JCR2014（2014.12.13@Novotel, Busan, South Korea）

Reverse Cholesterol Transport and Atherosclerosis

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Topics

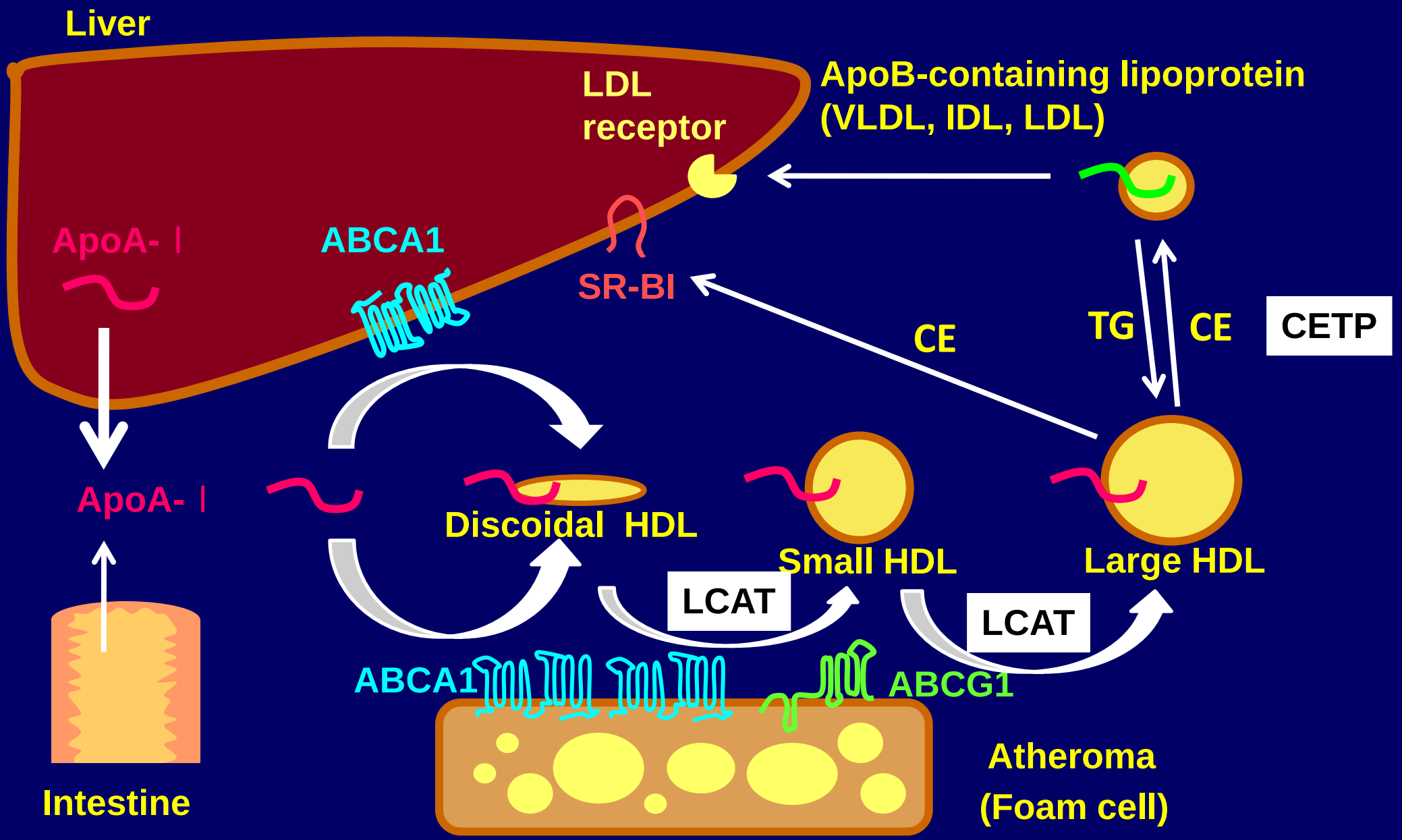
★ HDL-mediated reverse cholestrol transport and importace of HDL-C management

★ Novel functions of HDL and dysfunctional HDL

★ Anti-atherogenic effects of probucol and their mechanisms

★ Anti-atherogenic effects of probucol from human clinical studies

REVERSE CHOLESTEROL TRANSPORT



Familial HDL Deficiency

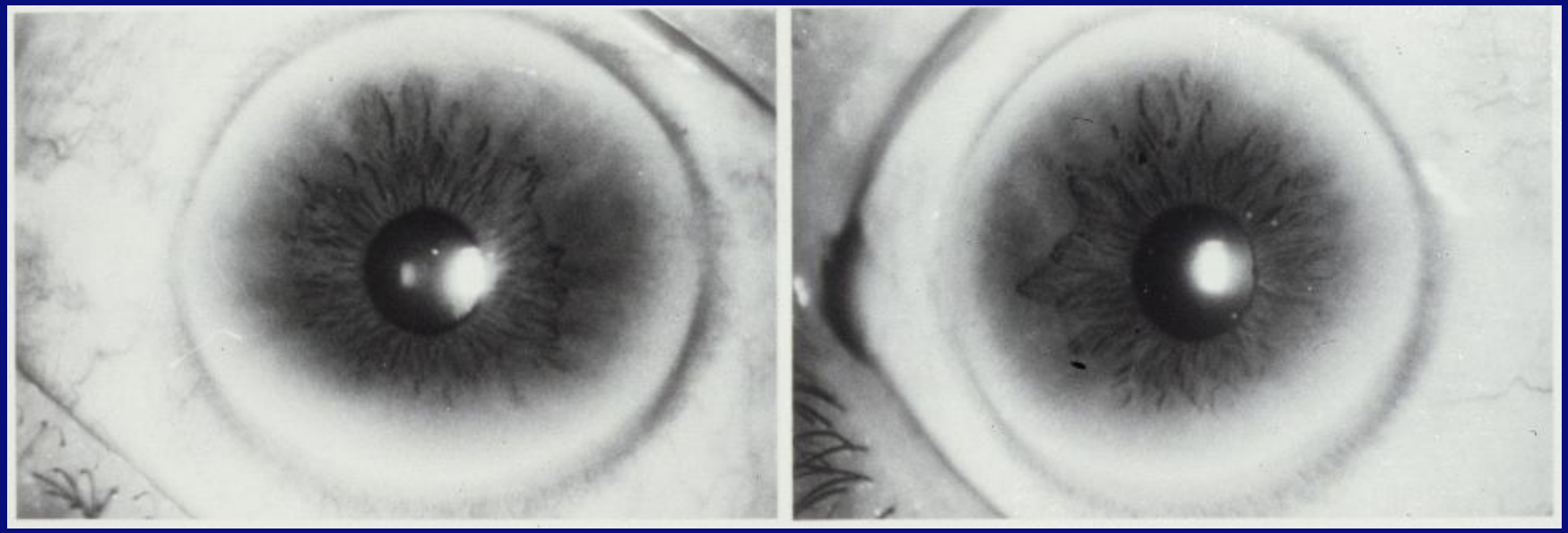
Tangier disease (Deficiency of ABCA1)
Familial LCAT deficiency / Fish eye disease
Familial apo A-I/C-III deficiency
Familial HDL deficiency with planar xanthoma
Familial apo A-I deficiency
Familial hypoalphalipoproteinemia

↓

Often associated with corneal opacity & premature coronary artery disease

Reduction of Serum HDL-cholesterol Alone Accelerates Atherosclerosis

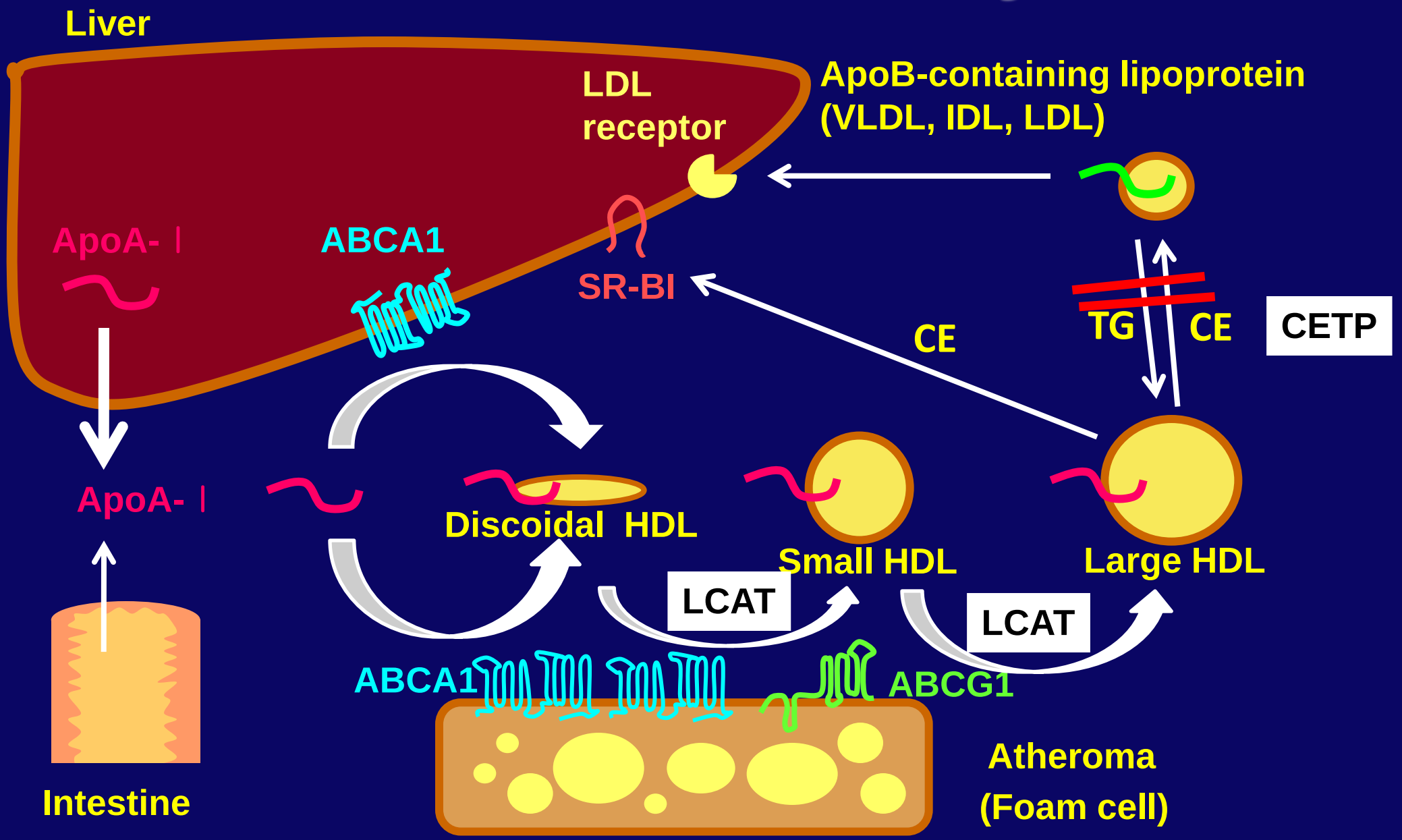
Two Cases of Marked Hyperalphalipoproteinemia with Premature Corneal Opacity



58 y.o. Male		61 y.o. Male	
TC	261 mg/dl	TC	238 mg/dl
TG	68	TG	64
HDL-C	154	HDL-C	138
CHD	(-)	CHD	(+)

Matsuzawa Y, Yamashita S, et al. Atherosclerosis 1984

CETP Deficiency

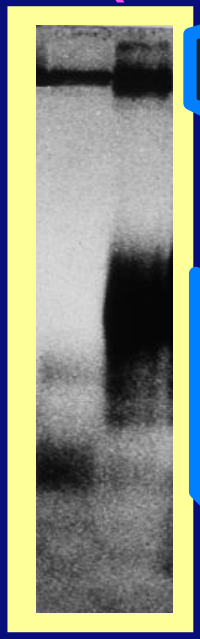


Lipoprotein Abnormalities of CETP Deficiency

Nondenaturing PAGE (4-30%)

Normal LDL

Normal HDL



Control

CETP Deficiency

LDL in CETP deficiency

Polydisperse LDL poor in CE

Low affinity to LDL receptors

Yamashita S et al, Atherosclerosis 1988

Sakai N et al, Arterioscler Thromb 1991 & Eur J Clin Invest 1995

HDL in CETP deficiency

Marked increase in HDL2

Large HDL particles enriched with CE & apo E

Reduced capacity of cholesterol efflux

Yamashita S et al, J Clin Invest 1990

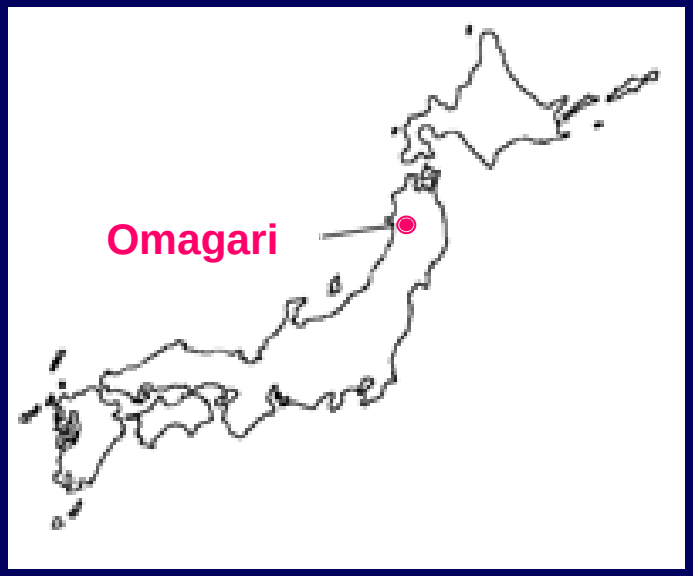
Ishigami M et al, J Biochem 1994

Hyperalphalipoproteinemia is a disorder of reverse cholesterol transport

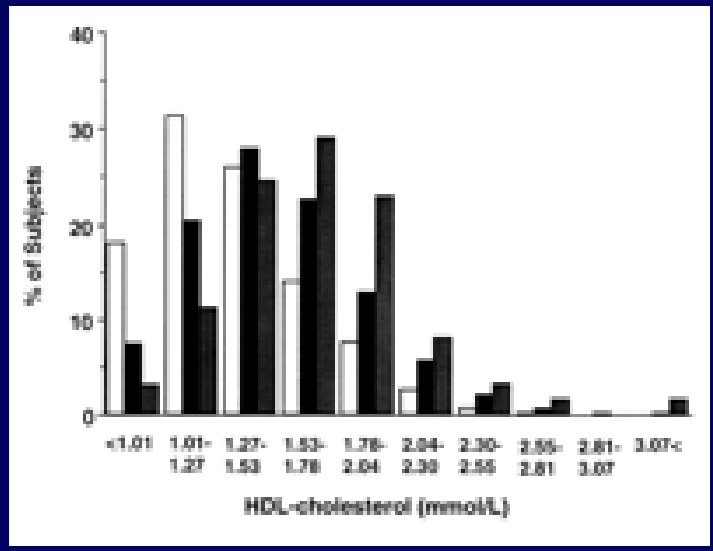
Epidemiological Study of Hyper-HDL-cholesterolemia in Omagari Area of Japan

