



# HDL functions - beyond reverse cholesterol transport

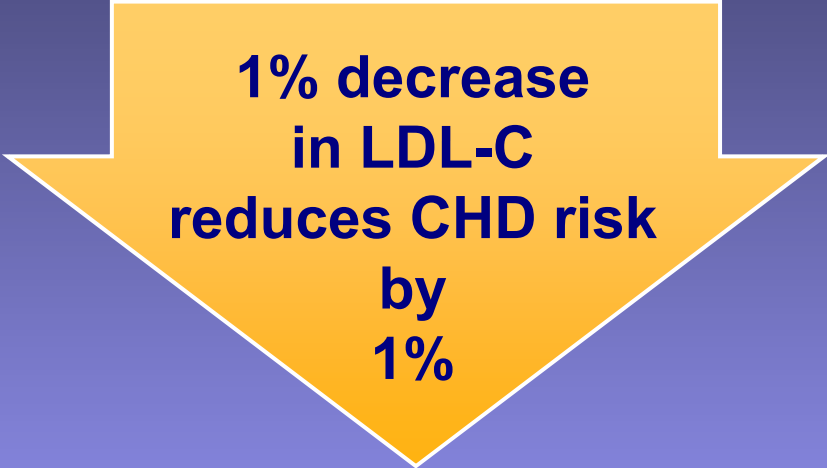
**Ulf Landmesser**

Department of Cardiology  
University Hospital Zurich

**EuroPrevent 2009**  
**Novel frontiers in cardiovascular prevention**

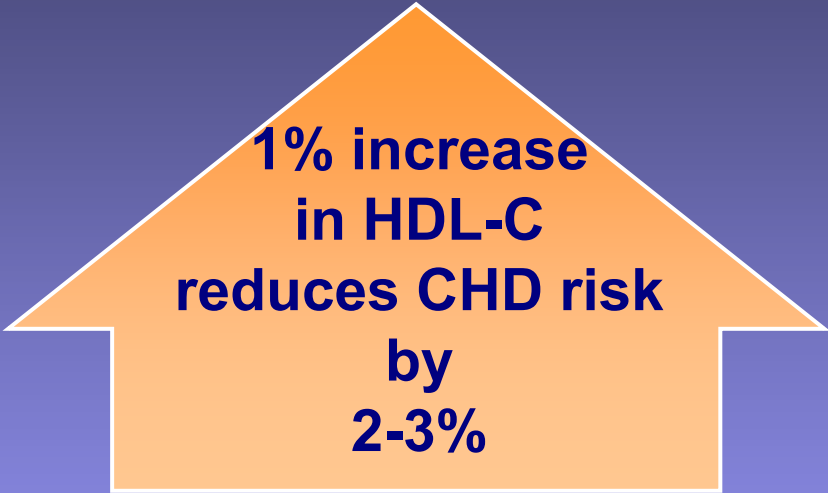
# LDL-Cholesterol/HDL-Cholesterol and risk of coronary disease

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1% decrease  
in LDL-C  
reduces CHD risk  
by  
1%

