

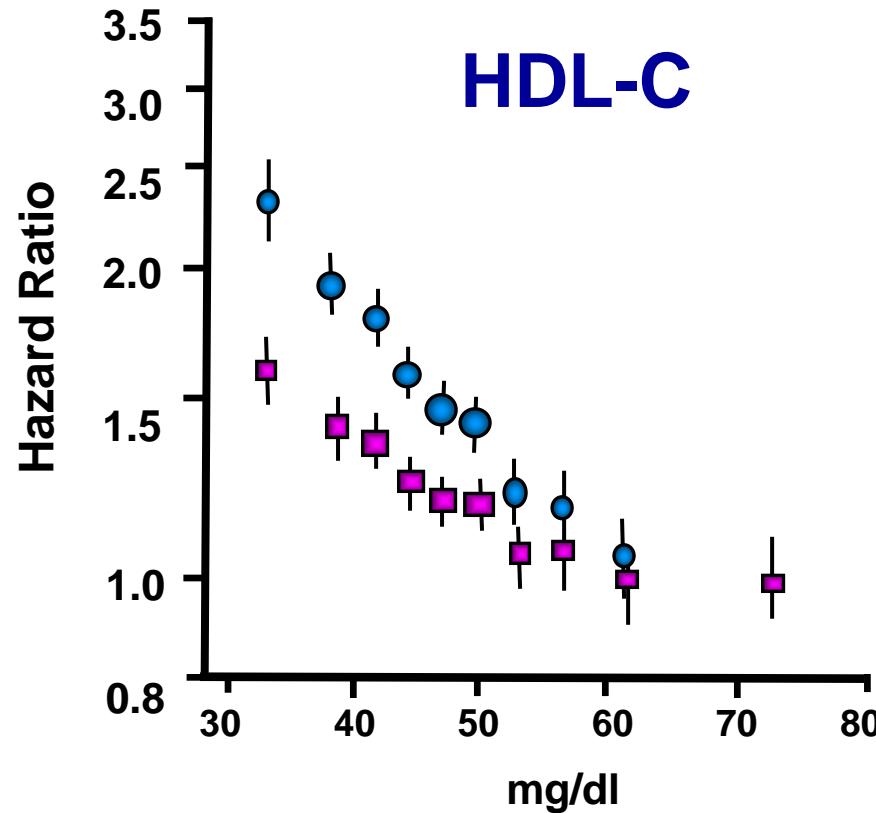
# NEXT STEPS IN ADDRESSING HDL CHOLESTEROL IN CLINICAL PRACTICE

Alberico L. Catapano

[Alberico.catapano@unimi.it](mailto:Alberico.catapano@unimi.it)

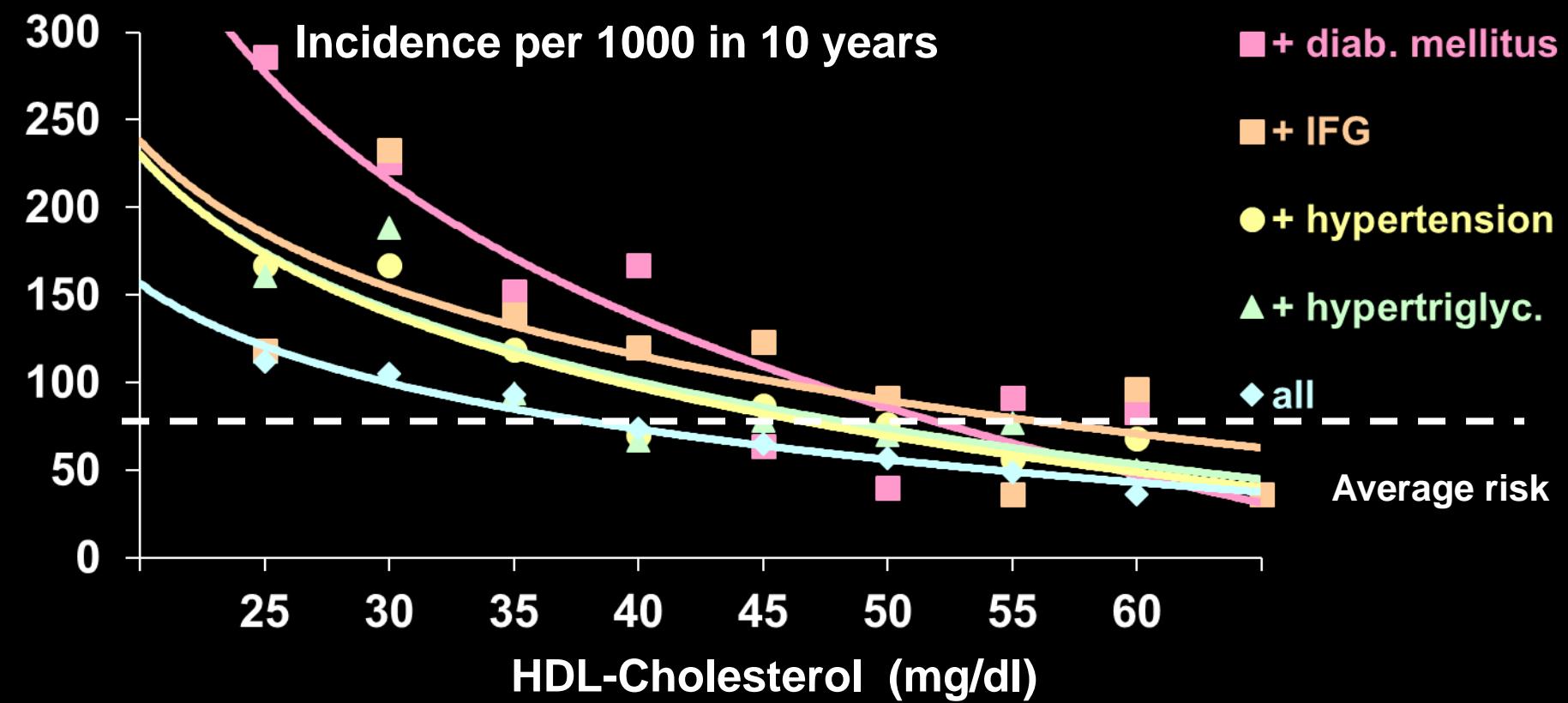
# Hazard Ratios for CHD Across Quantiles of HDL-Cholesterol

● Adjusted for age and sex only      ■ Further adjusted for several risk factors



From the Emerging Risk Factors Collaboration (68 studies in 302 430 participants)

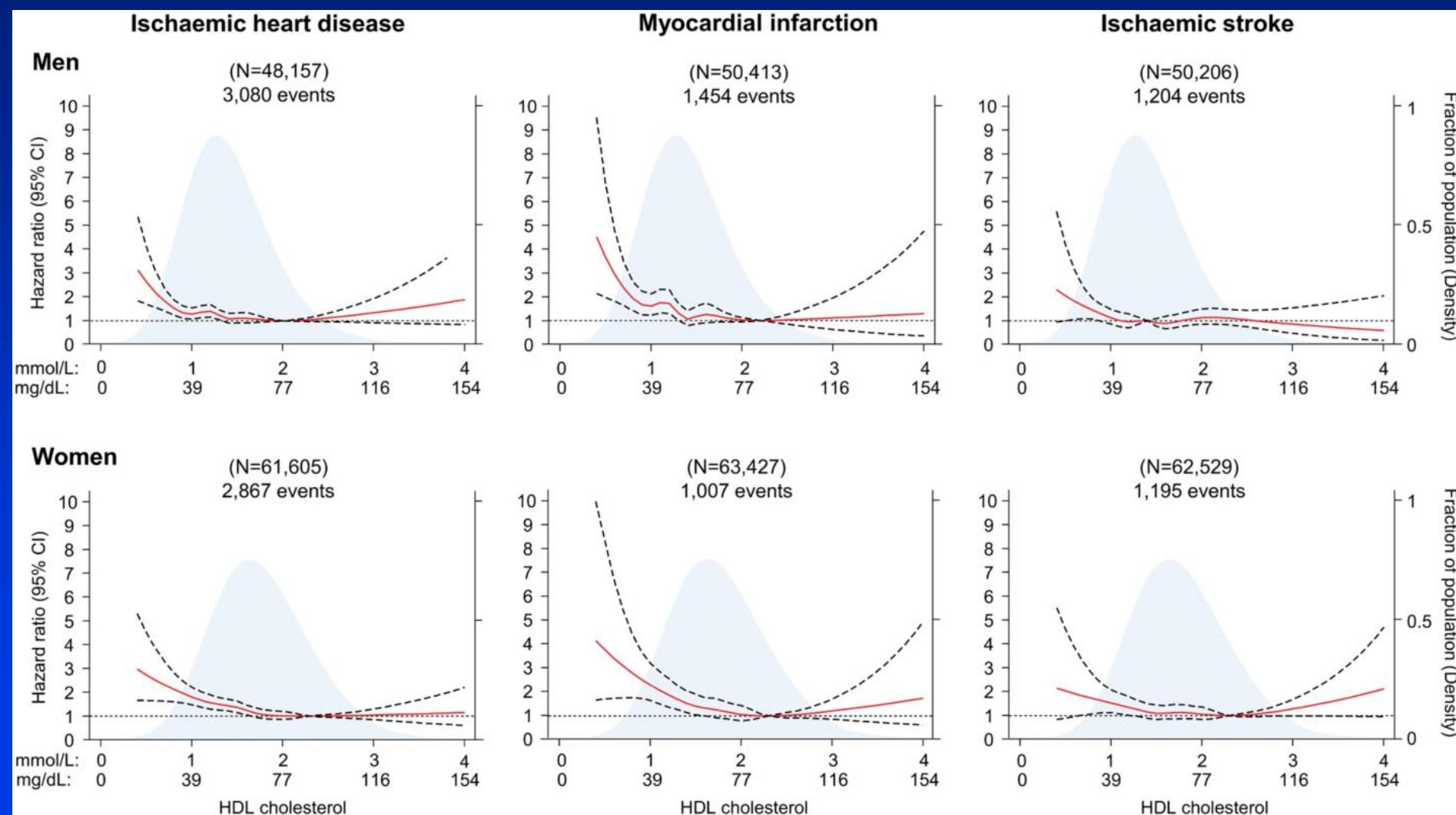
# Incidence of coronary events in men according to HDL-cholesterol and other risk factors



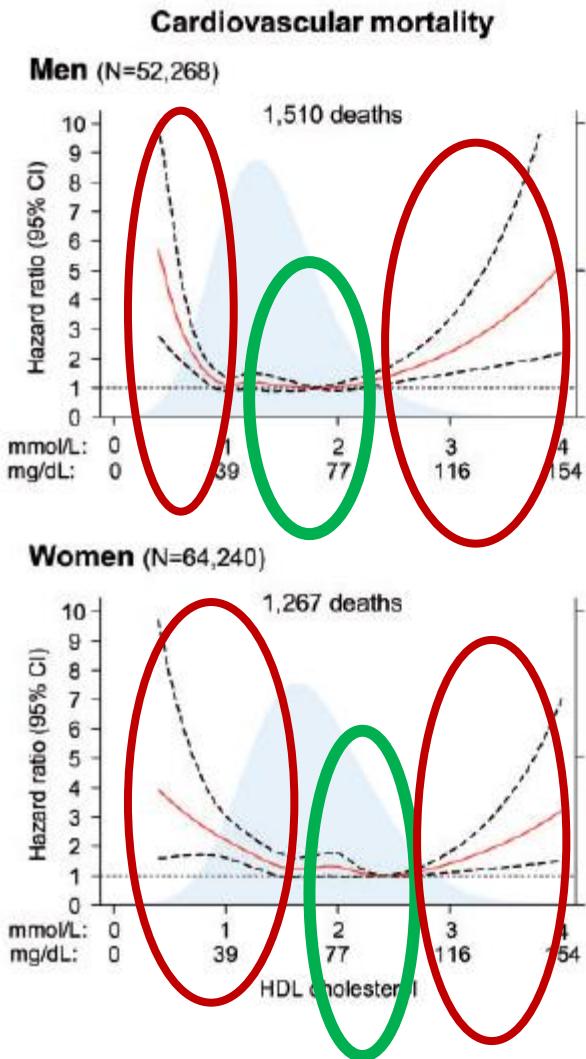
**PROCAM -Study:** 4818 men aged 35-65 years; 325 coronary events in 10 years  
(Curr. Opin. Lipidol. 2000; 11: 627ff; Drug Discovery Today Therapeutic Targets 2008; 3: e305ff)

# HDL-C and Cardiovascular Disease

## Copenhagen Studies



# CV Mortality and elevated values of HDL-C

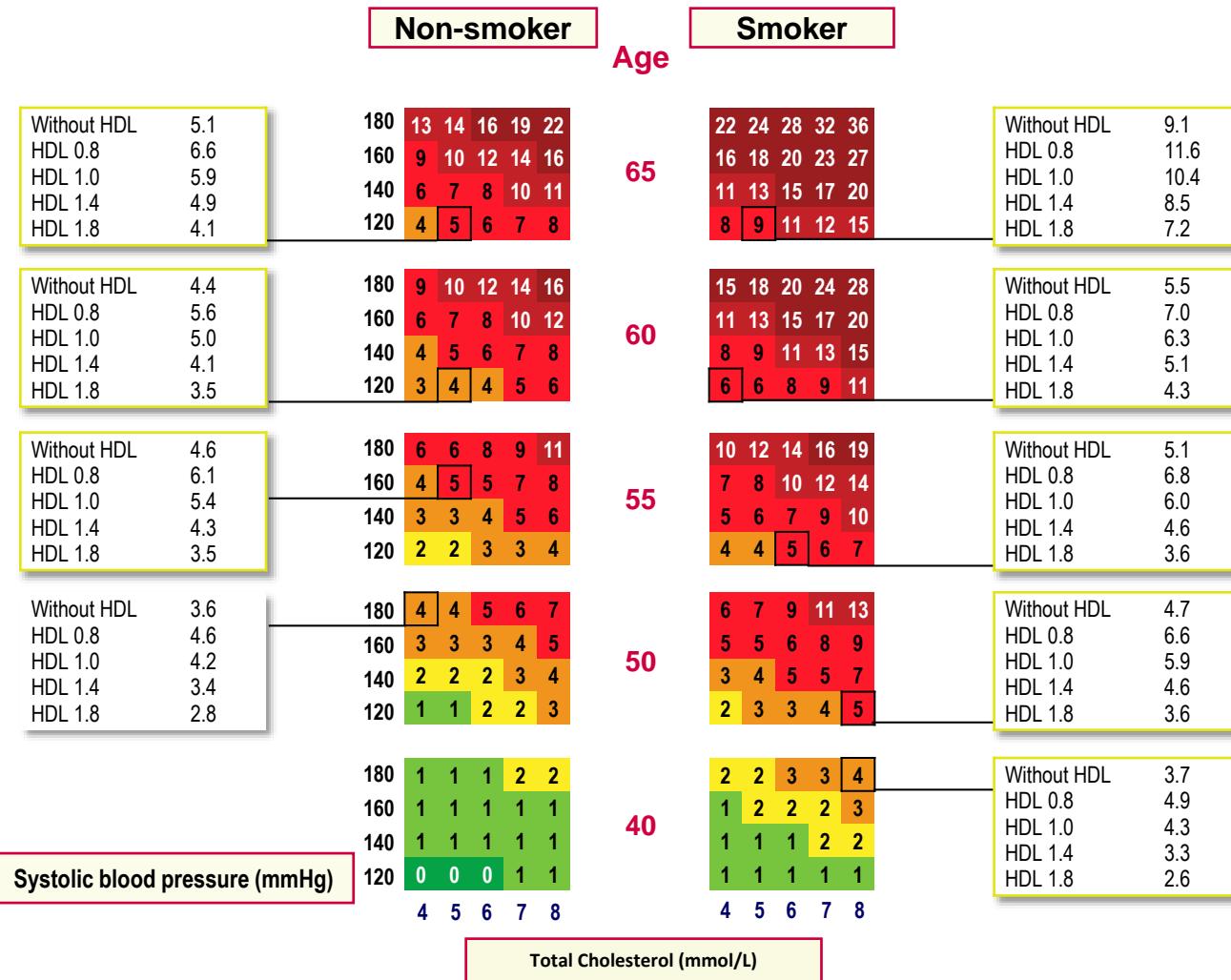


***HDL-C associated with lowest CV mortality***

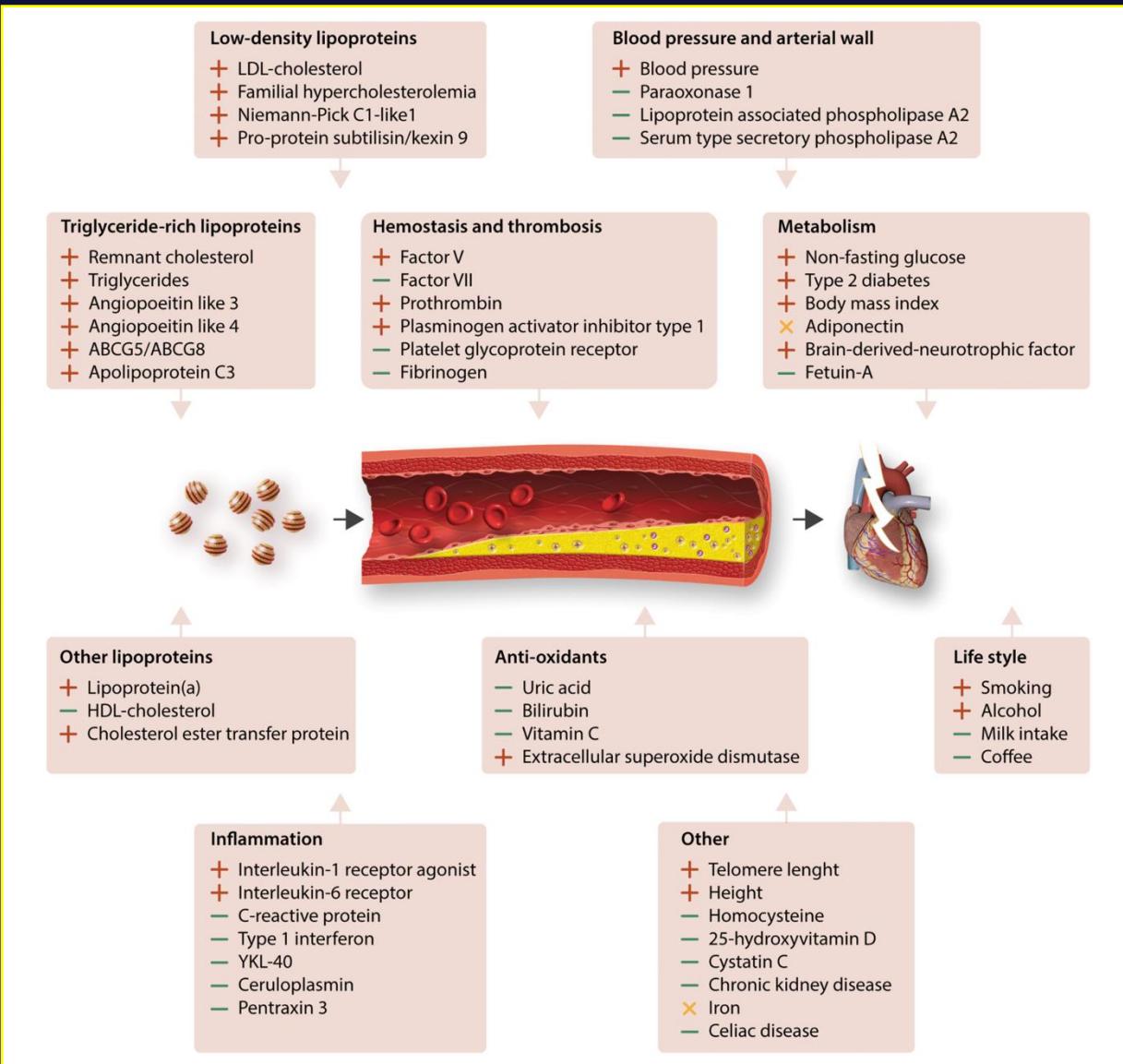
**Males : 50 – 80 mg/dL**

**Females : 70-90 mg/dL**

# Risk function without high-density lipoprotein-cholesterol (HDL-C) for men



# Overview of biomarkers and lifestyle factors examined for a causal effect on risk of cardiovascular disease using the Mendelian randomization design