Large Population-based study in Omagari, Japan
Subjects: Male=39567, Female=64938

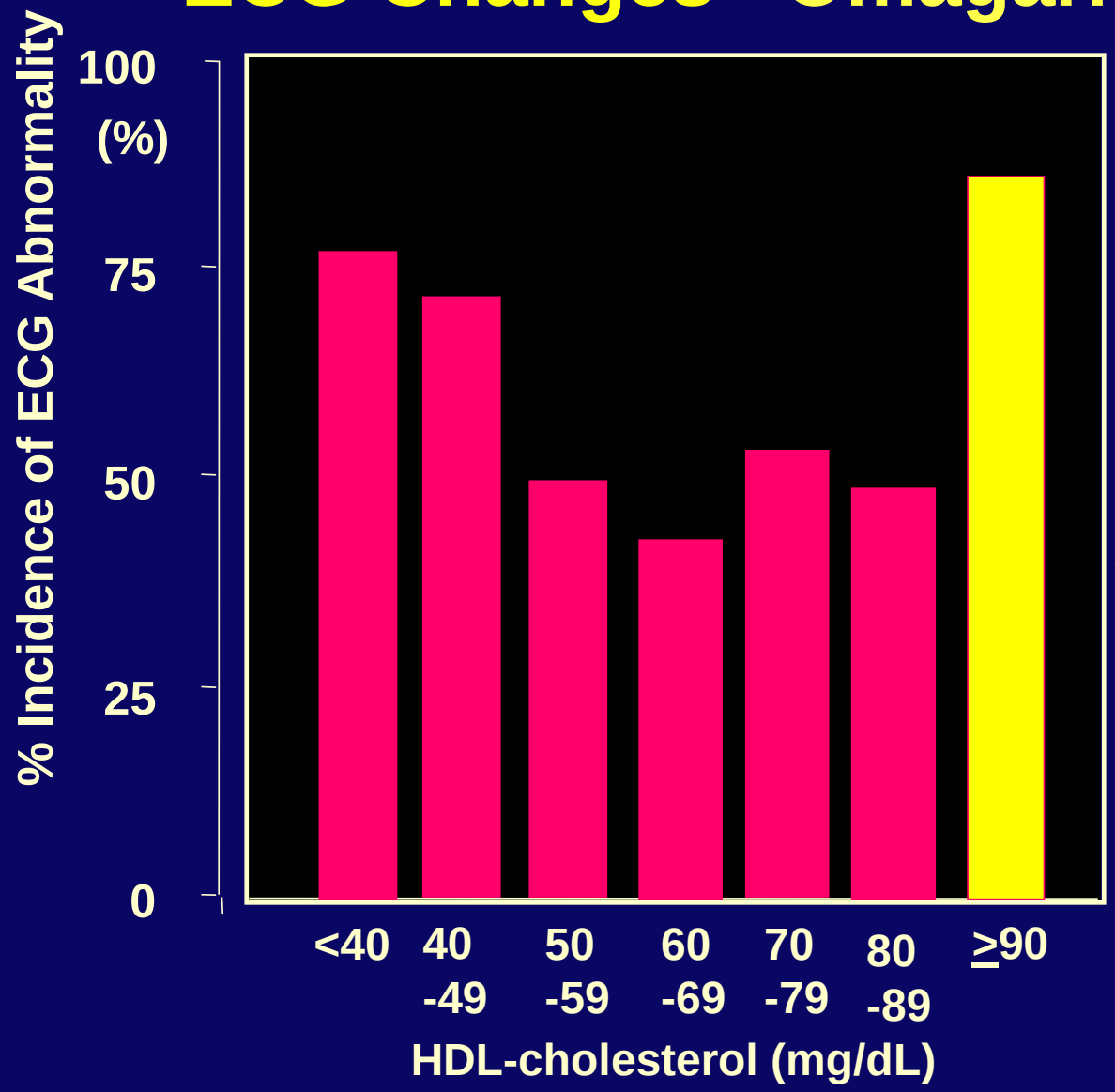
Prevalence of intron 14 splicing defect ---------- 20-fold higher
Marked hyperalphalipoproteinemia --- 10-fold higher
(HDL-C ≥ 100 mg/dl)



CETP Gene Mutation	
Omagari	29.7%
Osaka	1.0%
Tokyo	1.5%
Shizuoka	1.3%



Relationship between HDL-C and Ischemic ECG Changes −Omagari Study−



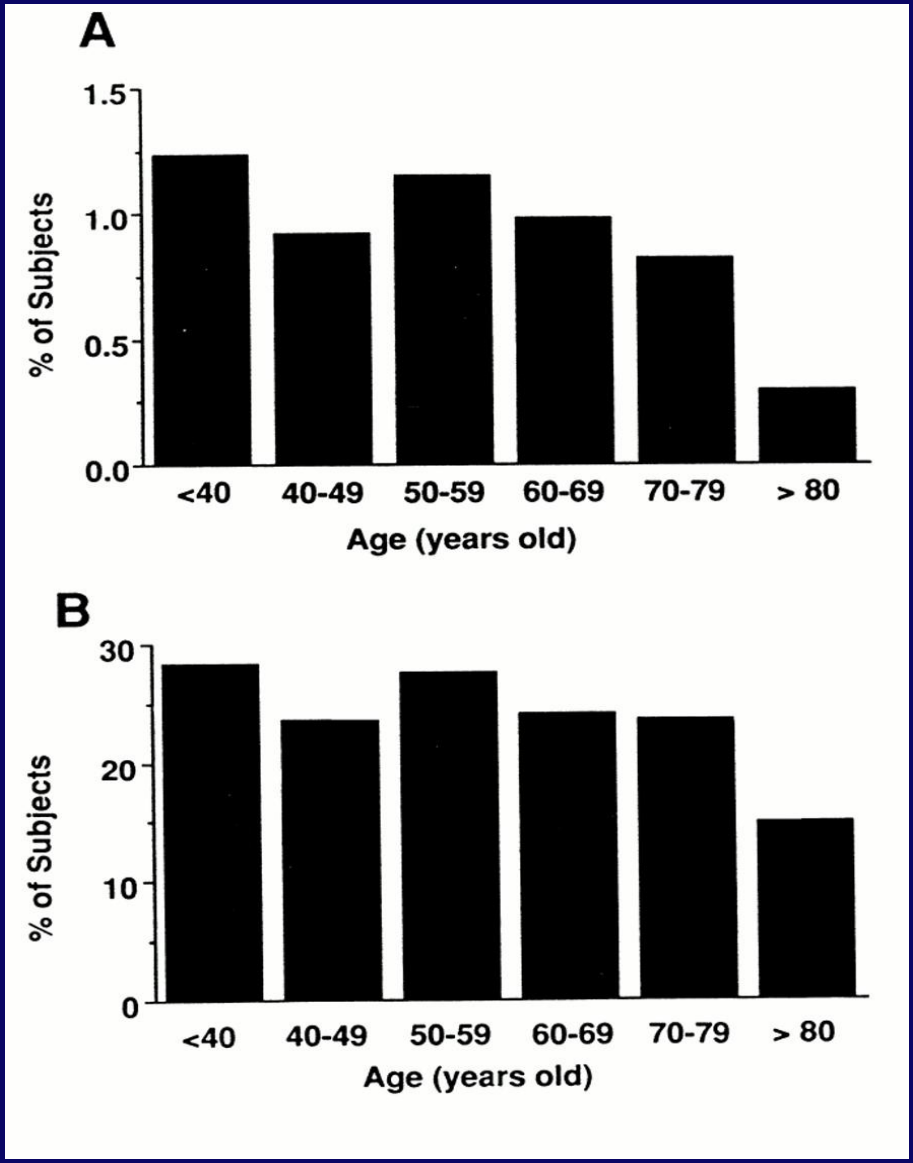
Arterioscler Thromb Vasc Biol 1997

Plaque score is increased In CETP deficiency

(Fushimi E and Yamashita S, unpublished)

Hyper-HDL-C by CETP deficiency is atherogenic ! Atherogenic Hyperalpha-lipoproteinemia
（Yamashita S: *Atherosclerosis 2000*）

Prevalence of a Marked HALP (HDL-C ≥100 mg/dL) and Intron 14 Splice Donor Site Mutation in Subgroups Divided by Every 10 Years of Age (Omagari Study)

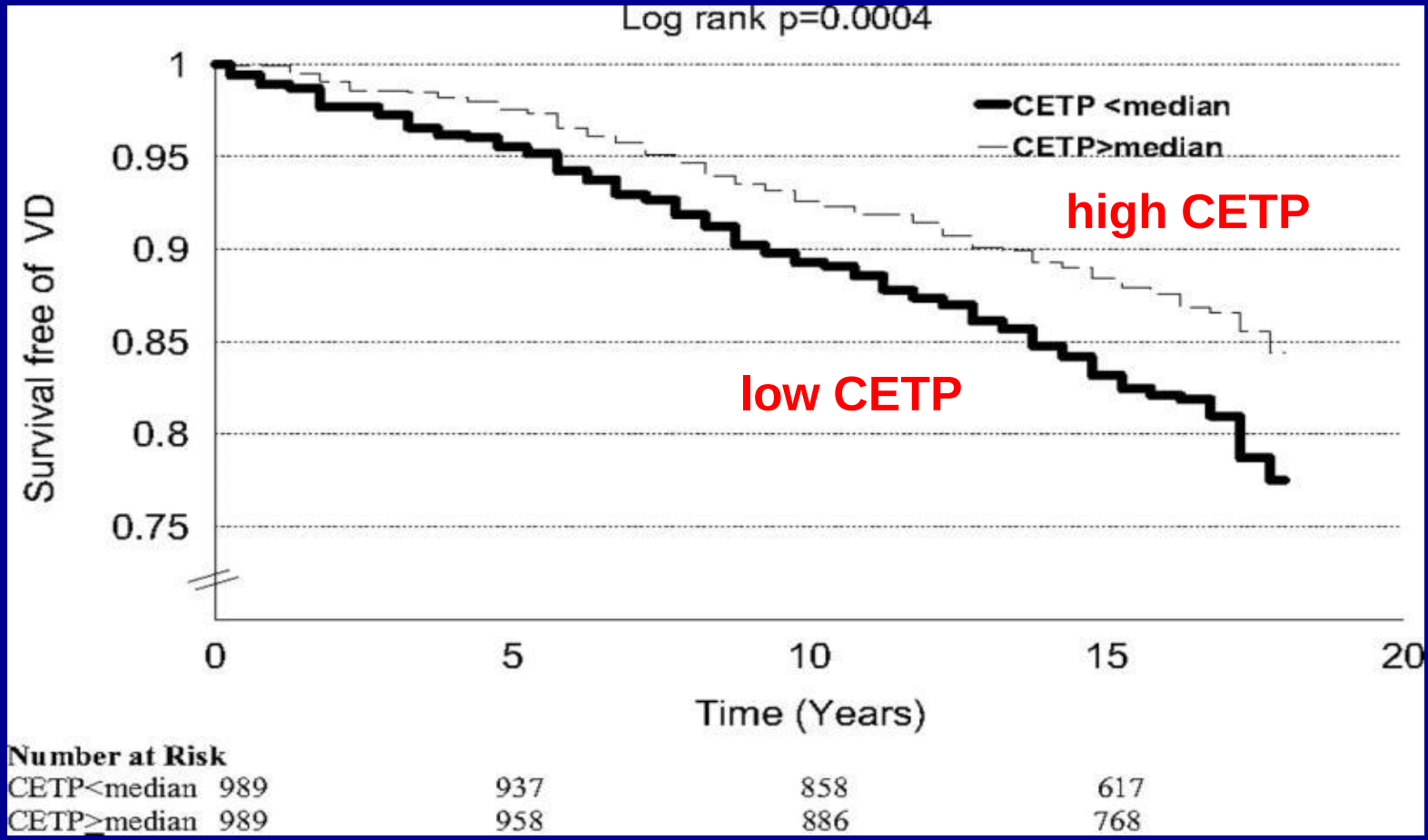


Marked HALP
(HDL-C ≥100 mg/dL)

Intron 14 Splice Donor Site Mutation

Hirano K et al: Arterioscler Thromb Vasc Biol 17:1053-1059, 1997

CETP activity and Cardiovascular Events



Subjects with low CETP activity had a higher risk for CV events than those with high CETP activity

Framingham Study

Vasan, R. S. et al. Circulation 2009;120:2414-2420

Lipid-loweing Effects of CETP Inhibitors/Modulators

% Change from Baseline

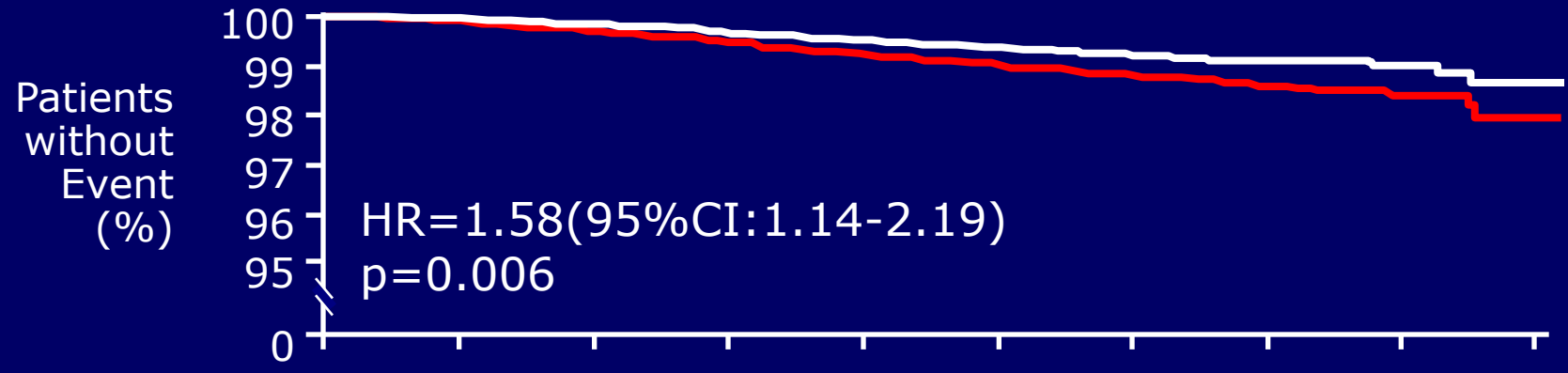
CETP Inhibitor	Torcetrapib	Dalcetrapib	Anacetrapib	Evacetrapib
dose (mg/day)	60	600	100	500
HDL-C (%)	+61	+31	+38	+29
LDL-C (%)	-24	-2	-40	-36
TG (%)	-9	-3	-7	-11

Adapted from Cannon C et al. *JAMA.* 2011;306:2153-2155.
Nicholls SJ et al. *JAMA.* 2011;306:2099-2109.