**Statin  
Therapy**

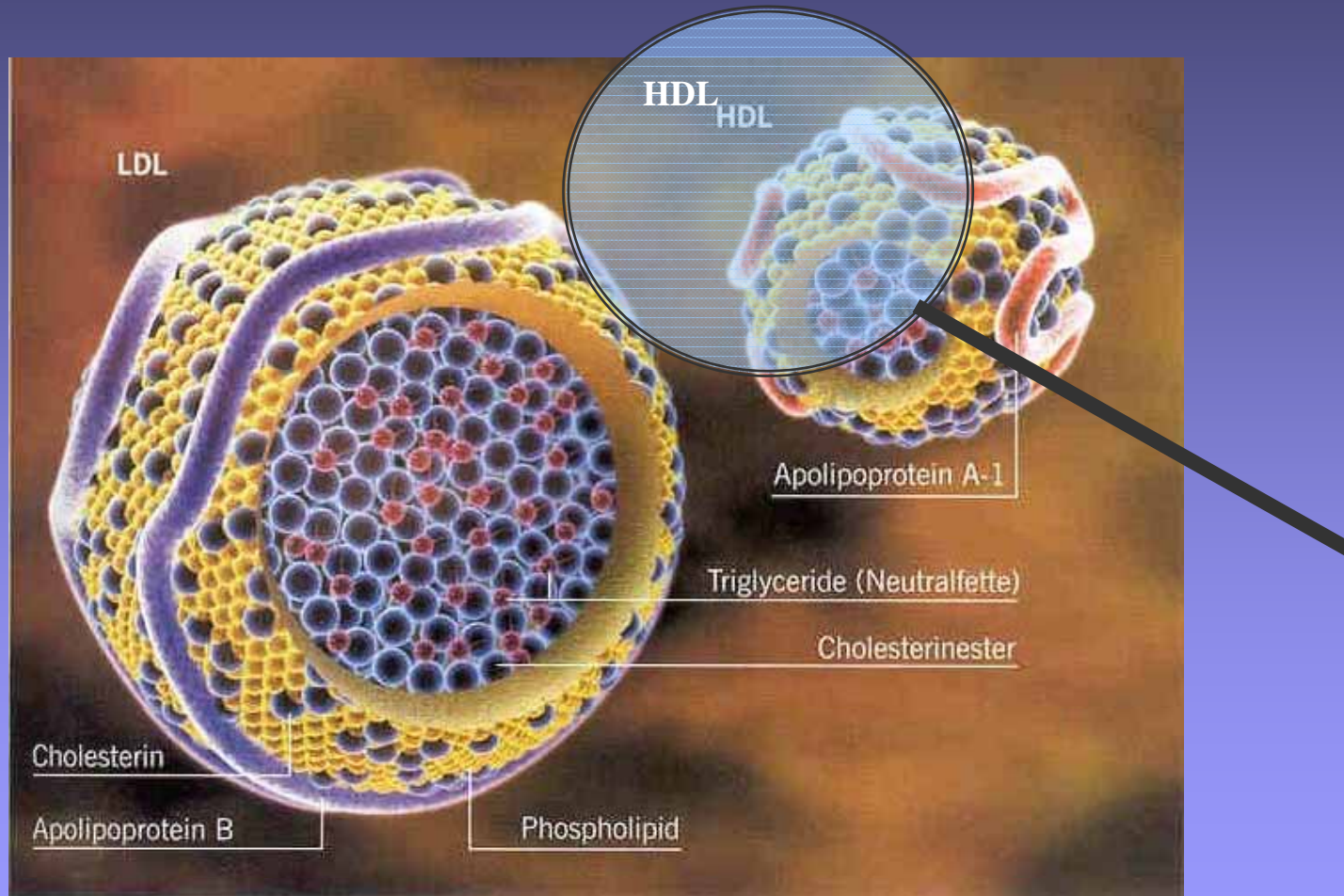


1% increase  
in HDL-C  
reduces CHD risk  
by  
2-3%

**Therapeutic  
Opportunity ?**

# HDL – a novel target in prevention ?

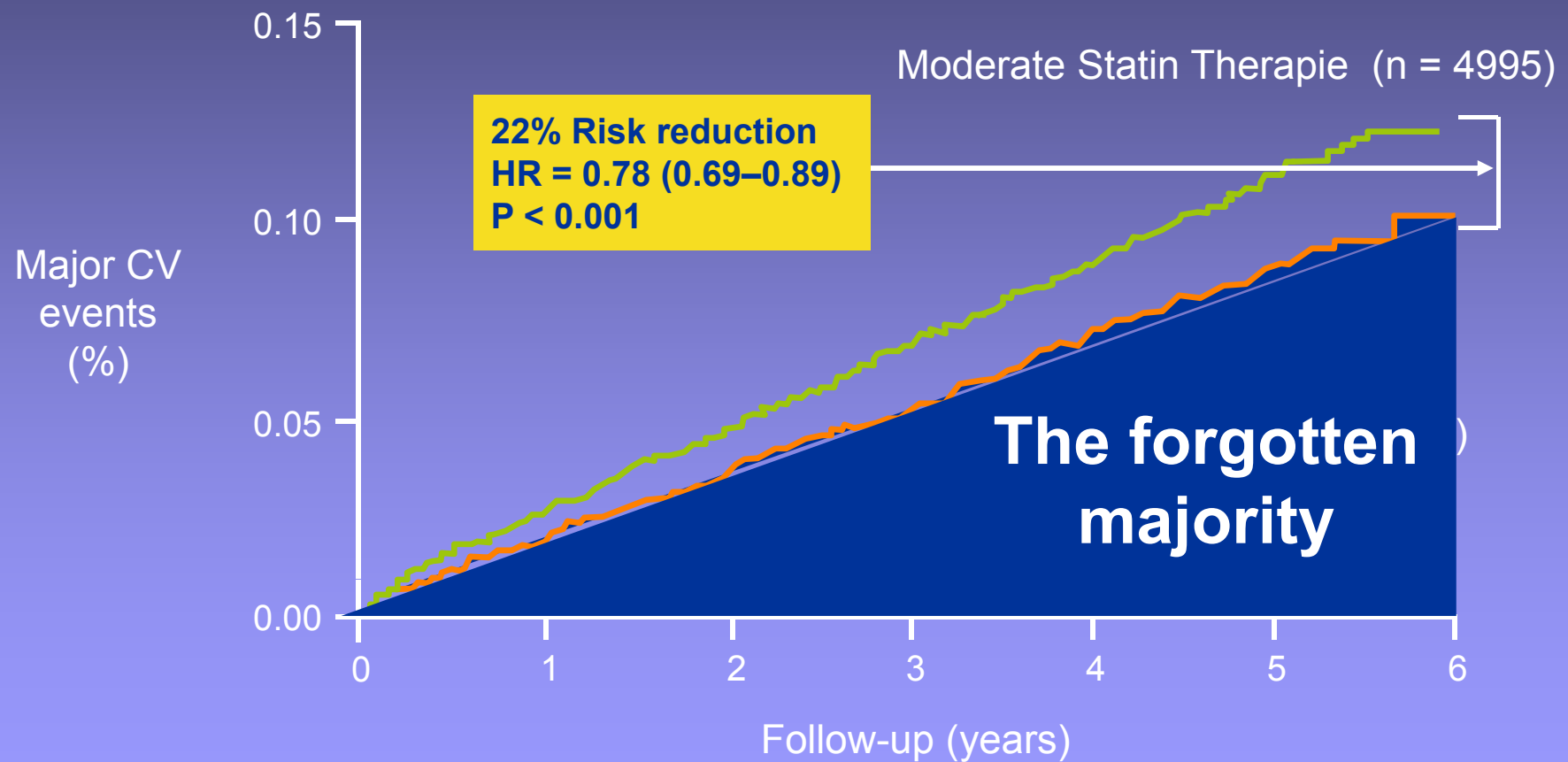
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# Intense statin therapy improves outcome –but still substantial residual risk

Treating to New Targets

N = 10,001



CHD death, MI, resuscitation after cardiac arrest, fatal/nonfatal stroke

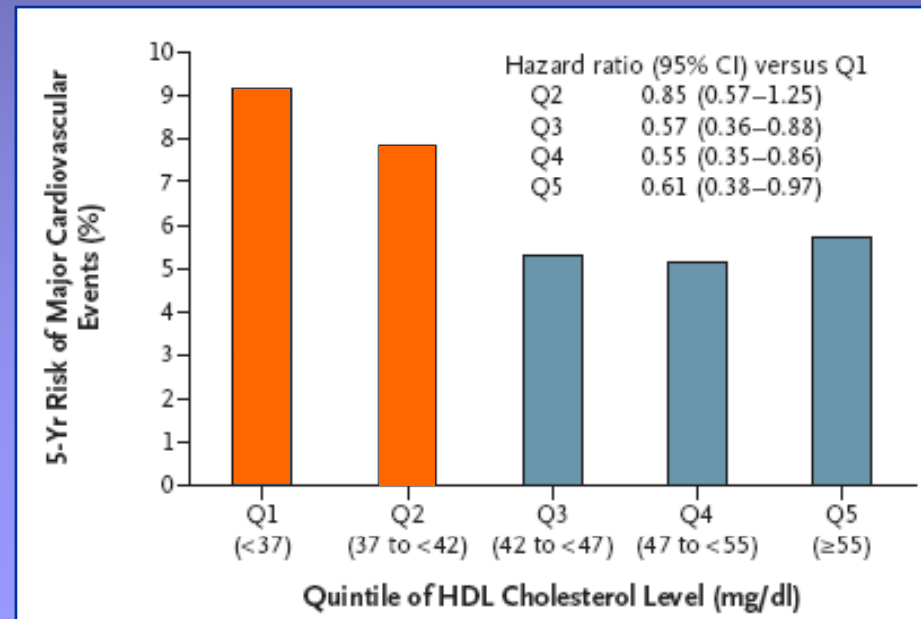
LaRosa JC et al. *N Engl J Med.* 2005;352:1425-35

# Low HDL remains a predictor of cardiovascular risk in statin-treated patients

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

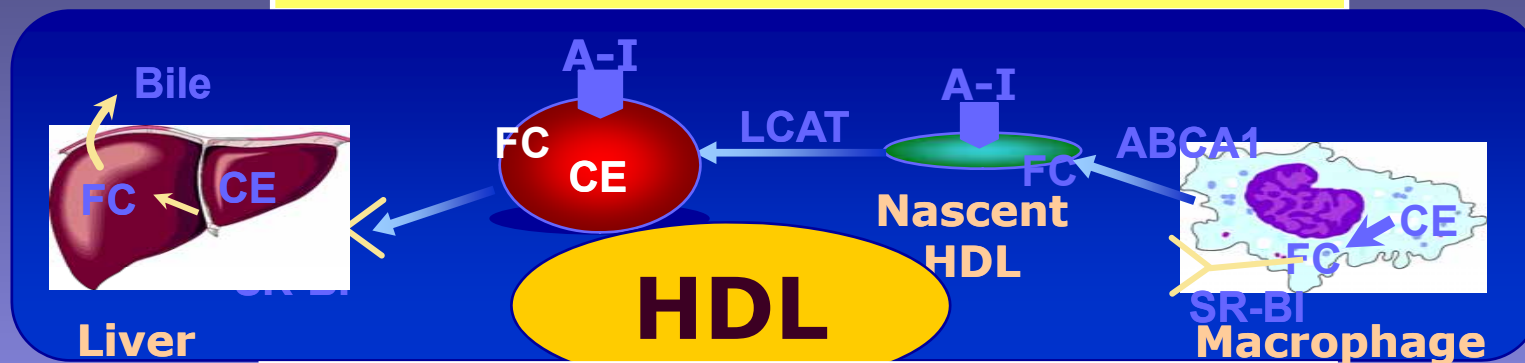
## HDL Cholesterol, Very Low Levels of LDL Cholesterol, and Cardiovascular Events



