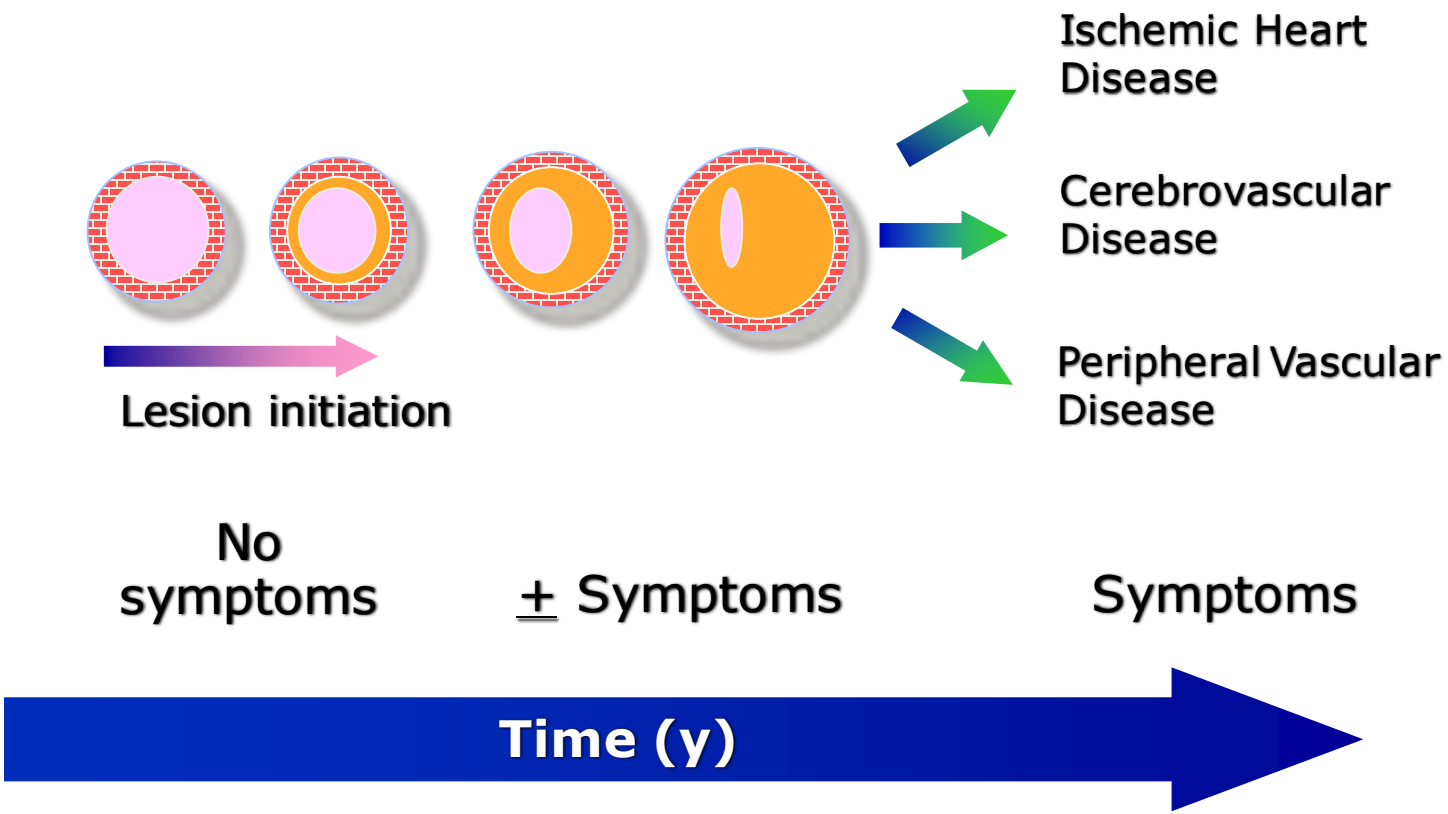
Early Inflammatory Mediators of Atherosclerosis



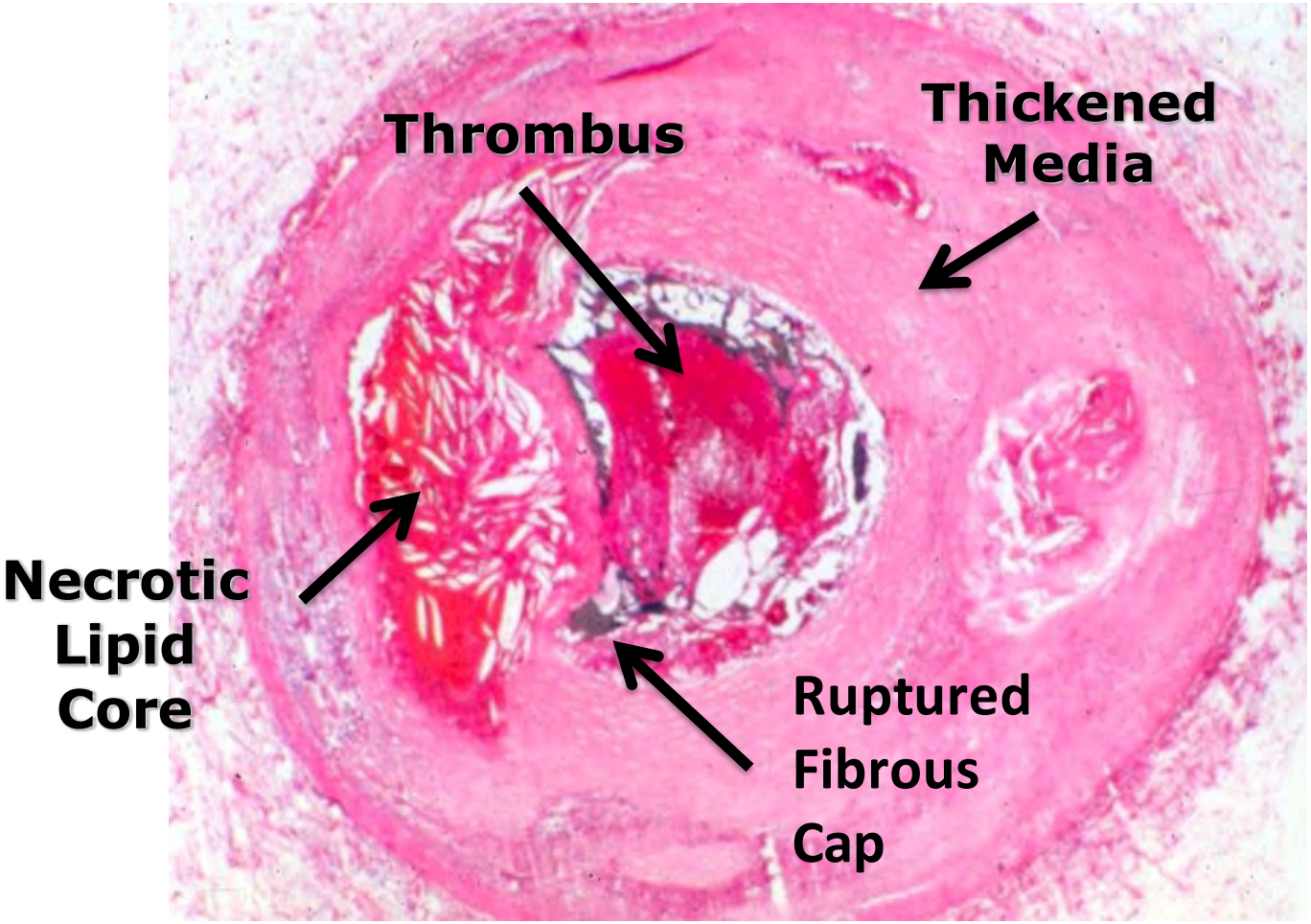
Features of a Complex Atherosclerotic Plaque