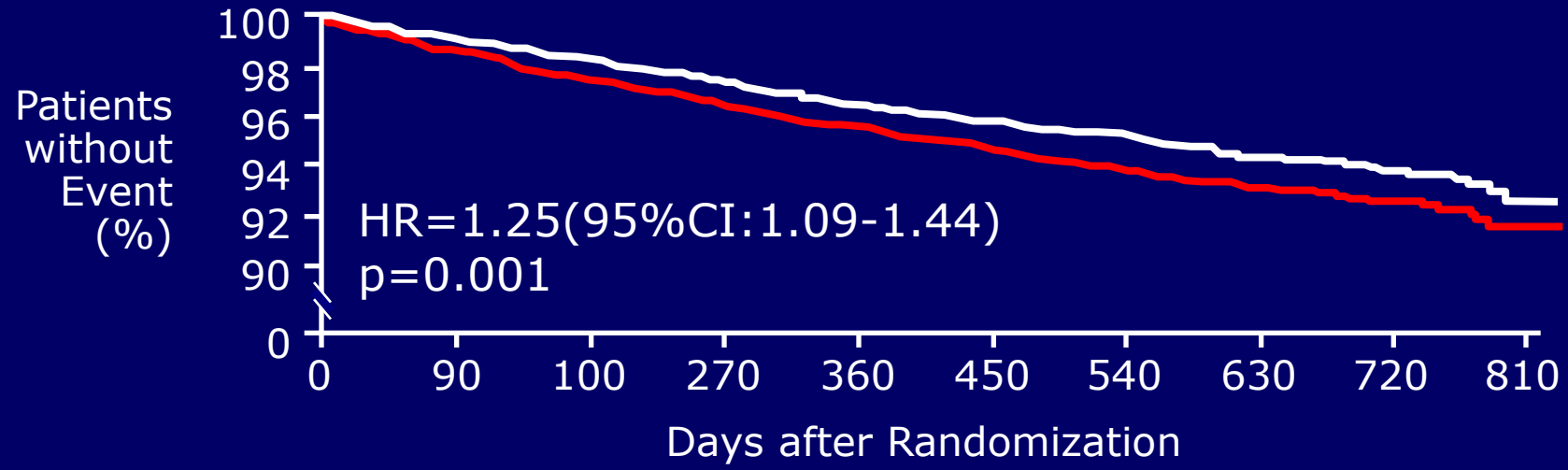
ILLUMINATE

Effects of Torcetrapib in Patients at High Risk for Coronary Events

◆Death from Any Cause



◆Primary composite outcome(Major Cardiovascular Events)



: Atorvastatin(n=7,534) : Atorvastatin+Torcetrapib(n=7,533)

Barter PJ et al for the ILLUMINATE Investigators: N Engl J Med 2007; 357 :2109-2122

Failure of CETP Inhibitor Torcetrapib

Torcetrapib reduces LDL-C by 20% and increases HDL by more than 60%, however:

ILLUMINATE Study: Combination of atorvastatin and torcetrapib increased total mortality, including cardiovascular mortality.

ILLUSTRATE Study: Torcetrapib had no effect on plaque volume

RADIANCE 1 & 2: Torcetrapib had no effect on IMT in FH heterozygotes and mixed hyperlipidemia

Torcetrapib elevated blood pressure due to increase of aldosterone

dal-OUTCOMES trial

dalcetrapib

eive daily

heartwire

LIPID/METABOLIC

Roche stops dalcetrapib trial for lack of benefit

MAY 7, 2012 Reed Miller

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23 Comments | Read later | Print | Font size | Cite

Basel, Switzerland– Roche has stopped the phase 3 **dal-OUTCOMES** trial of the cholesteryl ester transfer protein (CETP) inhibitor **dalcetrapib** after interim analysis of the study showed the HDL-cholesterol-boosting drug was not significantly reducing cardiovascular adverse events [1].

As reported by **heartwire**, the earlier **dal-PLAQUE** study showed that dalcetrapib reduced inflammation in the carotid artery and that there was an inverse relationship between HDL-cholesterol levels and markers of arterial inflammation in patients treated with the drug. Dal-OUTCOMES was a major morbidity and mortality study currently planned for about 16 000 stable coronary heart disease patients with recent acute coronary syndrome (ACS). Patients in the study were randomized to either 600 mg daily of dalcetrapib and standard medical therapy or placebo and standard medical therapy.

cardiac arrest, or atherothrom

end point events have occurr

least 2 years, and 80% of eva

years.

Roche

Roche provides update on Phase III study of dalcetrapib

Roche (SIX: RO, ROG; OTCQX: RHHBY) today announced that following the results of the second interim analysis of the dalcetrapib dal-OUTCOMES Phase III trial, the independent Data and Safety Monitoring Board (DSMB) has recommended stopping the trial due to a lack of clinically meaningful efficacy. The dal-OUTCOMES trial evaluated the efficacy and safety profile of dalcetrapib when added to existing standard of care in patients with stable coronary heart disease (CHD) following an acute coronary syndrome (ACS). No safety signals relating to the dal-OUTCOMES trial were reported from the DSMB.

Risk Reduction for CHD Events

As a Function of Changes in TC, LDL-C, and HDL-C

	PERCENT CHANGE	CHD EVENT RATE
* LDL-C	1% ↓	1% ↓
TC	1% ↓	2% ↓
** HDL-C	1% ↑	3% ↓

*4S, CARE, LIPID, WOSCOPS

**HELSINKI, VA-HIT,AFCAPS/TexCAPS

Failure of CETP Inhibitors

Torcetrapib:

Markedly increases serum HDL-C, and reduces LDL-C

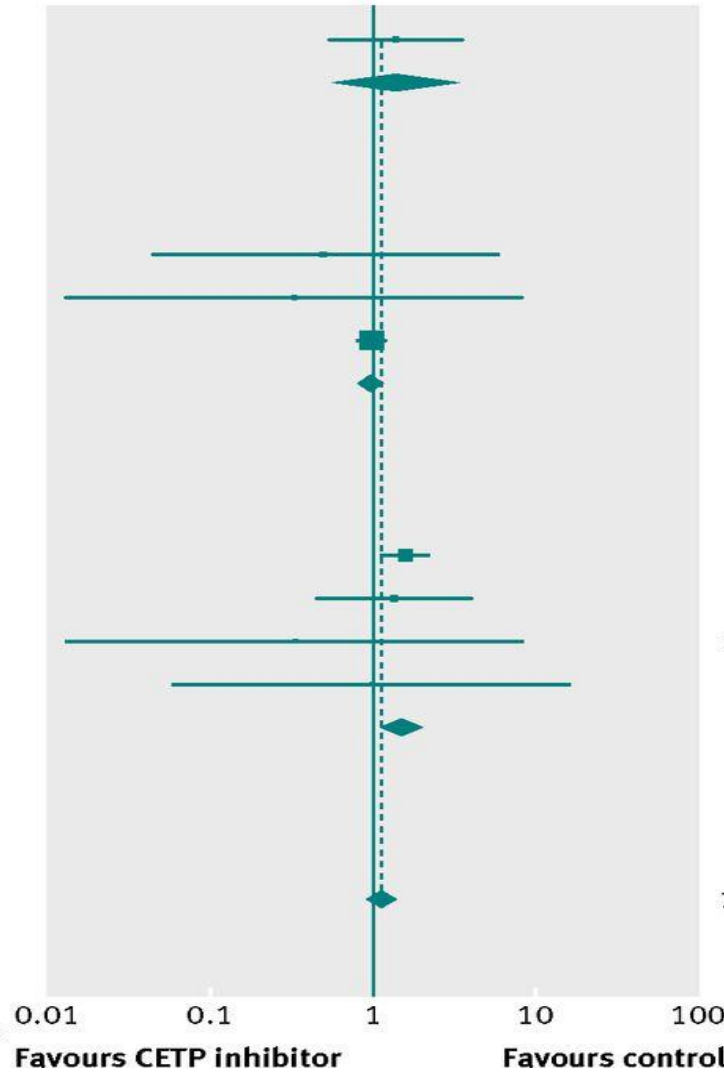
Dalcetrapib:

Increases serum HDL-C, but does not reduce LDL-C

→Elevation of HDL-C by CETP inhibition may not affect CVD or rather increase CVD. HDL functions and the efficiency of reverse cholesterol transport are more important for prevention and regression of atherosclerosis

Forest Plot Showing Effect of CETP Inhibitors on Risk of All Cause Mortality Stratified by CETP Inhibitors

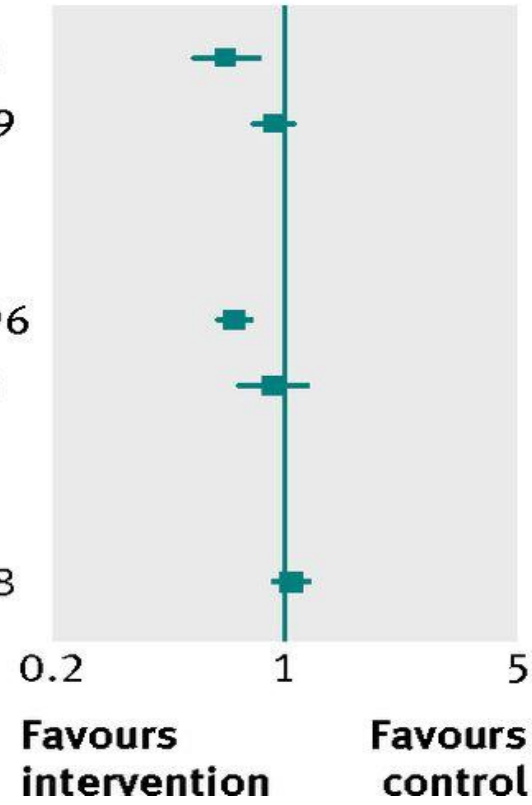
Study or subgroup	No of events/total: CETP inhibitor	No of events/total: Control	Weight (%)	Odds ratio M-H, random (95% CI)
Anacetrapib				
Define 2010	11/811	8/812	5.5	1.38 (0.55 to 3.45)
Subtotal	11/811	8/812	5.5	1.38 (0.55 to 3.45)
Test for heterogeneity: Not applicable				
Test for overall effect: z=0.69, P=0.49				
Dalcetrapib				
Dal-Vessel 2012	0/239	1/237	0.5	0.33 (0.01 to 8.12)
Dal-Plaque 2011	1/64	2/66	0.8	0.51 (0.04 to 5.74)
Dal-Outcomes 2012	226/7938	229/7933	57.2	0.99 (0.82 to 1.19)
Subtotal	227/8241	232/8236	58.4	0.98 (0.81 to 1.18)
Test for heterogeneity: τ^2=0.00, χ^2=0.73, df=2, P=0.69, I^2=0%				
Test for overall effect: z=0.23, P=0.82				
Torcetrapib				
Radiance 1 2007	0/450	1/454	0.5	0.34 (0.01 to 8.26)
Radiance 2 2007	1/377	1/375	0.6	0.99 (0.06 to 15.96)
Illuminate 2007	93/7533	59/7534	30.9	1.58 (1.14 to 2.20)
Illustrate 2007	8/591	6/597	4.1	1.35 (0.47 to 3.92)
Subtotal	102/8951	67/8960	36.1	1.53 (1.12 to 2.09)
Test for heterogeneity: τ^2=0.00, χ^2=1.05, df=3, P=0.79, I^2=0%				
Test for overall effect: z=2.69, P=0.007				
Total (95% CI)	340/18 003	307/18 008	100.0	1.16 (0.93 to 1.45)
Test for heterogeneity: τ^2=0.01, χ^2=7.91, df=7, P=0.34, I^2=12%				
Test for overall effect: z=1.31, P=0.19				
Test for subgroup difference: χ^2=6.12, df=2, P=0.05, I^2=67.4%				