Barter P et al., *N Engl J Med* 2007; 357: 1301-10

# HDL: proposed anti-atherogenic effects

## 1. Promotion of RCT (reverse cholesterol transport)



## 2. Direct vasoprotective effects

Endothelial NO  
Production

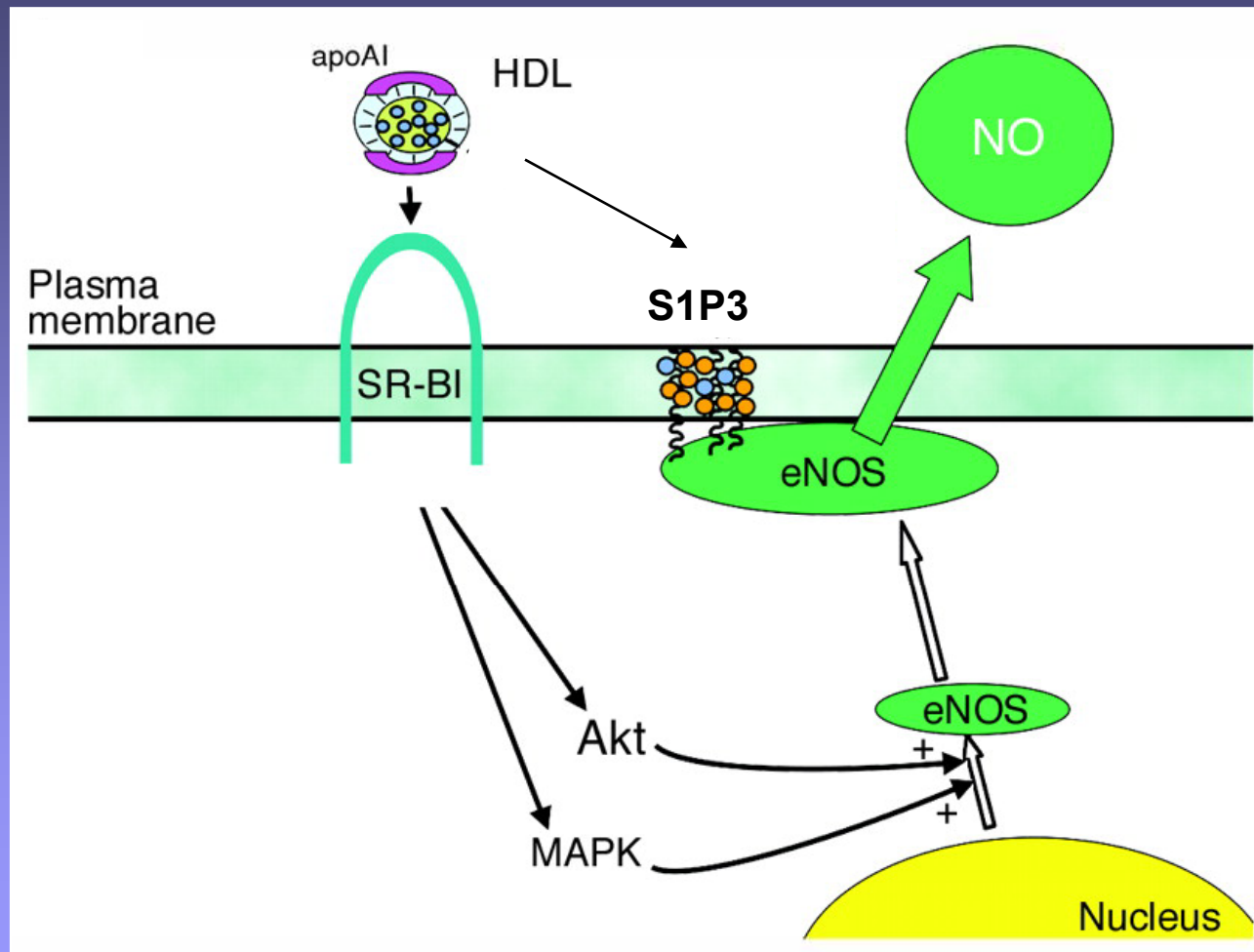
Antioxidant  
Effects

Anti-inflam.  
Effects

Endothelial  
Regeneration

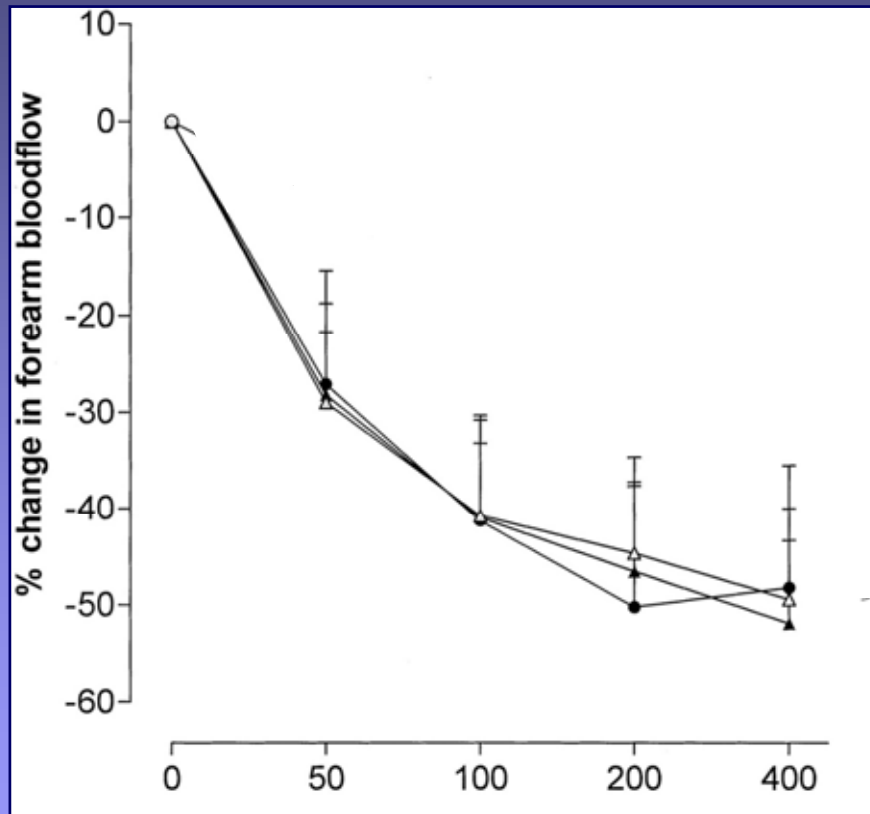
Anti-thromb.  
Effects

# Vasculoprotective effect of HDL: role of nitric oxide (NO)



Yuhanna IS et al.; *Nat Med* 2001  
Nofer et al.; *J. Clin. Invest.* 2004

# Reduced endothelial release of nitric oxide in patients with isolated low HDL (Tangiers disease)

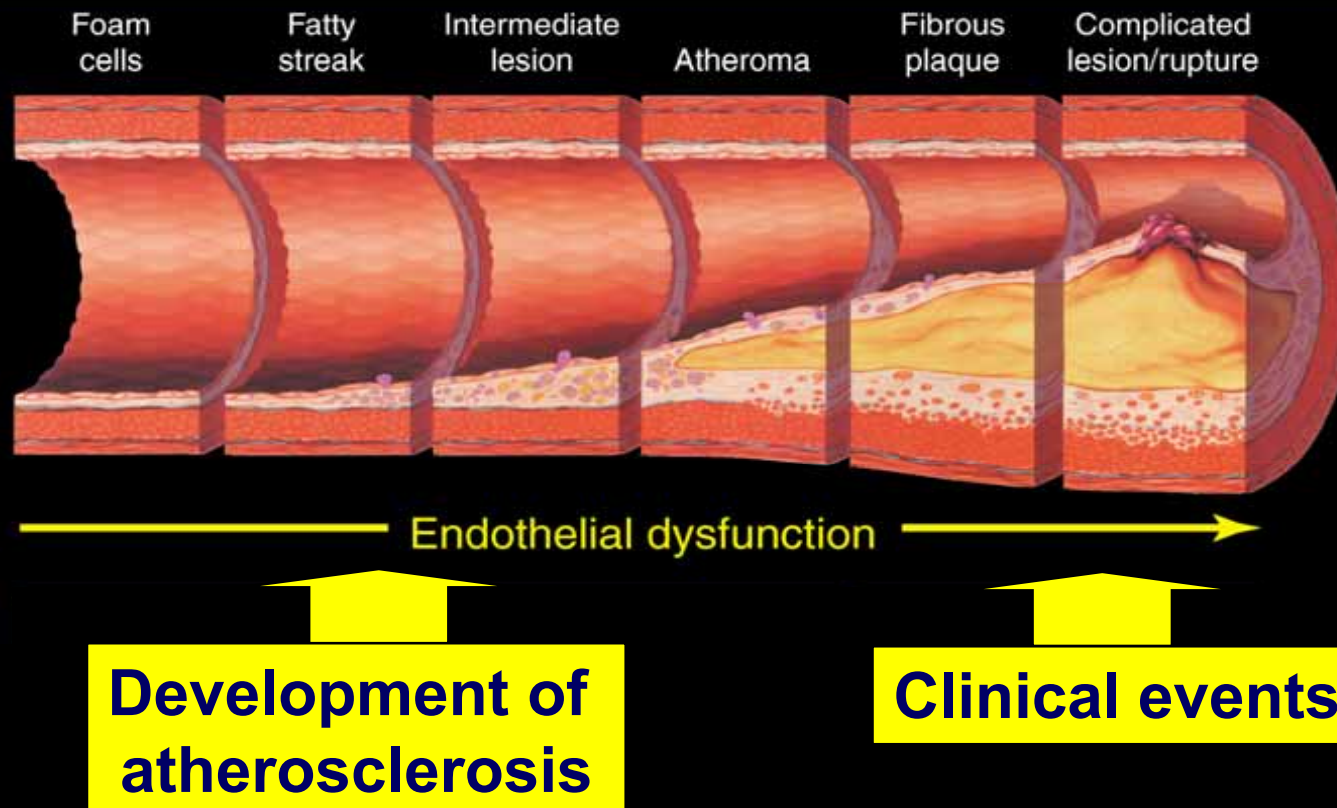


Isolated low HDL  
Tangiers Disease  
(ABCA-1 heterozygot)

After infusion of  
reconstituted HDL

# Endothelial dysfunction, nitric oxide and atherosclerosis

## Atherosclerosis timeline

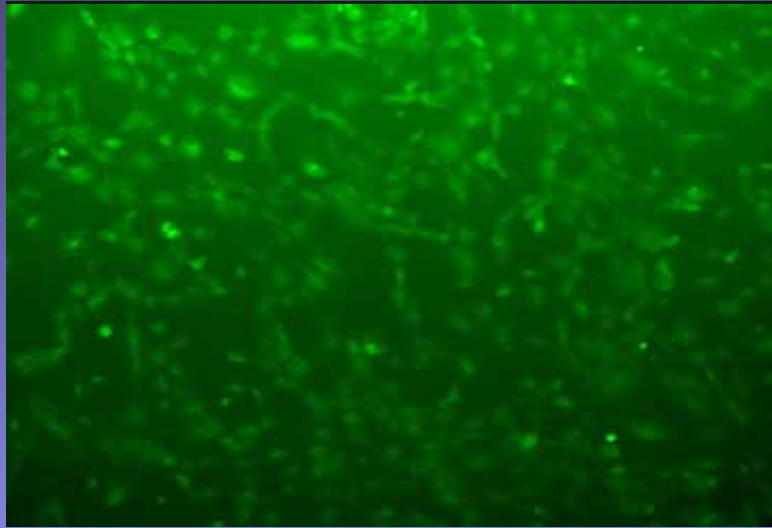


Summarized in:

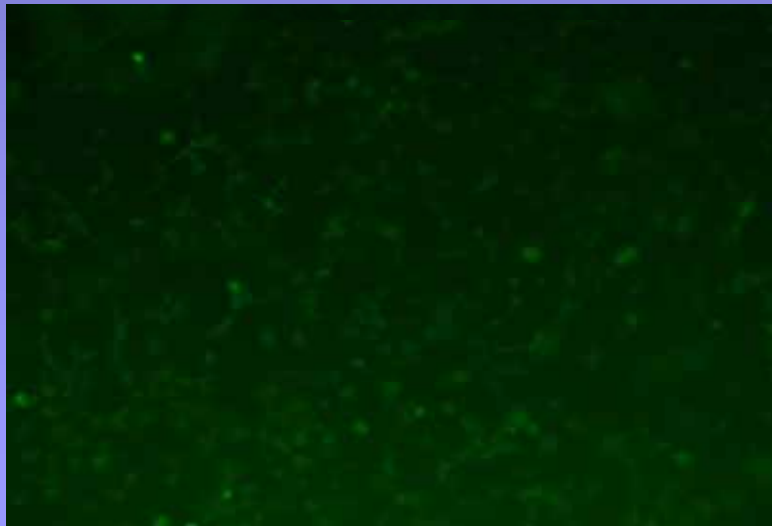
Landmesser et al. *Circulation* 2004; 109(21 Suppl 1):II27-33

# Effect of HDL on endothelial cell nitric oxide production ?

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HDL from  
Healthy subject



HDL from  
Patient with CAD

30 minutes

**Vascular effects of HDL  
in patients with stable  
coronary disease or  
acute coronary syndrome  
as compared to healthy  
subjects ?**

# Study setup

Patients with acute coronary syndrome (n=18)  
Patients with stable coronary disease (n=18)  
Healthy control subjects (n=16)

**Isolation of HDL<sub>2/3</sub>**  
(by sequential ultracentrifugation)

**Vascular effects**

**Endothelial Function**

(Endothelial cell NO production and vasoreactivity)

ESR spectroscopy  
Organ chamber

**Anti-oxidant effects**

(Endothelial cell superoxide production)

ESR spectroscopy

**Anti-inflammatory effects**

(Endothelial cell inflammatory activation)

Monocyte adhesion  
VCAM-1 expression

**Anti-thrombotic effects**

Tissue factor  
Arterial thrombosis

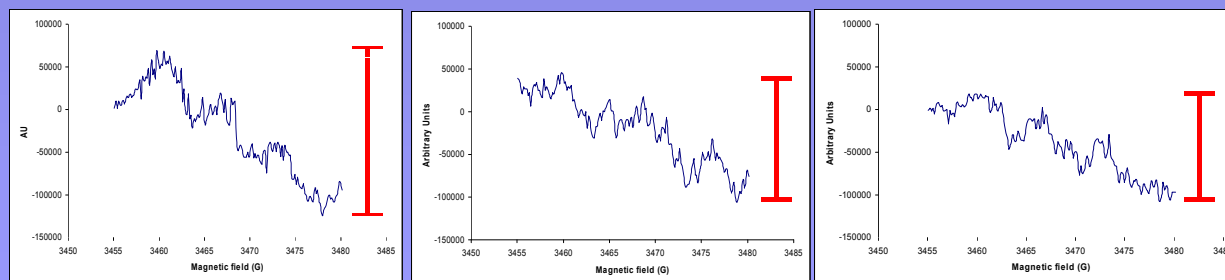
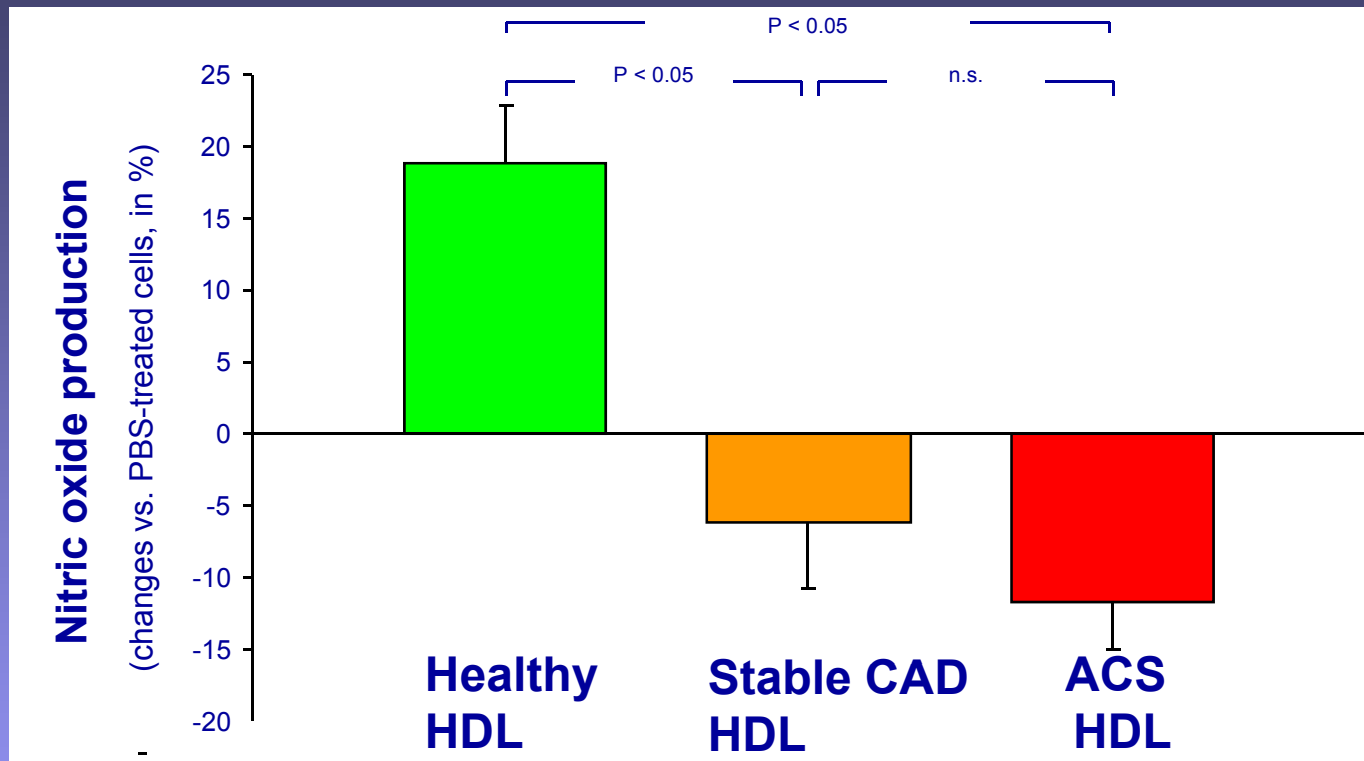
**Effects on Re-Endothelialization**