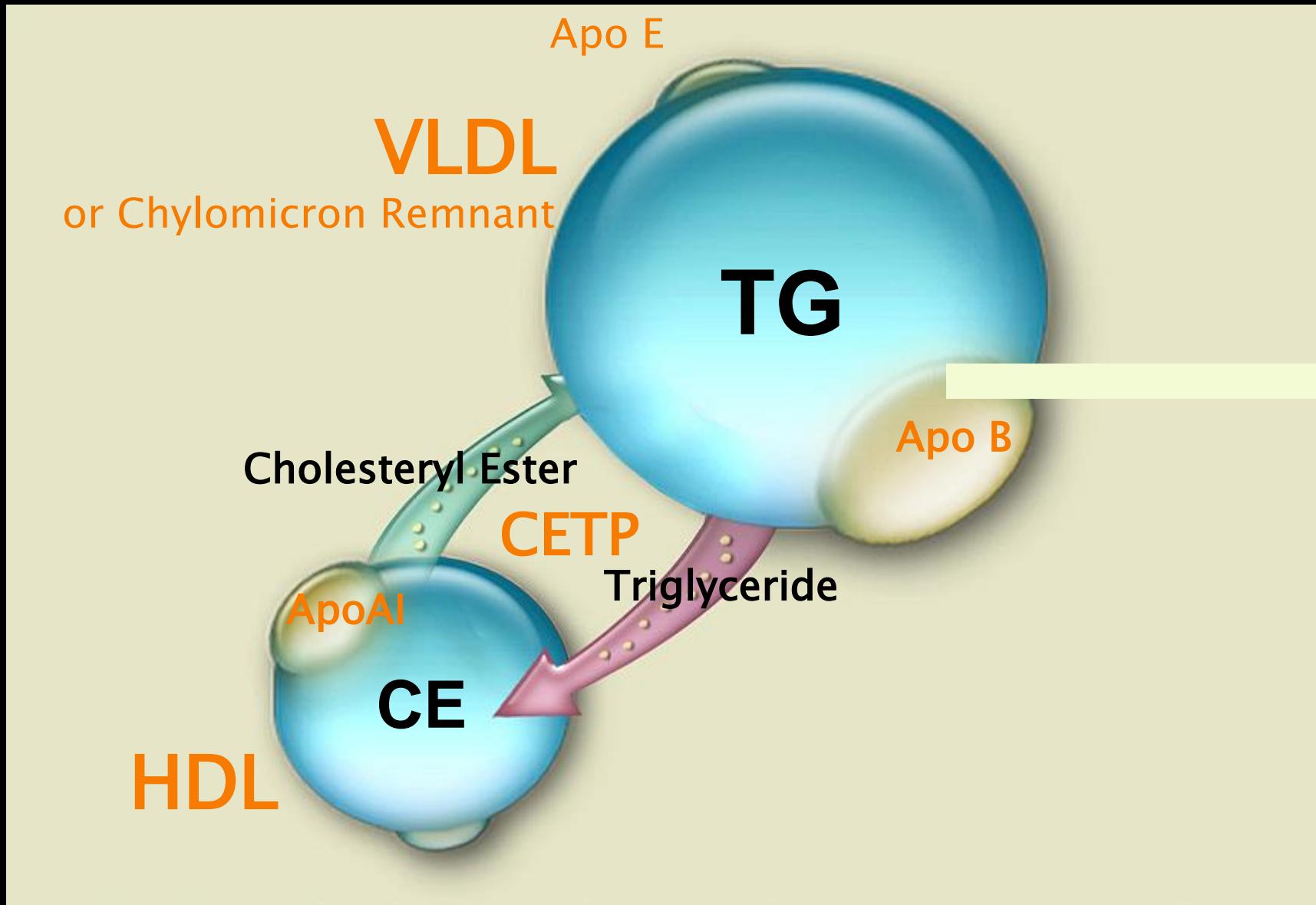
# Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study

	Chromosome	Gene(s) of interest within or near associated interval	Major allele, minor allele (minor allele frequency)*	Modelled allele	Effect of modelled allele on plasma HDL cholesterol (mmol/L)*	Effect of modelled allele on plasma triglycerides (mmol/L)*	Effect of modelled allele on plasma LDL cholesterol (mmol/L)*	Sample size (MI cases/MI-free controls)	For modelled allele, observed change in MI risk (%; 95% CI)	For modelled allele, p value for association with MI
rs17482753	8p21	LPL†	G, T (0.10)	T	0.08	-0.24	..	19 139/50 812	-12% (-16 to -7)	4x10 <sup>-7</sup> †
rs17321515	8q24	TRIB1†	A, G (0.45)	G	0.02	-0.11	-0.05	19 139/50 812	-7% (-9 to -4)	2x10 <sup>-8</sup> †
rs6589566	11q23	APOA1-APOC3-APOA4-APOA5†	A, G (0.07)	A	0.05	-0.27	-0.09	18 310/49 897	-10% (-15 to -5)	8x10 <sup>-7</sup> †
rs4846914	1q42	GALNT2†	A, G (0.40)	A	0.02	-0.03	..	19 139/50 812	-3% (-6 to -1)	0.02†
rs2967605	19p13	ANGPTL4†	C, T (0.16)	C	0.05	-0.07	..	13 595/16 423	-5% (-10 to -1)	0.03†
rs3764261	16q13	CETP†	C, A (0.32)	A	0.10	..	-0.03	16 503/46 576	-4% (-7 to 0)	0.04†
rs61755018 (Asn396Ser)	18q21	LIPG	A, G (0.015)	G	0.14‡	..	..	17 165/49 077	-6% (-18 to 9)	0.41
rs17145738	7q11	MLXIPL	C, T (0.11)	T	0.03	-0.15	..	19 139/50 812	-1% (-4 to 3)	0.61
rs3890182	9q31	ABCA1	G, A (0.14)	G	0.03	..	0.05	19 139/50 812	-1% (-5 to 4)	0.76
rs2338104	12q24	MMAB, MVK	G, C (0.46)	G	0.03	..	..	19 139/50 812	0% (-3 to 3)	0.85
rs471364	9p22	TTC39B	T, C (0.12)	T	0.03	..	..	15 693/47 098	0% (-5 to 5)	0.97
rs2271293	16q22	LCAT	G, A (0.11)	A	0.03	..	..	19 139/50 812	4% (-1 to 8)	0.10
rs174547	11q12	FADS1-FADS2-FADS3	T, C (0.33)	T	0.03	-0.06	..	19 139/50 812	3% (-1 to 6)	0.11
rs1800588	15q22	LIPC	C, T (0.22)	T	0.05	0.07	..	17 917/49 514	4% (0 to 7)	0.04
rs16988929	20q13	HNF4A	C, T (0.01)	T	0.01	..	..	17 041/20 137	31% (12 to 54)	9x10 <sup>-4</sup>

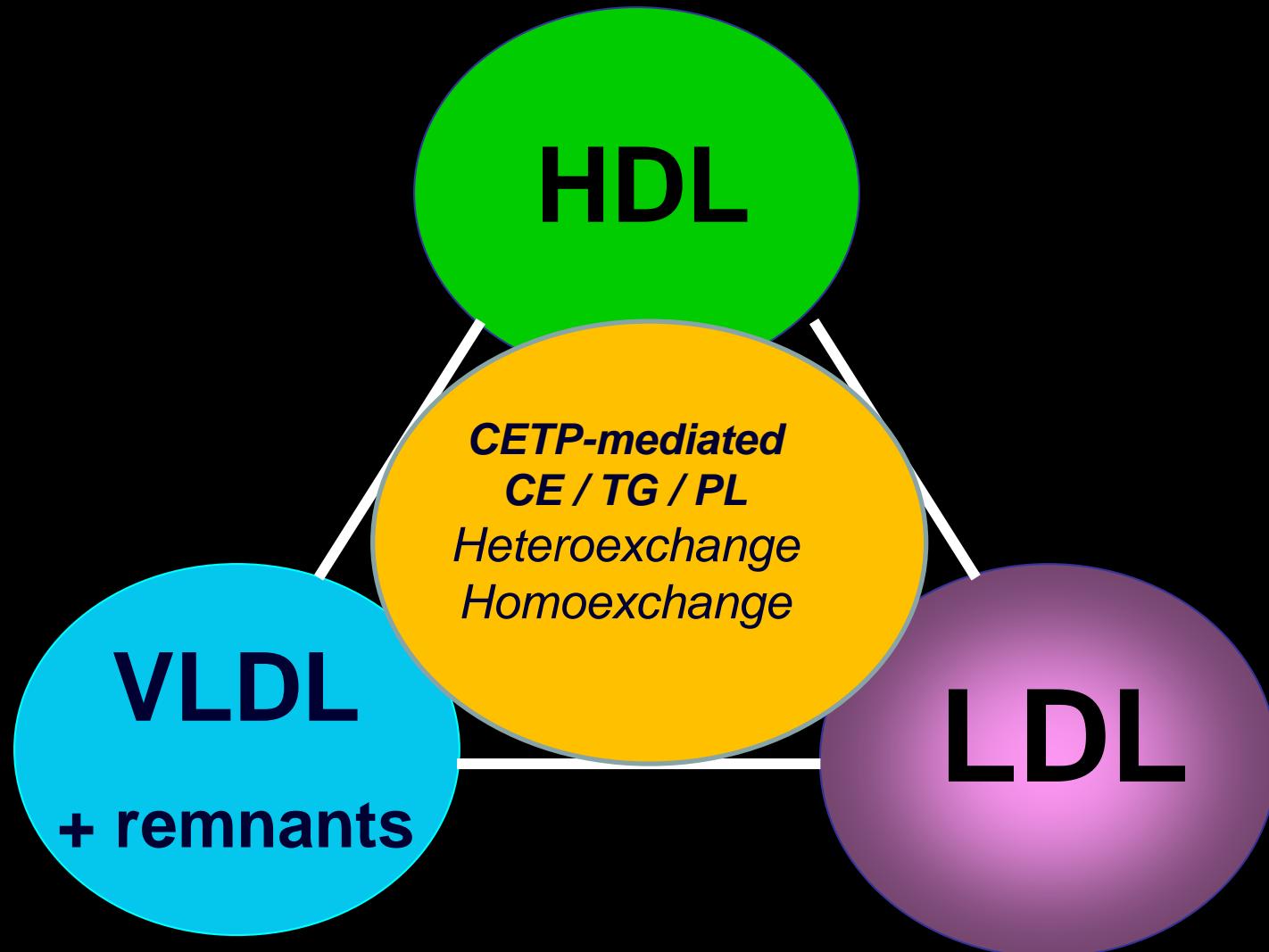
\*Data presented from a meta-analysis of seven cohorts (n up to 19 840) as presented in reference 16; the effect of each SNP on a lipid trait was modelled if the association of the SNP with a plasma lipid trait exceeded nominal significance ( $p<0.05$ ). †Loci and SNPs that exceeded nominal significance ( $p<0.05$ ) for association of modelled allele with MI; all modelled alleles increased HDL cholesterol. ‡Effect size presented is from the Atherosclerosis Risk in Communities Study.

Table 2: Association of myocardial infarction (MI) with single nucleotide polymorphisms (SNPs) previously found to relate to plasma HDL cholesterol

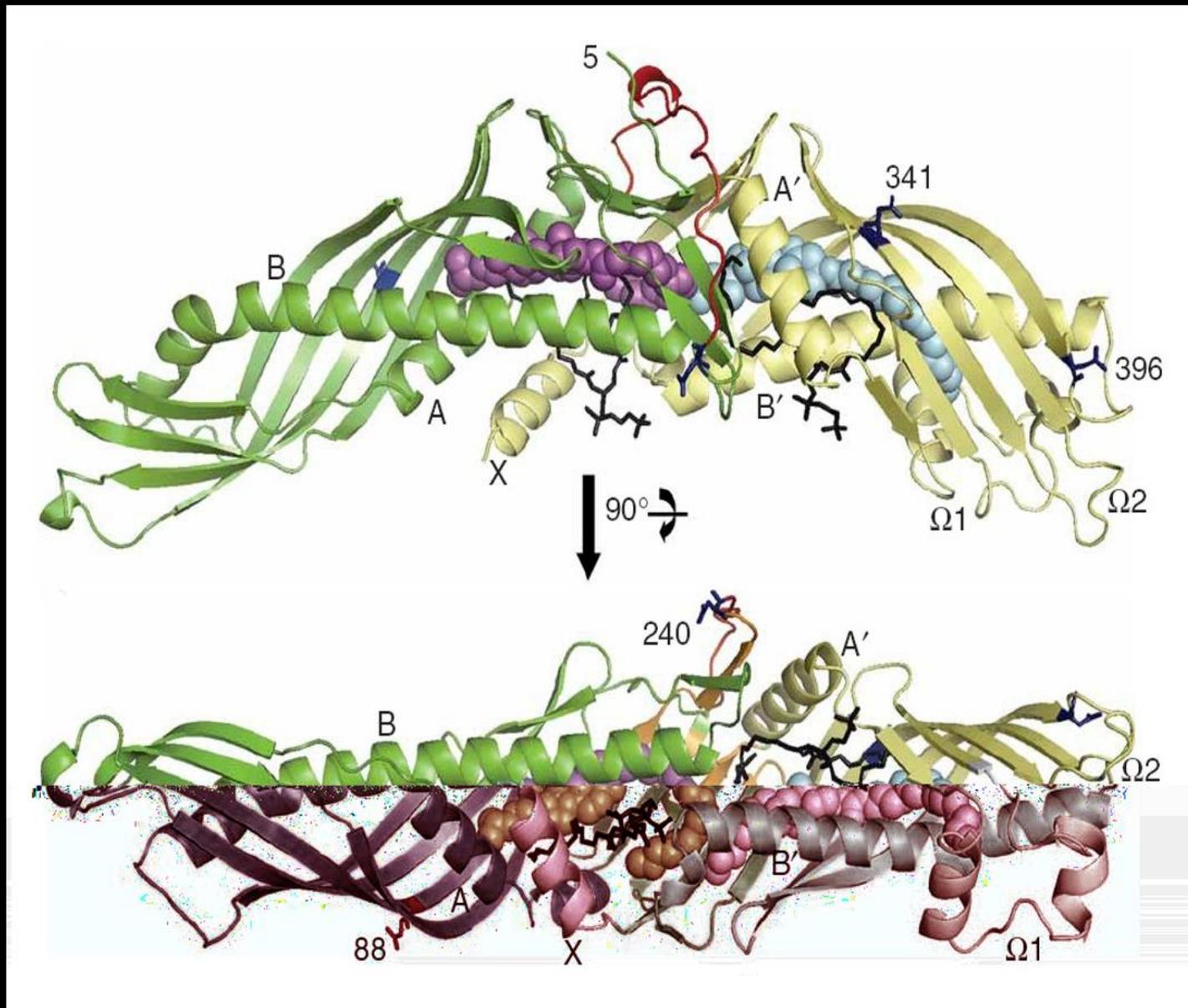
# Cholesteryl Ester Transfer Protein (CETP)