Keene D et al. BMJ 2014;349:bmj.g4379

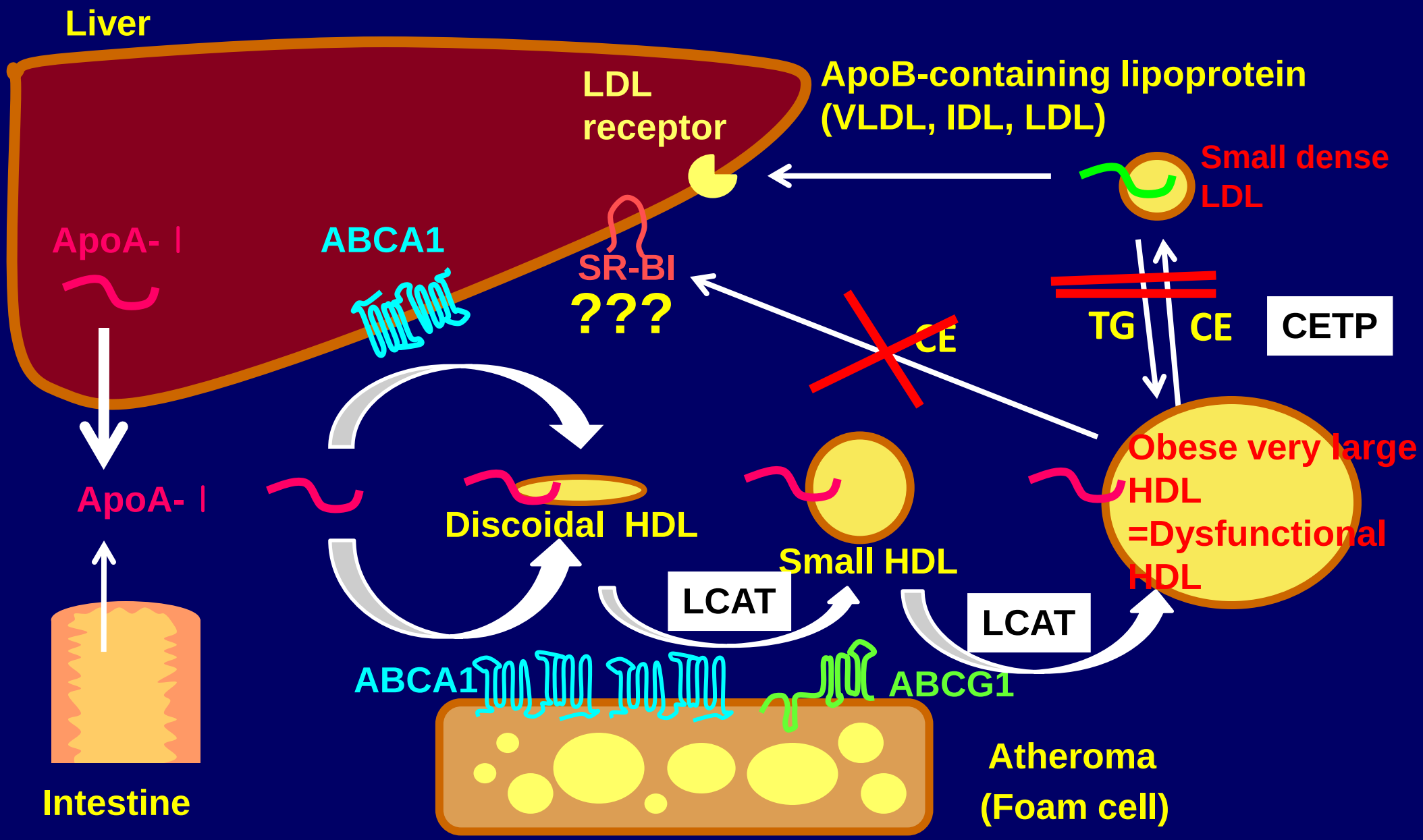
Without Background Statin Treatment, Fibrates and Niacin, But Not CETP Inhibitors Were Found to Reduce Non-fatal Myocardial Infarction

Non-fatal myocardial infarction	No of events/total Intervention	Control	Odds ratio M-H, random (95% CI)	Odds ratio M-H, random (95% CI)
Niacin				
No background statin	136/1659	394/3332		0.67 (0.54 to 0.82)
Background statin	509/15 371	527/14 939		0.94 (0.83 to 1.06)
Test for heterogeneity: I^2=87%				
Fibrate				
No background statin	773/14 236	1181/15 896		0.72 (0.65 to 0.79)
Background statin	173/2765	186/2753		0.92 (0.74 to 1.14)
Test for heterogeneity: I^2=78%				
CETP inhibitor				
Background statin	582/18 003	553/18 008		1.05 (0.93 to 1.18)



Keene D et al. BMJ 2014;349:bmj.g4379

Why CETP Inhibitors Do Not Work in Humans



Anti-atherogenic Actions of HDL

Cellular Cholesterol Efflux & Reverse Cholesterol Transport

Anti-inflammatory activity

Anti-diabetic

Anti-oxidative activity

Anti-apoptotic activity

Vasodilatory Activity

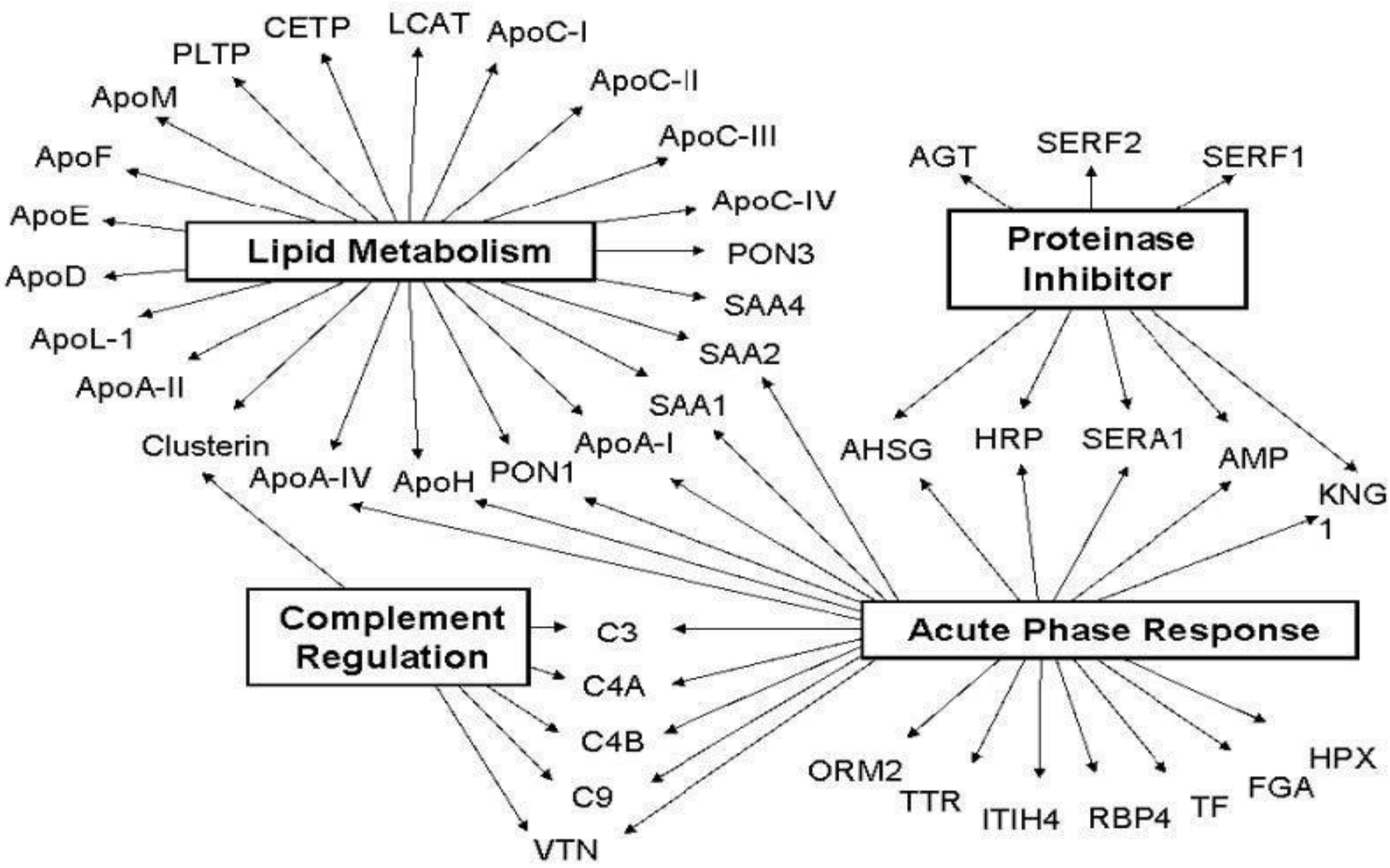
Endothelial Repair

Anti-thrombotic activity

Anti-infectious activity

Yamashita S: J Atheroscler Thromb 17:436-451, 2010

Functions of HDL-Associated Proteins



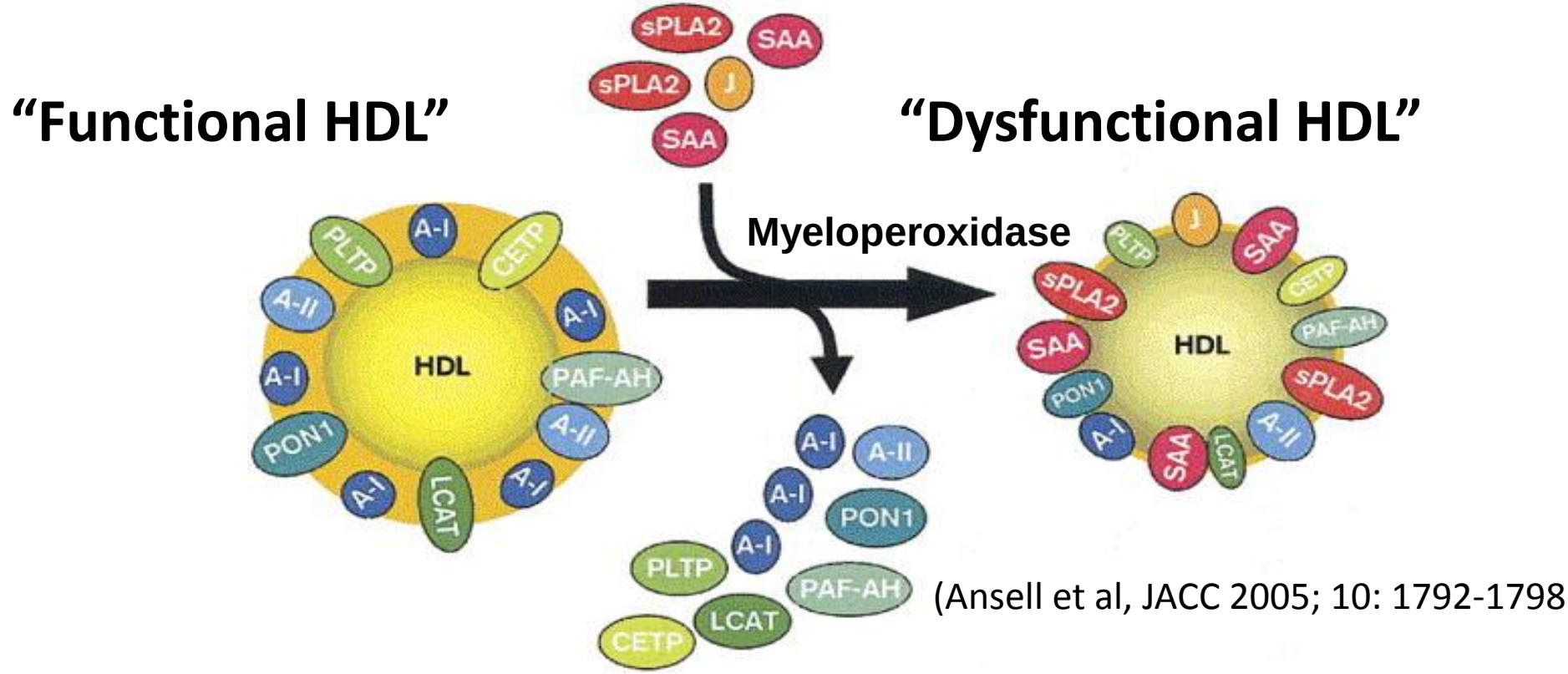
ATVB 2009

When good cholesterol goes bad?

(Fogelman AM et al, Nat Med 2004; 10: 902-903)

HDL: is it always atheroprotective?

(Ansell BJ et al, Curr Atheroscler Res 2006; 8: 405-411)

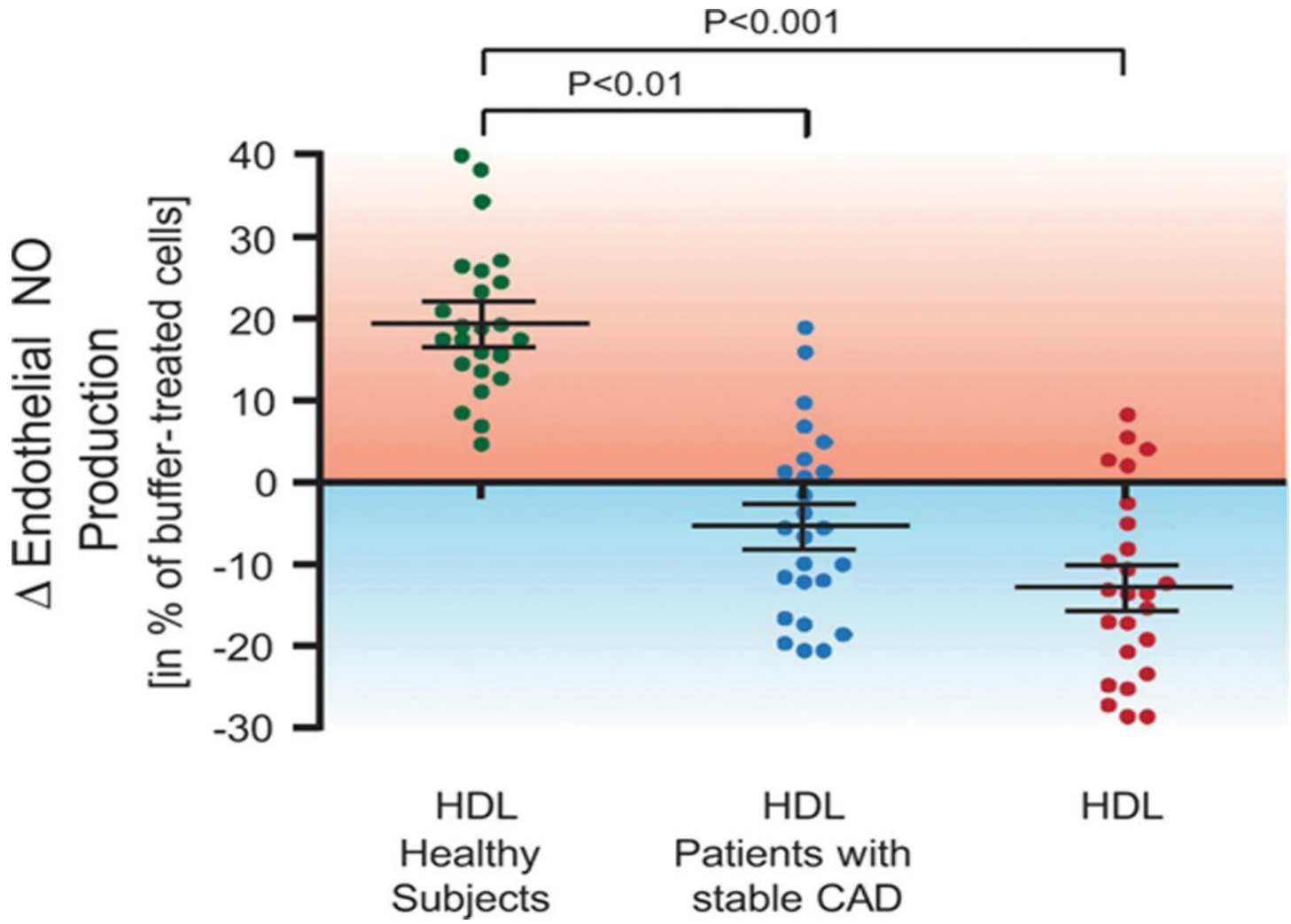


(Ansell et al, JACC 2005; 10: 1792-1798)

Quality is more important than Quantity?