Carotid artery injury model in nude mice

# Characteristics of the study population

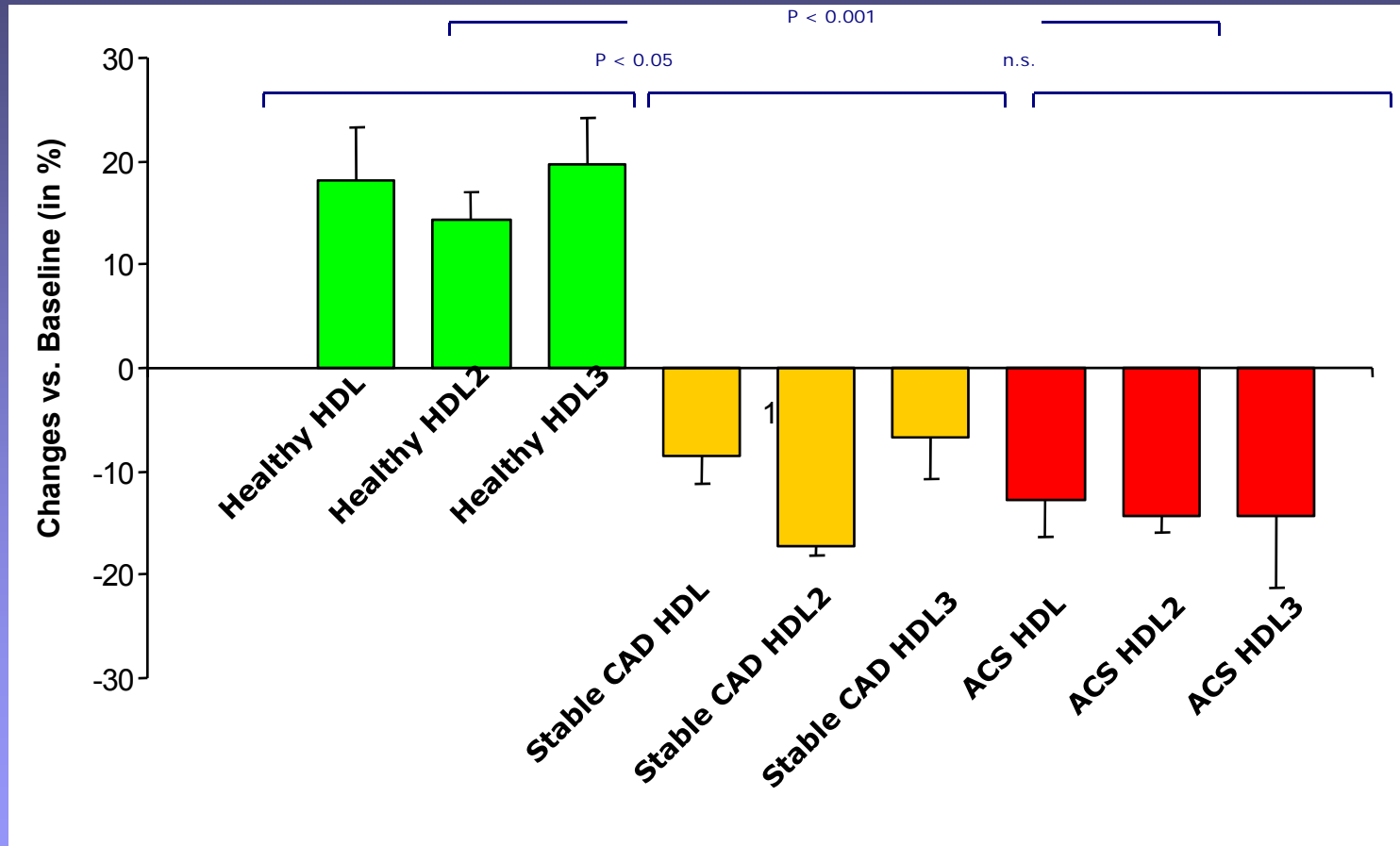
	Healthy subjects (n=16)	Stable coronary artery disease (n=18)	Acute coronary syndrome (n=18)
Age (years)	53±4	56±6	54±6
Sex (male/female)	10/5	11/5	11/4
Body mass index (kg/m <sup>2</sup> )	24.9±1.9	27.2±4.1	26.2±3.1
Total cholesterol (mmol/l)	4.9±0.8	4.2±1.1	4.5±0.7
HDL cholesterol (mmol/l)	1.47±0.47	1.27±0.25	1.21±0.32
LDL cholesterol (mmol/l)	2.8±0.8	2.6±0.9	2.8±0.6
C-reactive protein (µmol/l)	2±1	3±5	4±8

# HDL`s vascular effects in patients with coronary disease



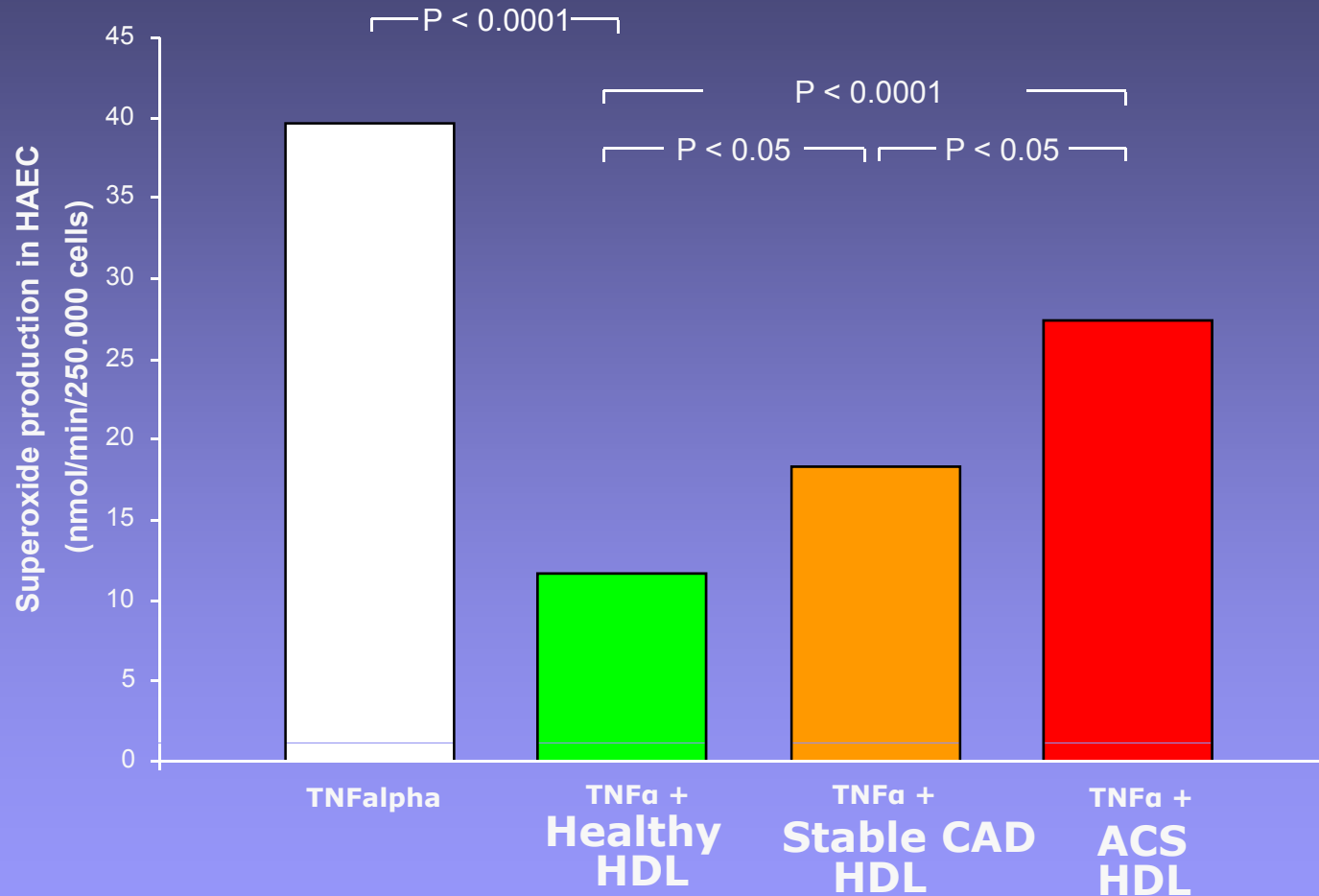
(ESR spectroscopy measurement)

# HDL: Effect on endothelial cell nitric oxide production



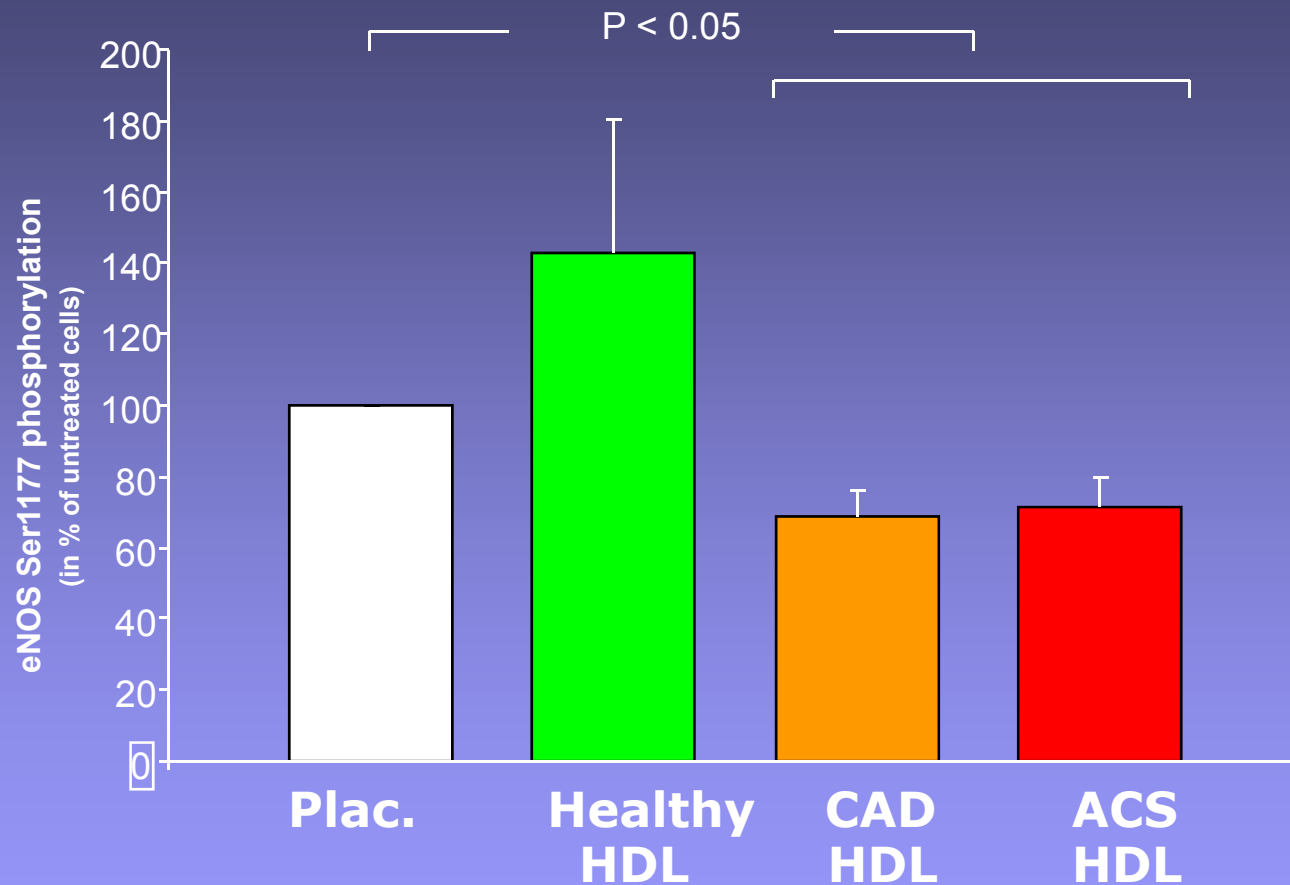
**NO production of human aortic endothelial cells  
(ESR spectroscopy measurement)**