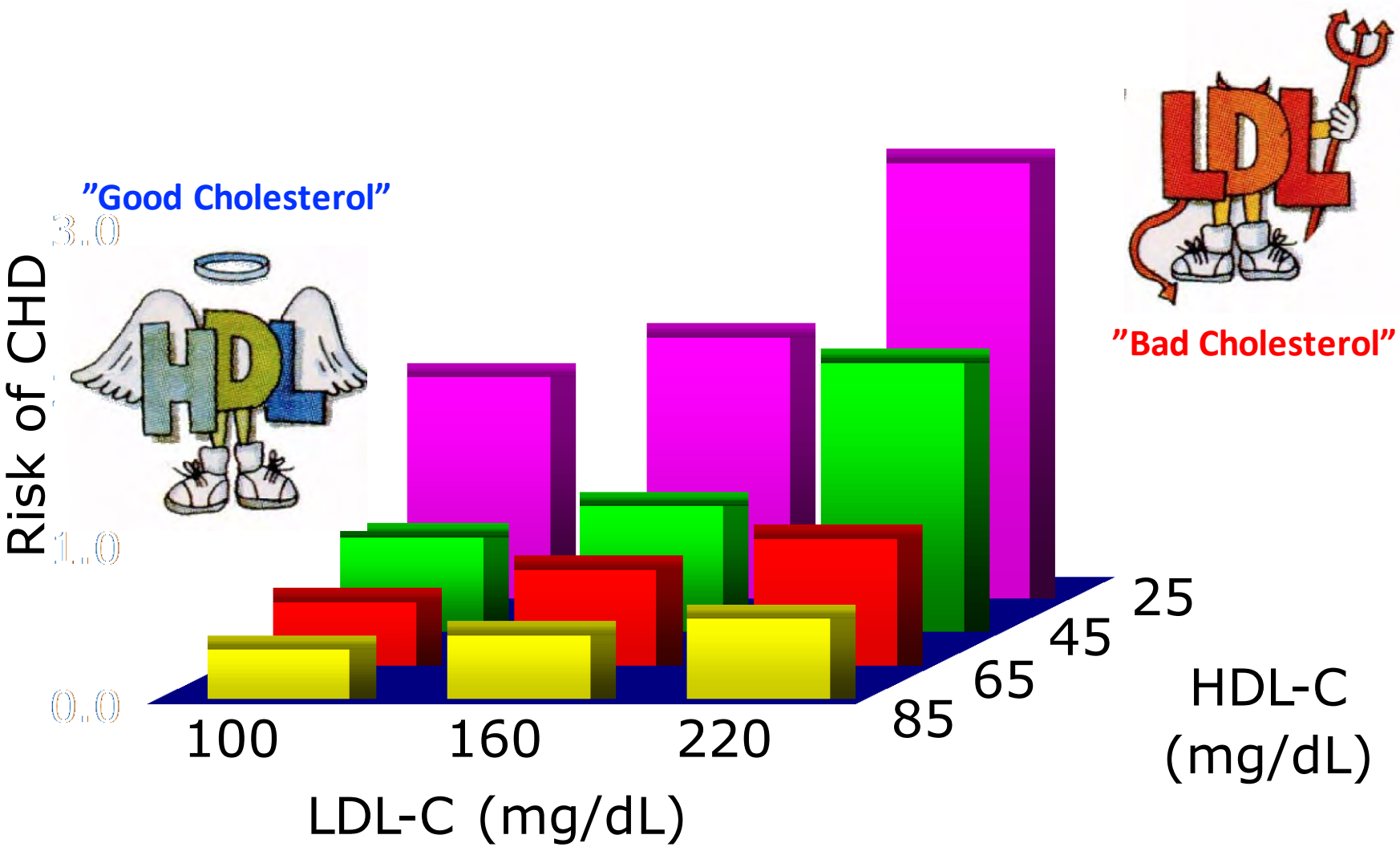
Schematic Timeline for Atherogenesis



Ruptured Atherosclerotic Plaque

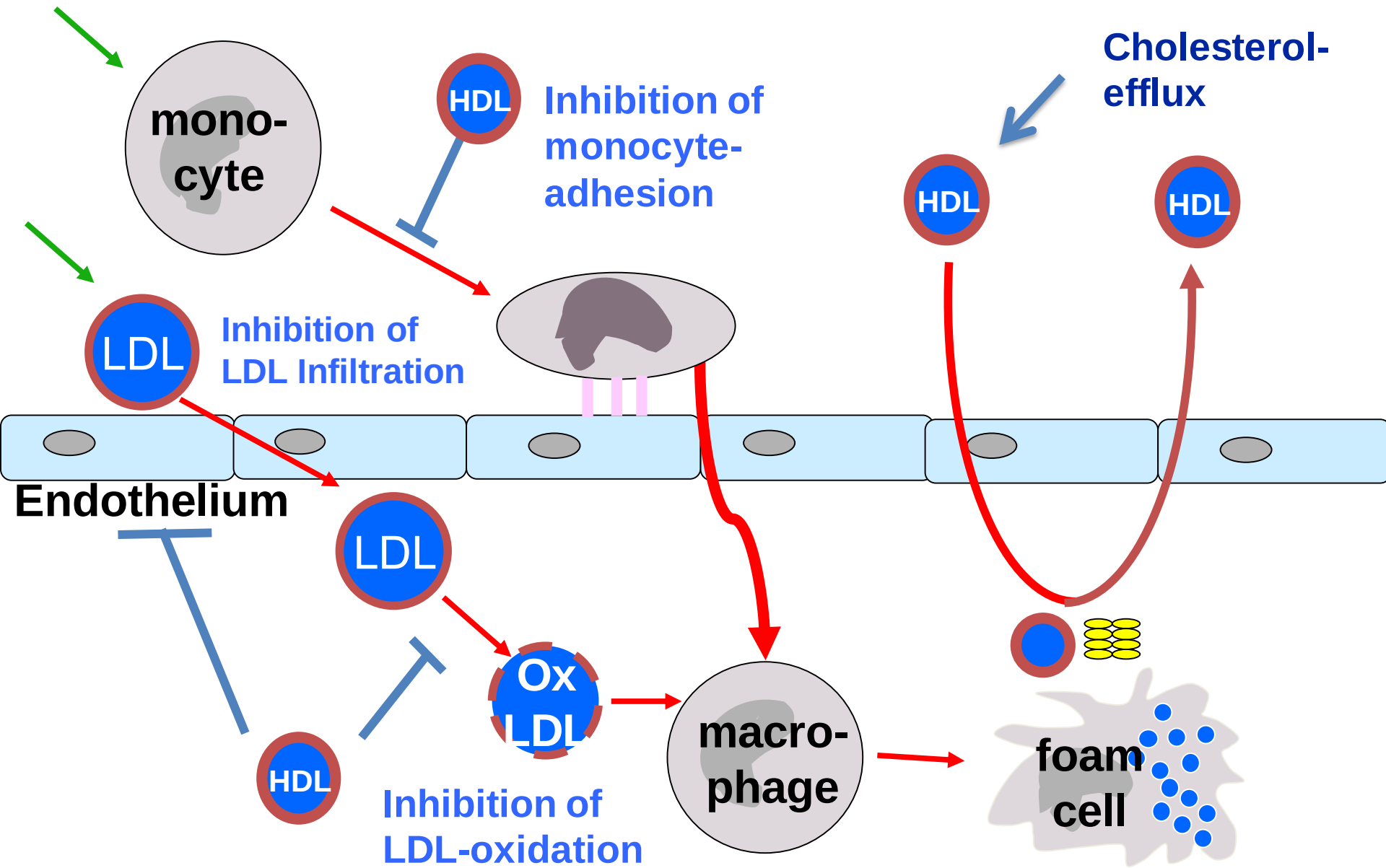


HDL-C is an Independent CVD risk factor

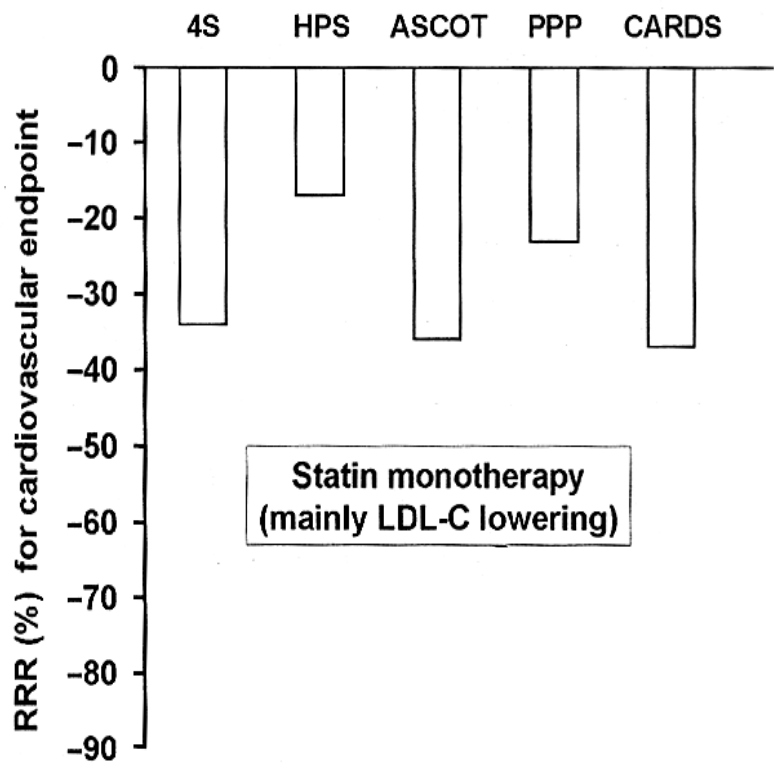


Gordon T et al. Am J Med 1977;62:707-714.

Multiple Anti-atherogenic Effects of HDL



Modulation of LDL-C versus HDL-C for CHD Risk Reduction



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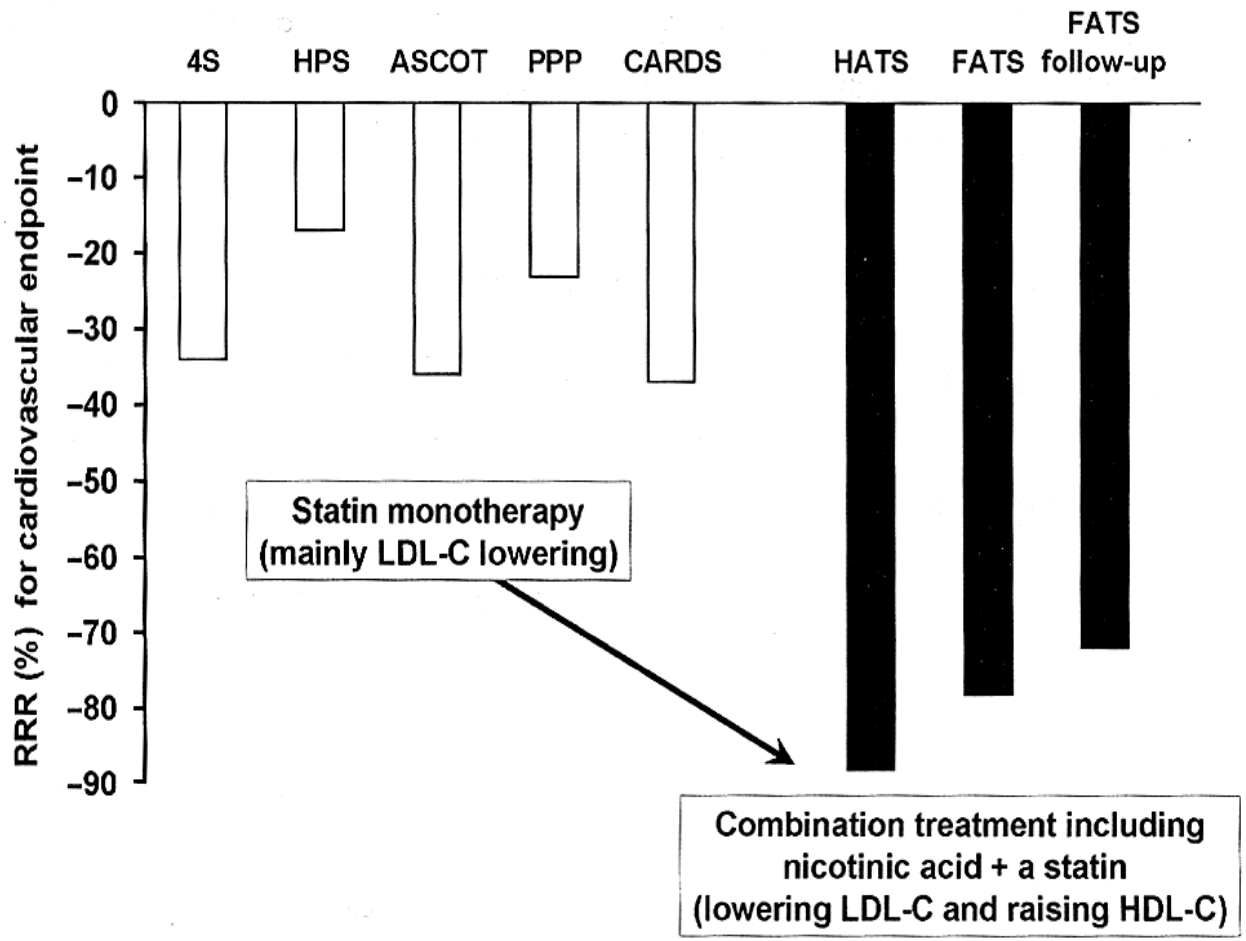


First Major Crack in HDL Hypothesis

Torcetrapib

Outfront

HEARTBREAK

Good Cholesterol Goes Bad

The drug industry makes $35 billion selling cholesterol-lowering drugs. But its attempts to come up with an encore have failed.

By Matthew Herper and Robert Langreth

DRUGS THAT LOWER CHOLESTEROL, like Lipitor, Zocor and Crestor, have long been the pharma industry's biggest moneymaker, generating cumulative sales well in excess of $200 billion over two decades. They have saved untold thousands of people from heart attacks and strokes. But the reign of these medical monoliths is coming to an end. Three once lucrative cholesterol-cutters have already lost patent protection (Merck's Mevacor and Zocor, and Bristol-Myers Squibb's Pravachol). Lipitor goes generic in 2010, erasing $8 billion in U.S. sales for Pfizer.

Which underscores why the drug industry is on an urgent hunt for a savior. For a few years it thought it had one: good cholesterol, also known as high-density lipoprotein.

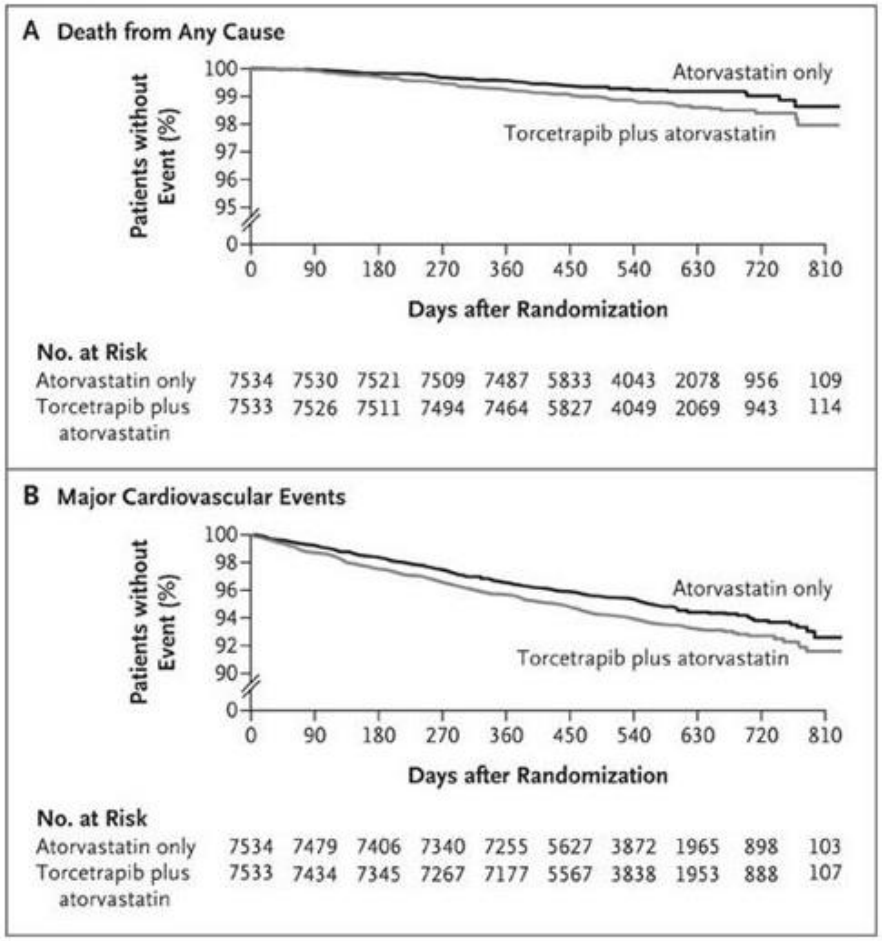
The mysterious HDL is thought to unclog arteries gunked up by too many french fries. People with high levels of good cholesterol appear to be somewhat protected against heart disease, even if their bad cholesterol levels are high. Over the last decade Pfizer and other drug companies have invested billions to concoct and test drugs to boost HDL.

Now the results of the first big trials of HDL-raising drugs are in, and they are a disaster. In December Pfizer stopped testing its experimental good-cholesterol drug, torcetrapib, after it boosted the death rate in a 15,000-patient trial—even though it raised good cholesterol by 60% in past studies. At a cardiology meeting last month heart researchers unveiled mediocre trial results for two other good-cholesterol-boosting drugs. Three more studies revealed that Pfizer's failed pill didn't unclog arteries at all. A fourth supposed artery unclogger from AstraZeneca and biotech company Atherogenics also failed to clearly mitigate heart problems.