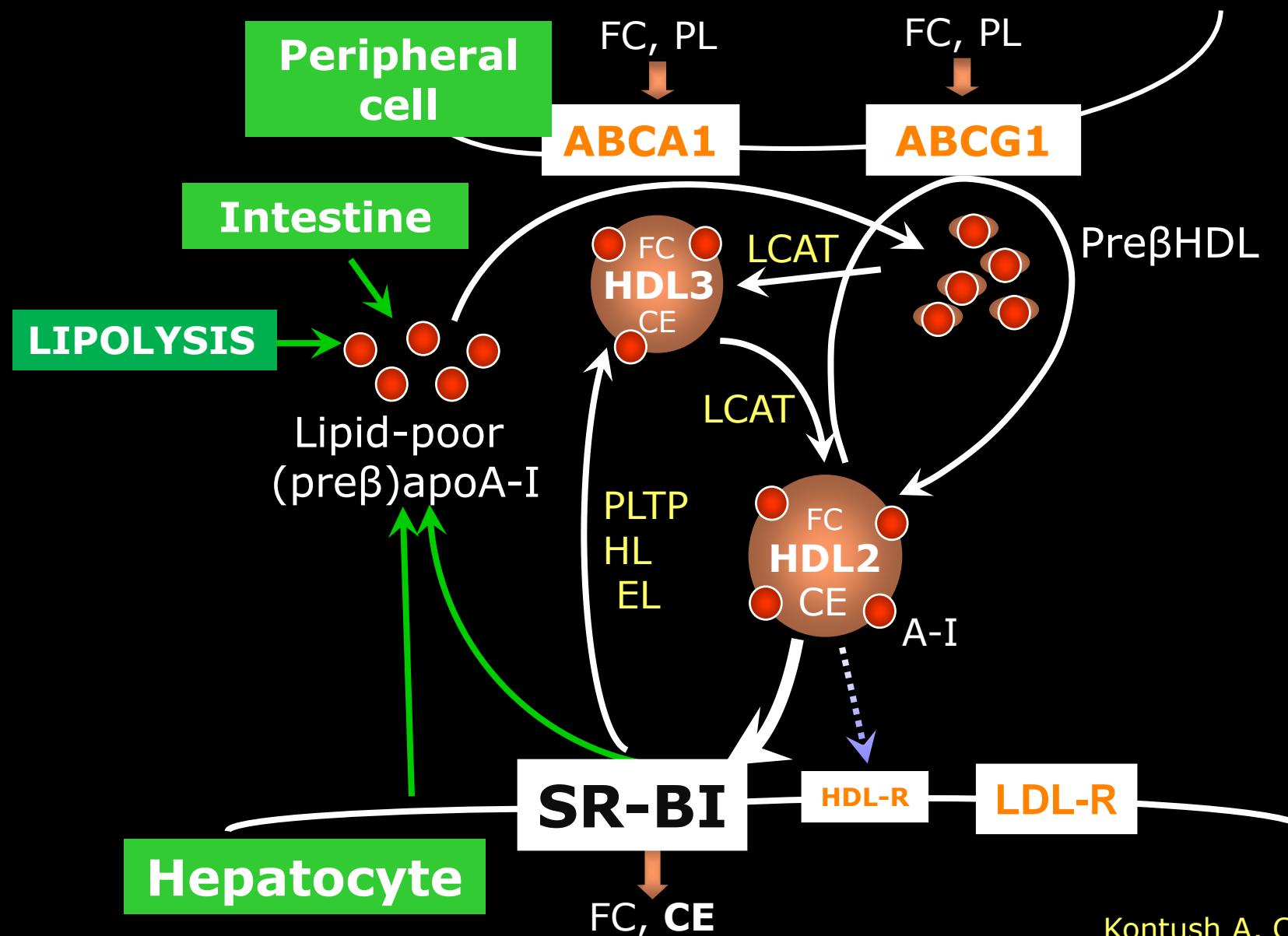
# CETP action



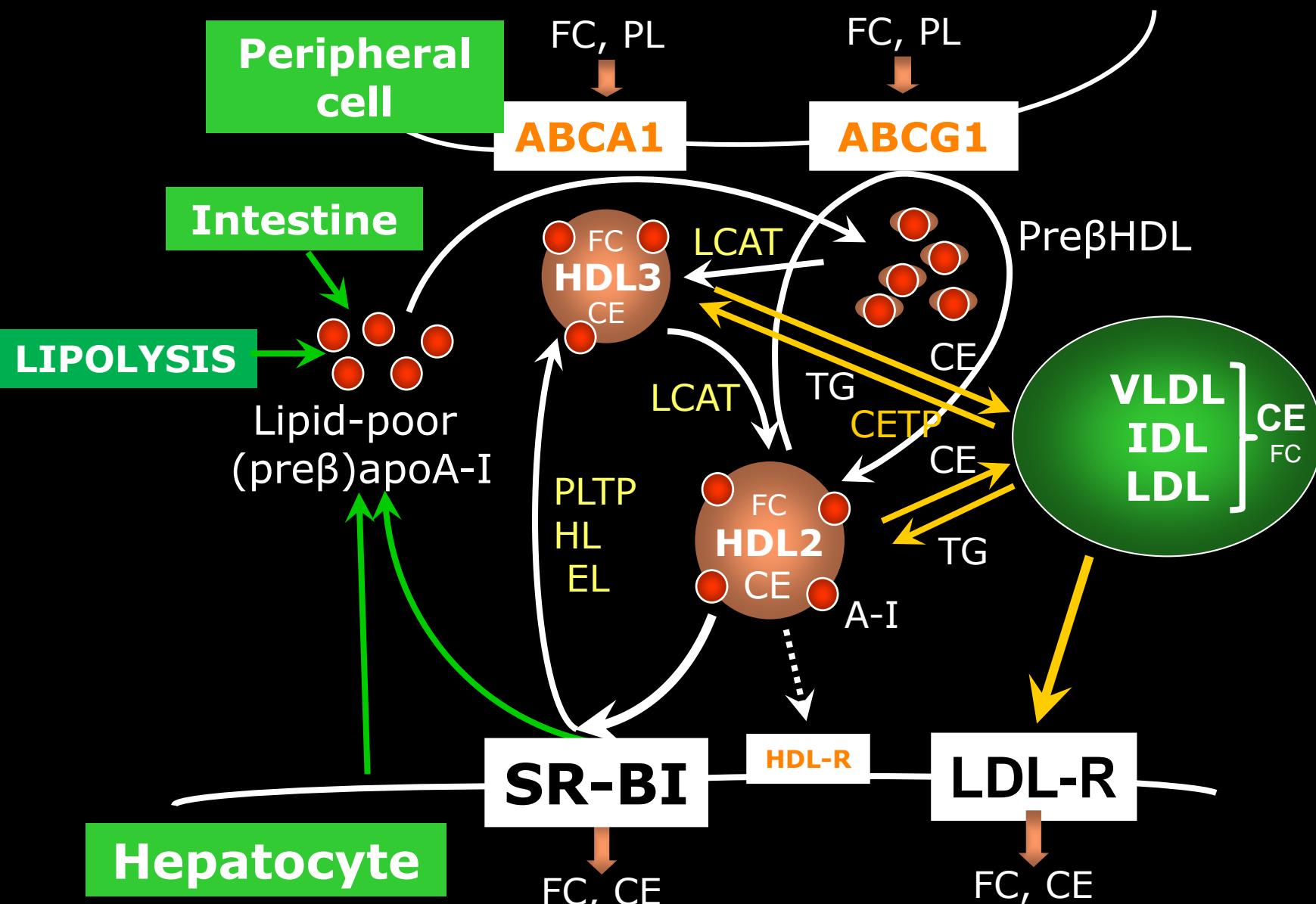
# Tertiary structure of CETP



# Formation and Metabolism of apoAI / HDL particles (RCT)



# Role of CETP

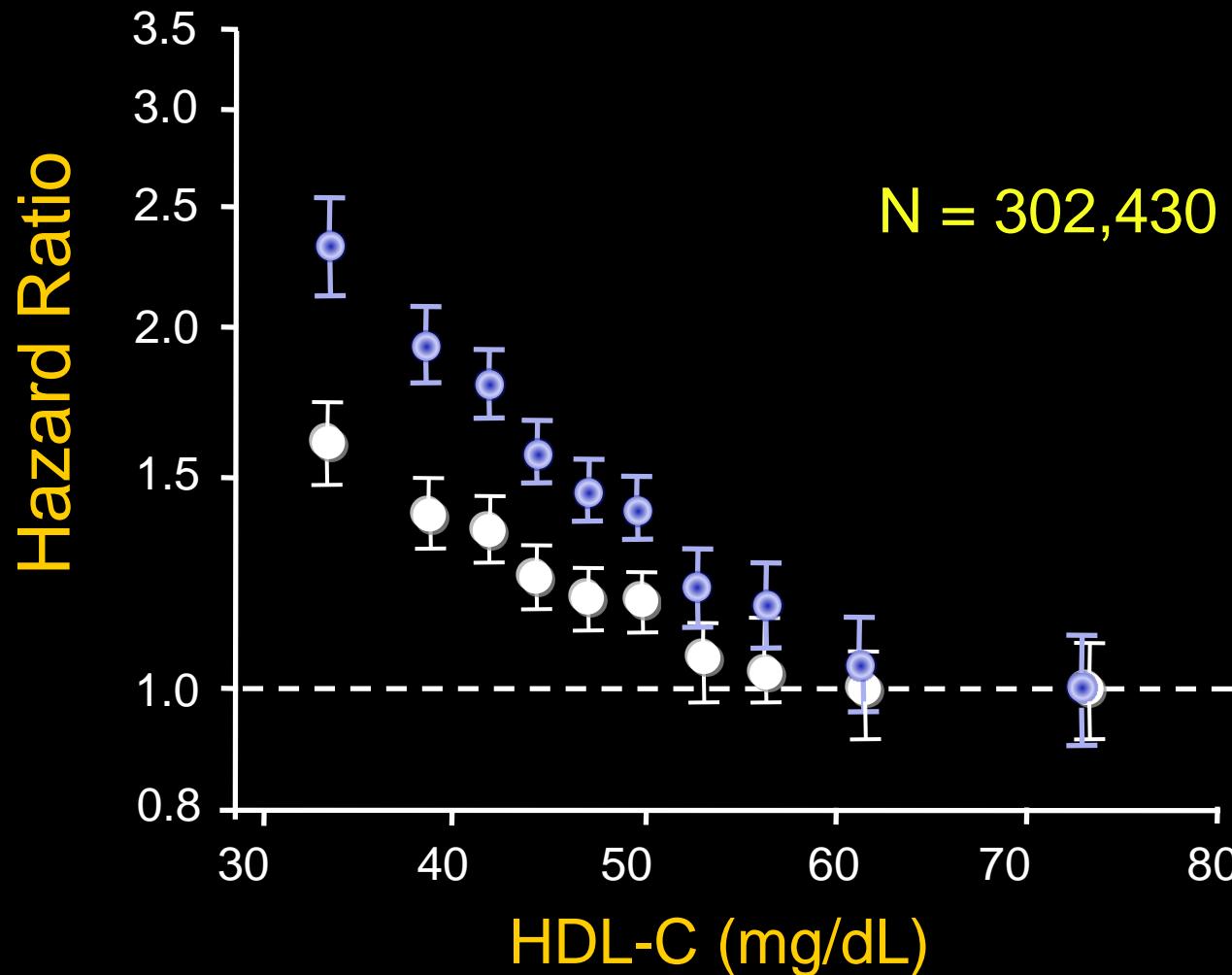


# CIRCA 2000 .....

It was widely accepted that  
subnormal levels of HDL-C  
were intimately linked with elevated CV risk

(causality ?)

# Coronary Heart Disease and Low HDL-C



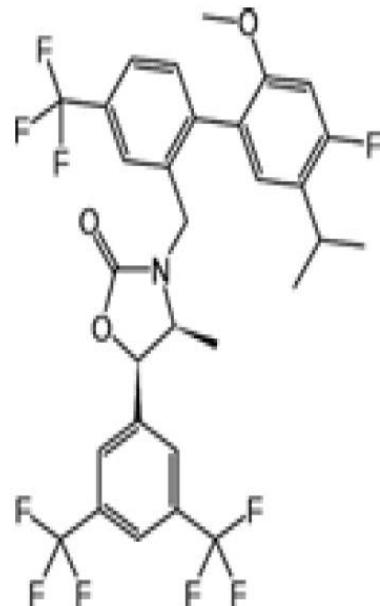
Lets raise HDL-C and reduce CV risk !!!

Lets inhibit CETP .....

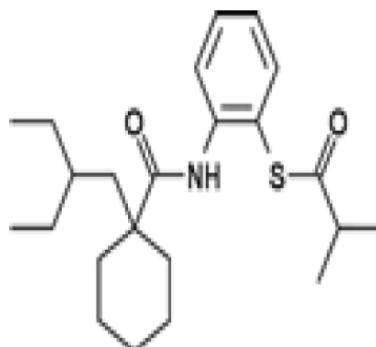
(Elevated CETP activity =  ASCVD risk)

# Chemical structures of CETP inhibitors

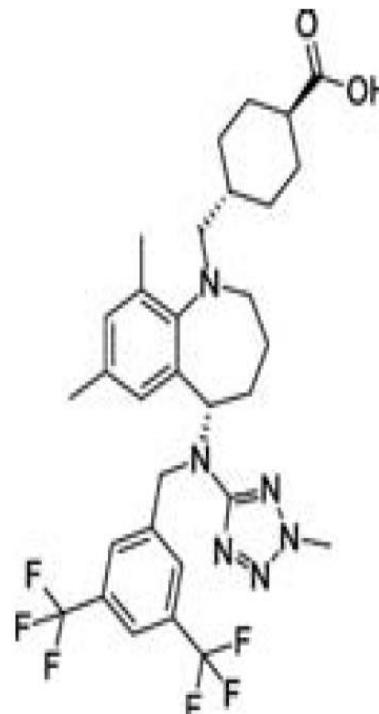
Anacetrapib



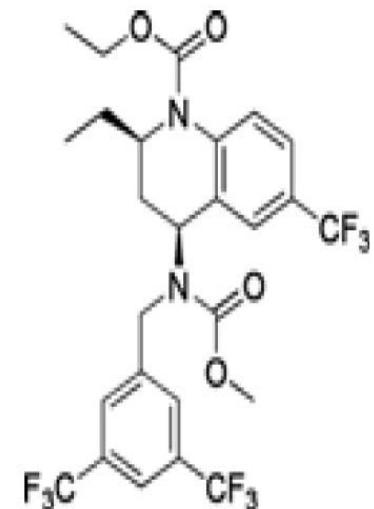
Dalcetrapib



Evacetrapib



Torcetrapib





The NEW ENGLAND  
JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease

The HPS3/TIMI55–REVEAL Collaborative Group\*

Available at [www.nejm.org](http://www.nejm.org)  
together with supplementary methods, analyses,  
and detailed tabulations of adverse events



# REVEAL trial design

Apixaban vs. Placebo in the REVEAL Trial  
A Randomized, Double-blind, Placebo-controlled Trial of Anacetrapib in Patients With Occlusive Vascular Disease (REVEAL) - A Pooled Analysis of Individual Patient Data From the REVEAL and REVEAL-CHINA Trials

Eligibility: 30,000 patients aged over 50 years with occlusive vascular disease

Background statin: Atorvastatin 20 or 80 mg daily (China: 10 or 20 mg)

Randomized: Anacetrapib 100 mg daily vs. matching placebo

Follow-up: ≥4 years and ≥1900 primary outcomes

Primary outcome: Major Coronary Event

(i.e. Coronary death, myocardial infarction, or coronary revascularization)

REVEAL Collaborative Group. Am Heart J 2017;187:182-90

# Baseline demographics

Characteristic	Total (30449)
Age (years)	Mean 67
Gender	Male 25534 (84%)
	Female 4915 (16%)
Region	Europe 15738 (52%)
	North America 6082 (20%)
	China 8629 (28%)

# Effects of anacetrapib on lipids at trial midpoint

ApoE-TIMI55  
REVEAL

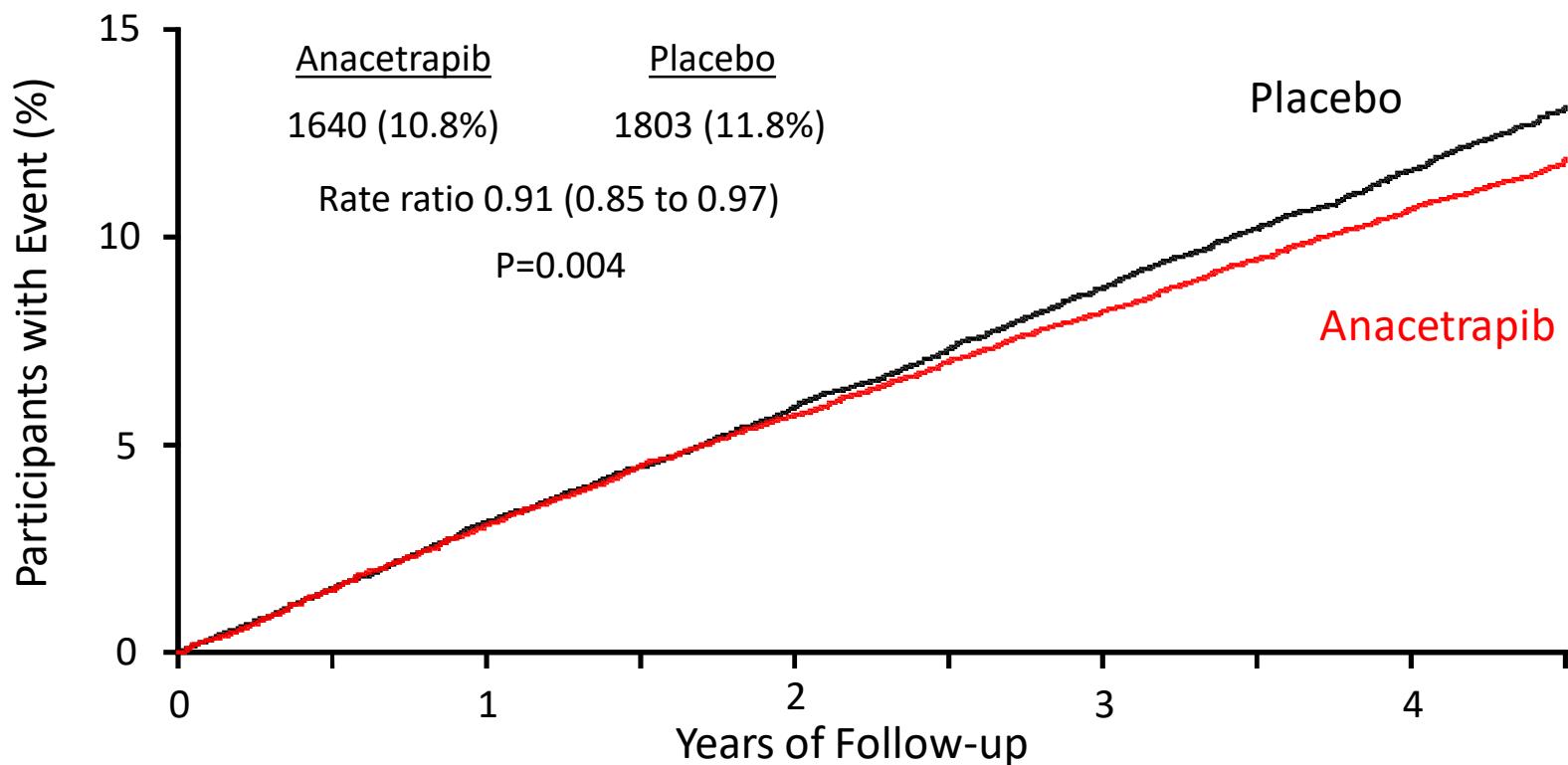
Measurement	Absolute difference		Proportional difference
	mg/dL	SI units	
HDL cholesterol	+43	+1.1 mmol/L	104%
Apolipoprotein AI	+42	+0.4 g/L	36%
LDL cholesterol			
- Direct (Genzyme)	-26	-0.7 mmol/L	-41%
- Beta-quantification*	-11	-0.3 mmol/L	-17%
Apolipoprotein B	-12	-0.1 g/L	-18%

\* measured in a random subset of 2000 participants

# Primary outcome: Major coronary events

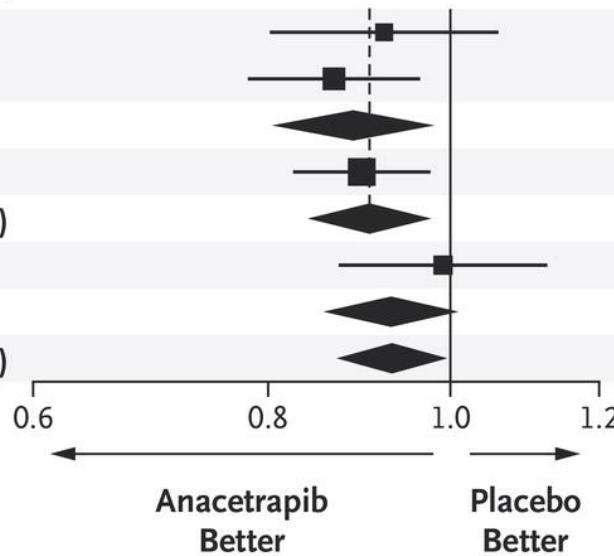
4p3-TIMI55  
REVEAL

(Coronary death, myocardial infarction, or coronary revascularization)

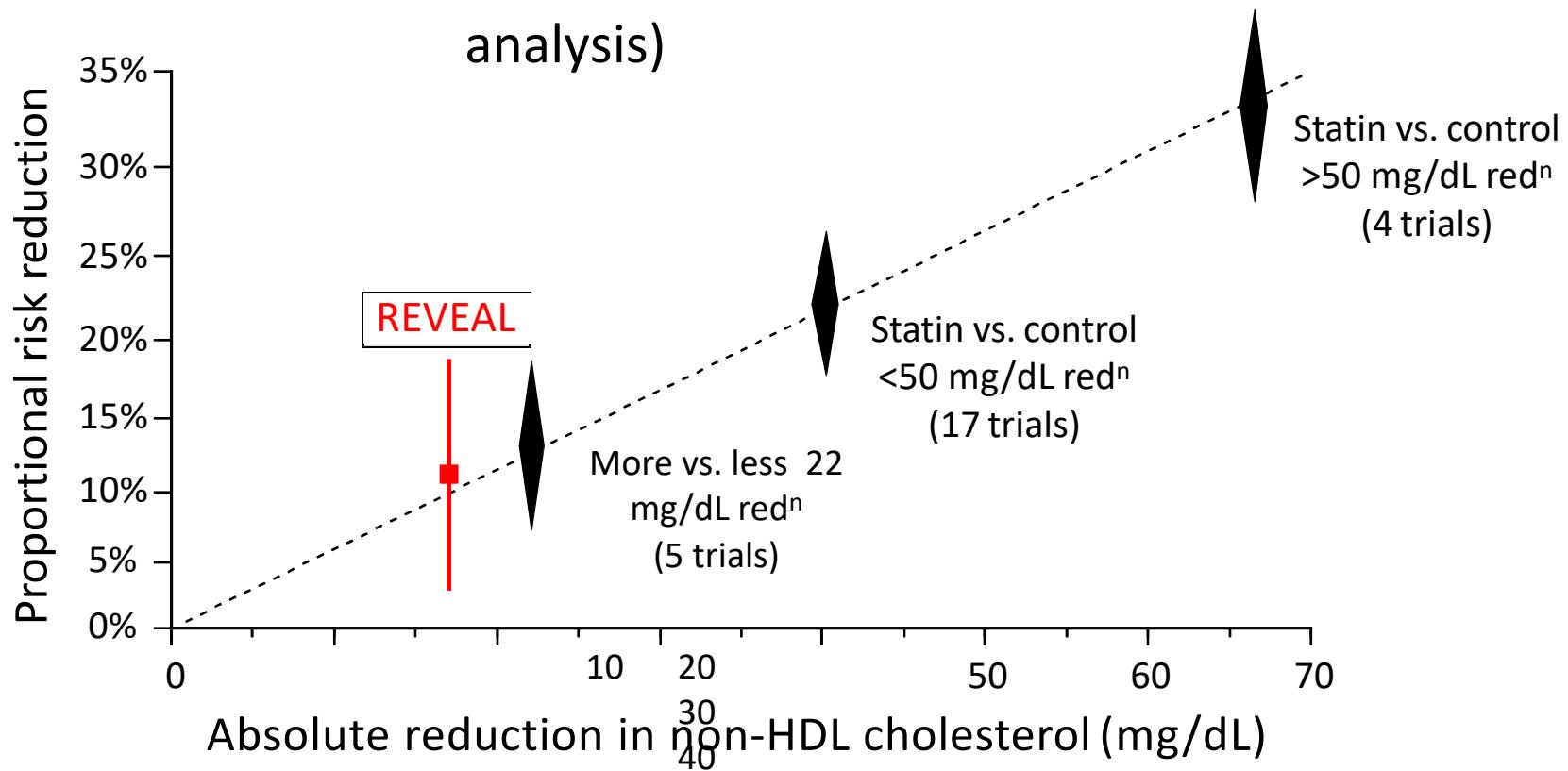


# The REVEAL Trial

Type of Event	Anacetrapib (N=15,225)	Placebo (N=15,224)	Rate Ratio (95% CI)	P Value
<i>no. of patients with event (%)</i>				
Coronary death	388 (2.5)	420 (2.8)	0.92 (0.80–1.06)	0.25
MI	669 (4.4)	769 (5.1)	0.87 (0.78–0.96)	0.007
<b>Coronary death or MI</b>	<b>934 (6.1)</b>	<b>1048 (6.9)</b>	<b>0.89 (0.81–0.97)</b>	<b>0.008</b>
Coronary revascularization	1081 (7.1)	1201 (7.9)	0.90 (0.83–0.97)	0.01
<b>Major coronary event</b>	<b>1640 (10.8)</b>	<b>1803 (11.8)</b>	<b>0.91 (0.85–0.97)</b>	<b>0.004</b>
Presumed ischemic stroke	485 (3.2)	489 (3.2)	0.99 (0.87–1.12)	NA
<b>Major atherosclerotic event</b>	<b>1383 (9.1)</b>	<b>1483 (9.7)</b>	<b>0.93 (0.86–1.00)</b>	<b>0.052</b>
<b>Major vascular event</b>	<b>2068 (13.6)</b>	<b>2214 (14.5)</b>	<b>0.93 (0.88–0.99)</b>	<b>0.02</b>



# Proportional reduction in Coronary death or MI vs. absolute reduction in non-HDL cholesterol (derived from published CTT meta-analysis)