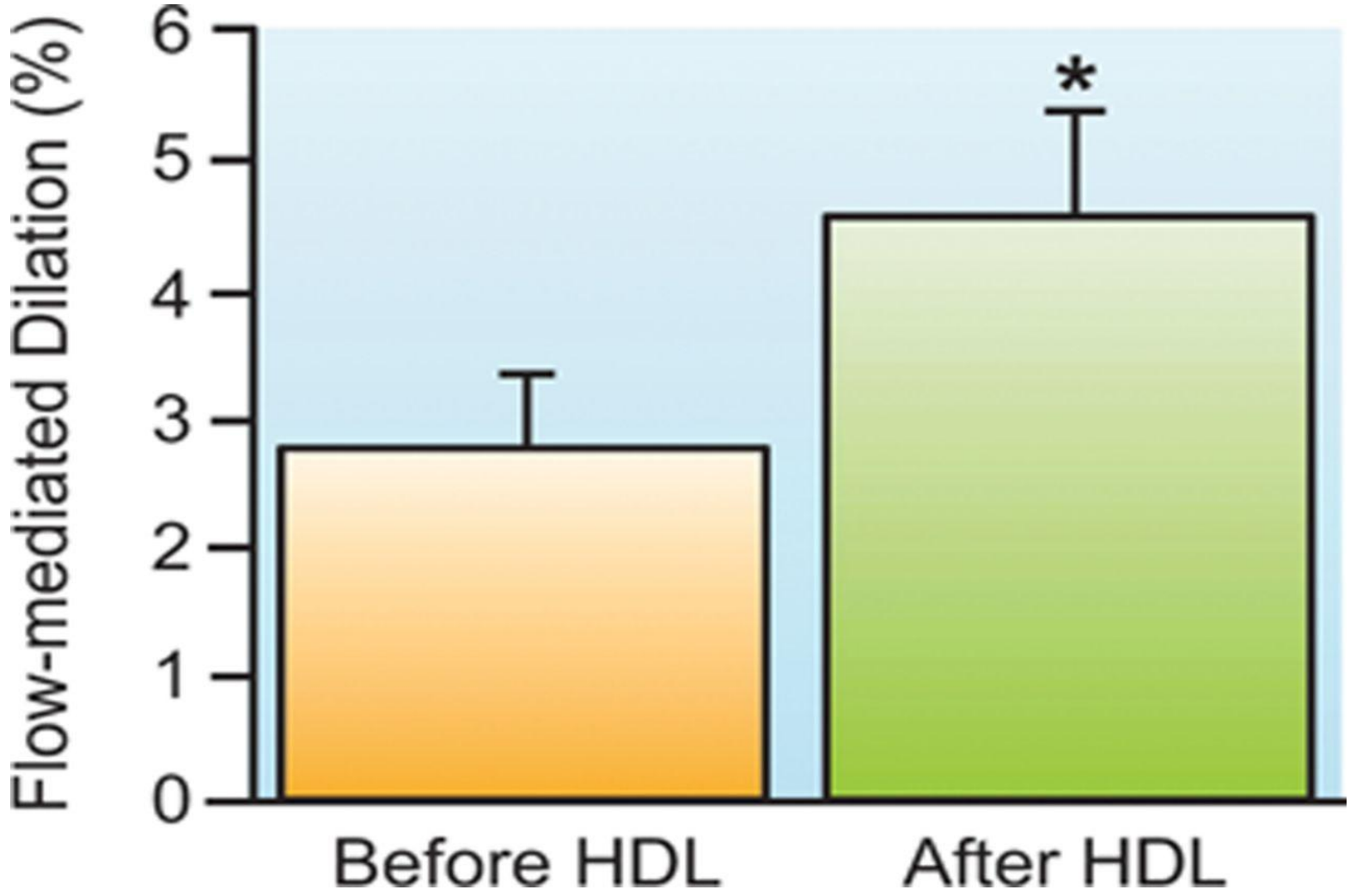
Composition of HDL is important for playing its proper role?

Effects of HDL Obtained from Healthy Subjects, Patients with CAD or Acute Coronary Syndrome on NO Release from Human Aortic Endothelial Cells



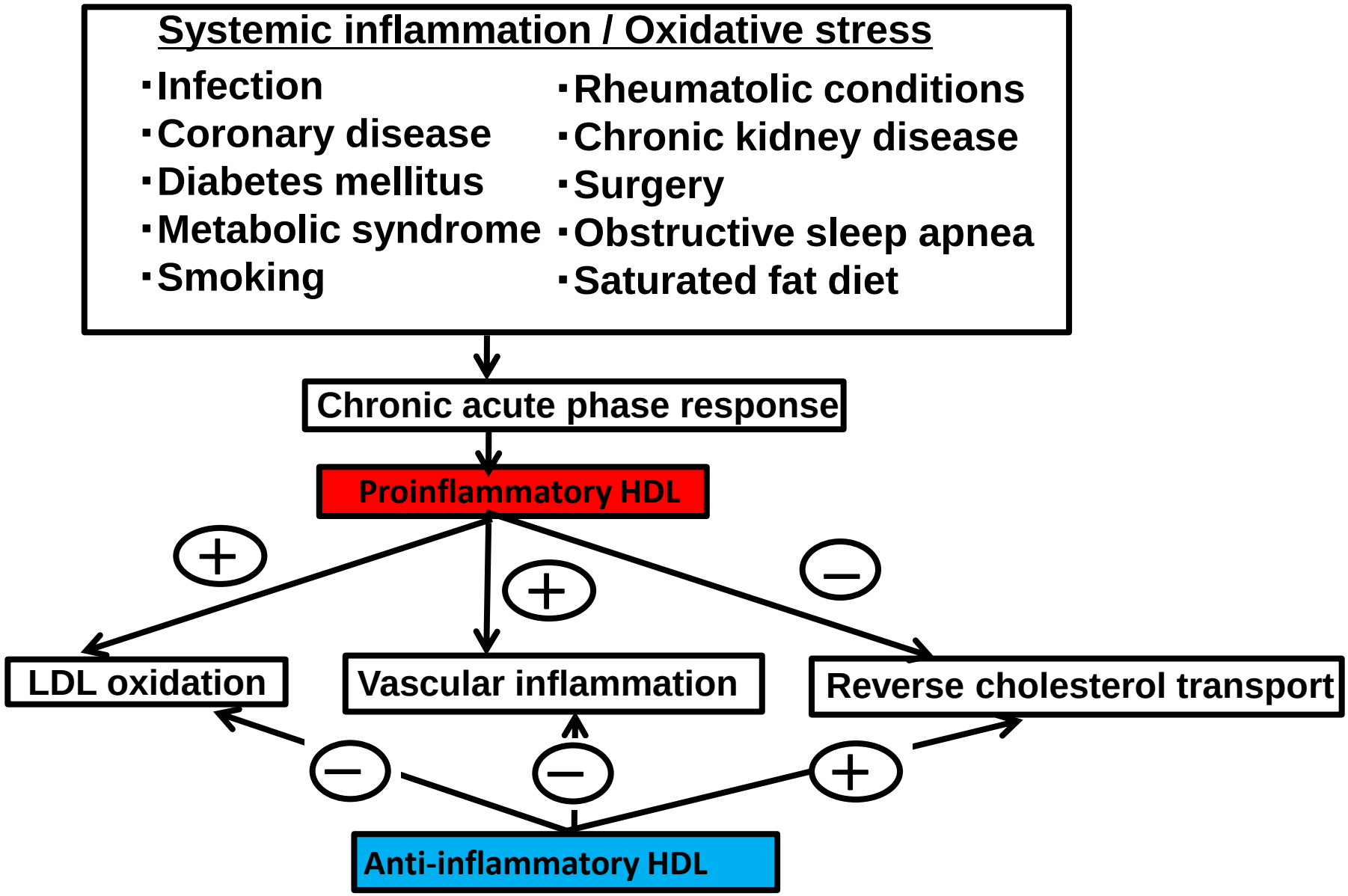
Lüscher T et al: Circulation Research 114:171-182, 2014

HDL Infusion Improves Endothelial Function in Humans



Lüscher T et al: Circulation Research 114:171-182, 2014

Functional HDL and Dysfunctional HDL



Ansell et al, Curr Opin Lipidol 18: 157-163, 2007

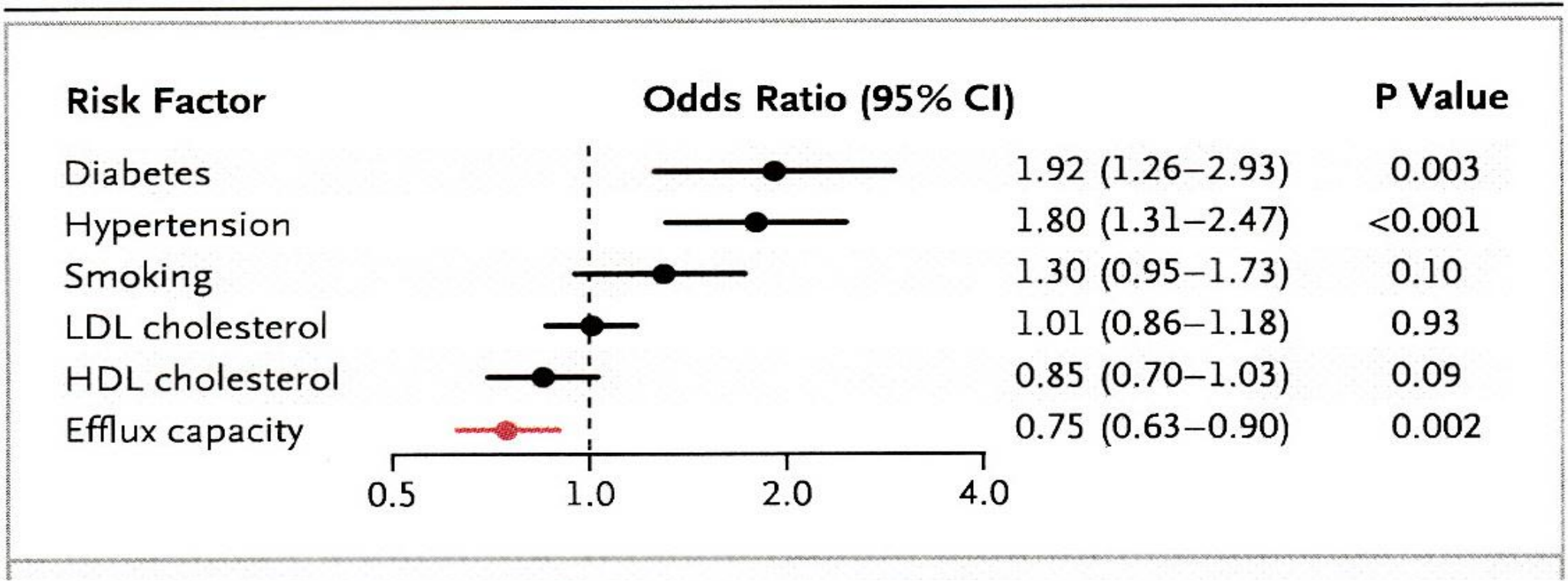
Odds Ratios for Coronary Artery Disease According to Cholesterol Efflux Capacity

Variable	No. of Patients	Odds Ratio for Coronary Artery Disease (95% CI)*		
		Adjusted for Cardiovascular Risk Factors	Adjusted for Cardiovascular Risk Factors and HDL Cholesterol	Adjusted for Cardiovascular Risk Factors and Apolipoprotein A-I
Quartile 1	198	1.00	1.00	1.00
Quartile 2	198	0.75 (0.48–1.16)	0.79 (0.51–1.24)	0.77 (0.49–1.21)
Quartile 3	198	0.58 (0.37–0.89)	0.64 (0.41–1.00)	0.63 (0.40–0.99)
Quartile 4	199	0.40 (0.25–0.63)	0.48 (0.30–0.78)	0.46 (0.28–0.75)
P value for trend		<0.001	0.002	0.002

* Cardiovascular risk factors included in the logistic-regression model were age, sex, smoking status, presence or absence of diabetes, presence or absence of hypertension, and low-density lipoprotein cholesterol. HDL denotes high-density lipoprotein.