The results are so bad that they call into question Big Pharma's ability to come up with a new crop of cholesterol blockbusters. This is forcing doctors to reassess whether high good-cholesterol levels in blood test results are always good. "We've hit a terrible dry spell. It's very dis-

Illuminate Phase 3 trial
halted for increased CVD events
Forbes, April 2007

HDL-C: 72% increase
LDL-C: 25% decrease



Bartrer P et al. NEJM 2007

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Potential Anti-inflammatory Mechanisms of HDL

- Cholesterol Removal-Both cellular and extracellular
- Sequestration of oxidized lipids/LPS/cytokines
- Delivery of Anti-inflammatory Proteins and Lipid Cargo

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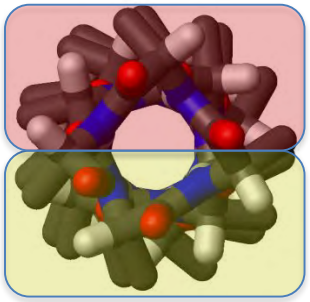
5A ApoA-I Mimetic Peptide

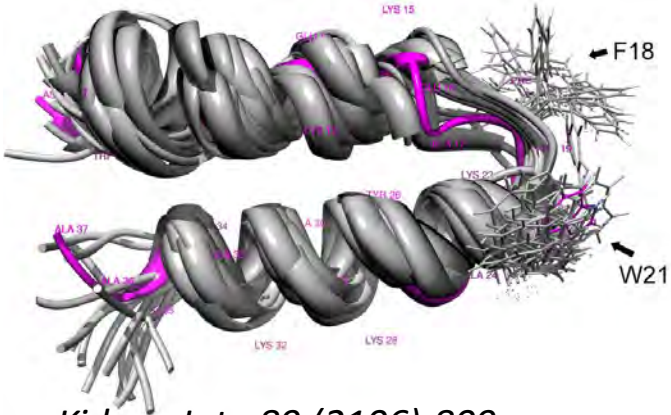
5A peptide



Hydrophilic

Alpha-helix
cross-section

Hydrophobic



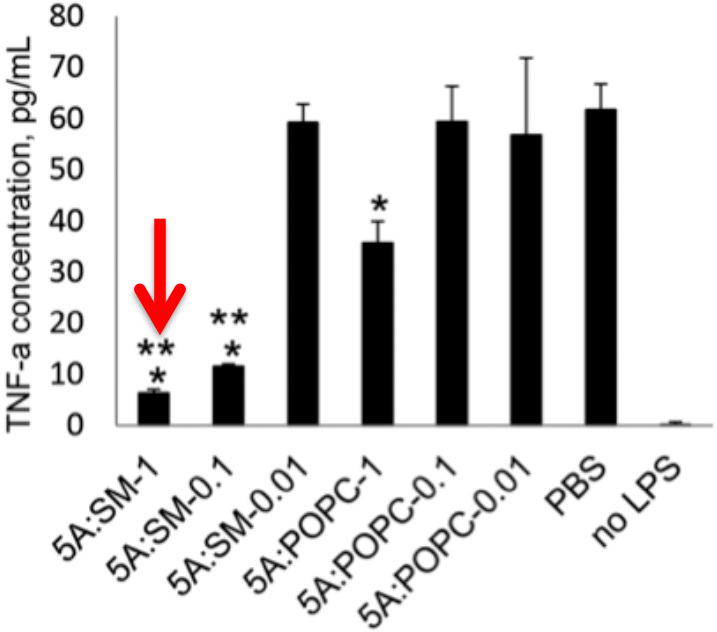
Kidney Int. 89 (2106) 809
JIMD Rep. 2 Dec (2016)
J Lipid Res. 56 (2015) 1727
PLoS One. 8 (2013) e68802
J Biol Chem. 287 (2012)43730
J Pharm Exp Ther. 344 (2013) 50
Am J Respir Cell Mol Biol. 47 (2012) 186

Control

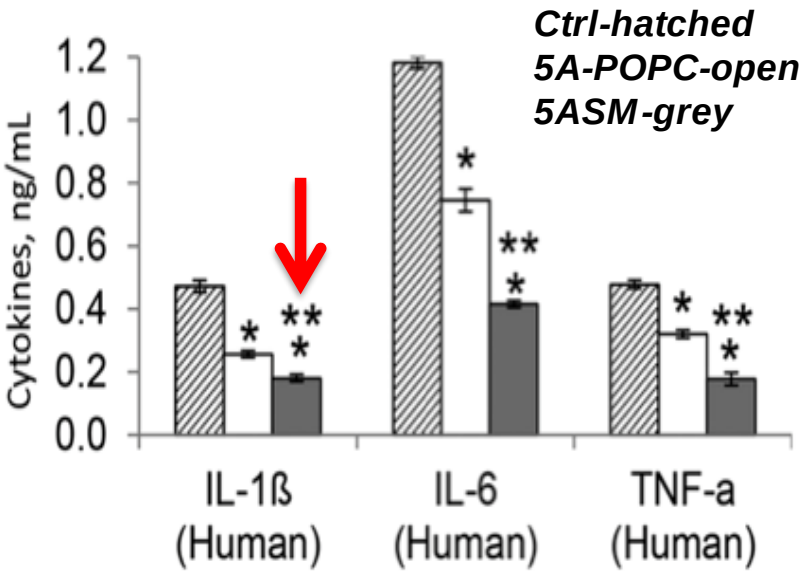
5A-POPC

5A Inhibits Cytokine Release by Macrophages

TNF-alpha release murine macrophages



Cytokine release PHA-treated whole blood



Anna Schwendeman et al. J. Lipid Res. 2015;56:1727-1737

Features of LCAT Deficiency

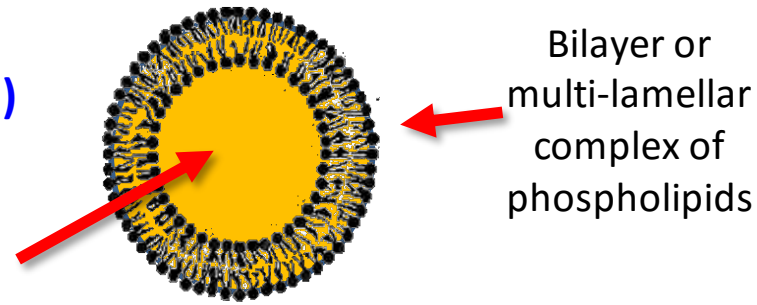
Partial deficiency: Fish Eye Disease (FED)

- Cloudy cornea

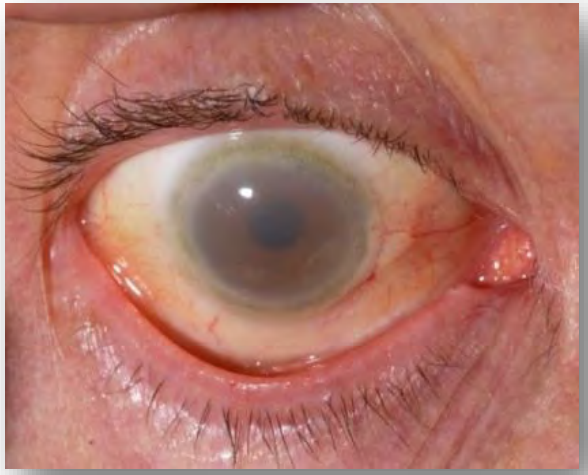
Complete deficiency: Familial LCAT Deficiency (FLD)

- Cloudy cornea
- Normochromic normocytic anemia
- Proteinuria/ESRD
- Low HDL-C
- Presence of LpX

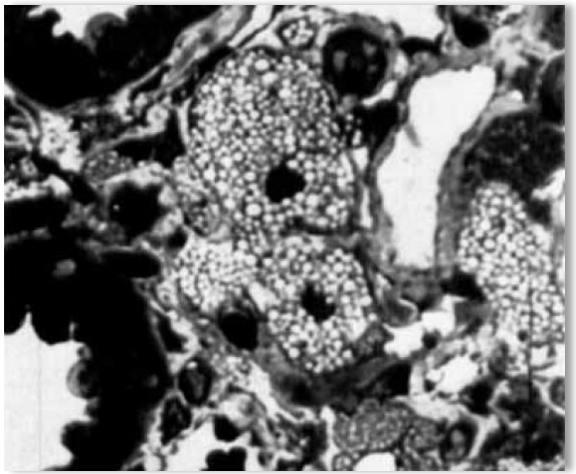
LpX



Cloudy Cornea

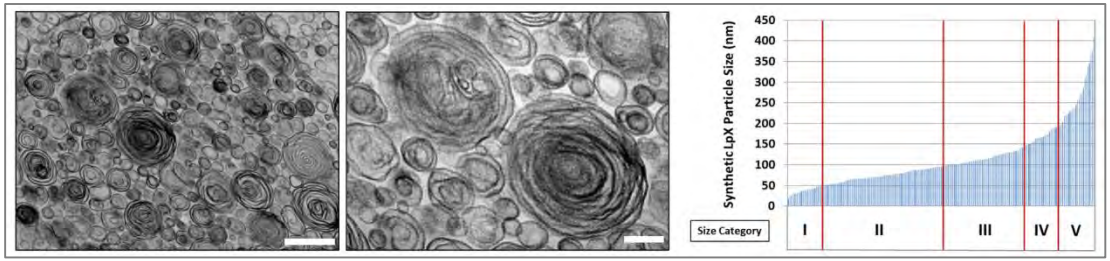


Renal Lipid Deposits

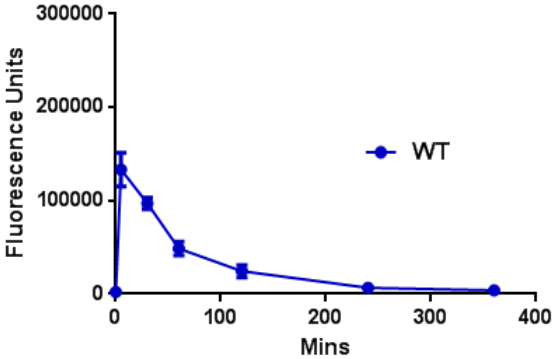


LpX Causes Renal Disease in LCAT Deficiency

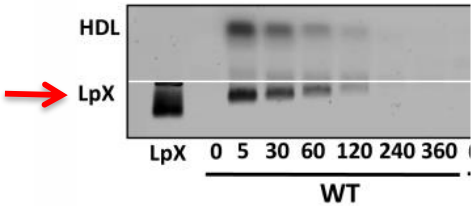
Synthetic Fluorescent LpX



Plasma Clearance of LpX is Delayed in *Lcat*$^{-/-}$ Mice



Agarose gel
Plasma PE fluorescence



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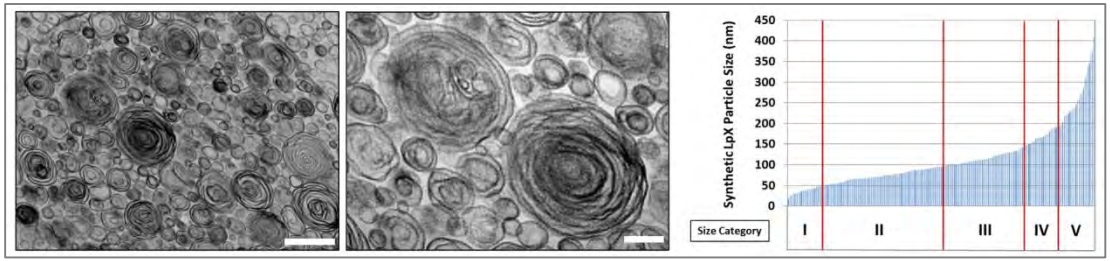

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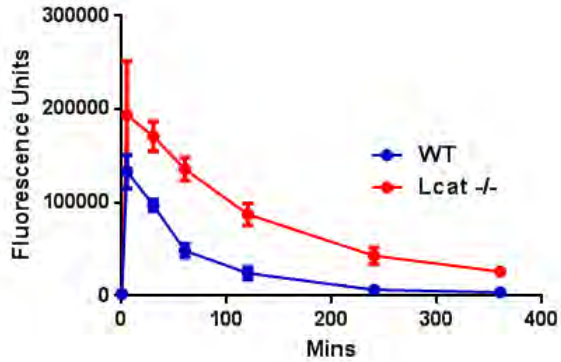

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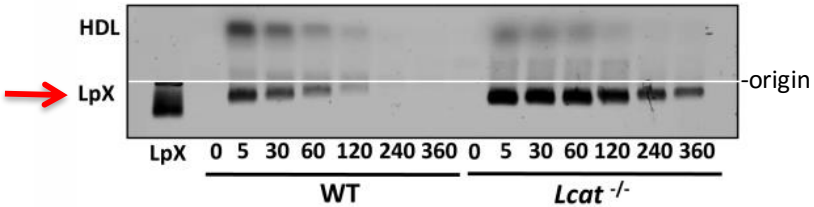
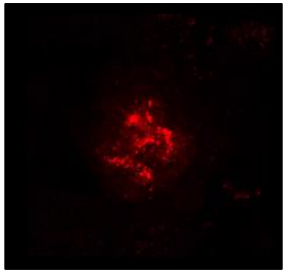
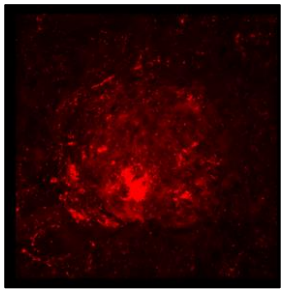