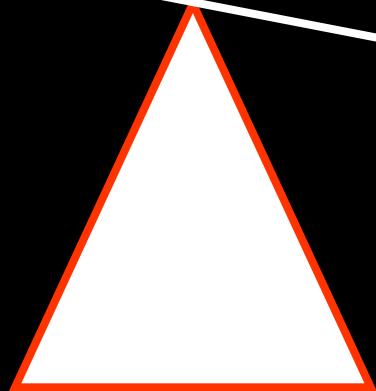
## Effects of adding anacetrapib to intensive statin therapy

- Significant 9% proportional reduction in major coronary events (effect appears to be greater in later years of treatment)
- Small reduction in risk of new-onset diabetes mellitus
- No excess of symptomatic side-effects with anacetrapib (levels in adipose tissue rise with continued treatment)
- No excess of mortality, cancer or other serious adverse events (small increase in BP and small reduction in kidney function)
- Post-trial follow-up of all consenting participants (off-drug) to assess longer-term efficacy and safety of anacetrapib

# Benefit / Risk ratio for Anacetrapib in the REVEAL trial

**Adverse Events**  
SBP; eGFR;  
**Adipose tissue  
accumulation**

9% RRR for primary  
endpoint (MACE)



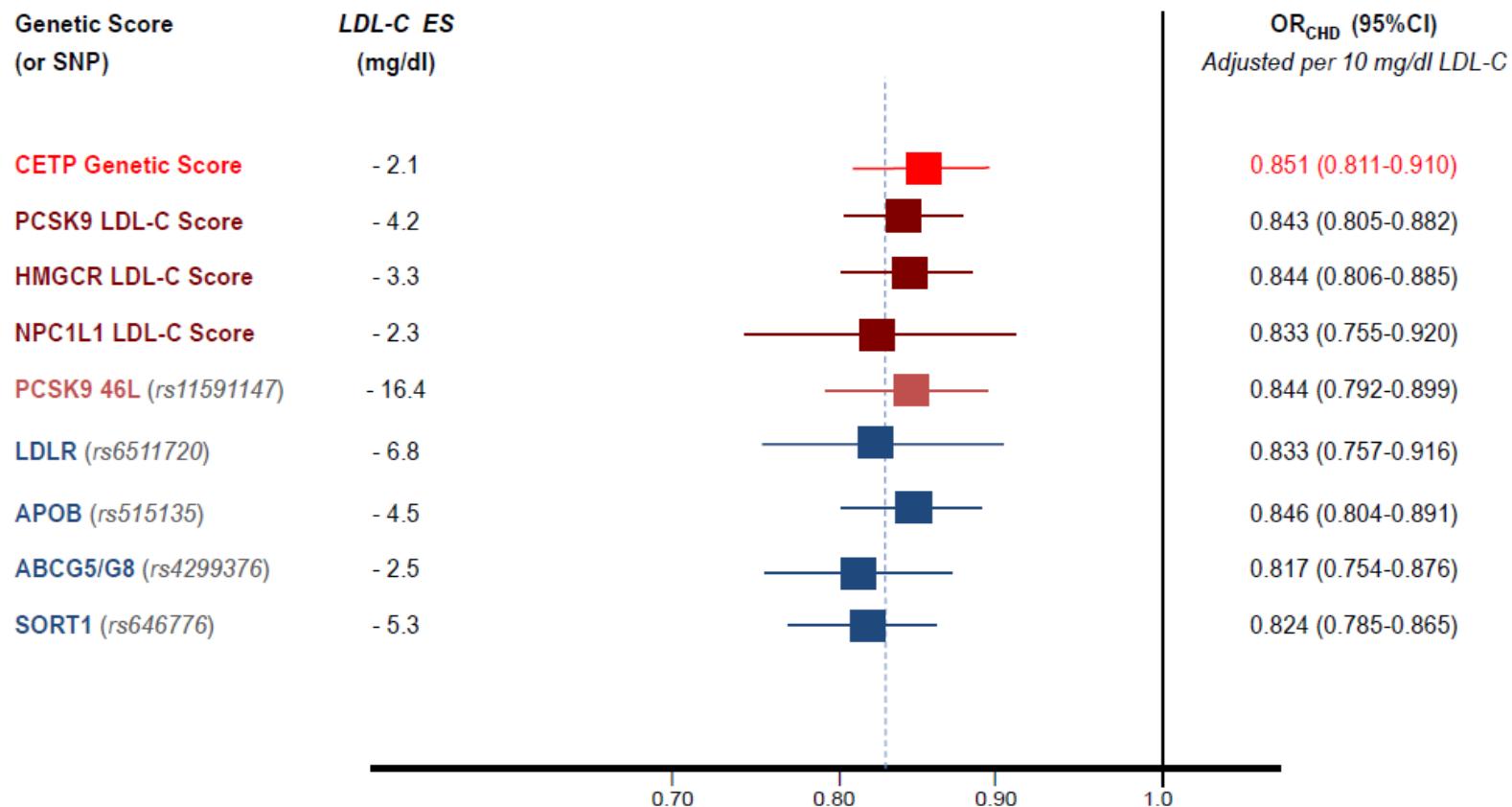
For the first time, inhibition of the CETP mechanism has translated into clinical benefit

Is there still a place for a CETP inhibitor in our therapeutic arsenal for lipid management, and if so, with what pharmacological profile, matched to which patient population ?

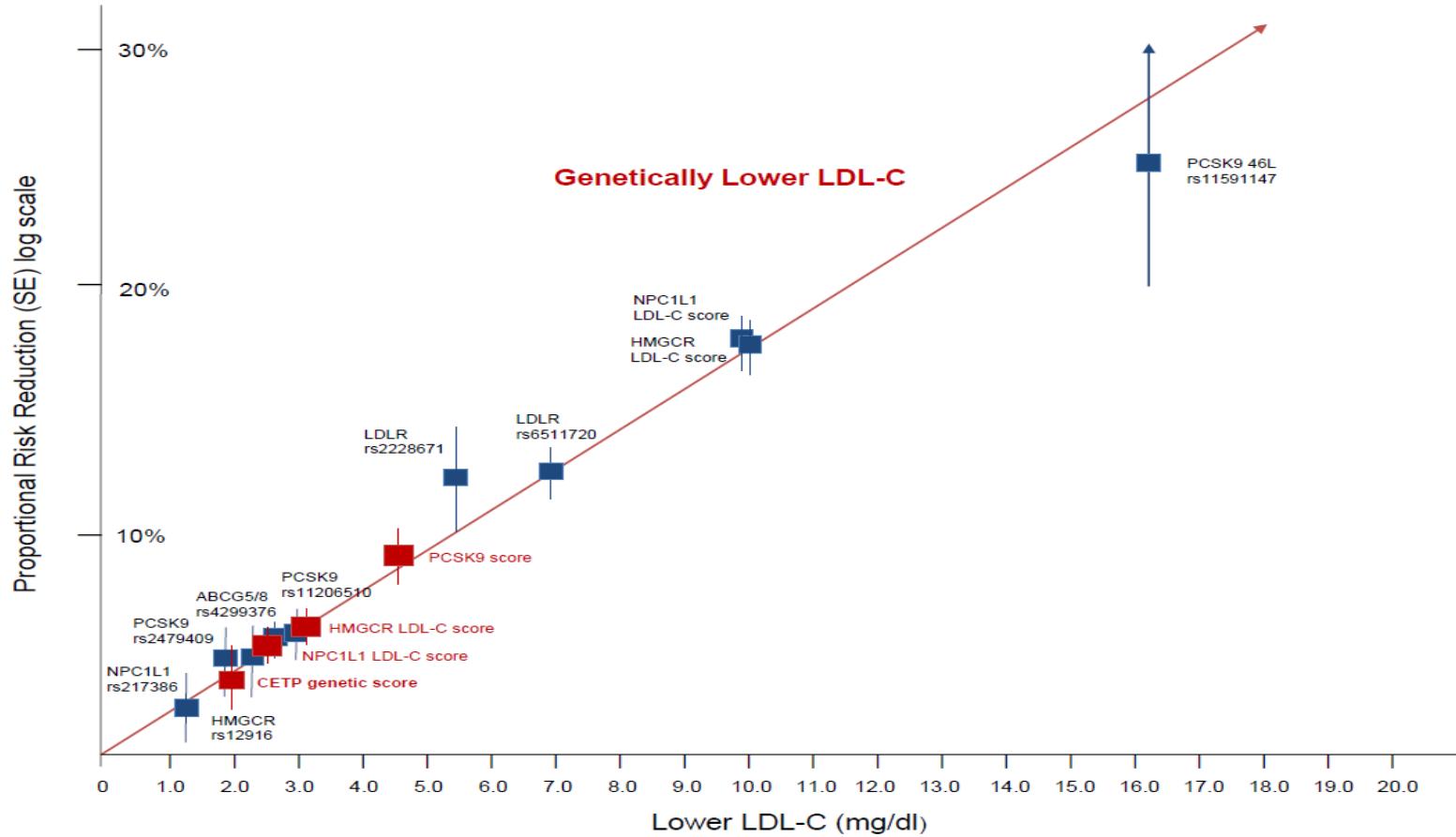
# Of 15 variants that alter HDL-C, 6 also affect MI risk

Gene(s) of interest within or near associated interval	Major allele, minor allele (minor allele frequency)*	Modelled allele	Effect of modelled allele on plasma HDL cholesterol (mmol/L)*	Effect of modelled allele on plasma triglycerides (mmol/L)*	Effect of modelled allele on plasma LDL cholesterol (mmol/L)*	Sample size (MI cases/MI-free controls)	For modelled allele, observed change in MI risk (%; 95% CI)	For modelled allele, p value for association with MI
LPL†	G, T (0·10)	T	0·08	-0·24	..	19 139/50 812	-12% (-16 to -7)	4×10 <sup>-7</sup>
TRIB1†	A, G (0·45)	G	0·02	-0·11	-0·05	19 139/50 812	-7% (-9 to -4)	2×10 <sup>-6</sup> †
APOA1-APOC3-APOA4-APOA5†	A, G (0·07)	A	0·05	-0·27	-0·09	18 310/49 897	-10% (-15 to -5)	8×10 <sup>-5</sup> †
GALNT2†	A, G (0·40)	A	0·02	-0·03	..	19 139/50 812	-3% (-6 to -1)	0·02†
ANGPTL4†	C, T (0·16)	C	0·05	-0·07	..	13 595/16 423	-5% (-10 to -1)	0·03†
CETP†	C, A (0·32)	A	0·10	..	-0·03	16 503/46 576	-4% (-7 to 0)	0·04†
LIPG	A, G (0·015)	G	0·14‡	..	..	17 165/49 077	-6% (-18 to 9)	0·41
MLXIPL	C, T (0·11)	T	0·03	-0·15	..	19 139/50 812	-1% (-4 to 3)	0·61
ABCA1	G, A (0·14)	G	0·03	..	0·05	19 139/50 812	-1% (-5 to 4)	0·76
MMAB, MVK	G, C (0·46)	G	0·03	..	..	19 139/50 812	0% (-3 to 3)	0·85
TTC39B	T, C (0·12)	T	0·03	..	..	15 693/47 098	0% (-5 to 5)	0·97
LCAT	G, A (0·11)	A	0·03	..	..	19 139/50 812	4% (-1 to 8)	0·10
FADS1-FADS2-FADS3	T, C (0·33)	T	0·03	-0·06	..	19 139/50 812	3% (-1 to 6)	0·11
UPC	C, T (0·22)	T	0·05	0·07	..	17 917/49 514	4% (0 to 7)	0·04
HNF4A	C, T (0·01)	T	0·01	..	..	17 041/20 137	31% (12 to 54)	9×10 <sup>-4</sup>

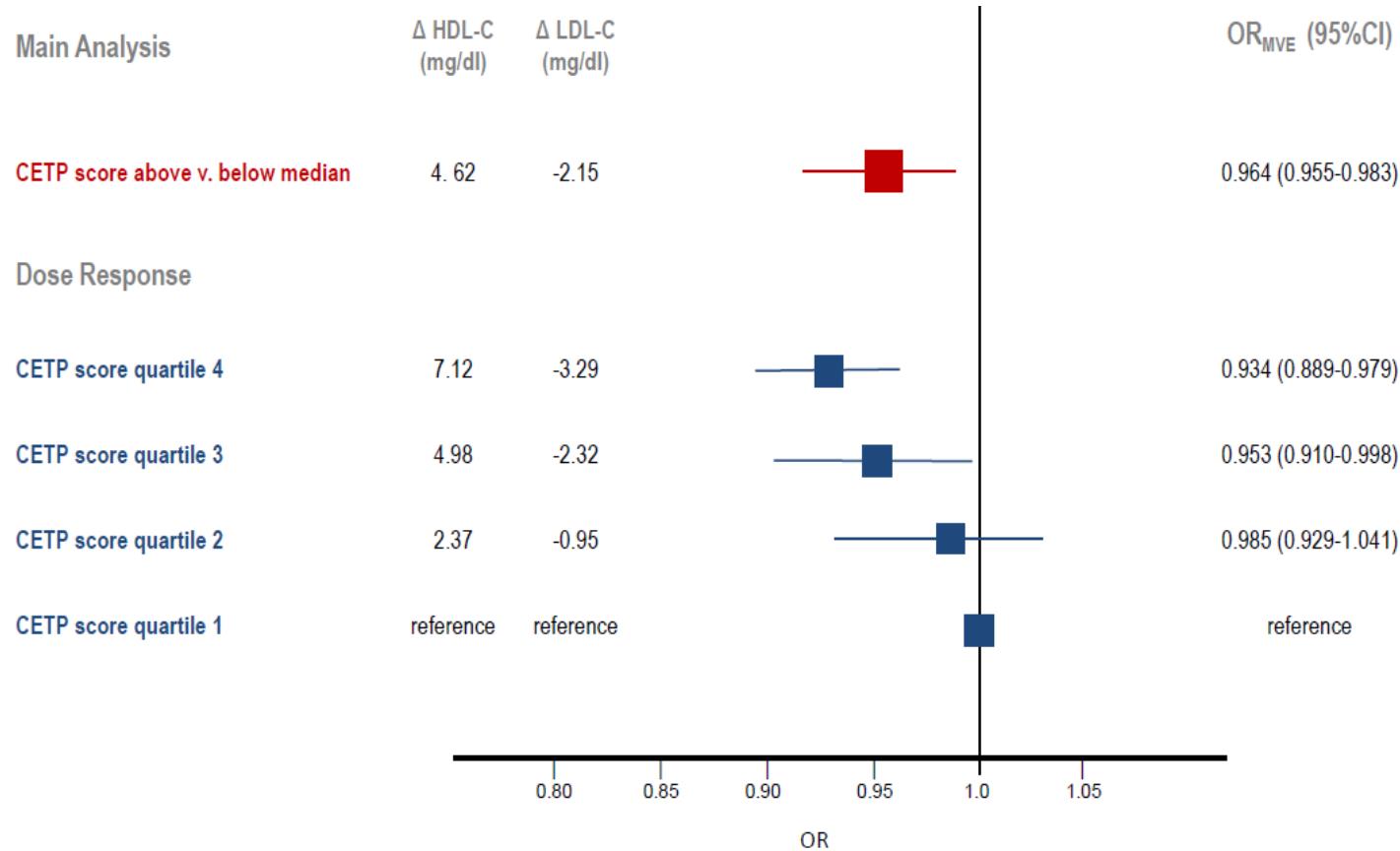
# Comparison of effect of CETP score and LDL-C variants on risk of CHD per unit change in LDL-C



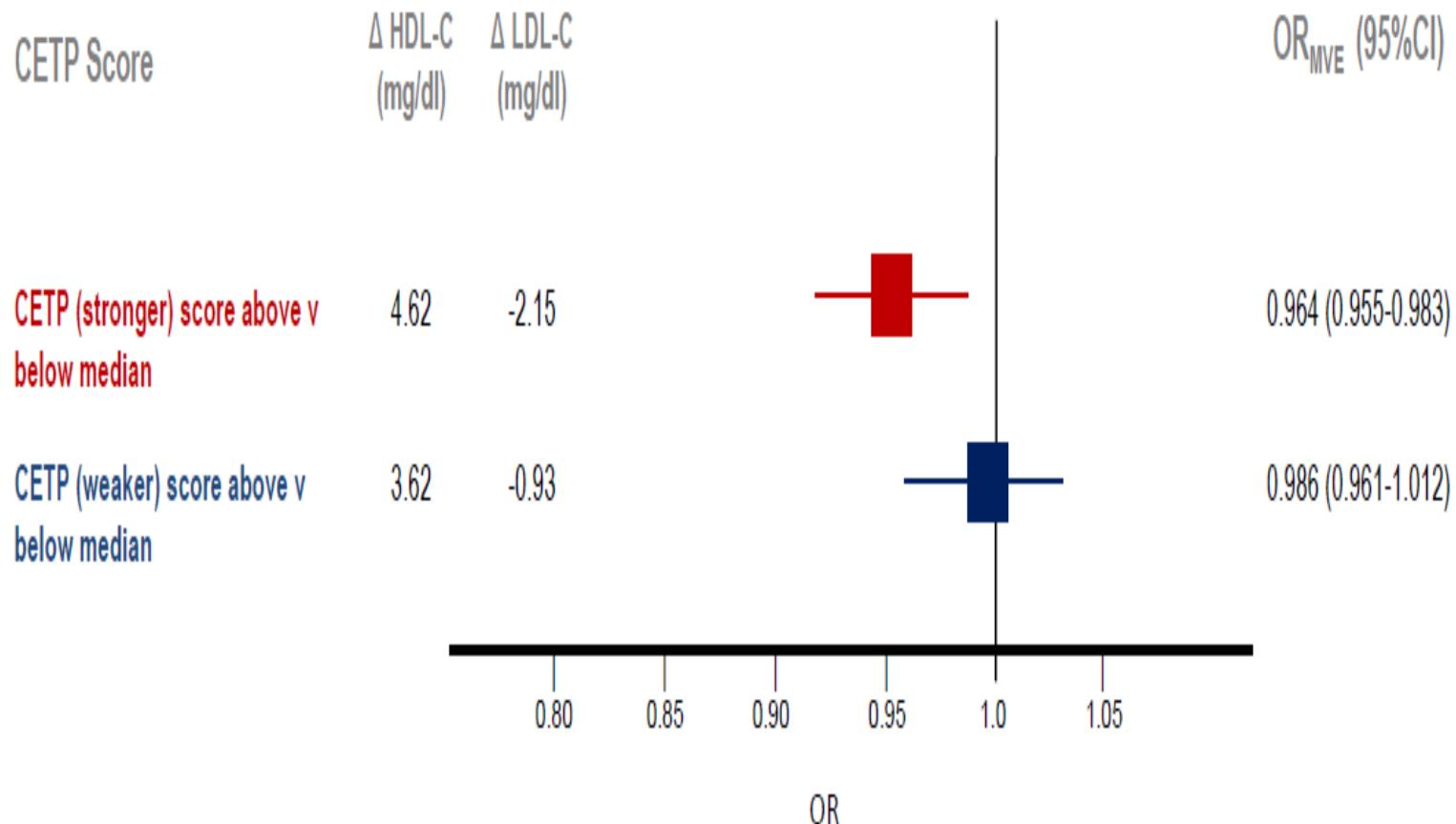
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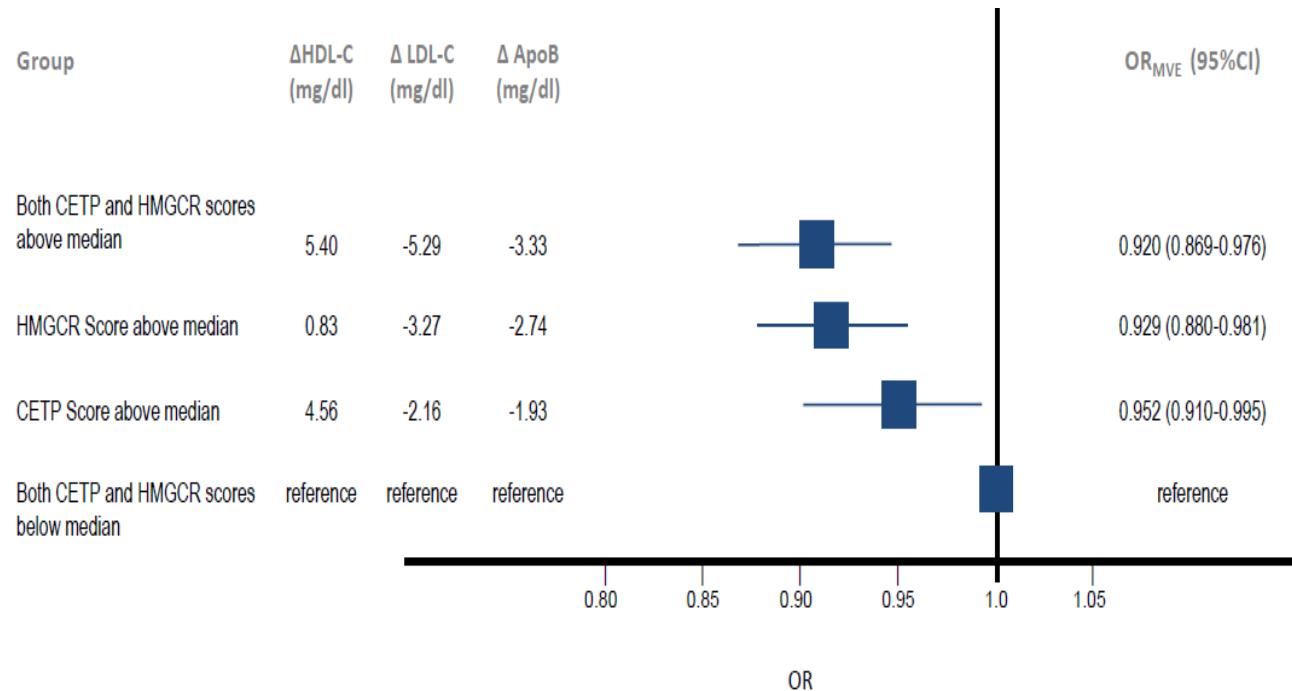
# Association of CETP score with risk of Major Vascular Events