# Effects of HDL on endothelial cell superoxide production



Superoxide production in TNF $\alpha$ -stimulated human aortic endothelial cells (ESR spectroscopy analysis)

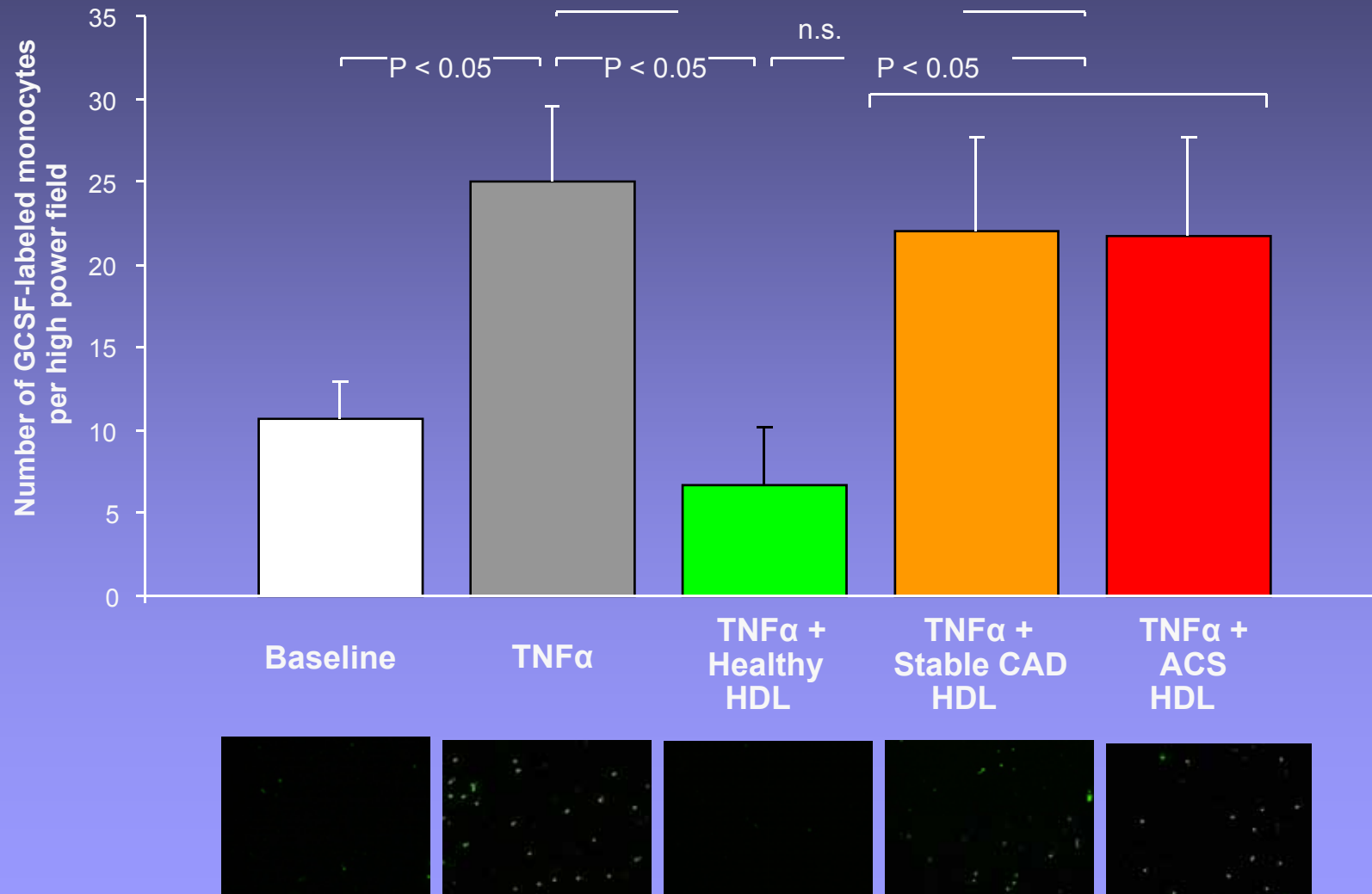
# Effects of HDL on eNOS activation: eNOS phosphorylation in endothelial cells



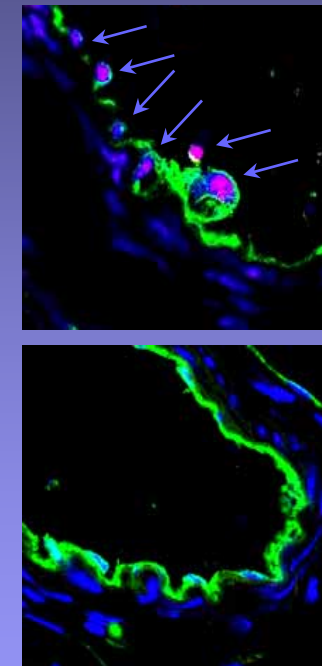
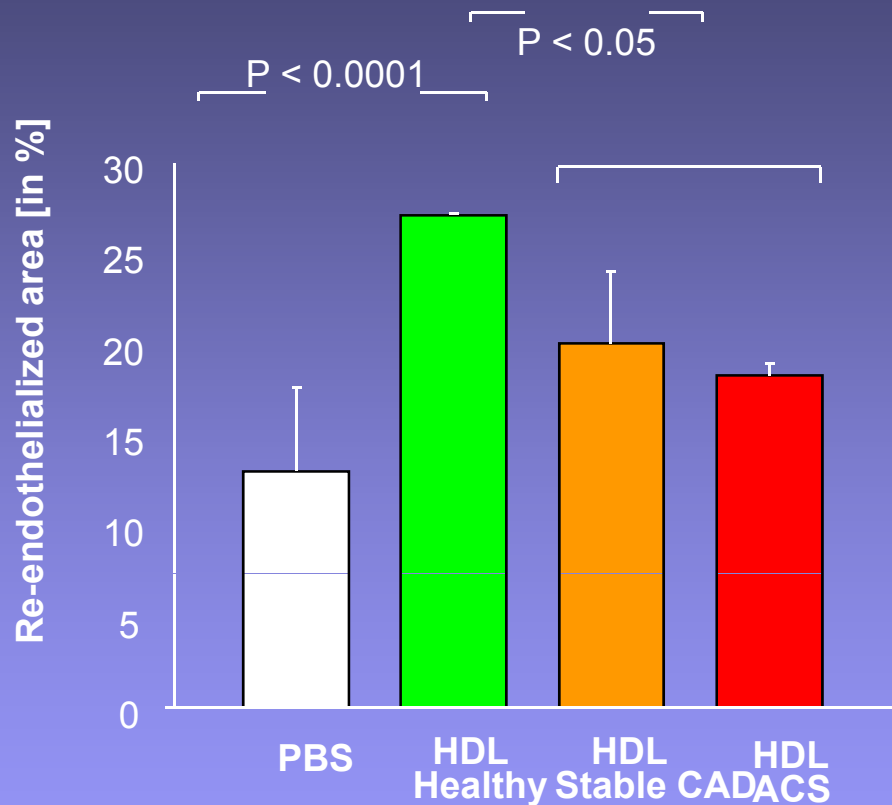
**eNOS phosphorylation (Se1177)**

(Western Blot analysis)

# Effects of HDL on vascular inflammation: monocyte adhesion on TNF $\alpha$ -stimulated endothelial cells



# Effects of HDL on endothelial repair after arterial injury



Quantification of re-endothelialized area 3 days after induction of carotid injury by Evan`s blue staining