Glomerular Deposition of LpX

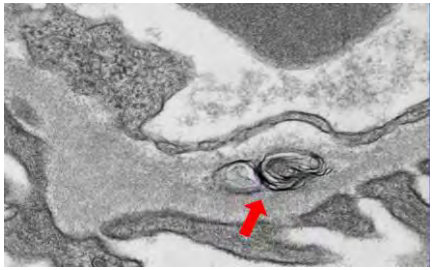
WT



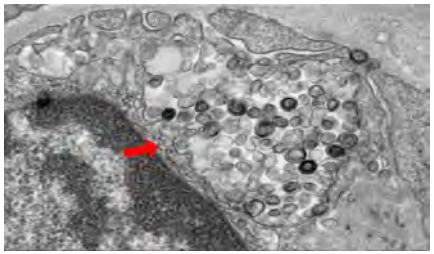
***Lcat*$^{-/-}$**



Basement membrane



Mesangium



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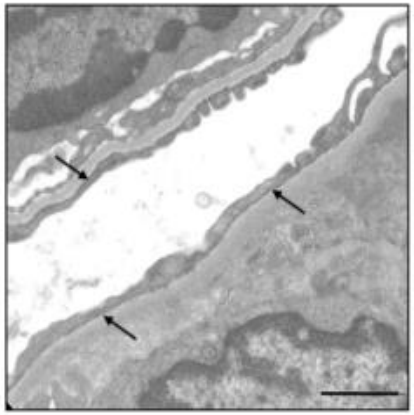
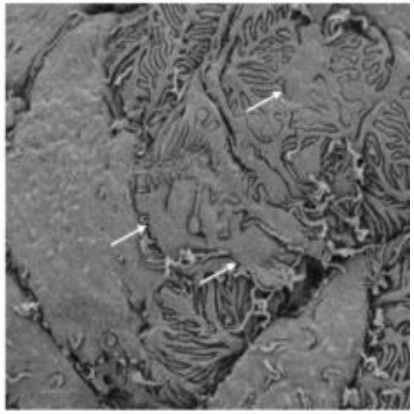
LpX Causes Podocyte Effacement and Proteinuria