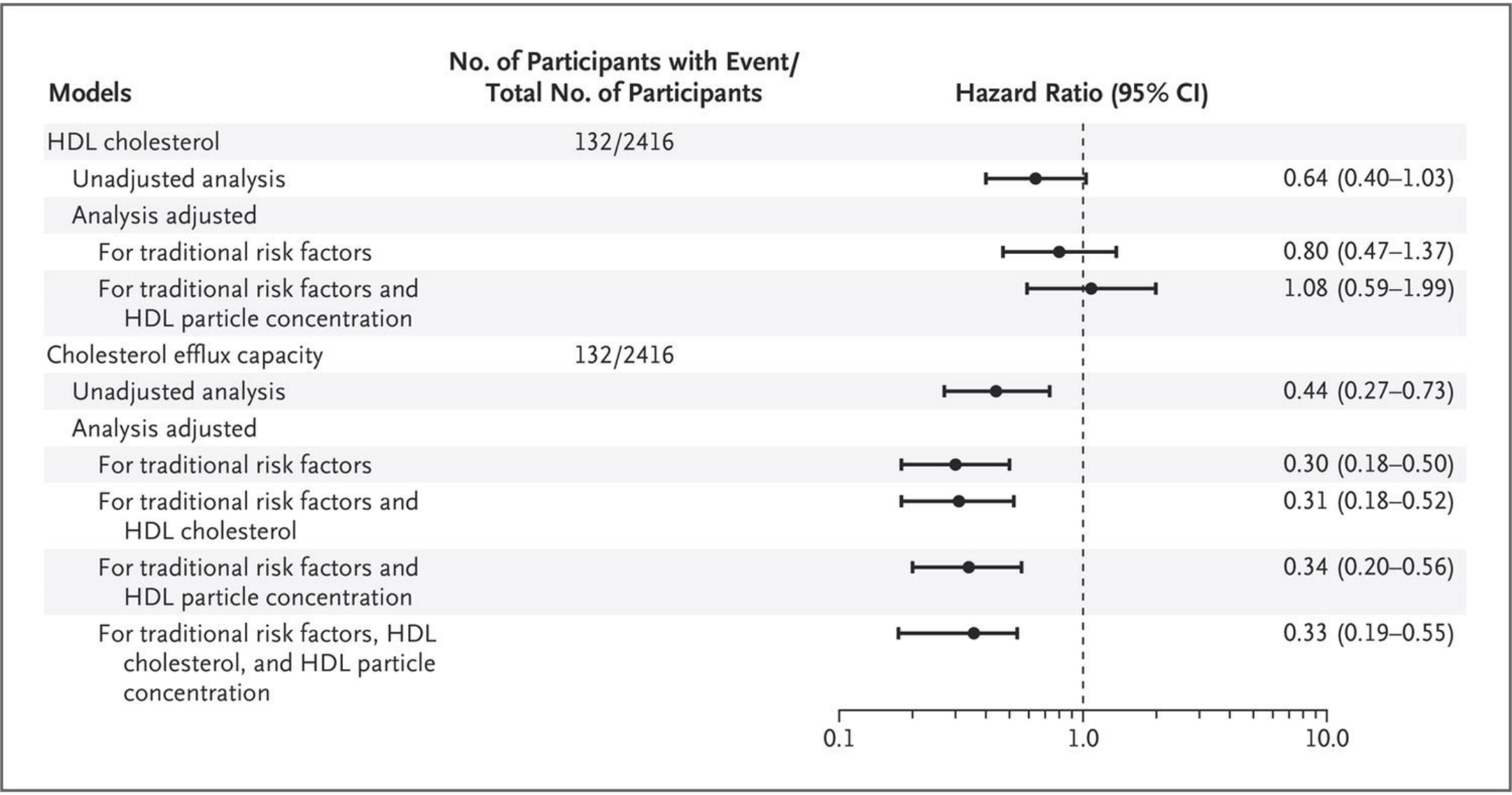
Rader et al: N Engl J Med 2011

Odds Ratios for Coronary Artery Disease According to Cholesterol Efflux Capacity and Selected Risk Factors



Cholesterol efflux capacity is more important for reduction of coronary artery disease than serum HDL-C levels.

HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events

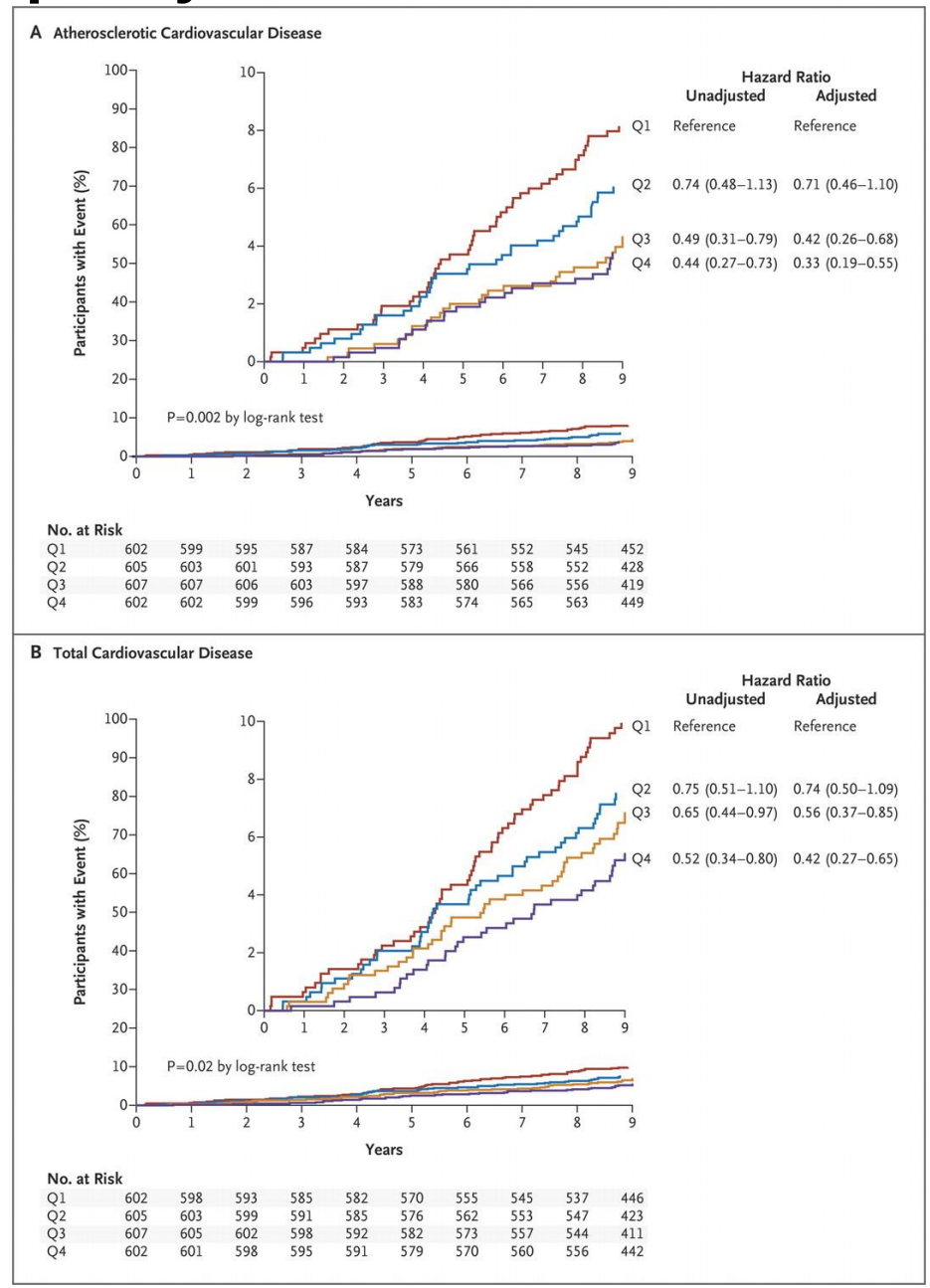


Rohatgi A, et al: N Engl J Med, Nov 18, 2014 Epub

HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events

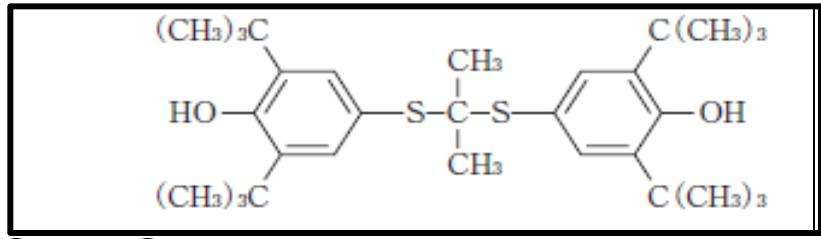
Atherosclerotic Cardiovascular Disease

Total Cardiovascular Disease



Rohatgi A, et al: N Engl J Med, Nov 18, 2014 Epub

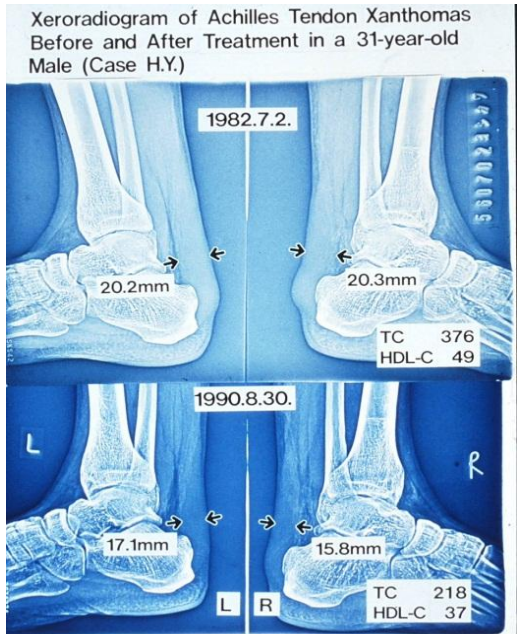
Unique Characteristics of Probucol

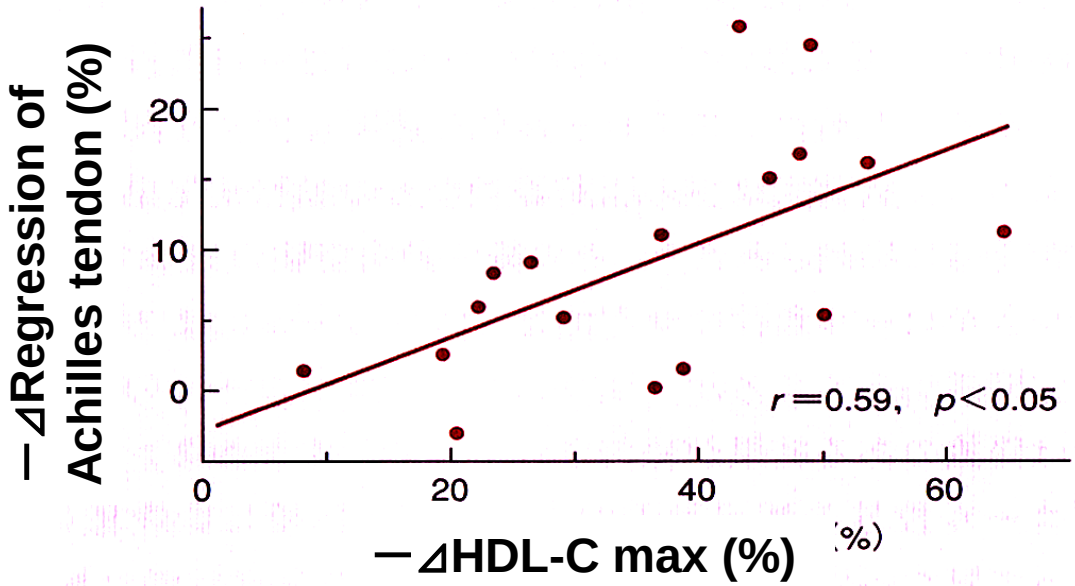


- Lowers HDL-C as well as LDL-C
- HDL is small and poor in CE
- Enhances HDL-mediated cholesterol efflux from mφ
- Accelerates HDL-mediated reverse cholesterol transport *in vivo*

by enhancement of CETP and SR-BI

- Strong anti-oxidative effect
- Reduces xanthomas (Achilles tendon, xanthelasma, etc)

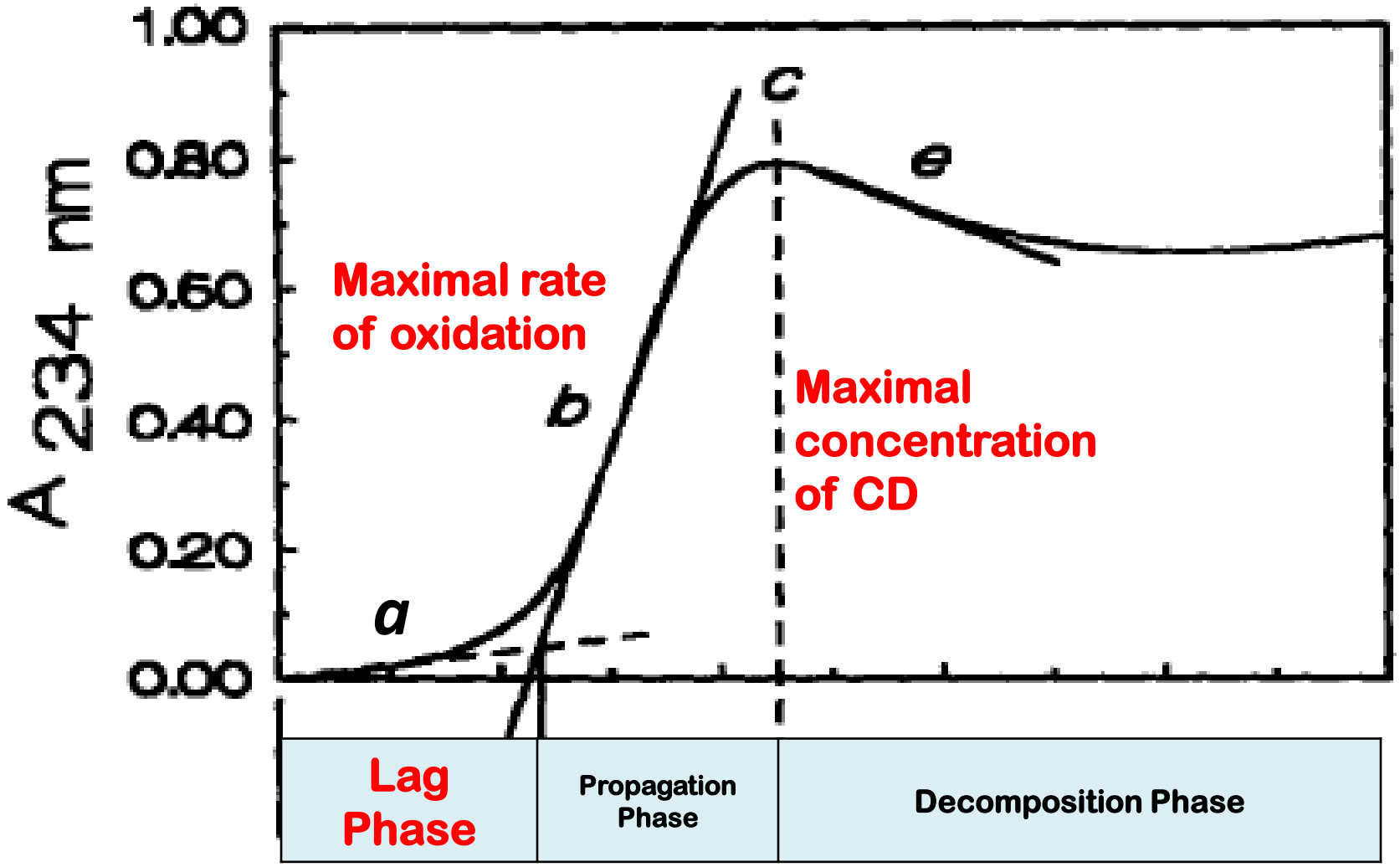




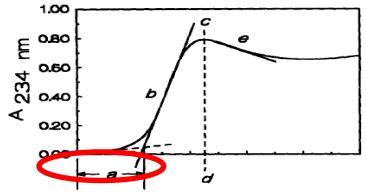
Effects of Probucol on HDL

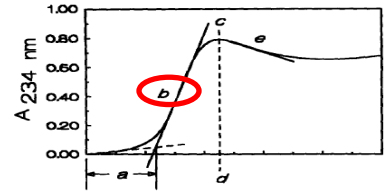
- Probucol reduces HDL-C
- HDL of probucol-treated patients is small and poor in cholesteryl ester
- HDL of probucol-treated patients has more capacity for cholesterol efflux
- HDL of probucol-treated patients has a strong anti-oxidative activity