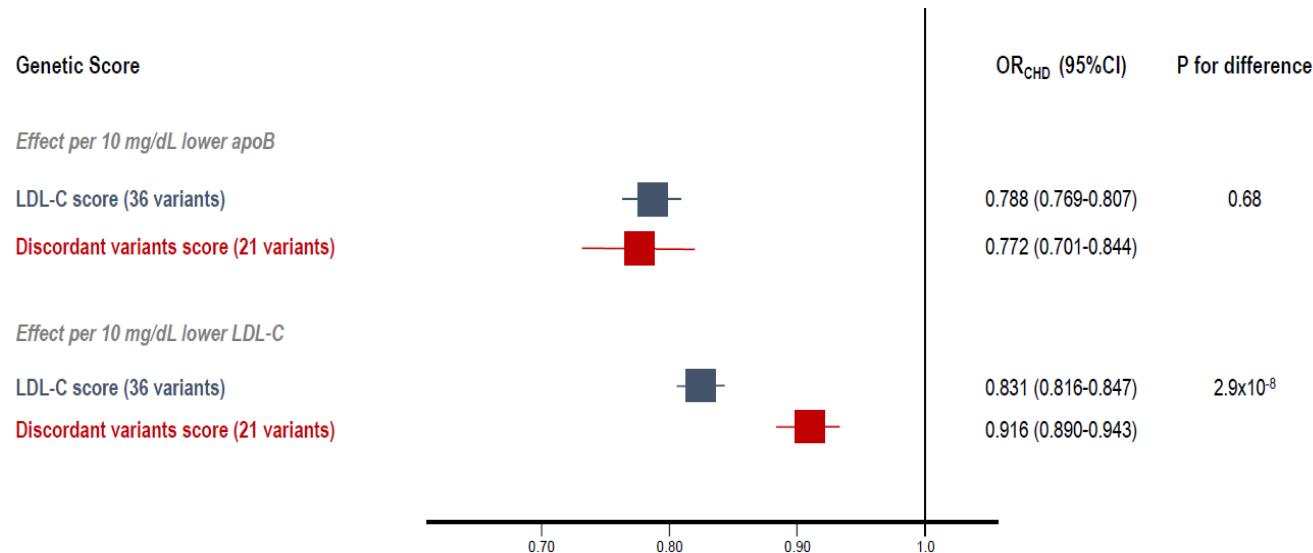
# Comparison of stronger and weaker CETP scores



## 2x2 Factorial Analysis of *CETP* and *HMGCR* genetic scores



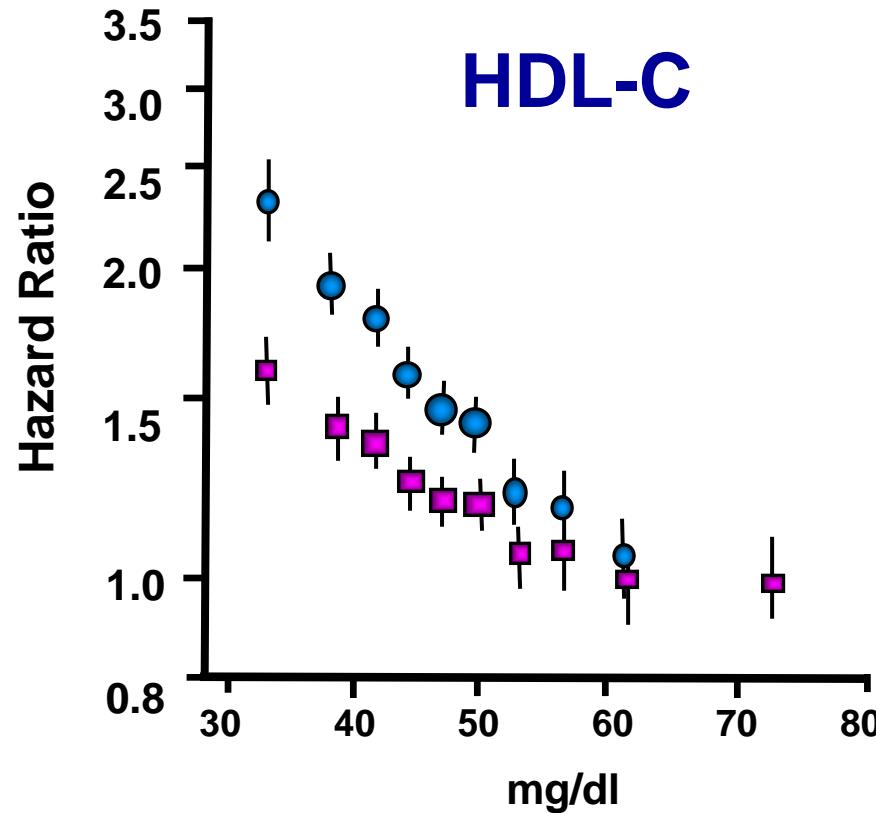
# Association of genetic variants with naturally occurring discordance between changes in LDL-C and apoB and the risk of CHD 2x2 Factorial Analysis of *CETP* and *HMGCR* genetic scores



JAMA. 2017;318(10):947-956

# Hazard Ratios for CHD Across Quantiles of HDL-Cholesterol

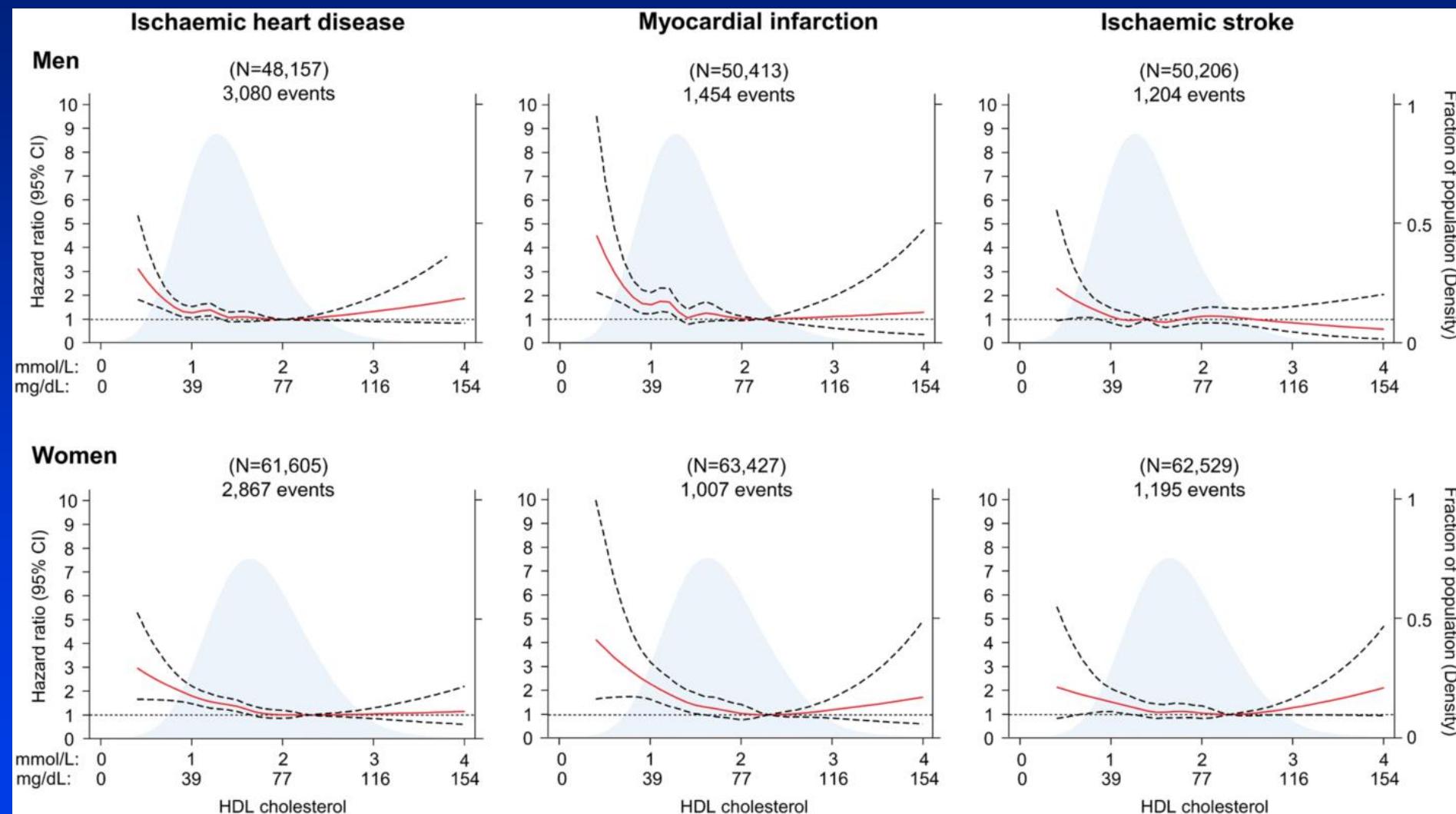
● Adjusted for age and sex only      ■ Further adjusted for several risk factors



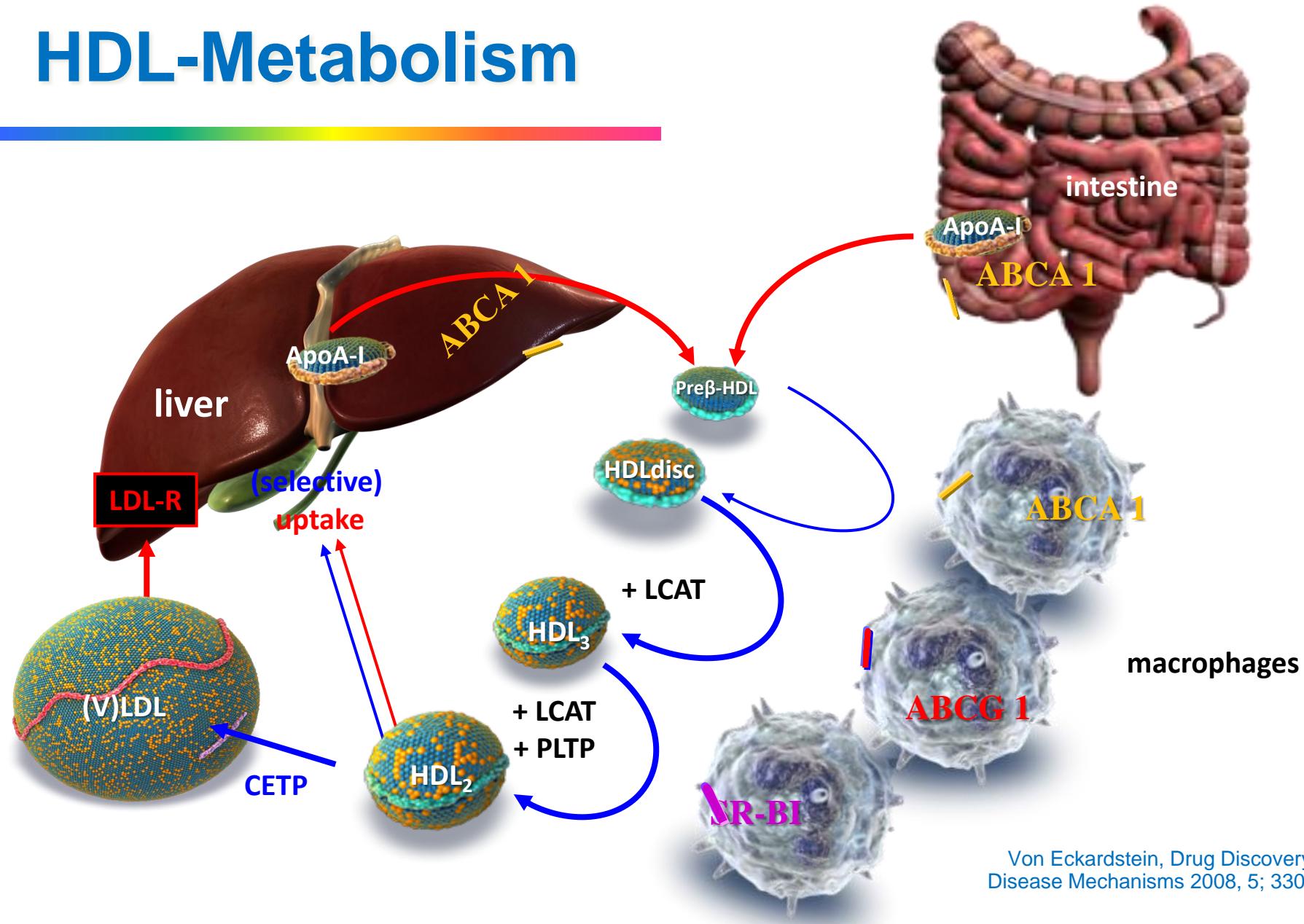
From the Emerging Risk Factors Collaboration (68 studies in 302 430 participants)

# HDL-C and Cardiovascular Disease

## Copenhagen Studies

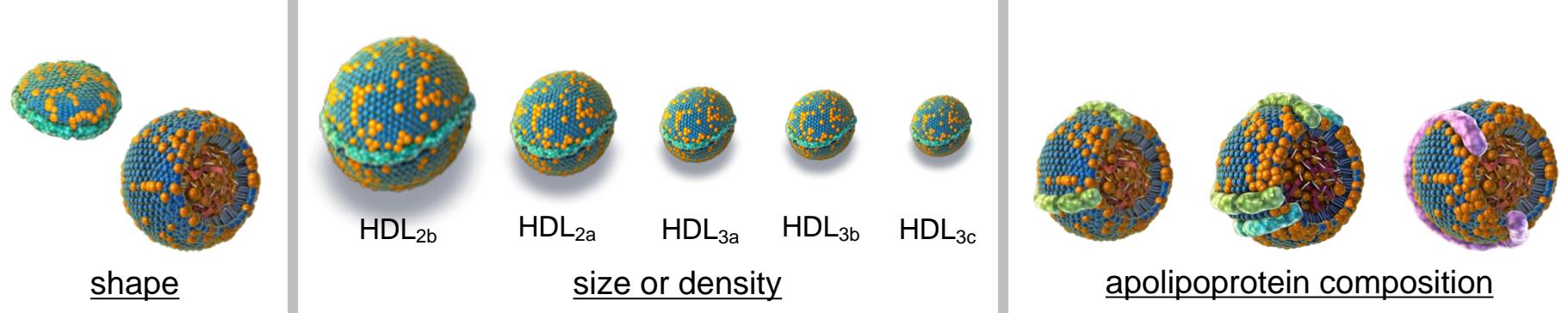


# HDL-Metabolism



Von Eckardstein, Drug Discovery Today  
Disease Mechanisms 2008, 5; 3305-e324.

## HDLs consist of many subclasses and carry many components



### > 80 proteins or peptides

Apolipoproteins and lipocalins: apoA-I, apoA-II, apoA-IV apoA-V, apoC-I, apoC-II, apoC-III, apoC-IV, apoD, apoE, apoF, apoH, apoJ, apoL-I, apoM

Enzymes: LCAT, CETP, PLTP, PON1, PON3, GPx, Lp-PLA<sub>2</sub>, MPO

Other proteins: SAA1, SAA2, SAA4, Coe, Hp, Hb, HRP, hPBP, PSP, C3, C4,  $\alpha_1$ -AT,  $\alpha_2$ -HS-GP,  $\alpha_2$ -AP, TTR,  $\alpha$ Fib,  $\alpha_1$ -PI

### > 200 lipid species

Isoprenoids: esterified and unesterified cholesterol, oxysterols, bile acids, steroid hormones

Acylglycerols: triglycerides

Glycerophospholipids: (lyso)-phosphatidylcholines, -ethanolamines, - serines, -inositols, plasmalogens, cardiolipins

Sphingolipids: sphingomyelins, ceramides, glycolipids, sphingosine-1-phosphate

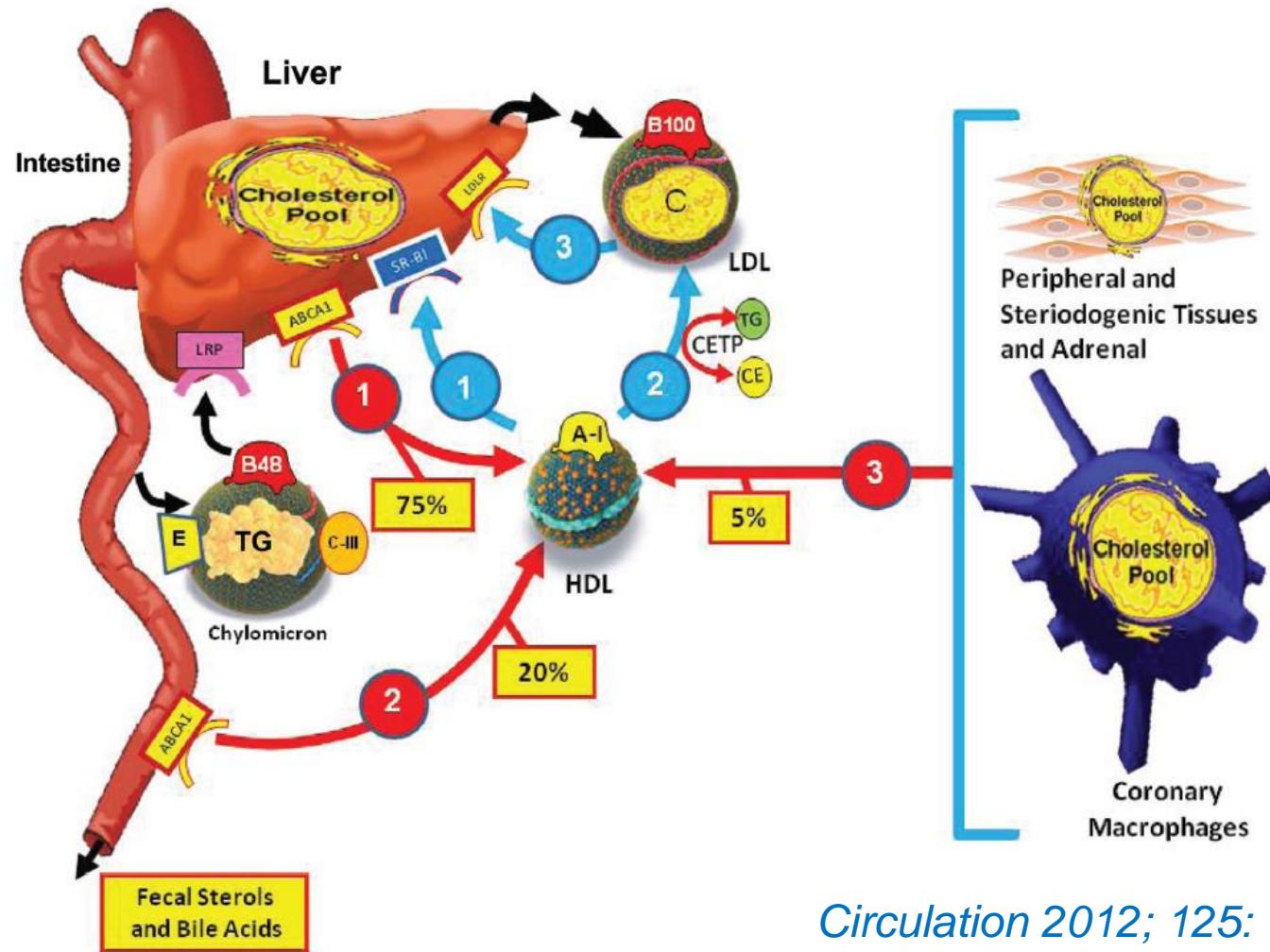
Vitamins:  $\alpha$ -tocopherol, carotenoids, retinoids