LpX Induces Podocyte Effacement

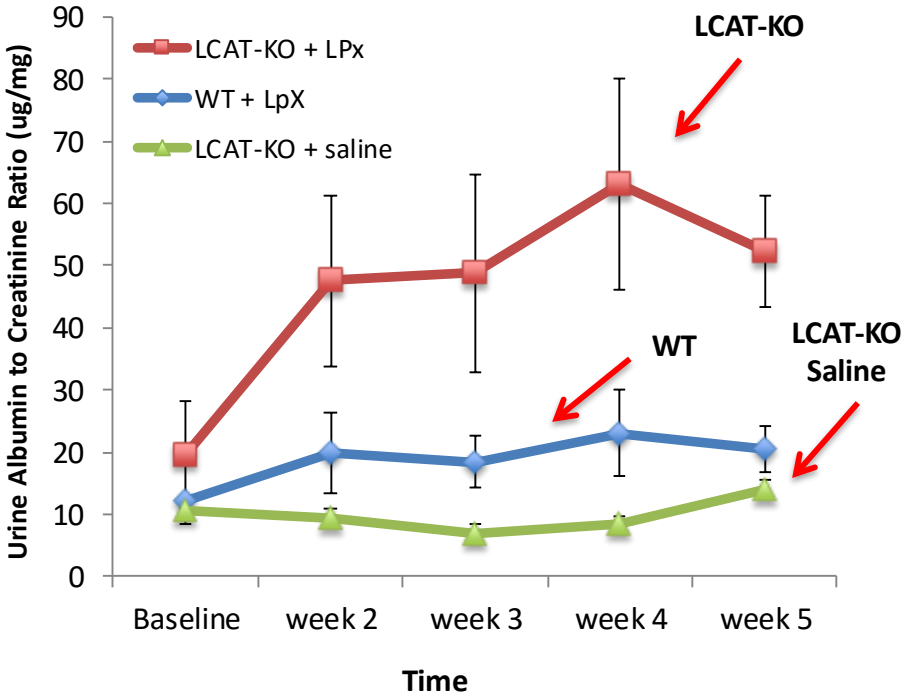
TEM



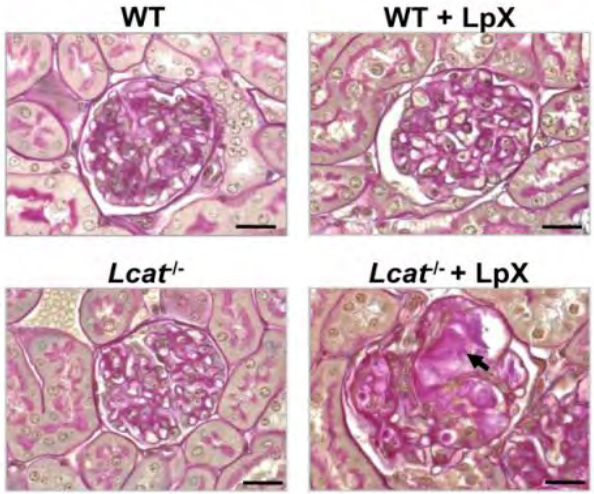
SEM



LpX Causes Proteinuria

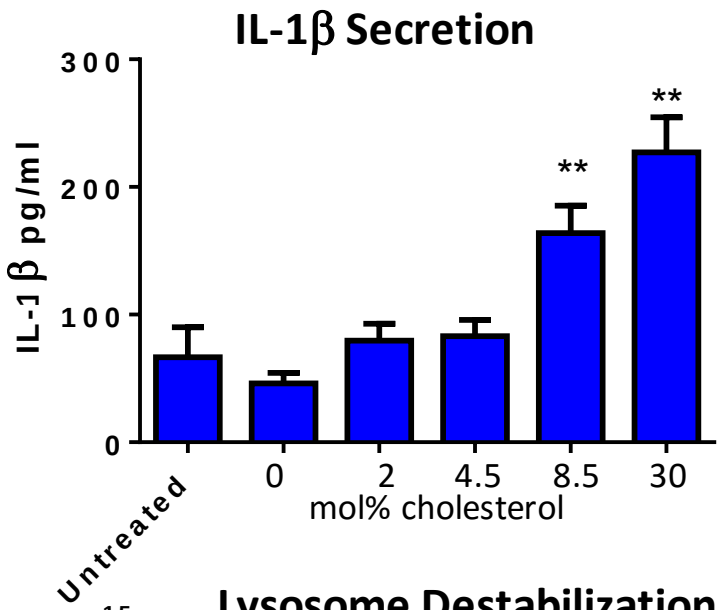


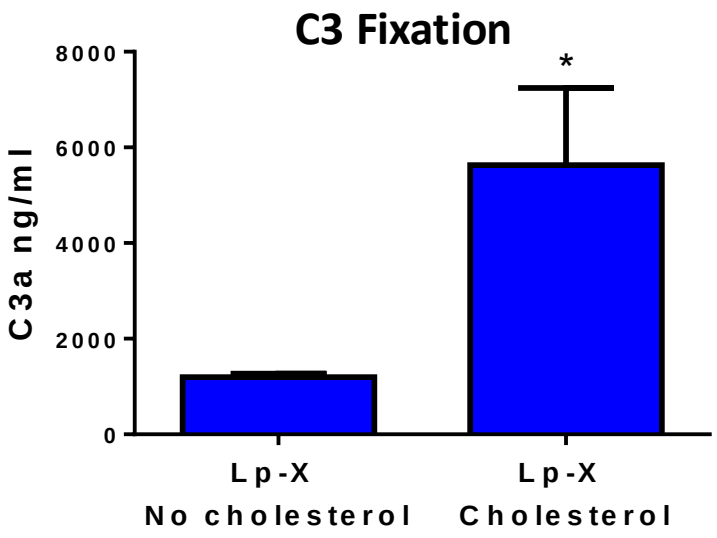
LpX Causes Glomerulosclerosis

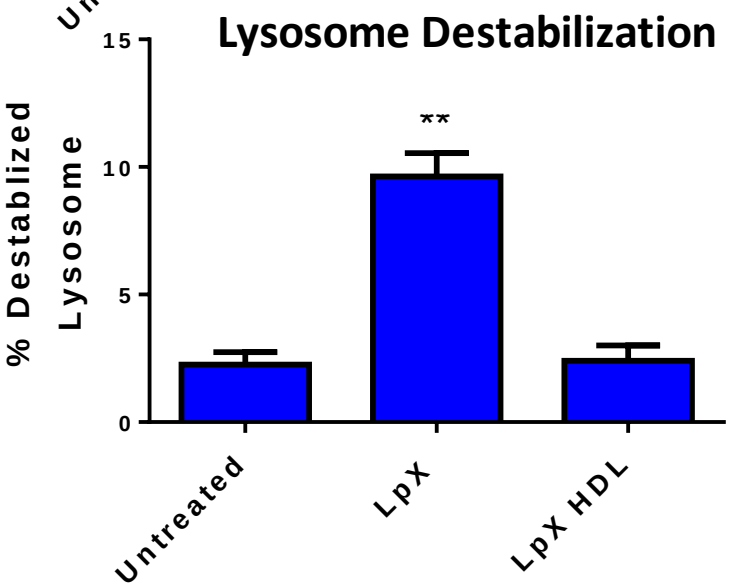


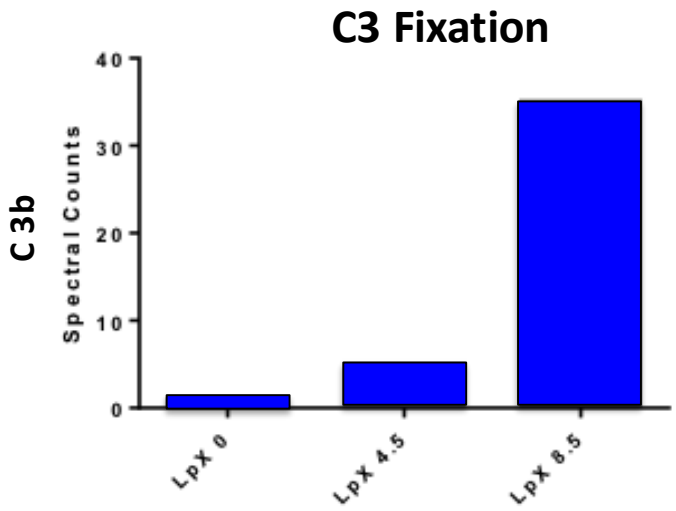
PLoSOne 26 (2016) e0150083

LpX Activates the Inflammasome and Fixes Complement

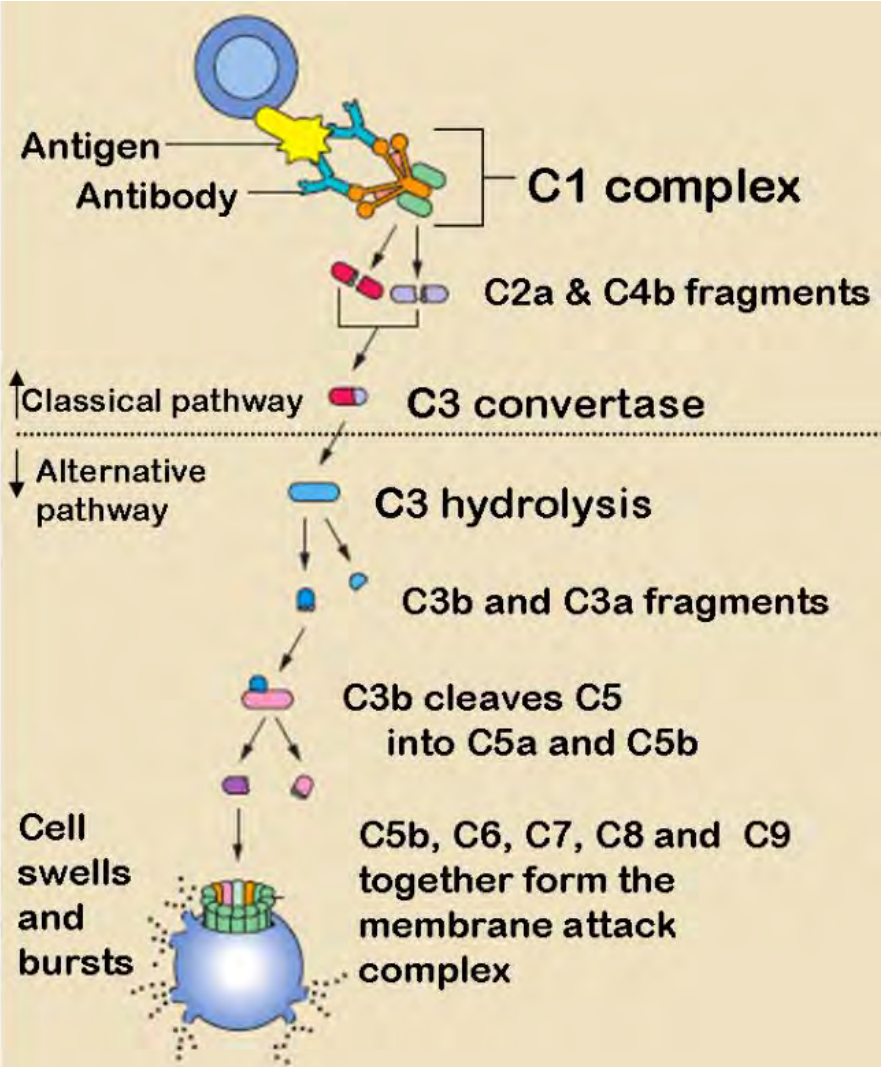




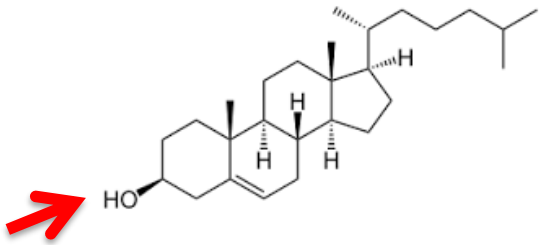




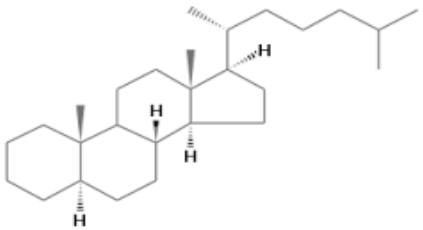
Complement Fixation of Cholesterol



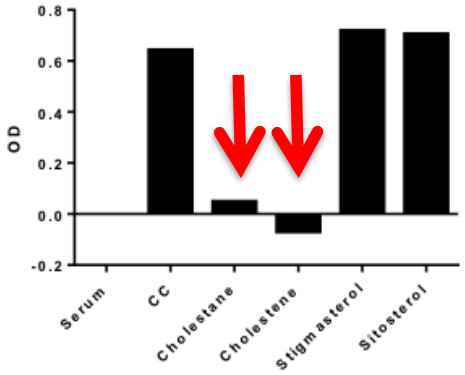
Cholesterol



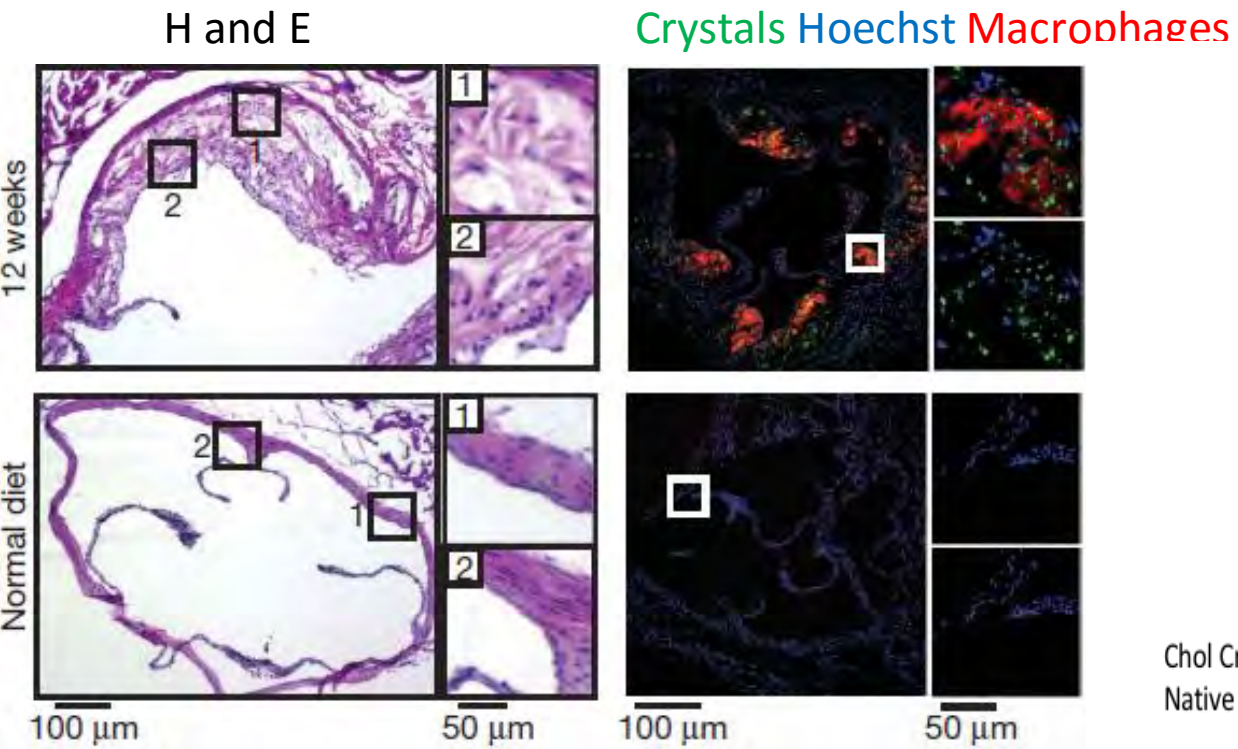
Cholestane

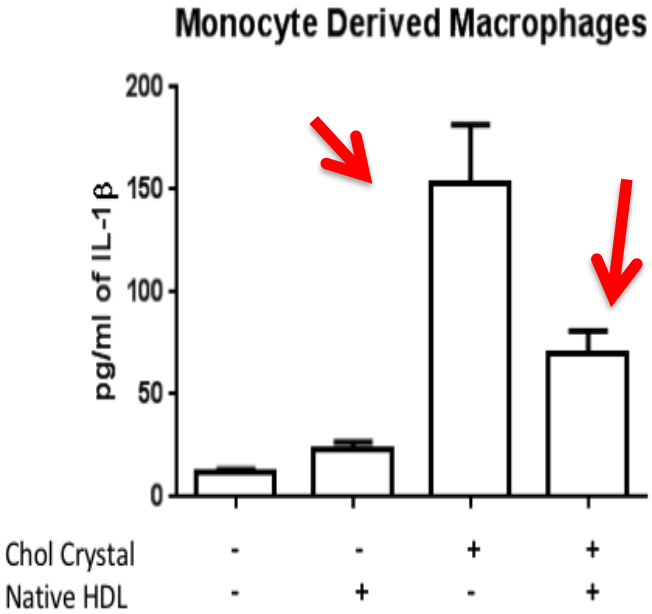


C3 Fixation



Cholesterol crystals Induce Inflammation



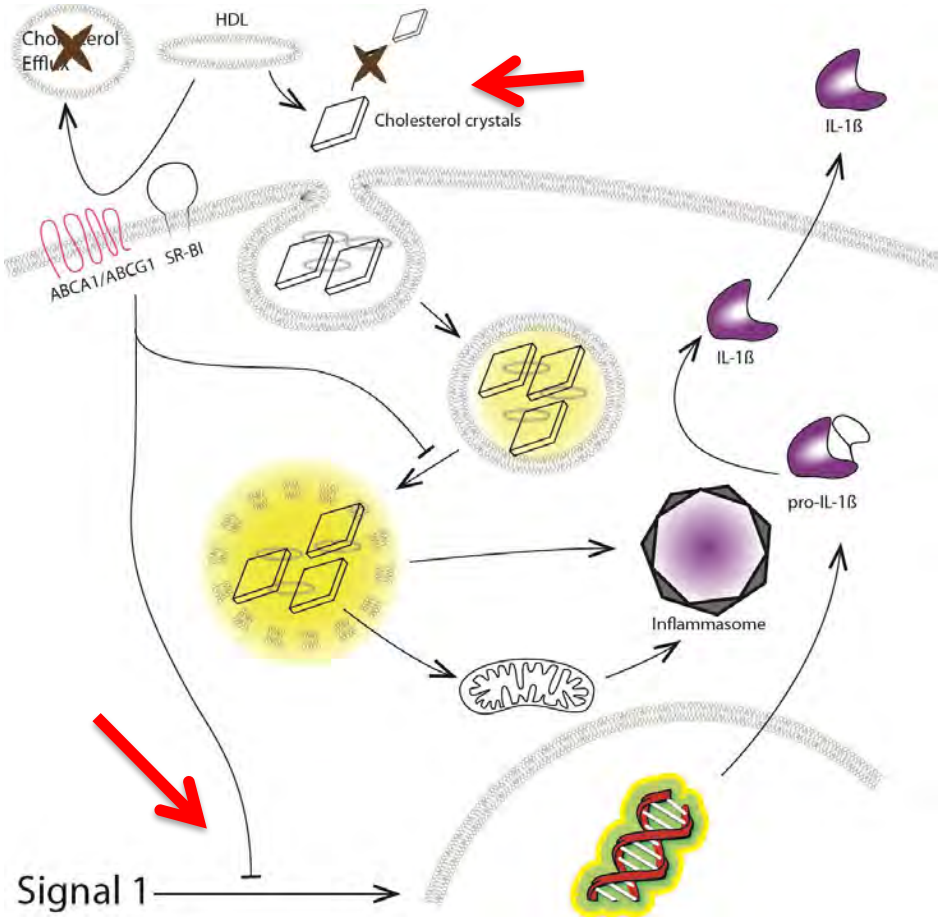


- Crystals can be seen as early as 2 weeks

Immunol 149 (2016) 306

NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Peter Duewell Eicke Latz

HDL Suppresses Inflammasome Activation by Cholesterol Crystals

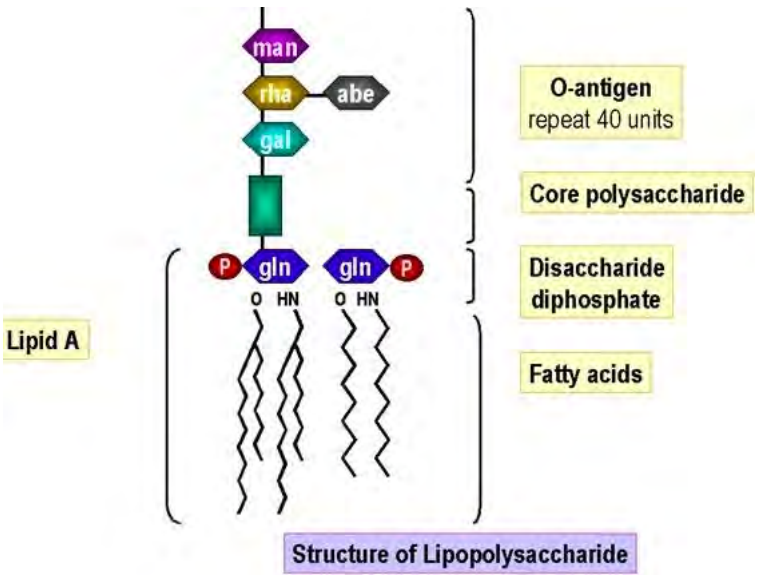


Immunol 149 (2016) 306

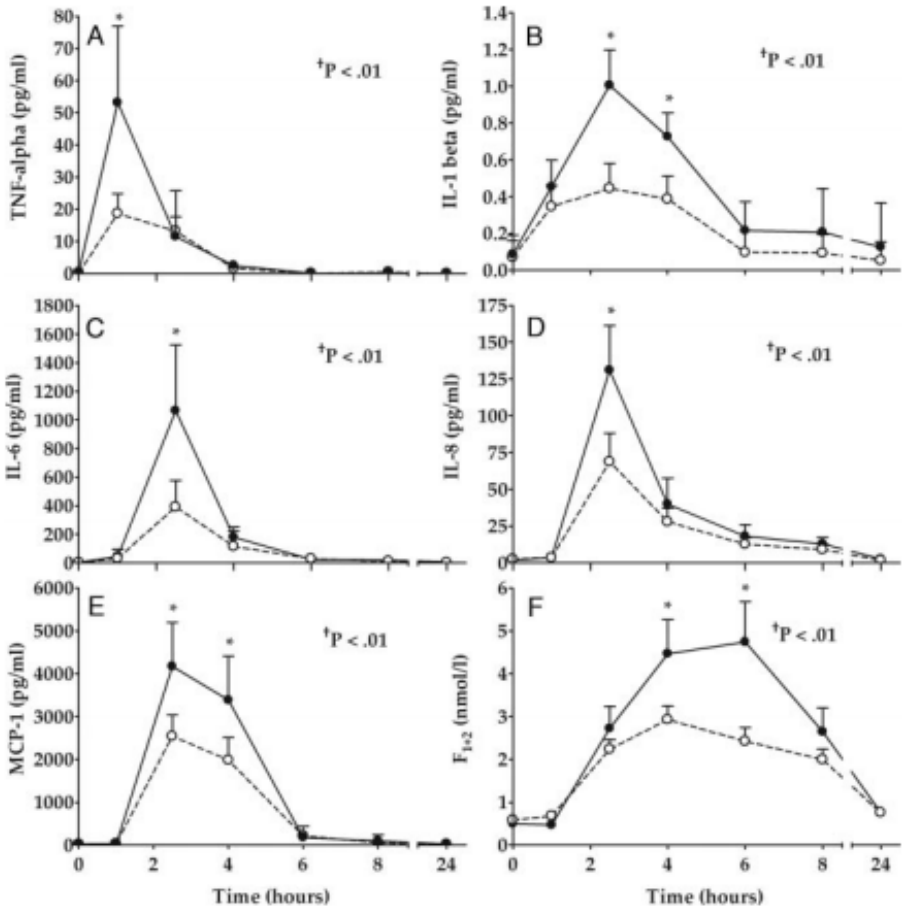
Potential Anti-inflammatory Mechanisms of HDL