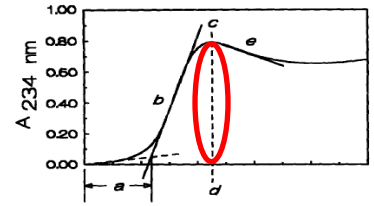
Typical Absorption Profiles (Conjugated Diene Formation) Produced during Oxidation of LDL by AAPH



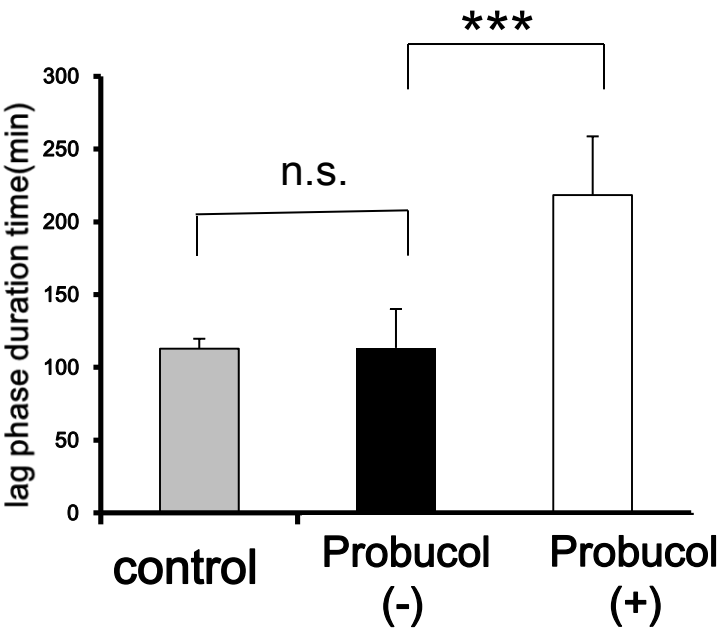
Influence of HDL Derived from FH Patients on LDL Oxidation by AAPH



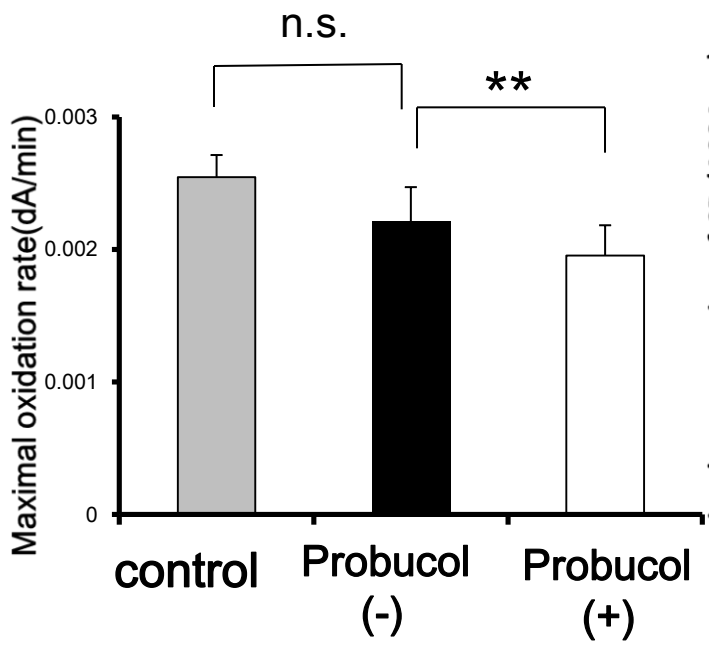




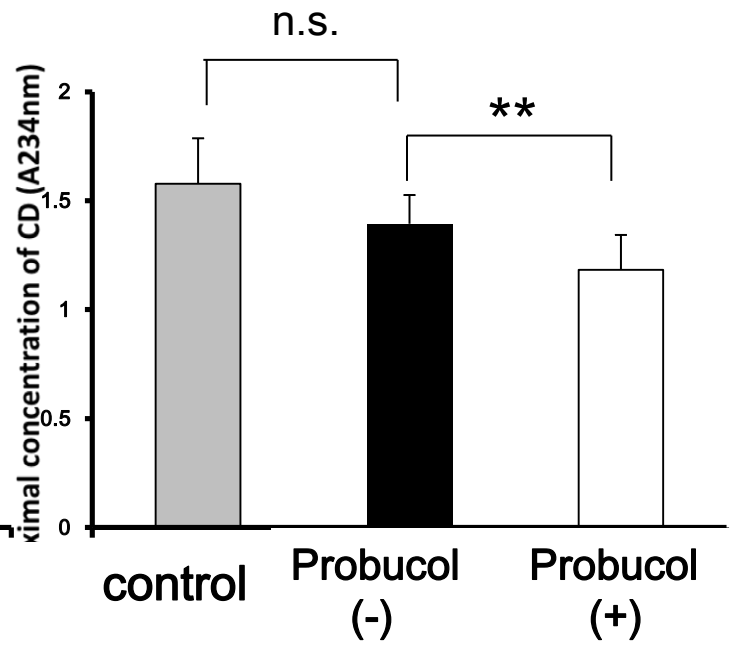
Lag phase duration



Oxidation rate

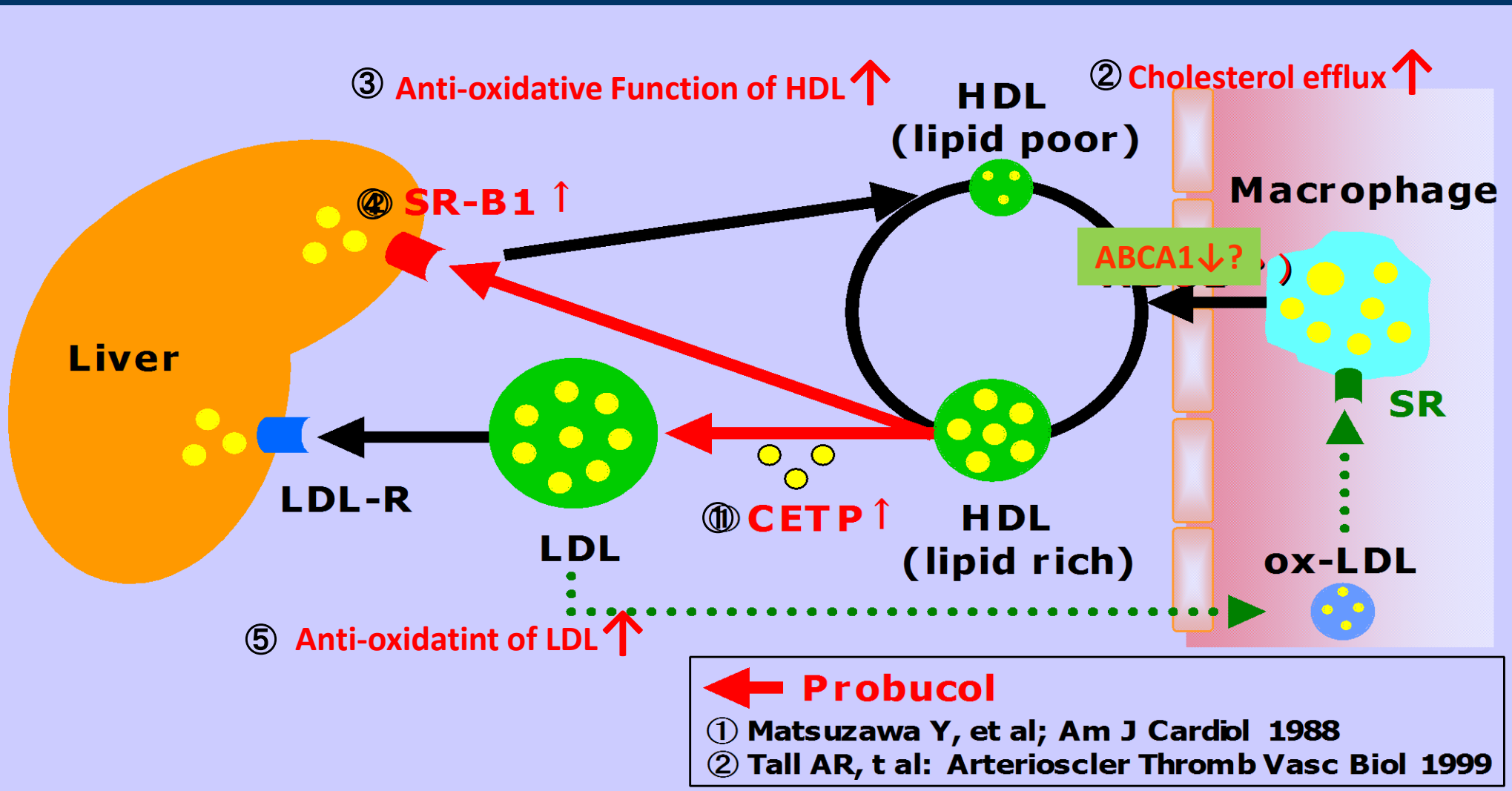


Maximum CD



**p<0.01. ***p<0.001

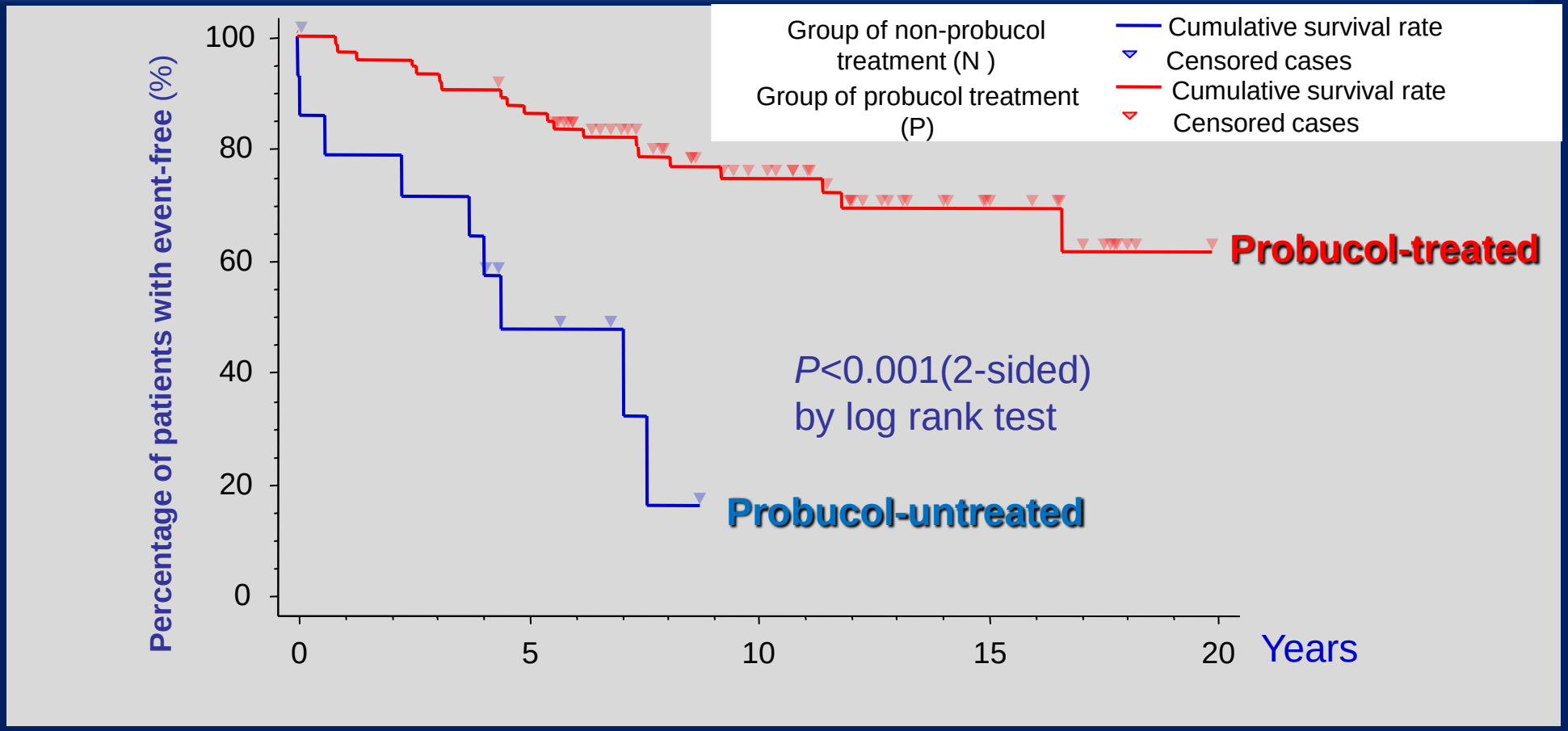
Anti-atherogenic Functions of Probucol



Randomized Clinical Trials of Probucol

PQRST	FA	P vs Placebo	lumen volume	n.s.	Walldius 1994
PART	CoroA	P vs Con	restenosis rate	23% vs 58% (p<0.001)	Yokoi 1997
MVP	CoroA	P vs MV	repeated PTCA	13% vs 26% (p<0.009)	Tardif 1997
FAST	CarotA	P vs Pra vs Diet	CV event	2.4% vs 4.8% vs 13.6% P vs Diet(p<0.001)	Sawayama 2002
PAB	FemorA	P vs Con	restenosis rate	23% vs 58% (p<0.001)	Gallino 2004
SAKURA	DiabNeph	P vs Con	interval to HD	27mo vs 11mo (p<0.02)	Endo 2006
POSITIVE	FH	P vs Con	CV event	27% vs 64% (p<0.001)	Yamashita 2008