### microRNAs

miR-223  
miR-126  
miR-92a  
miR-150

miR-30c\*  
miR-145\*  
miR-146a\*  
miR-155  
miR-378\*

# The updated view of HDL's classical anti-atherogenicity: Cholesterol efflux and reverse cholesterol transport



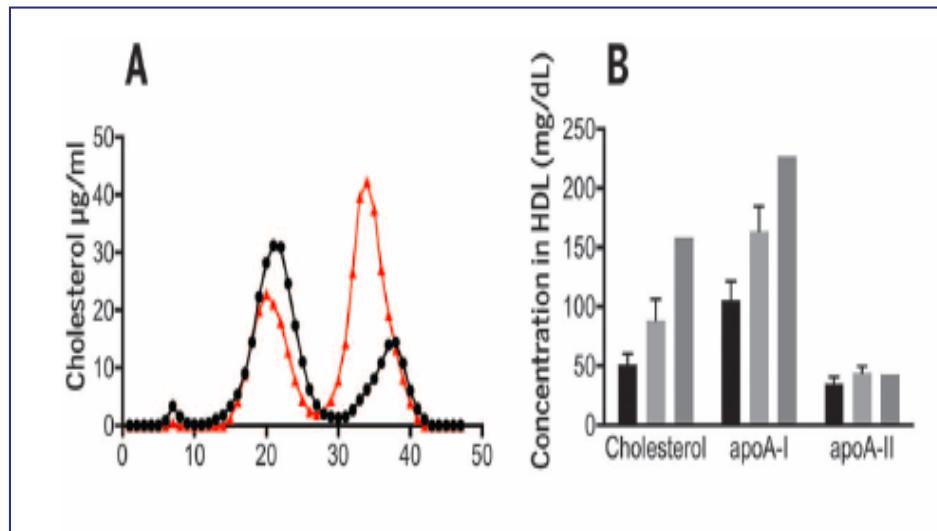
Circulation 2012; 125: 1905-1919

# Increased HDL-C due to SR-BI variant P376L associated with increased risk of coronary disease

## RESEARCH ARTICLES

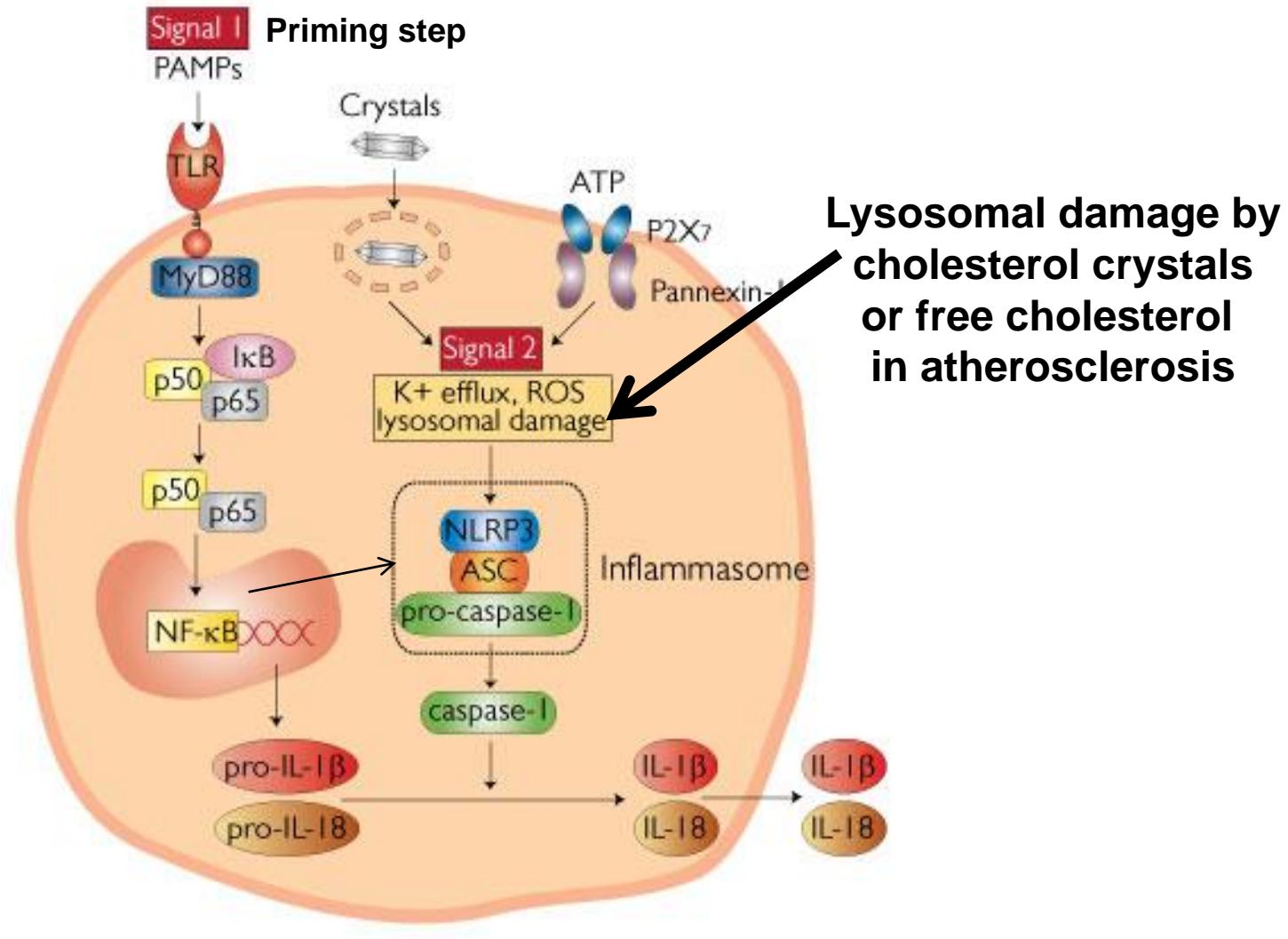
### HEART DISEASE

## Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease

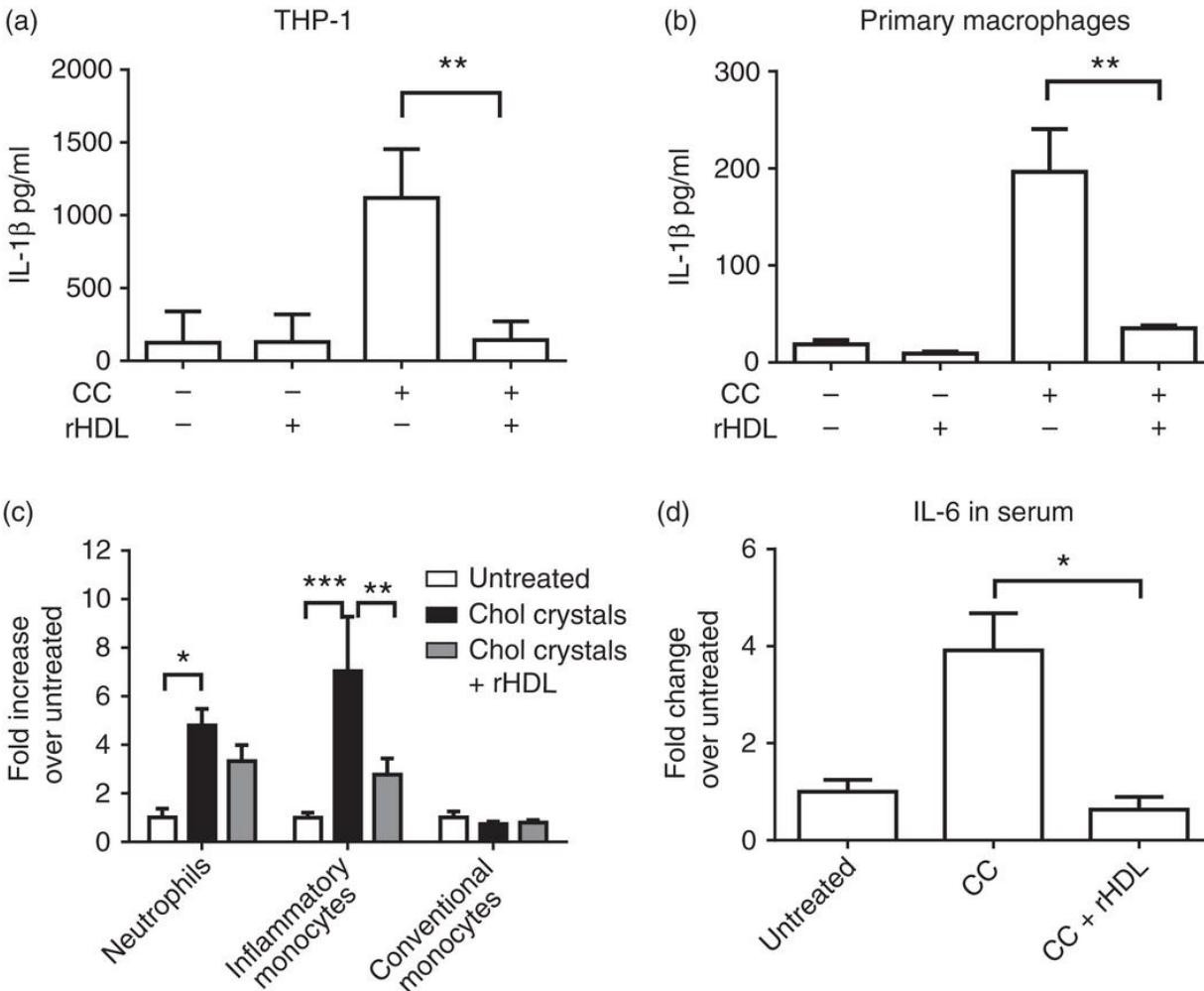


Across 49,846 CHD cases and 88,149 CHD controls, we found that P376L carriers had a significantly higher risk of CHD compared with noncarriers  
**(odds ratio for disease among carriers = 1.79;  $P=0.018$ )**

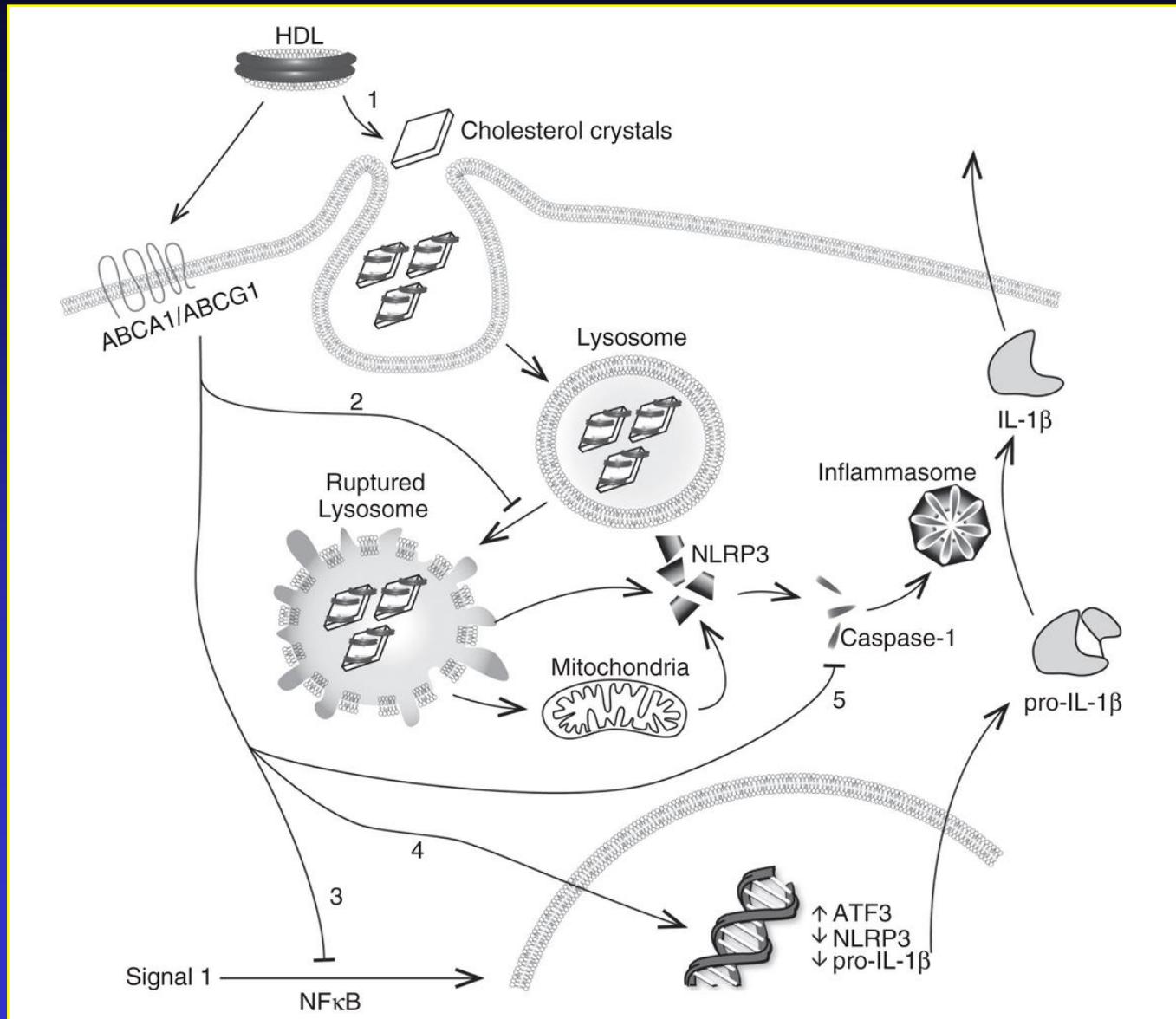
# NLRP3 Inflammasome Activation



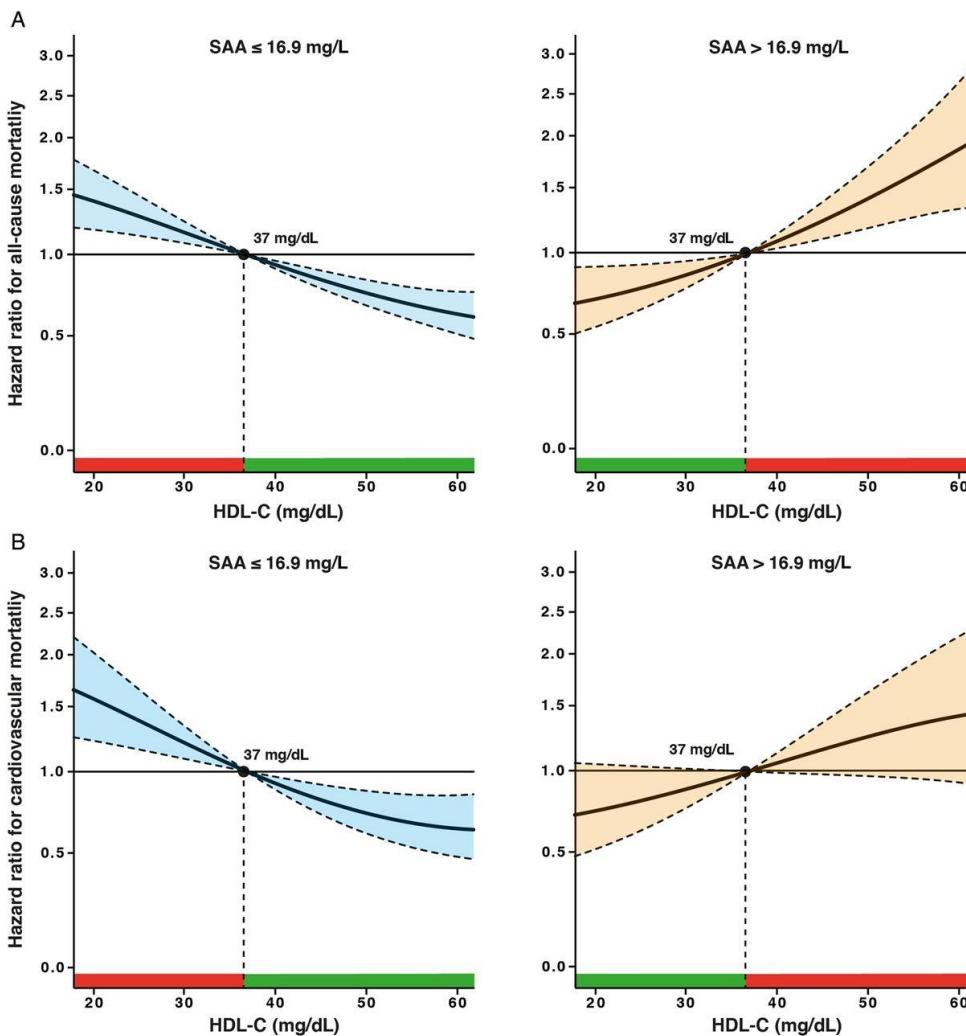
# High-density lipoprotein (HDL) blocks interleukin-1 $\beta$ (IL-1 $\beta$ ) secretion and inflammatory cell infiltration in response to cholesterol crystals CC



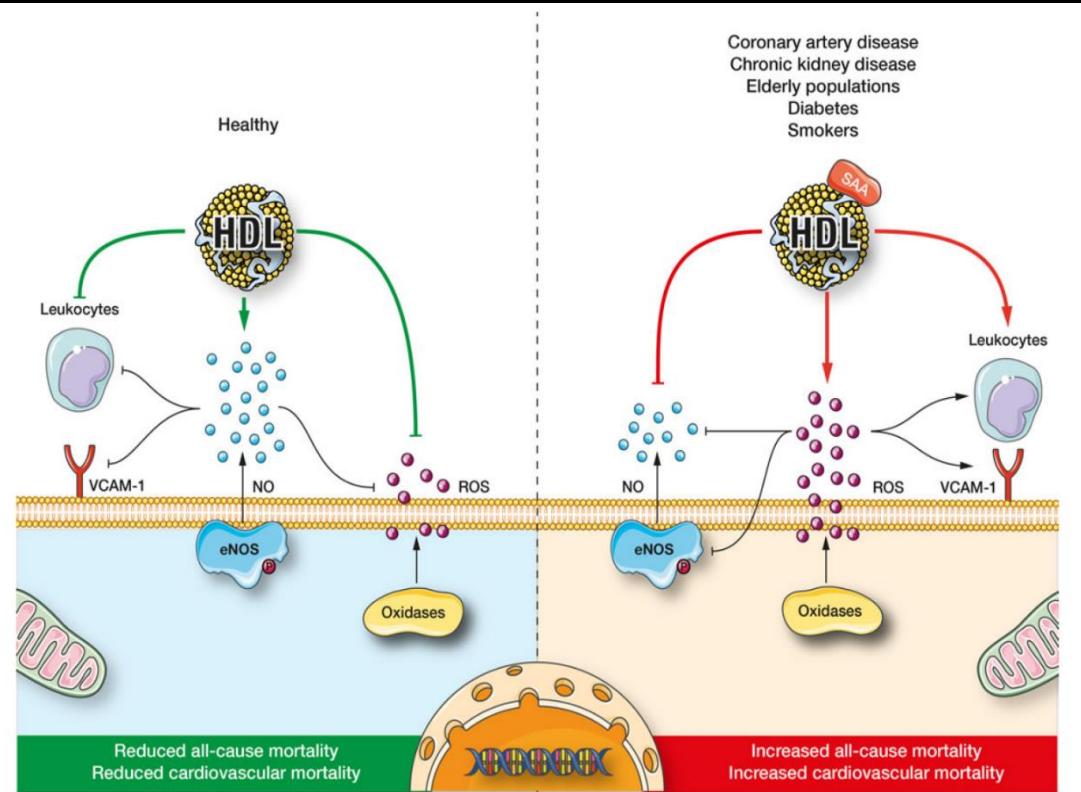
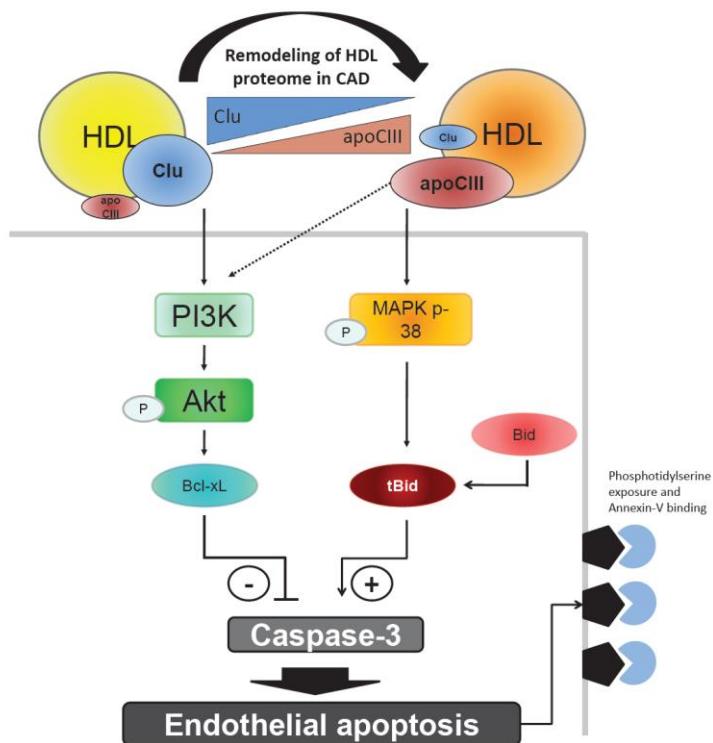
# Model of high-density lipoprotein (HDL) suppression of the inflammasome