- Cholesterol Removal-Both cellular and extracellular
- Sequestration of oxidized lipids/LPS/cytokines
- Delivery of Anti-inflammatory Proteins and Lipid Cargo

HDL Dampens LPS Inflammatory Response



https://microbeonline.com/lipopolysaccharide



*ATVB 27(*2007) 1153

Potential Anti-inflammatory Mechanisms of HDL

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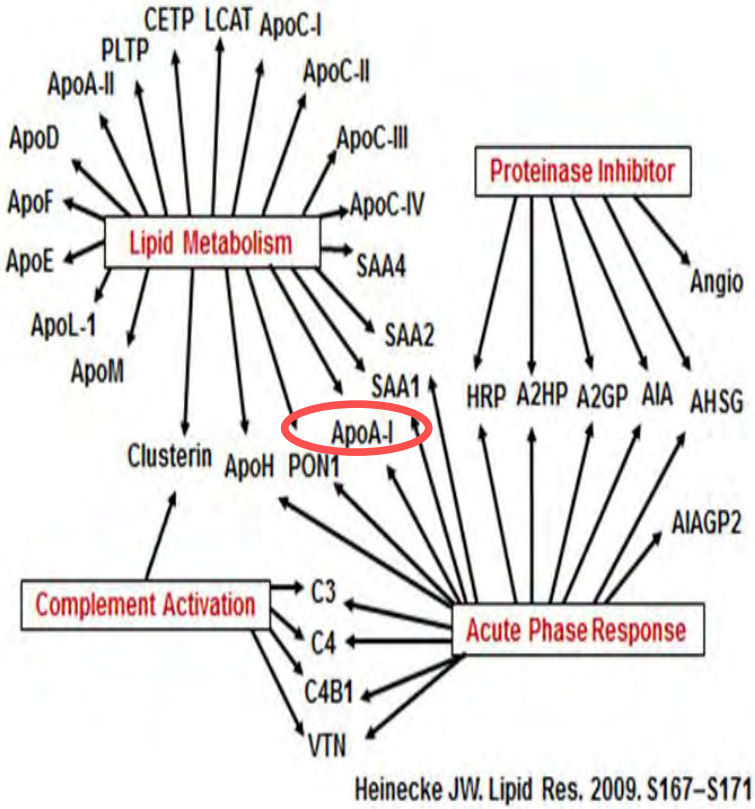
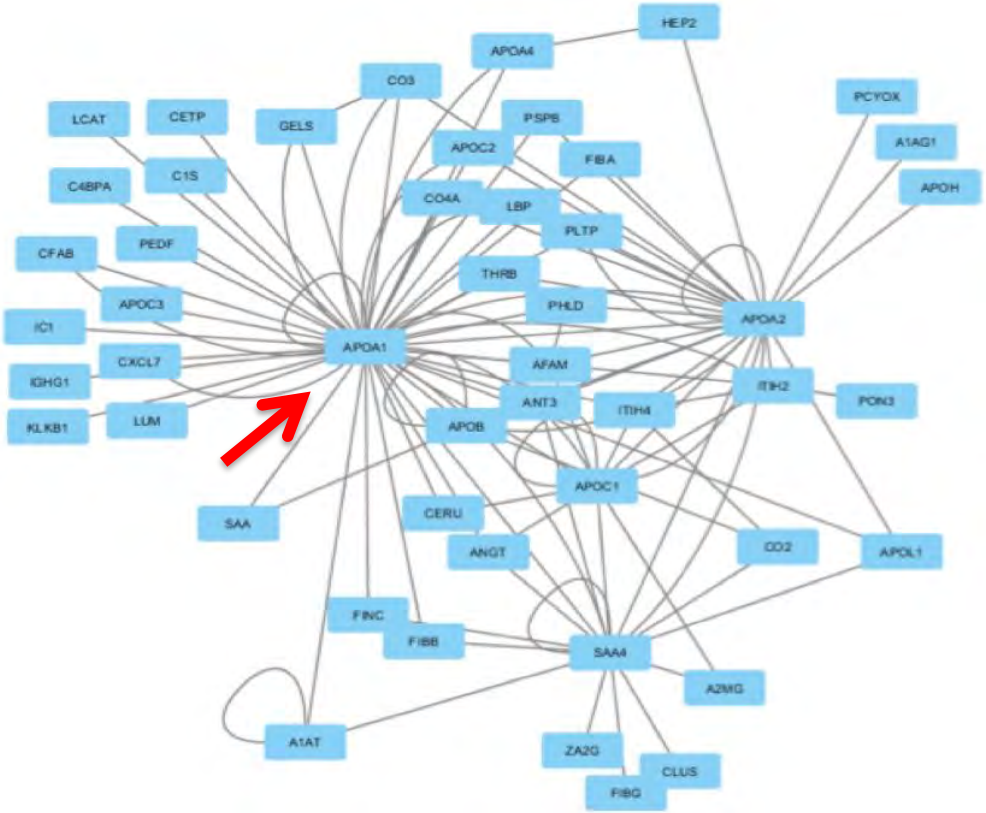
HDL-Proteome

Functional Map



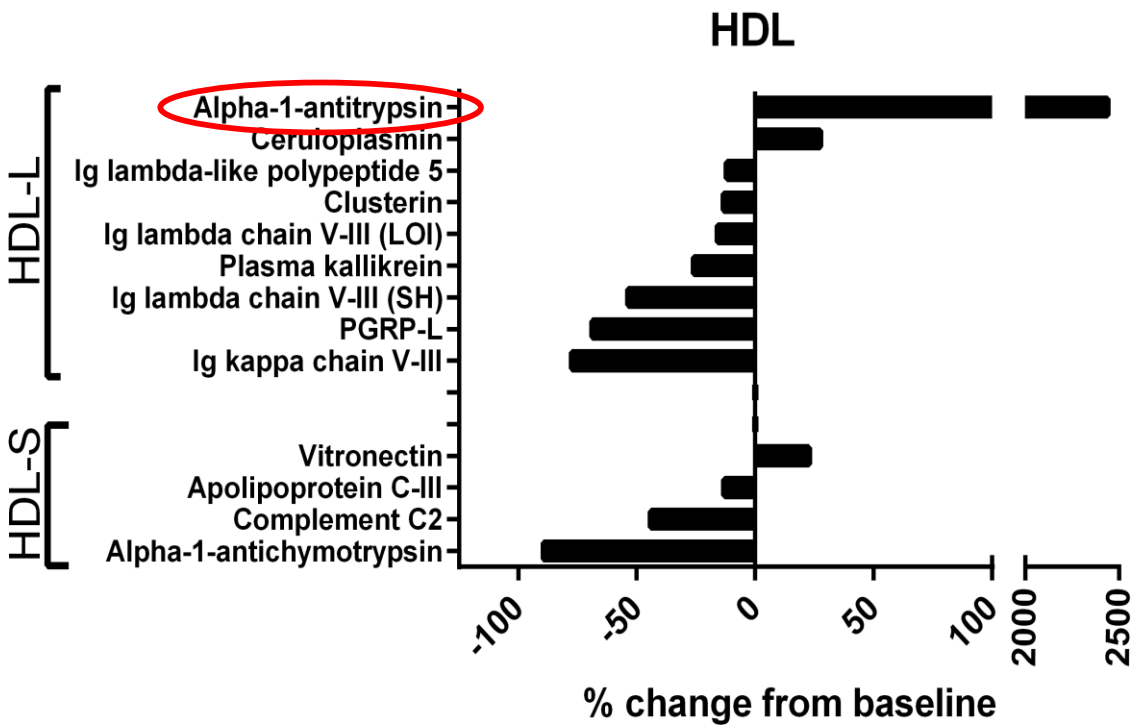
Interactome Map



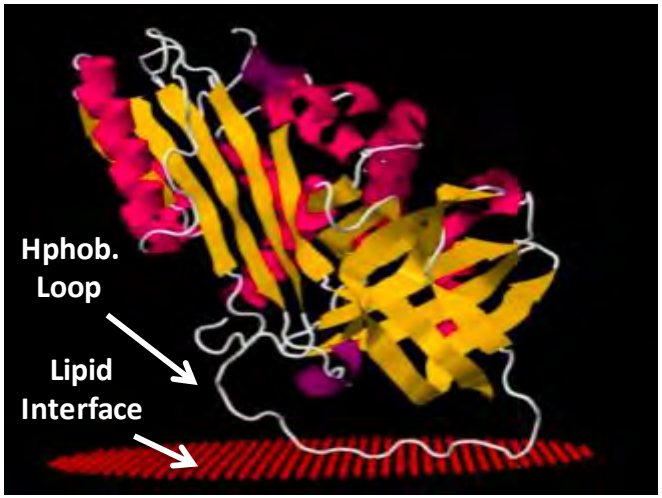
J Clin Invest 2007; 117: 746

Rosuvastatin Increases A1AT on HDL

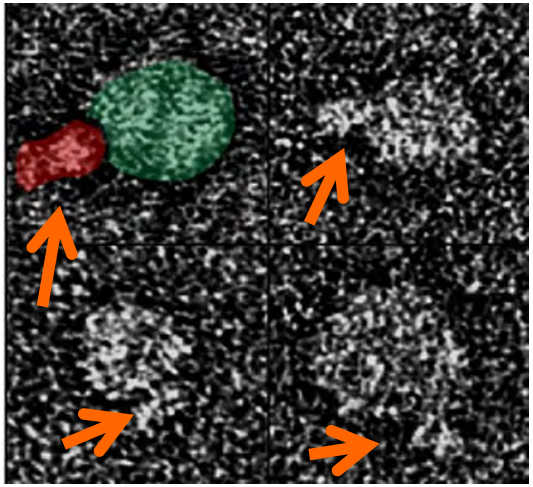
Protein Spectral Count Changes



Molecular Modeling



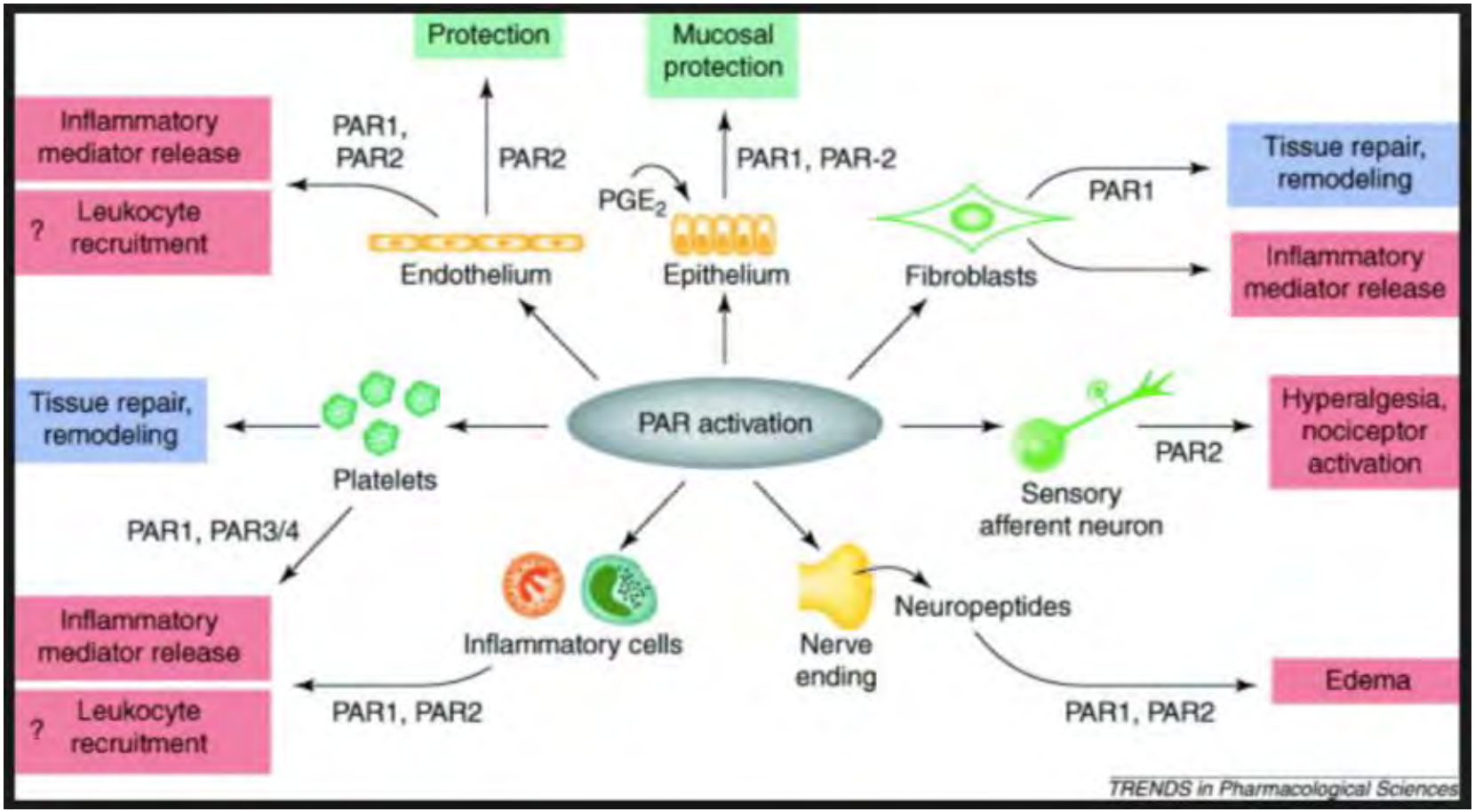
Cryoelectron Microscopy



A1AT (Red), HDL (Green)