POSITIVE:
Kaplan-Meier Survival Curve (Secondary prevention)



Year	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
P	74	71	70	68	66	62	54	50	42	38	34	30	25	19	17	13	12	8	3	1	0
N	14	11	11	10	9	5	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0

Yamashita S, et al: J Atheroscler Thromb 15:292-303, 2008

Probucol therapy improves long-term (>10-year) survival after complete revascularization

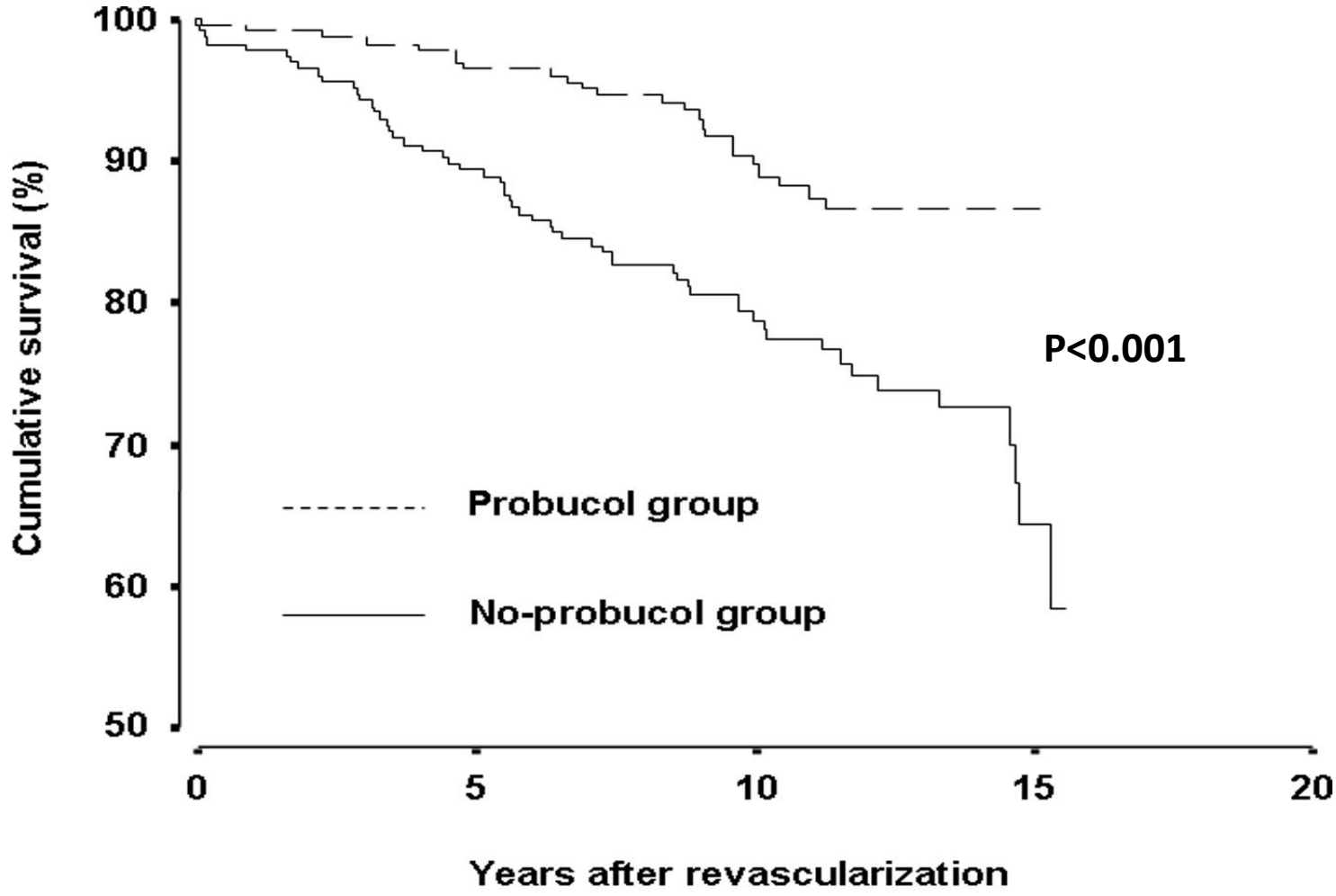
Hazard ratio of probucol use mortality

No-probucol (n=225) vs Probucol (n=225)

	HR (95% CI)	p
All-case	0.45 (0.27-0.75)	0.002

Kasai T et al: Atherosclerosis 220:463-469, 2012

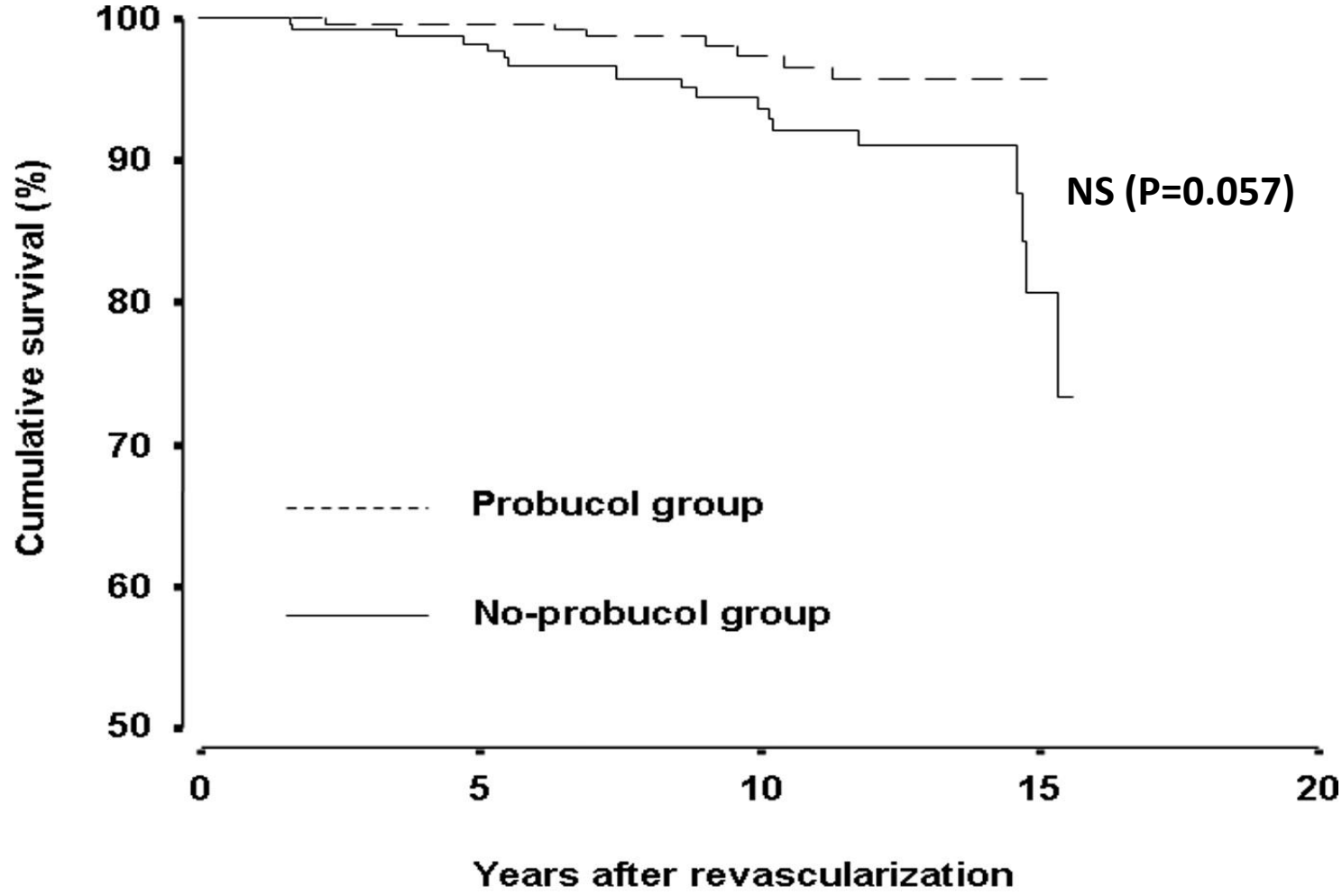
All-cause Death in the Matched Dataset



Probucol group	225	213	123	1
No-probucol group	225	200	119	16

Atherosclerosis 220:463-469, 2012

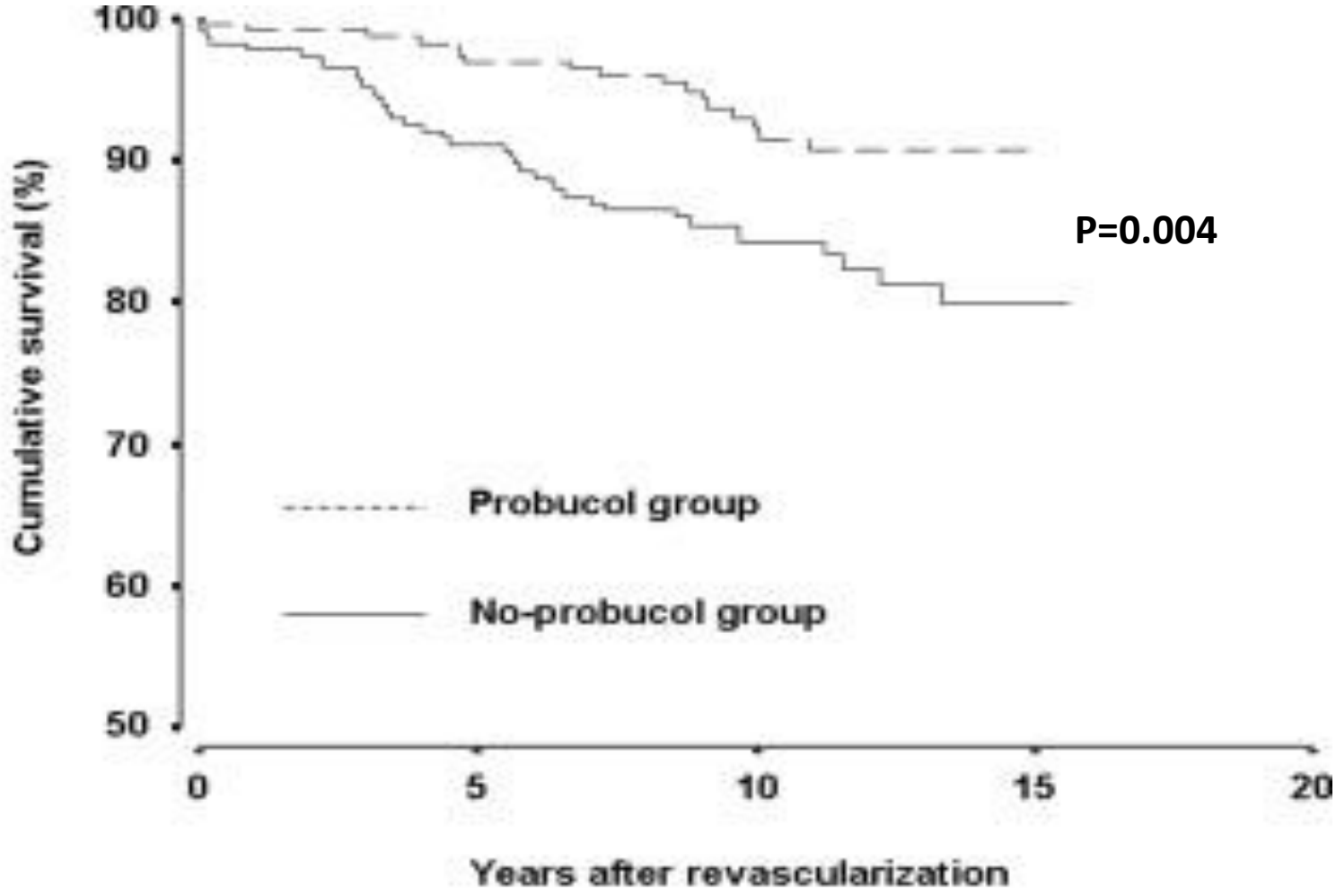
Cardiac Death in the Matched Dataset



Probucol group	225	213	123	1
No-probucol group	225	200	119	16

Atherosclerosis 220:463-469, 2012

Non-cardiac Death in the Matched Dataset



Probucol group	225	213	123	1
No-probucol group	225	200	119	16

Atherosclerosis 220:463-469, 2012

Content of secondary prevention study of probucol

TRI
（Translational Research Informatics Center）

Secondary prevention study
（Number of 860）Japan
Entry period: 2 years、
Follow-up period: 3 years
Open、Prospective,
Random、Multiple

Meta analysis of three countries of cardiovascular

Effect confirmation of secondary prevention of three countries

Japan(Number of patients:860)

article

Primary endpoint：Cardiovascular events,
Secondary endpoint：IMT

data

Korea（Number of patients:150）

China（Number of patients:192）

Primary endpoint：IMT,
Secondary endpoint：Cardiovascular events

article

Three countries Meta analysis

Take Home Messages

- HDL-cholesterol level is important, but marked hyper-HDL-cholesterolemia is not always protected from atherosclerosis
- The functions of HDL such as cholesterol efflux capacity, anti-oxidant activity and anti-inflammatory property need to be tested
- Enhancement of reverse cholesterol transport (RCT) by CETP may protect CV events rather than inhibition of CETP
- Probucol reduces HDL-C by enhancing CETP and RCT, preventing atherosclerotic cardiovascular diseases and coronary restenosis after PCI