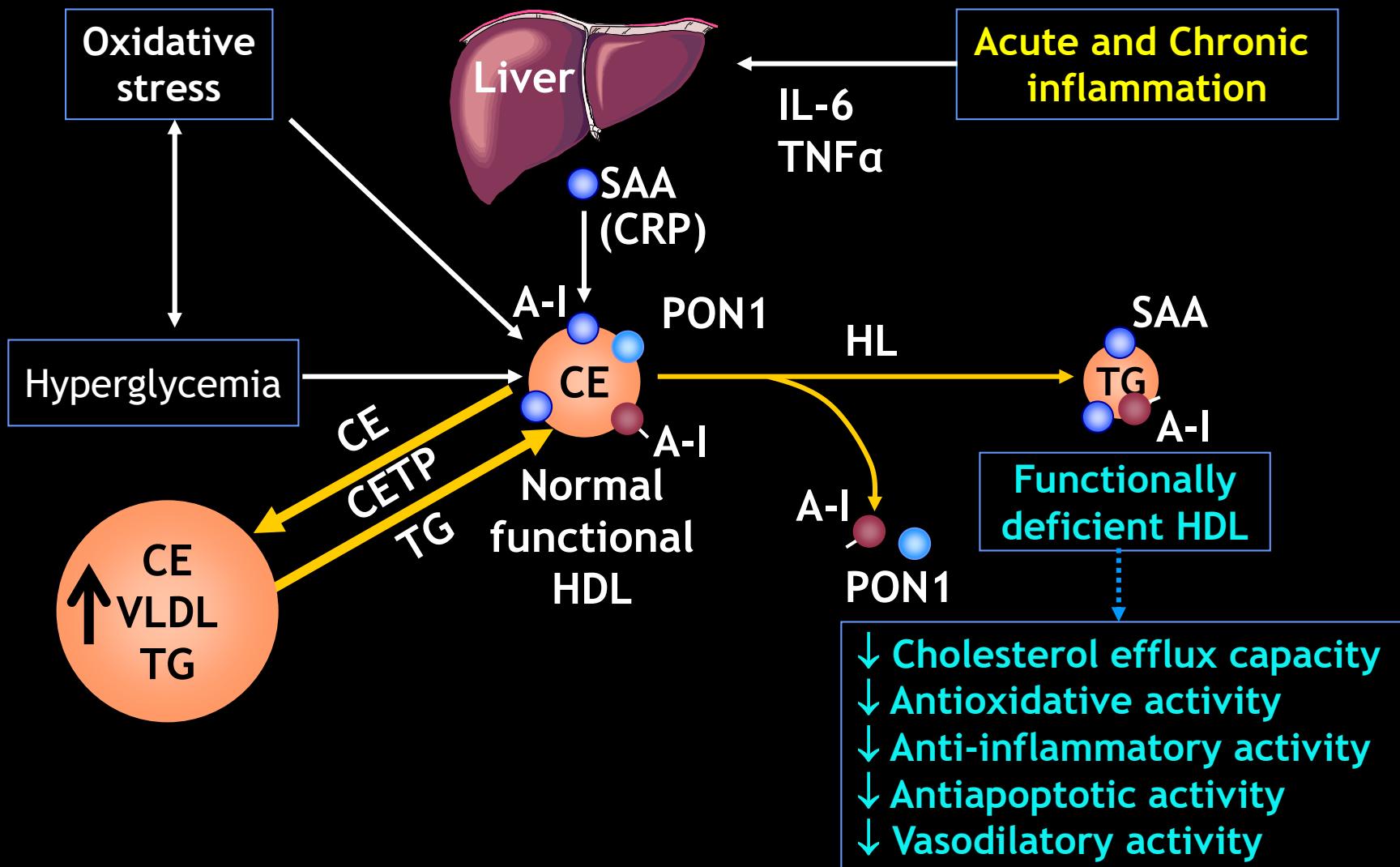
# All-cause and cardiovascular mortality according to HDL-C levels at high and low SAA concentrations



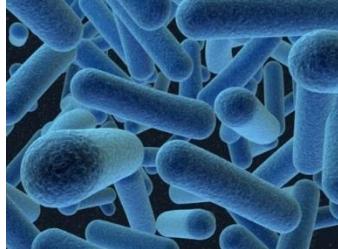
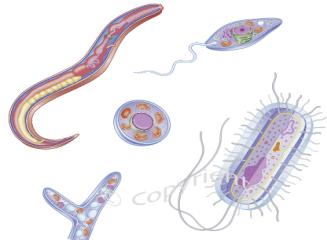
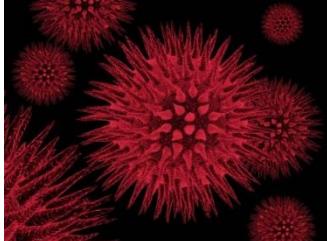
# Enrichment with ApoC-III or SAA lead to endothelial HDL-dysfunctions



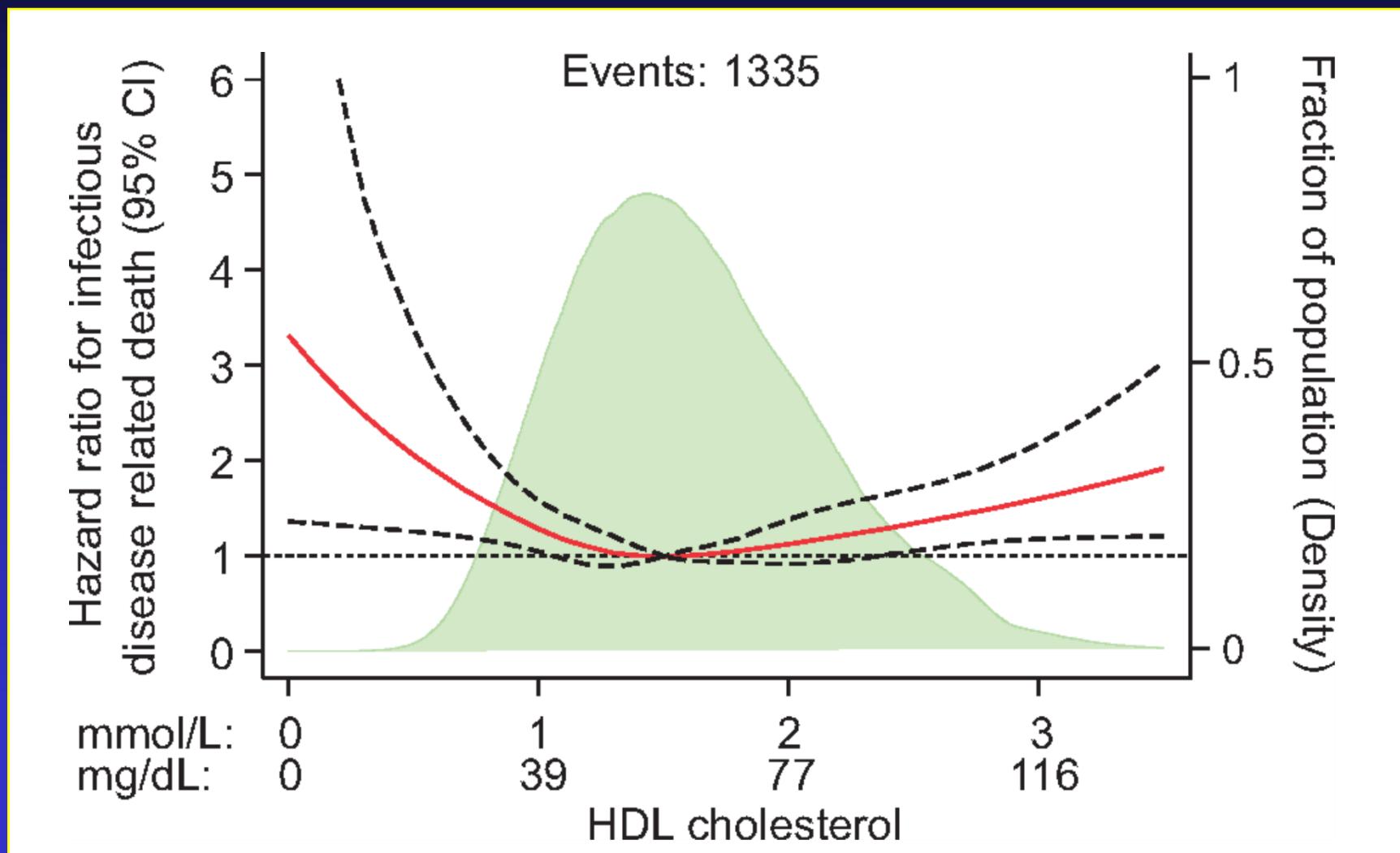
# Abnormal Metabolism and Defective Function of HDL



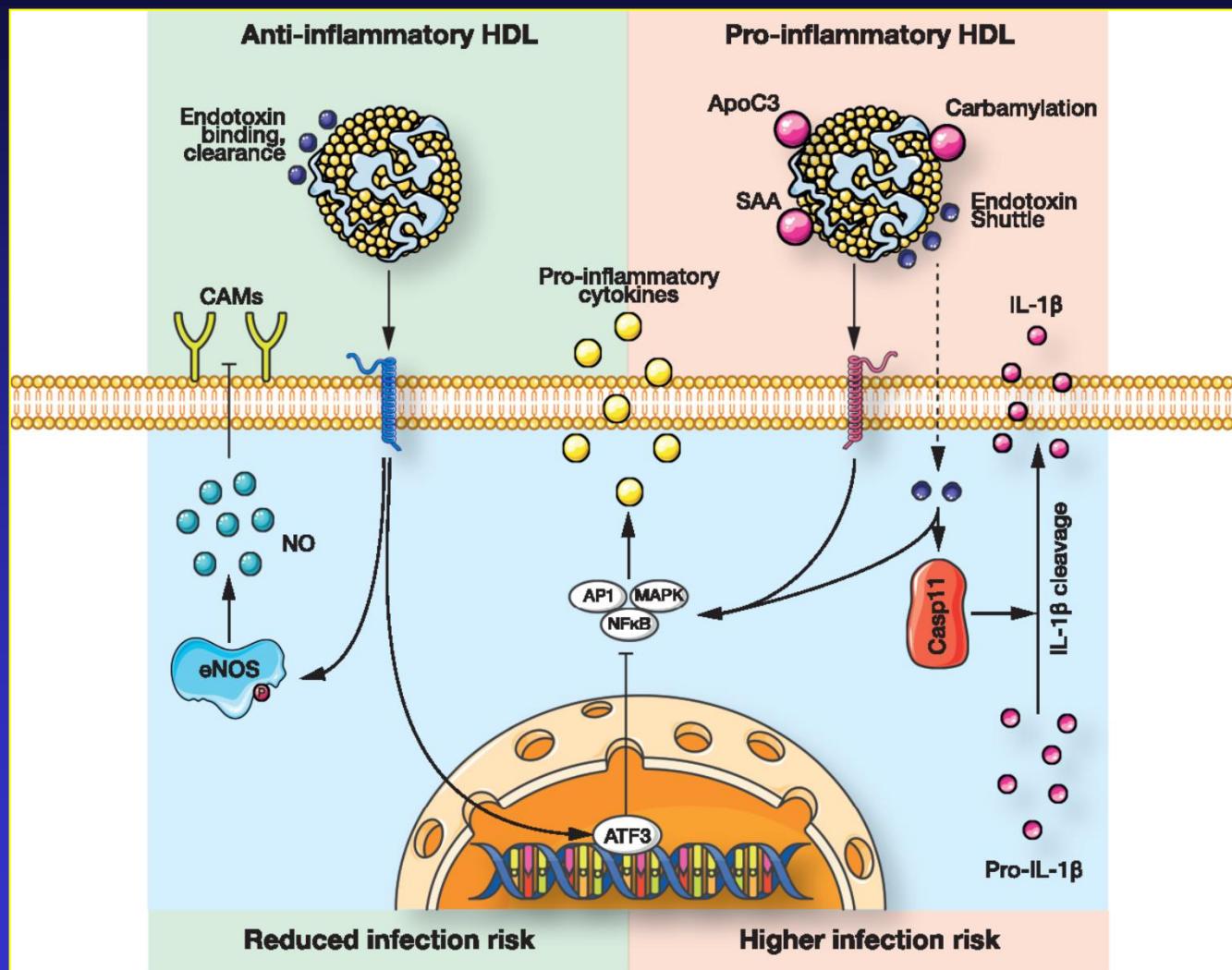
# HDL and INFECTIONS

TYPE OF INFECTION	HDL-MEDIATED EFFECT
<b>Bacteria</b> 	<ul style="list-style-type: none"><li>•Favor LPS/LTA binding and neutralization.</li><li>•Favor LPS/LTA clearance</li><li>•Inhibit LPS (LTA)-induced cytokine release</li><li>•Inhibit of LPS (LTA)-induced cell activation</li><li>•Induce an early inflammatory response</li></ul>
<b>Parasite</b> 	<ul style="list-style-type: none"><li>•Support ApoL1, ApoA-I and HRP interaction to form the trypanosoma lytic factor-1 (TLF-1). complex. ApoL1 then traffics to the trypanosomal lysosome, where causes swelling which kills the trypanosome.</li></ul>
<b>Virus</b> 	<ul style="list-style-type: none"><li>•Dampen (ApoA-1 mimetic peptides) the ABCA-1 impairment induced by the HIV-1 Nef protein.</li><li>•Inhibit cell fusion, both in HIV-1-infected T cells and in recombinant vaccinia-virus-infected CD4+ HeLa cells.</li><li>•Compete with Hepatitis C virus on SRBI interaction to dampen virus entry?</li></ul>

# 6 HDL cholesterol on a continuous scale and risk of infectious disease-related death in 97166 individuals from the Copenhagen General Population Study



# Distinct anti-inflammatory and pro-inflammatory effects of HDL modulating risk of infectious disease

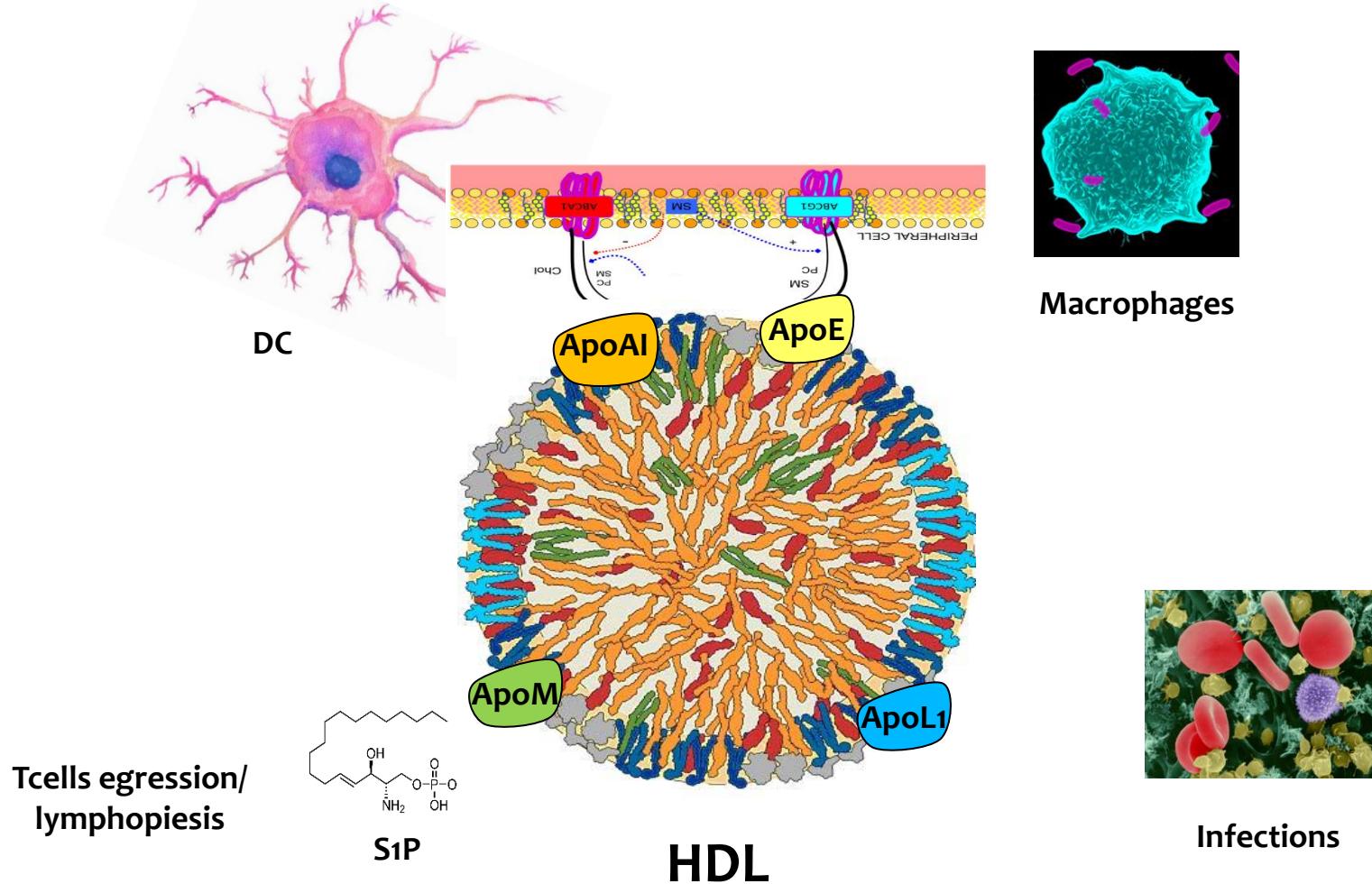


## HDL and IMMUNITY

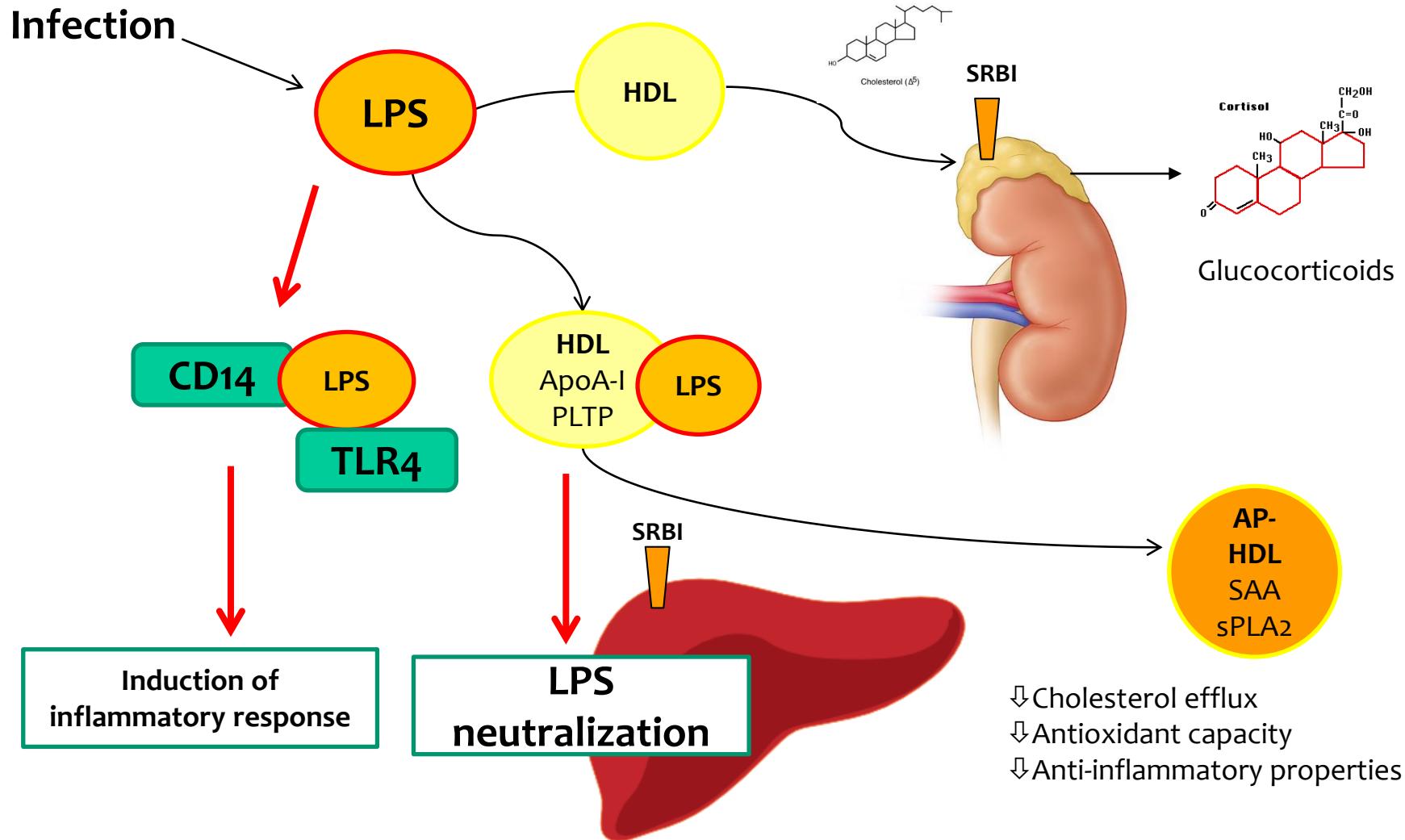
- Protection from endotoxemia
- Anti parasitic function
- Modulation of pentraxins
- Cholesterol efflux and immune cells
- Monocyte subsets
- APCs activity and autoimmunity