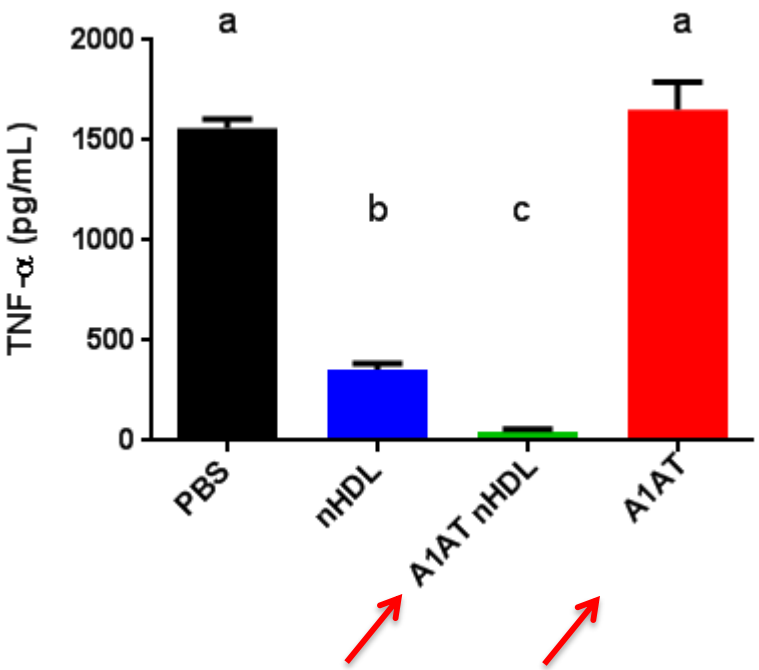
Mol Cell Proteomics 14 (2015) 3247

Role of PAR in Inflammation



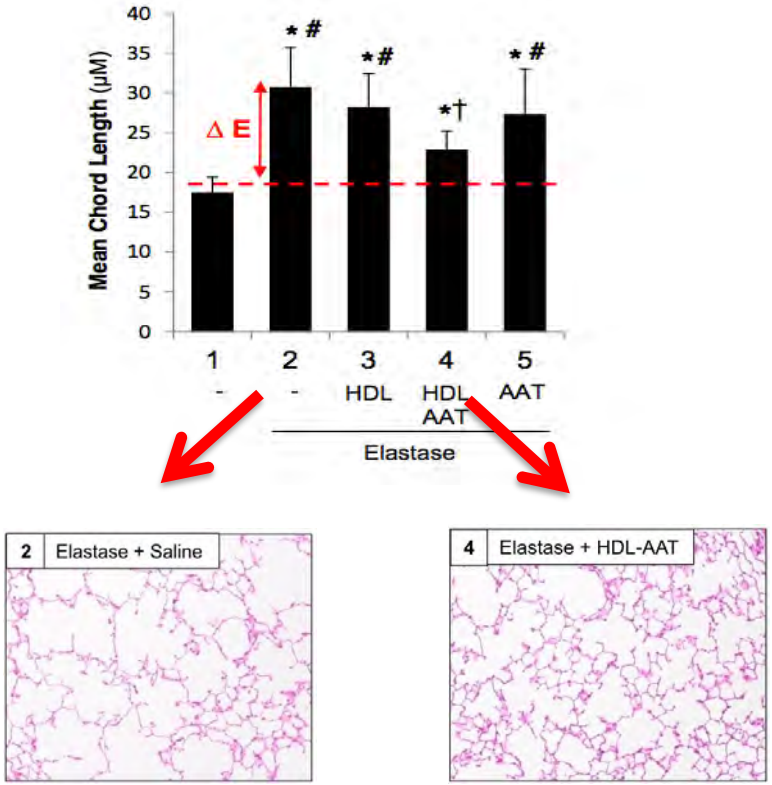
A1AT-HDL Inhibits Elastase-induced Inflammation