# **HDL function** (vascular effects)

- **The true therapeutic target ?**

# **HDL function** (vascular effects)

**Which changes of  
HDL are mediating  
differences in HDL's  
vascular effects ?**

# HDL characterisation: Functionally relevant changes

**Mechanisms of altered function of HDL ?**

**„Lipidomics“**

**Proteomics**

**HDL binding to  
endothelial cells**

**HDL-  
cargo**

**A.von  
Eckardstein**

**Analysis of HDL-  
associated Lipids**

**Functional  
genomis center**

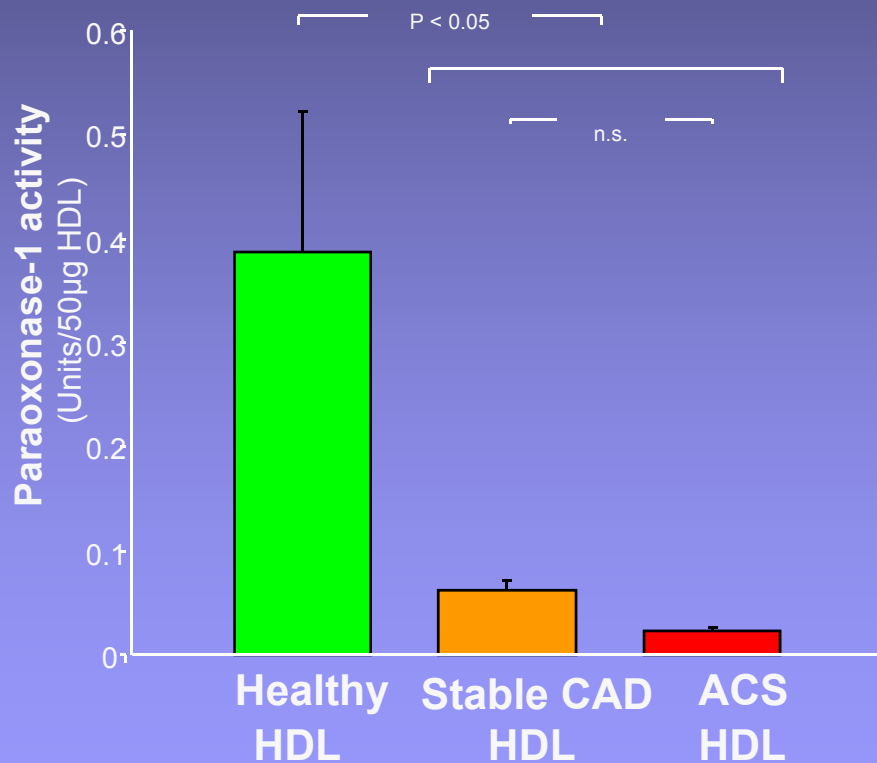
**Analysis of HDL-  
associated proteins**

**Binding assays**

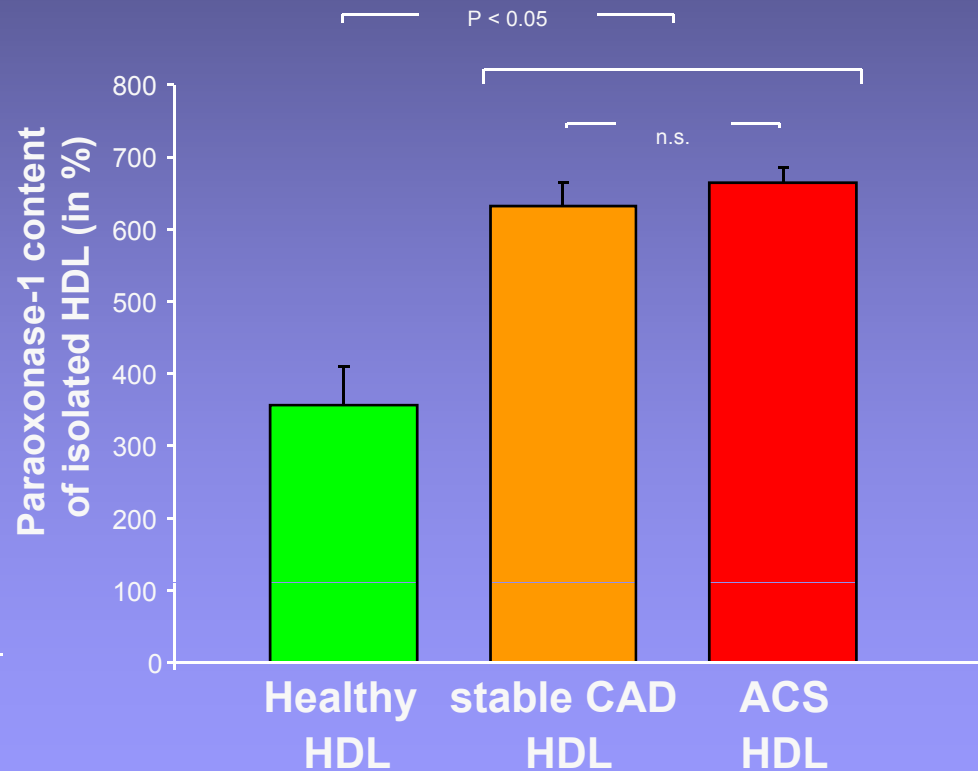
**HDL endothelial  
binding  
Reverse Cholesterol-  
transport**