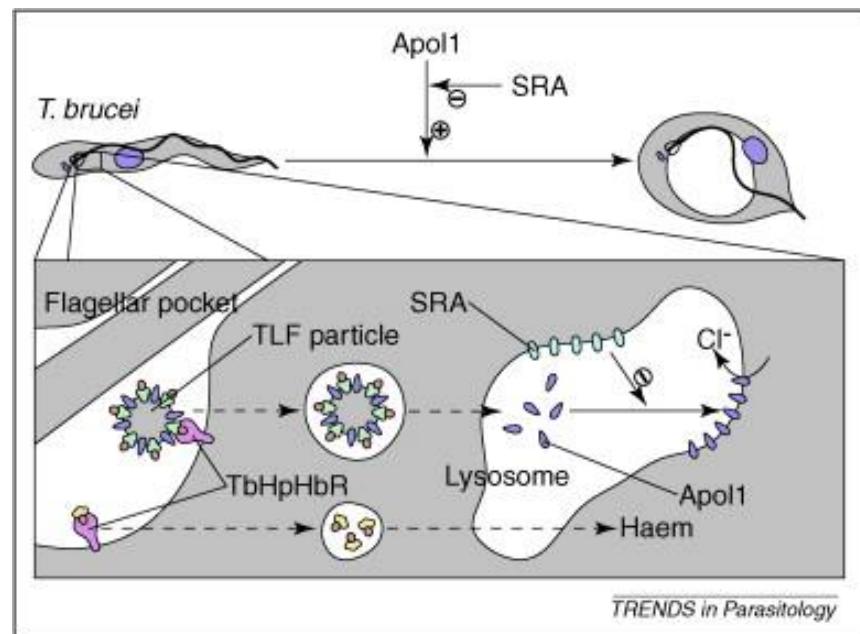
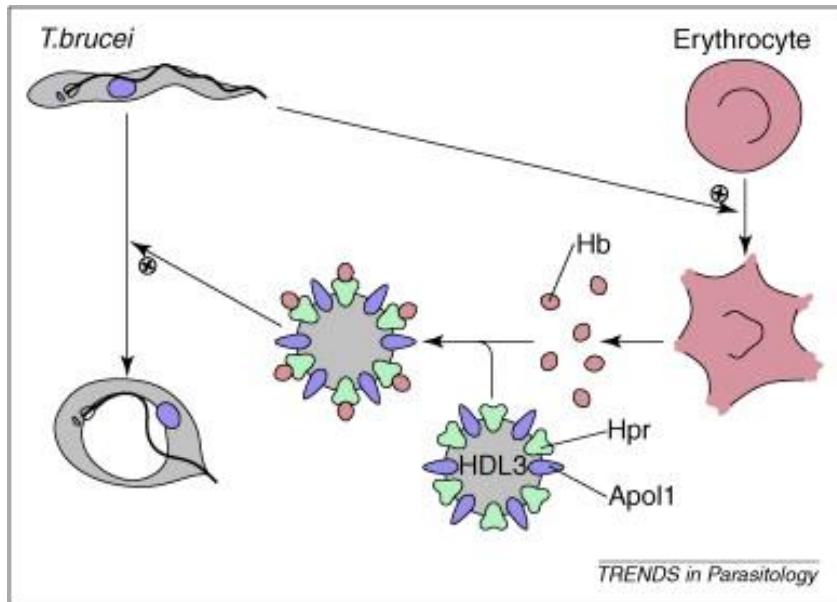
# HDL are a shuttle for apolipoproteins which play a key role in Innate and Adaptive Immune Responses



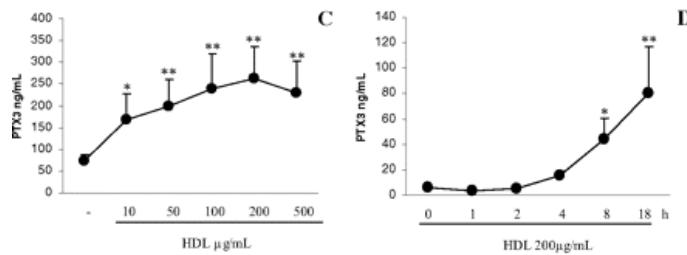
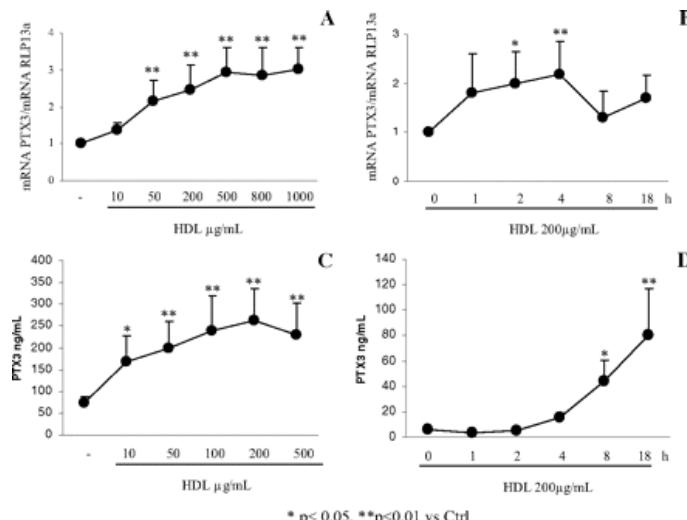
# HDL and endotoxemia



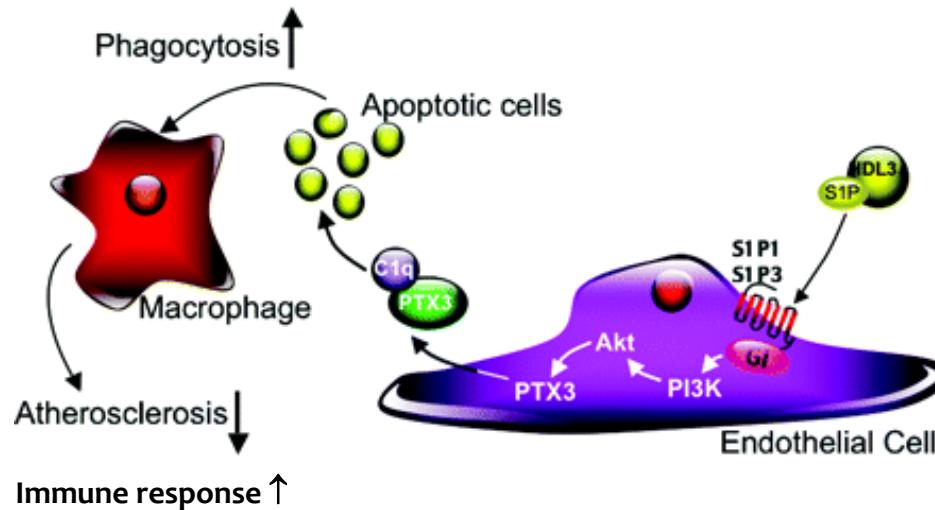
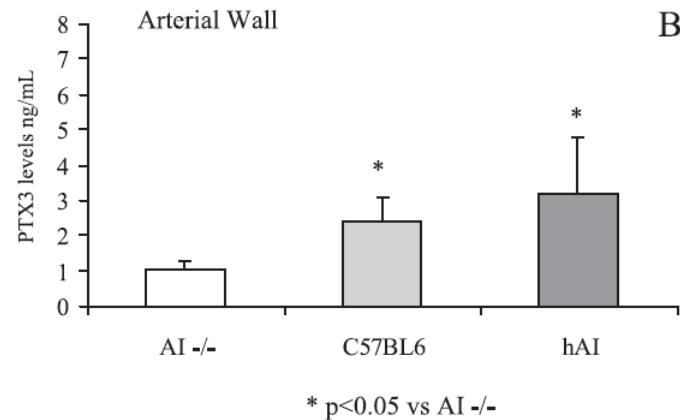
# HDL anti-parasitic function (ApoL1)



# HDL and the long pentraxin PTX3

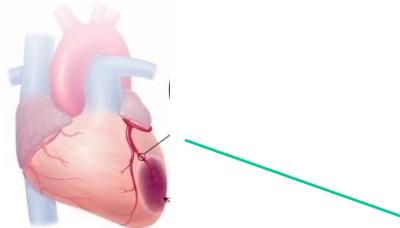


\* p<0.05, \*\*p<0.01 vs Ctrl

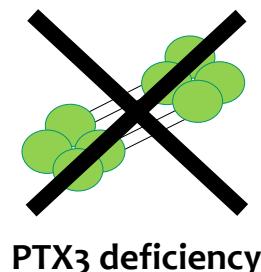


# PTX3 IN CVD

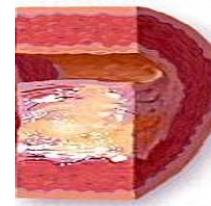
Myocardial infarction



Vascular atherosclerosis,  
inflammation or damage



PTX3 deficiency



↑ Atherosclerosis  
↑ Vascular inflammation  
↑ Macrophage recruitment

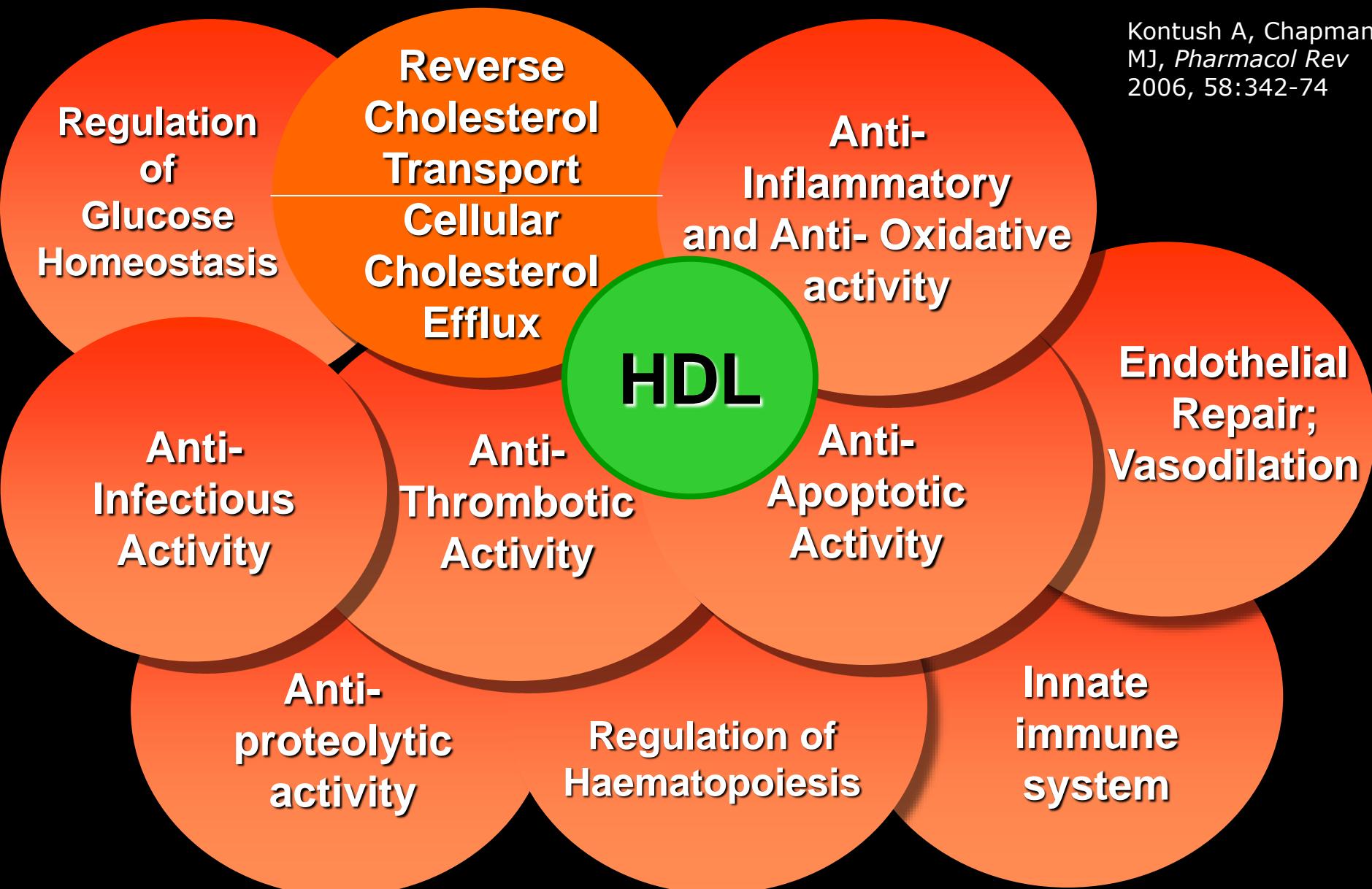


↑ Arterial  
thrombosis

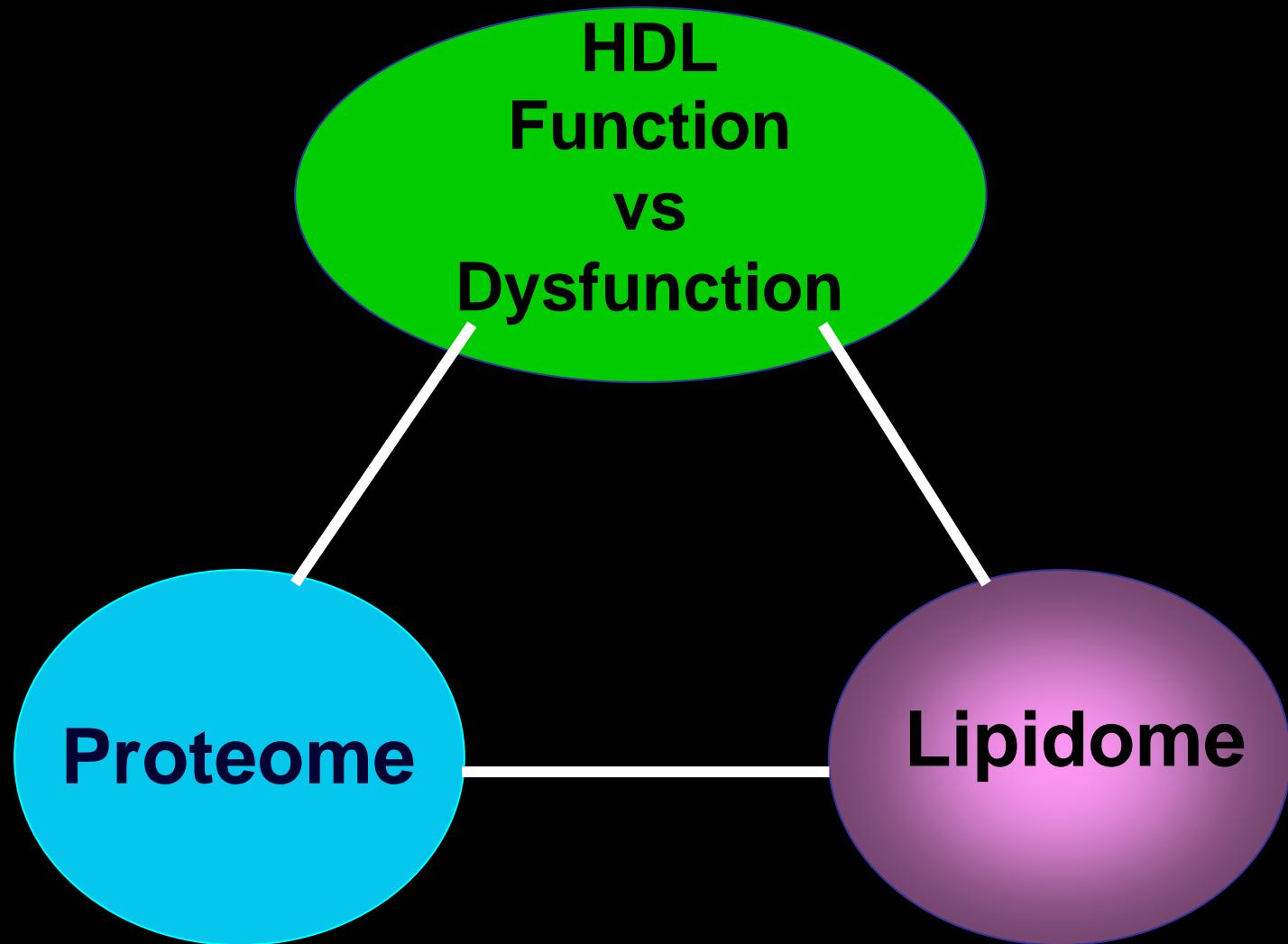
Perhaps we may look to the increase of acute phase proteins in atherothrombosis not as a harmful response but as a further attempt to protection of our body.

# Atheroprotective & Vasculoprotective Activities of HDL

Kontush A, Chapman MJ, *Pharmacol Rev* 2006, 58:342-74



# HDL : The Future



# Effects of compositional changes of HDL on HDL function

Modification	Functional impact
SAA enrichment	<ul style="list-style-type: none"> <li>↓ Cholesterol efflux capacity &amp; macrophage reverse cholesterol transport</li> <li>↓ Anti-oxidative activity</li> <li>↓ Inhibition of MCP-1</li> <li>Disturbed endothelial function (↓ VCAM inhibition, ↓ eNOS stimulation)</li> </ul>
ApoC-III enrichment	Disturbed endothelial function (↓ VCAM inhibition, ↓ apoptosis inhibition)
Triglyceride enrichment	<ul style="list-style-type: none"> <li>↓ Cholesterol efflux capacity, ↓ SR-BI-mediated selective uptake of HDL-CE</li> <li>↓ Inhibition of IL-8 release from ECs</li> </ul>
Sphingomyelin depletion	<ul style="list-style-type: none"> <li>↓ Cholesterol efflux capacity</li> <li>↓ Anti-oxidative activity</li> <li>↓ Inhibition of cytokine release</li> </ul>
Phosphatidylserine depletion	<ul style="list-style-type: none"> <li>↓ Cholesterol efflux capacity</li> <li>↓ Inhibition of LDL oxidation</li> <li>↓ Inhibition of platelet activation</li> <li>Disturbed endothelial function (↓ VCAM inhibition, ↓ apoptosis inhibition)</li> </ul>
PC-depletion	↓ Cholesterol efflux capacity
LPC-enrichment	<ul style="list-style-type: none"> <li>↓ Cholesterol efflux capacity</li> <li>↓ Inhibition of LDL oxidation</li> </ul>
PA-enrichment	↓ Cholesterol efflux capacity
Plasmalogen depletion	↓ Inhibition of endothelial apoptosis
S1P-depletion	Disturbed endothelial function (↓ eNOS activation, ↓ Inhibition of endothelial apoptosis, ↓ Endothelial barrier function)

# Risk function without high-density lipoprotein-cholesterol (HDL-C) for men