Elastase Induced Inflammation



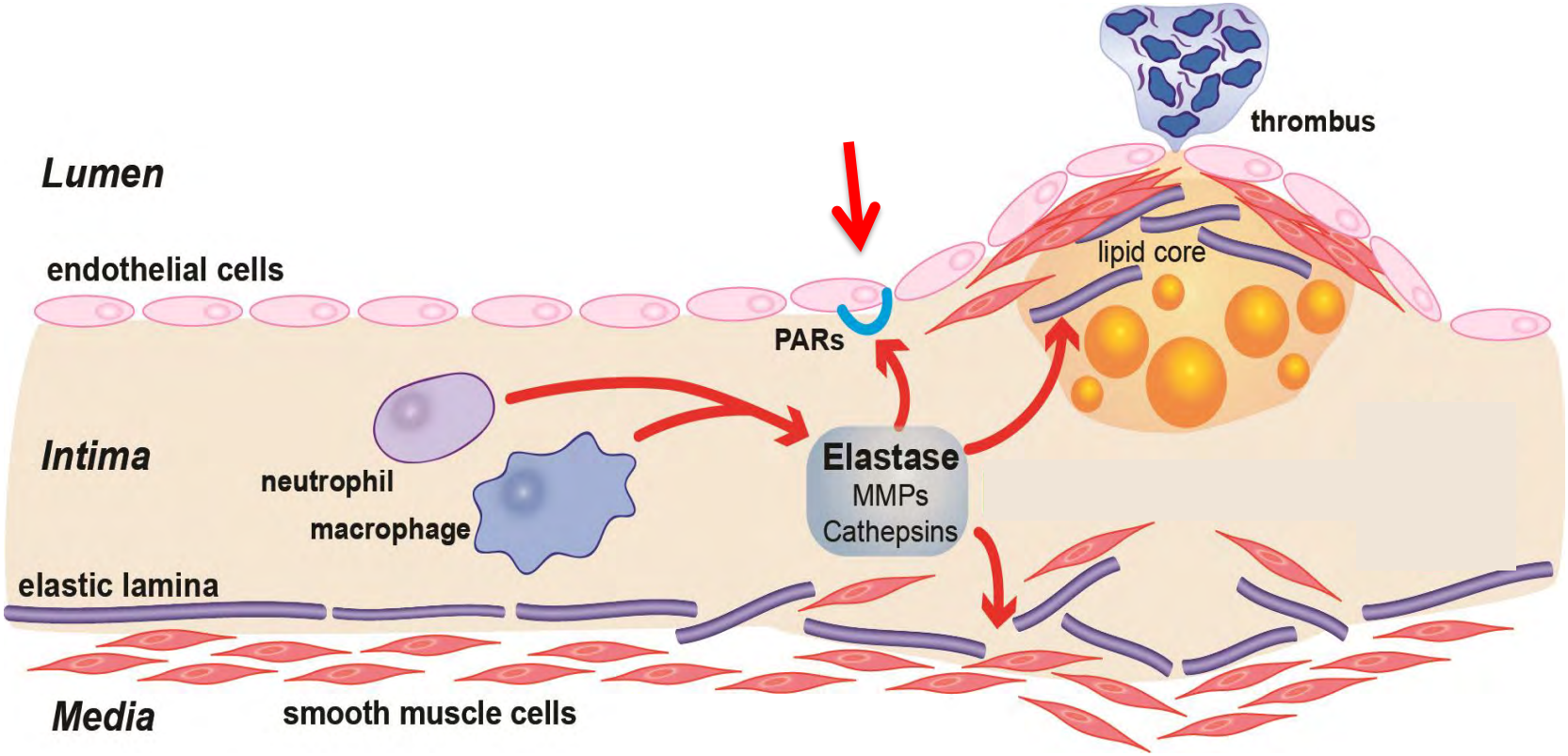
Mol Cell Proteomics 14 (2015) 3247

HDL Delivers A1AT to Lung

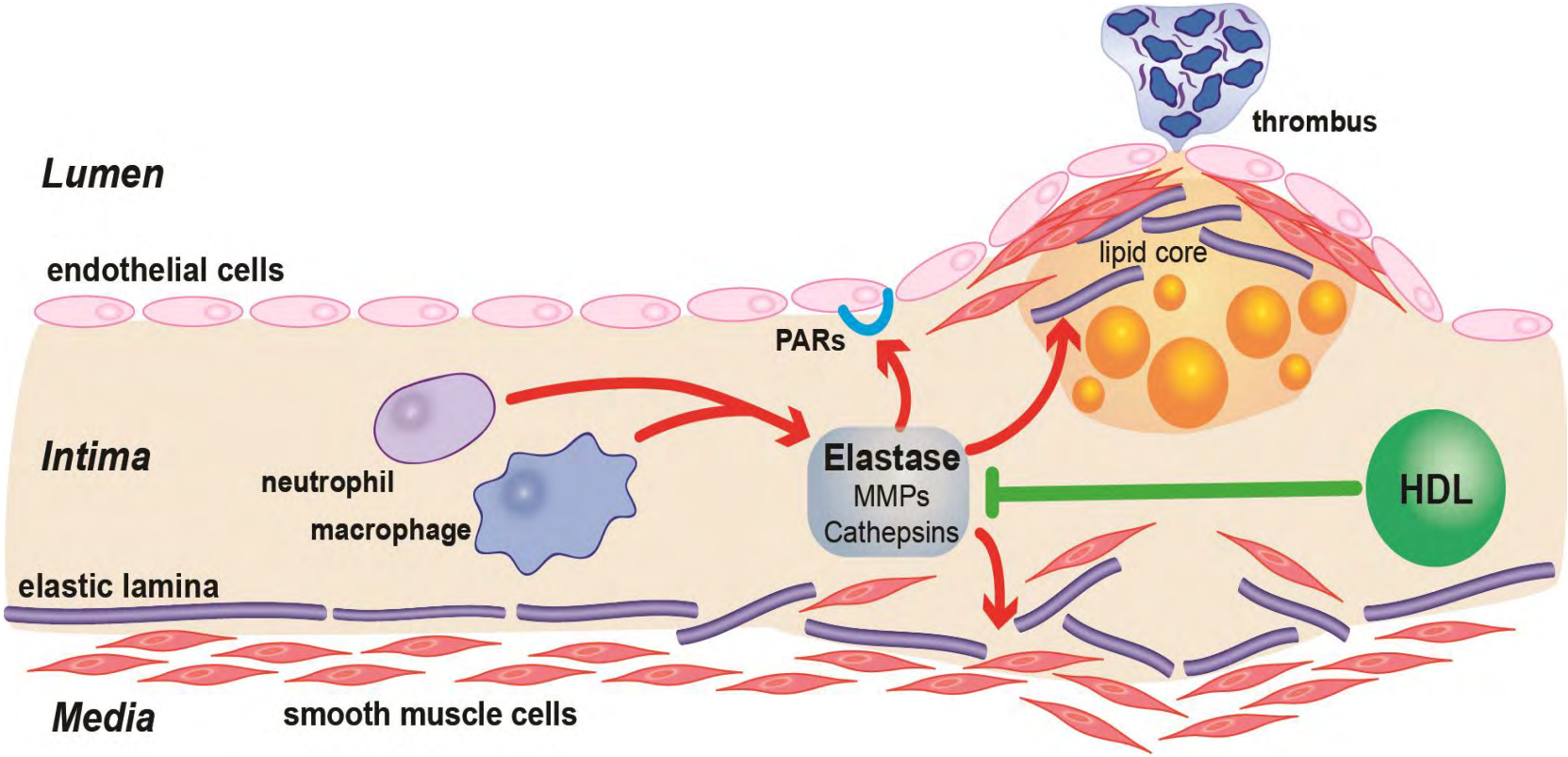


Am J Resp Cell Mol Biol 51 (2015) 4

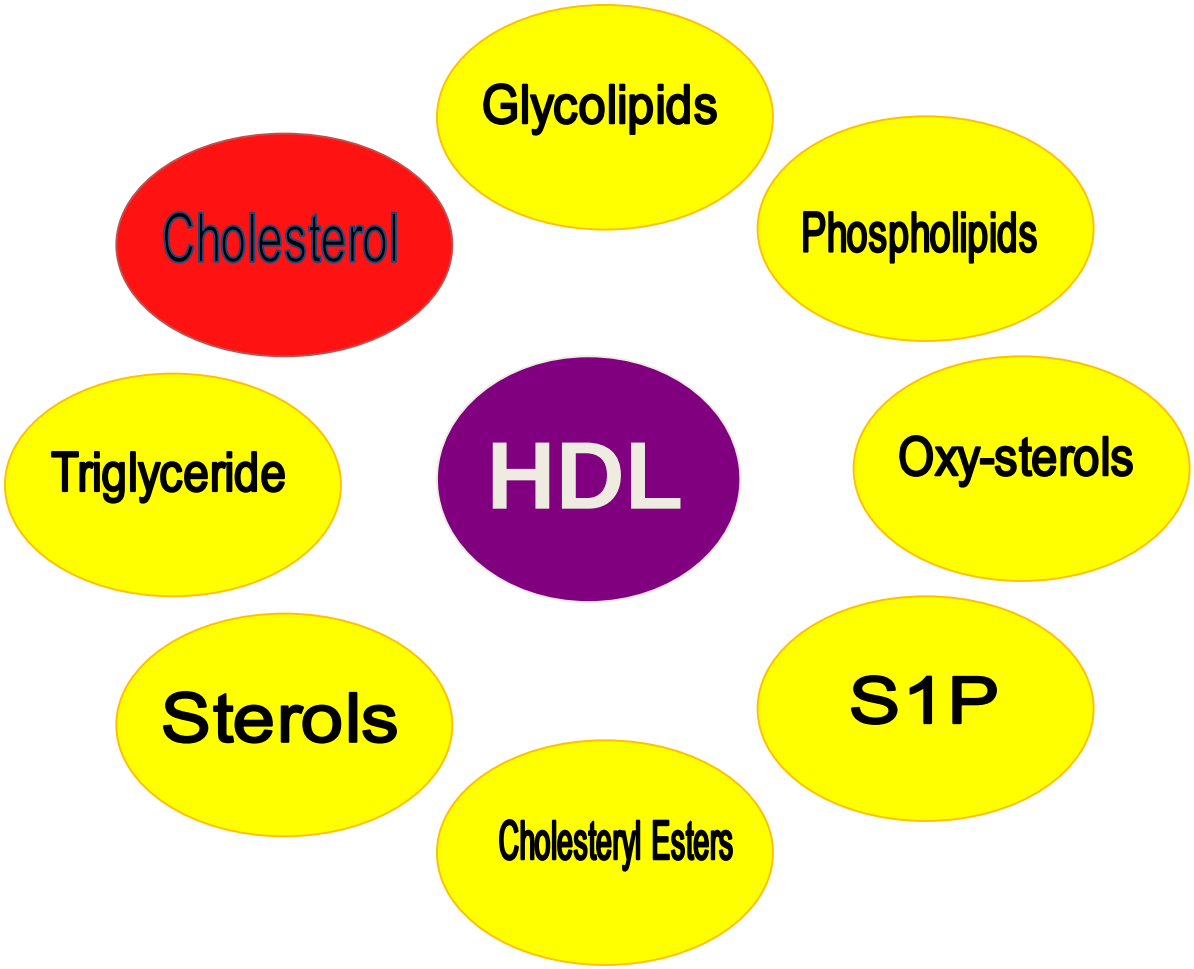
Proposed Role of A1AT-HDL in Atherogenesis



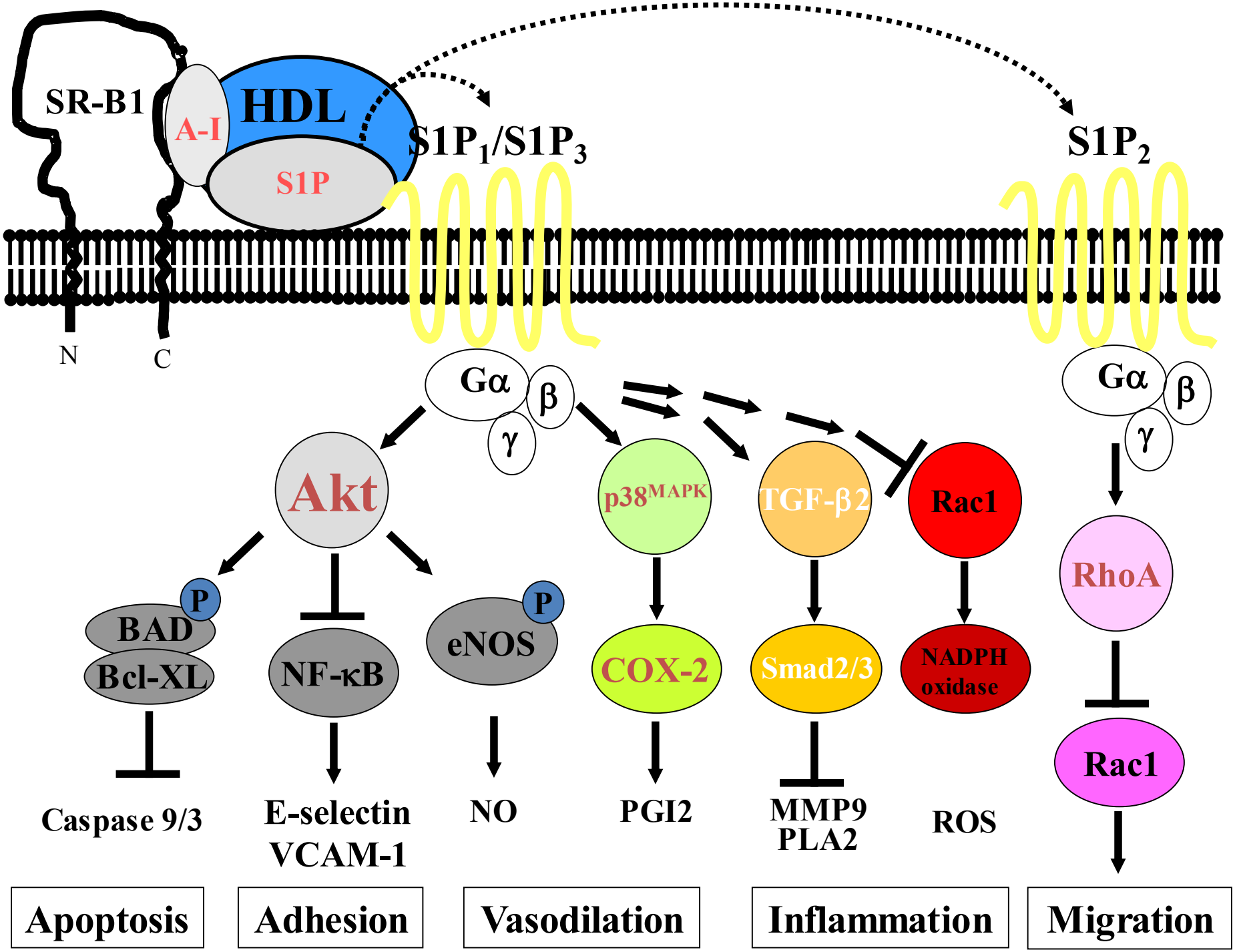
Proposed Role of A1AT-HDL in Atherogenesis



HDL-Lipidome: Major Lipid Classes

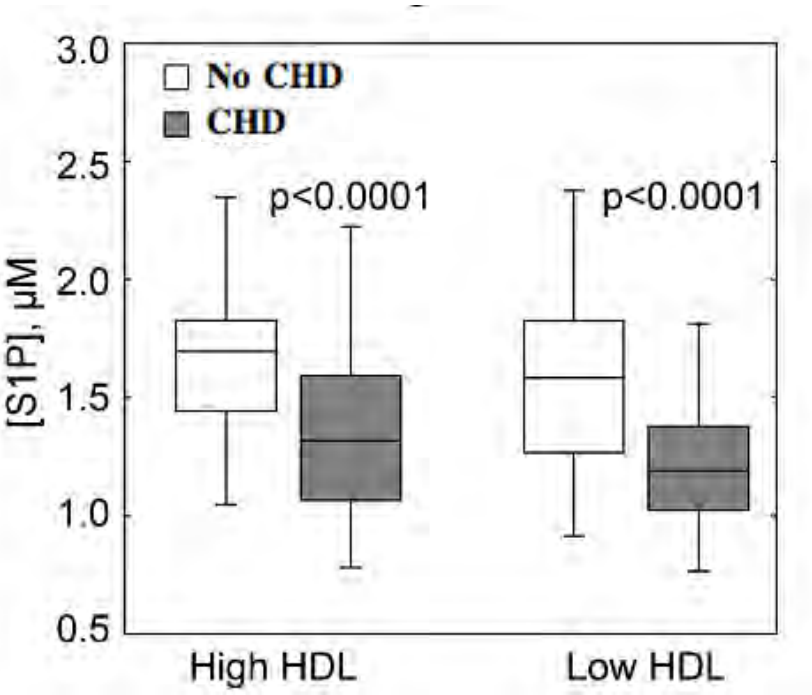


>200 species of specific lipids


SR-B1
A-I
HDL
S1P
S1P1/S1P3
S1P2
N
C
Gα
β
γ
Gα
β
γ
Akt
p38MAPK
TGF-β2
Rac1
RhoA
P
BAD
Bcl-XL
NF-κB
P
eNOS
COX-2
Smad2/3
NADPH oxidase
Rac1
Caspase 9/3
E-selectin
VCAM-1
NO
PGI2
MMP9
PLA2
ROS
Apoptosis
Adhesion
Vasodilation
Inflammation
Migration

HDL-S1P Inversely Related CHD

S1P Content of HDL

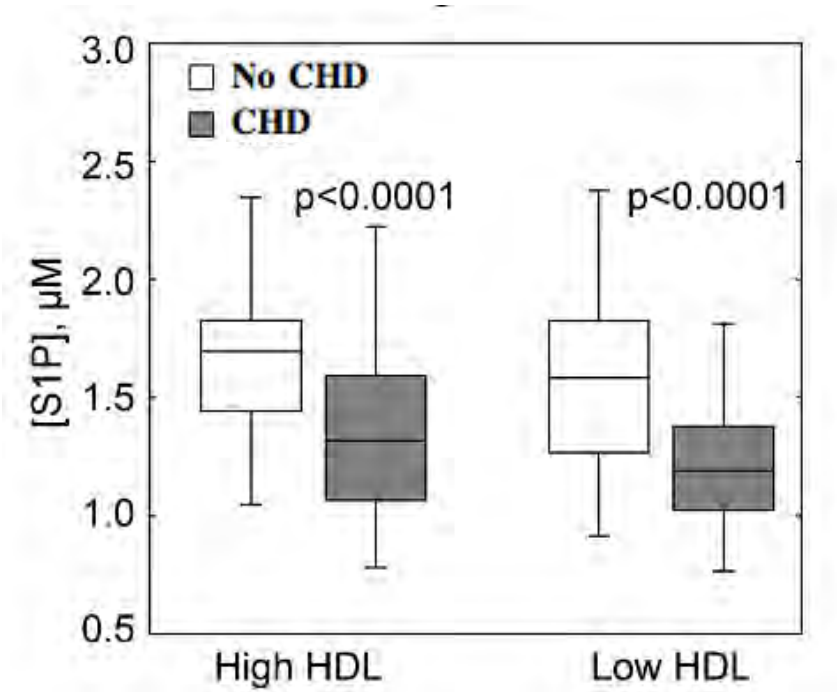


Subjects with CHD have decreased S1P on HDL.

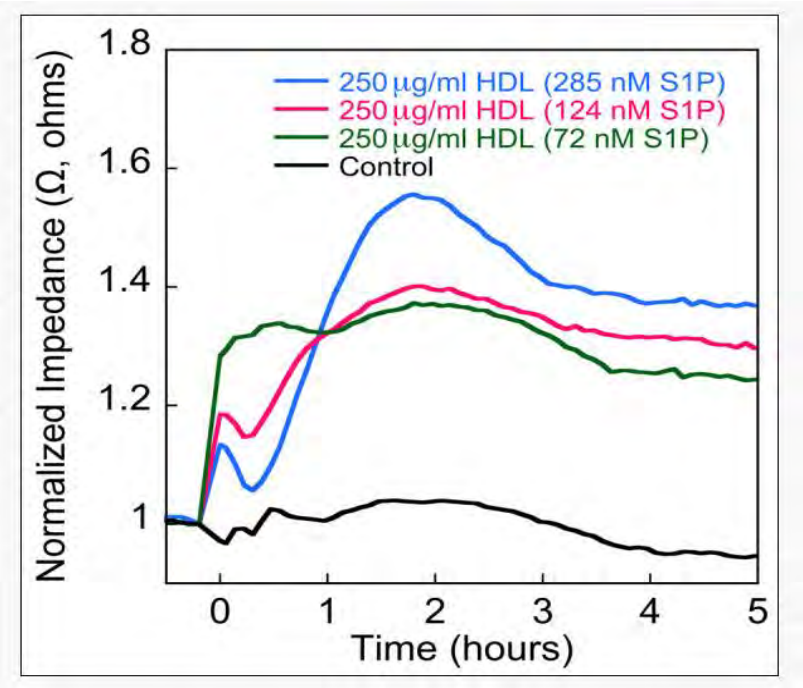
Lipids Health Disease 2011; 10: 70

HDL-S1P Inversely Related CHD

S1P Content of HDL



Endothelial Integrity



Subjects with CHD have decreased S1P on HDL.
S1P on HDL improves endothelial barrier function.

Lipids Health Disease 2011; 10: 70

Major Questions in HDL Field

- Is it involved in the pathogenesis of CVD?

  Most likely, but maybe not RCT.

- Should we measure it?

  Yes, but need better assays.

- Is it a target of therapy or just a CVD biomarker?

  No for HDL-C, but can be under some conditions.

- What is the future of HDL research?

  Composition studies and how relate to function.

HDL research is still valuable despite recent clinical trial failures and more research *not less* is needed to harness its potential.

National Institutes of Health

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