# HDL-associated paraoxonase activity and content

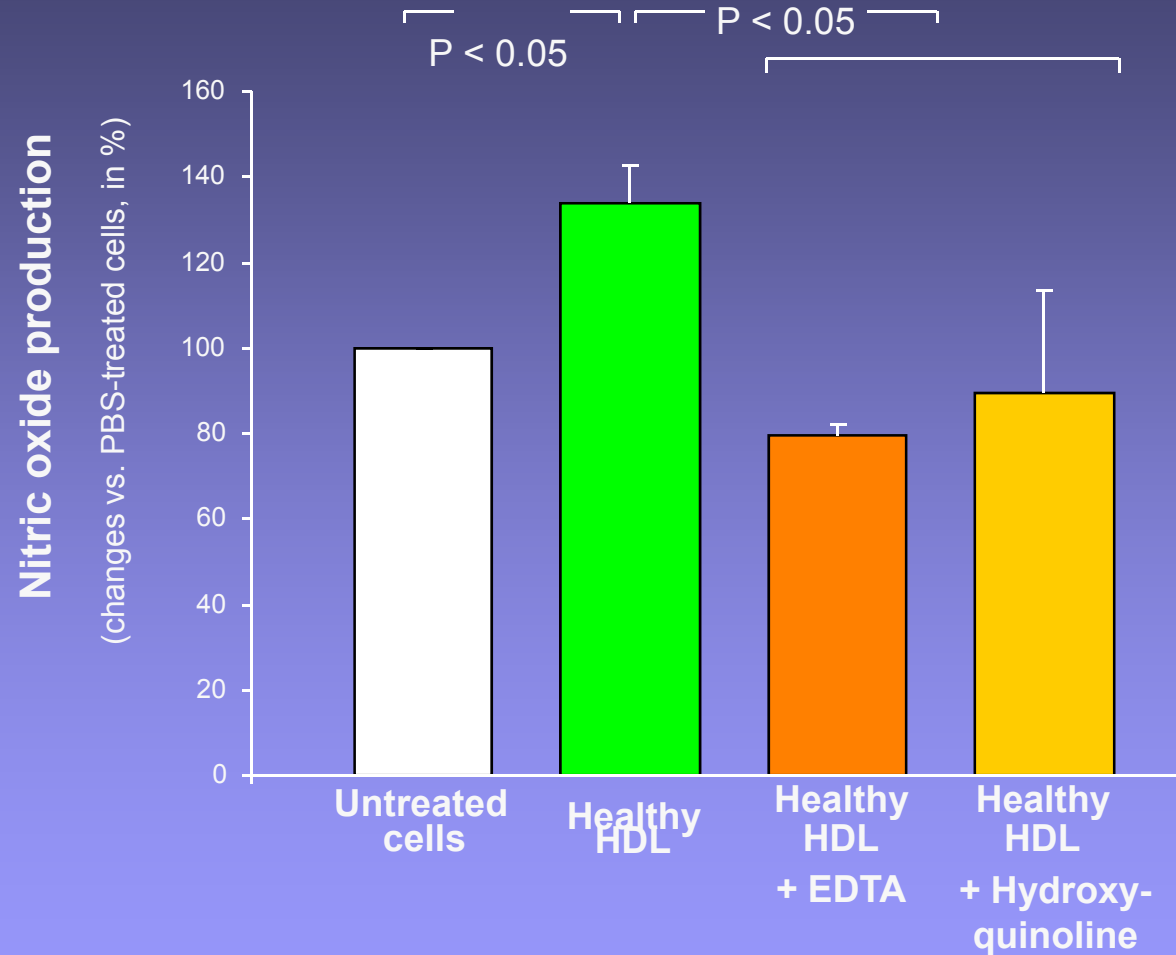
## Paraoxonase activity



## Paraoxonase-1 content



# Effect of paraoxonase inhibition on nitric oxide production in endothelial cells



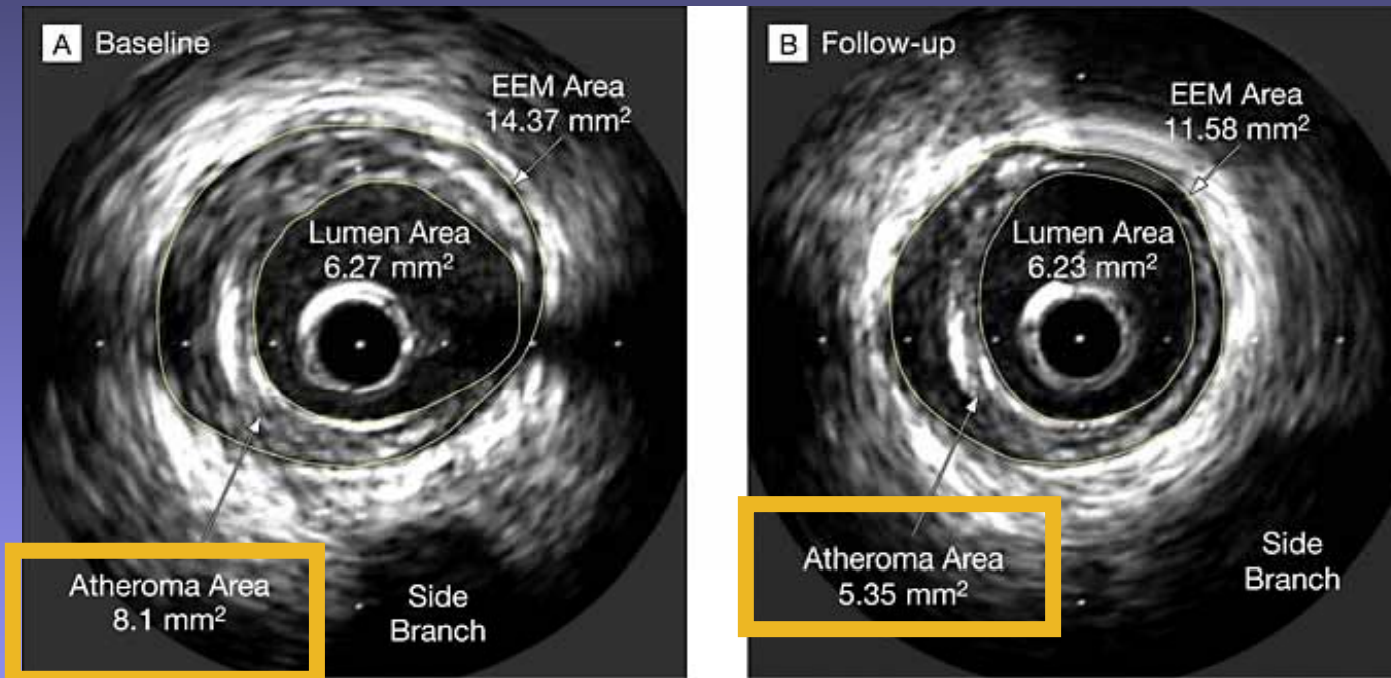
(ESR spectroscopy measurement)

# **HDL in clinical trials - where are we now ?**

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- 1. ApoA1 - infusion - Proof of concept ?**
- 2. Life style and HDL**
- 3. Niacin - an old drug with new hope ?**
- 4. CETP inhibitors - a painful lesson ?**

# Infusion of ApoA1 Milano reduced coronary plaque-volume



**Regression of atheroma in a patient who received high-dose ETC-216 (apoA1-Milano)**

# **HDL in clinical trials - where are we now ?**

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**1. ApoA1 - infusion - Proof of concept ?**

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**3. Niacin - an old drug with new hope ?**

**4. CETP inhibitors - a painful lesson ?**

# 1. Life style change has limited effect on HDL levels

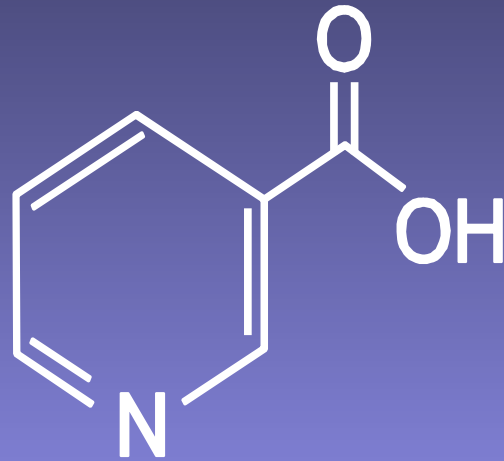
Therapeutic Intervention	Increase in HDL-C Levels, %	Mechanism of Action
Aerobic exercise <sup>24,25,42,43</sup>	5-10	Increases pre- $\beta$ -HDL, reverse cholesterol transport, LPL, and atheroprotective subpopulations
Tobacco cessation <sup>19,26,27</sup>	5-10	Increases LCAT and reverse cholesterol transport Decreases CETP
Weight loss <sup>28-32</sup>	0.35 mg/dL per kilogram of weight lost	Increases LCAT, LPL, and reverse cholesterol transport

# **HDL in clinical trials - where are we now ?**

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- 2. Life style and HDL**
- 3. Niacin - an old drug with new hope ?**
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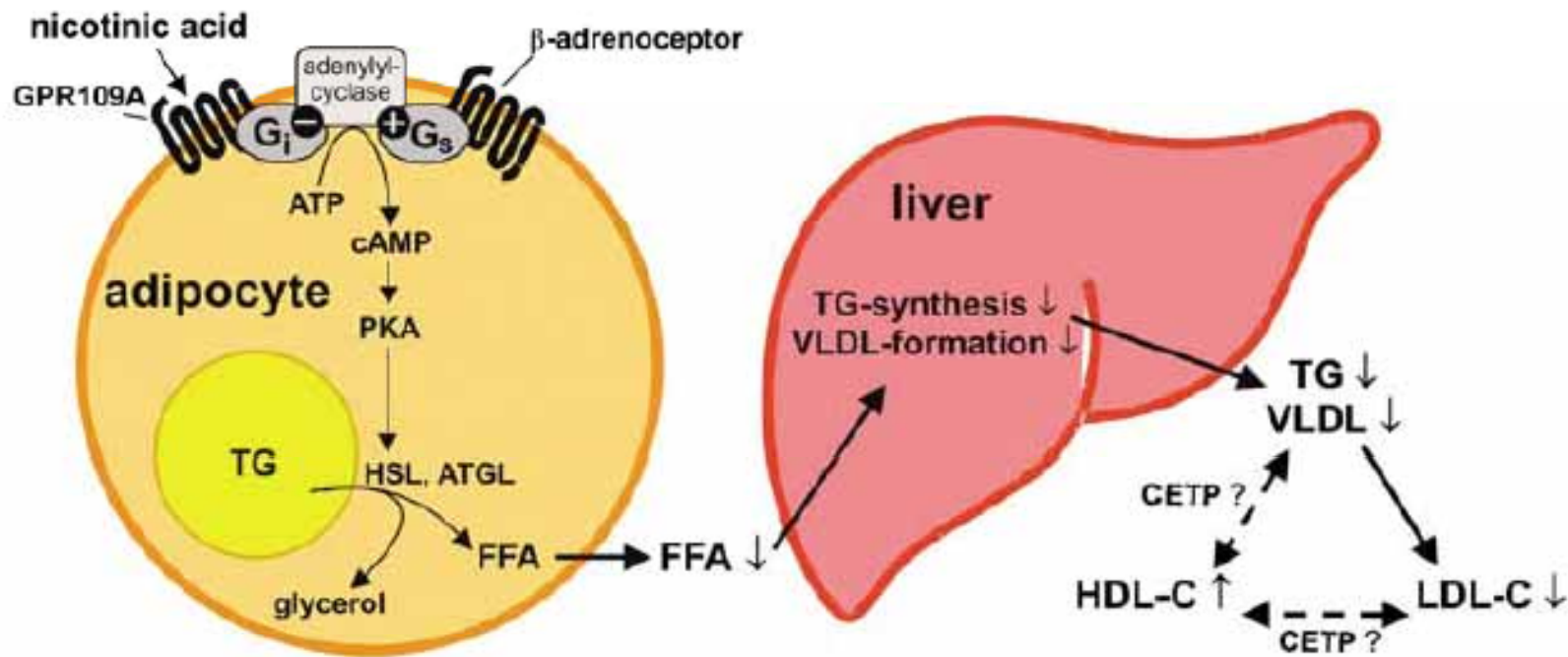
# Niacin (Nicotinic acid)



**“Wasserlösliches B-Vitamin”**

Altschul R, Hoffer A, Stephen JD (1955).  
Influence of nicotinic acid on serum cholesterol in man.  
*Arch Biochem* 54: 558–559

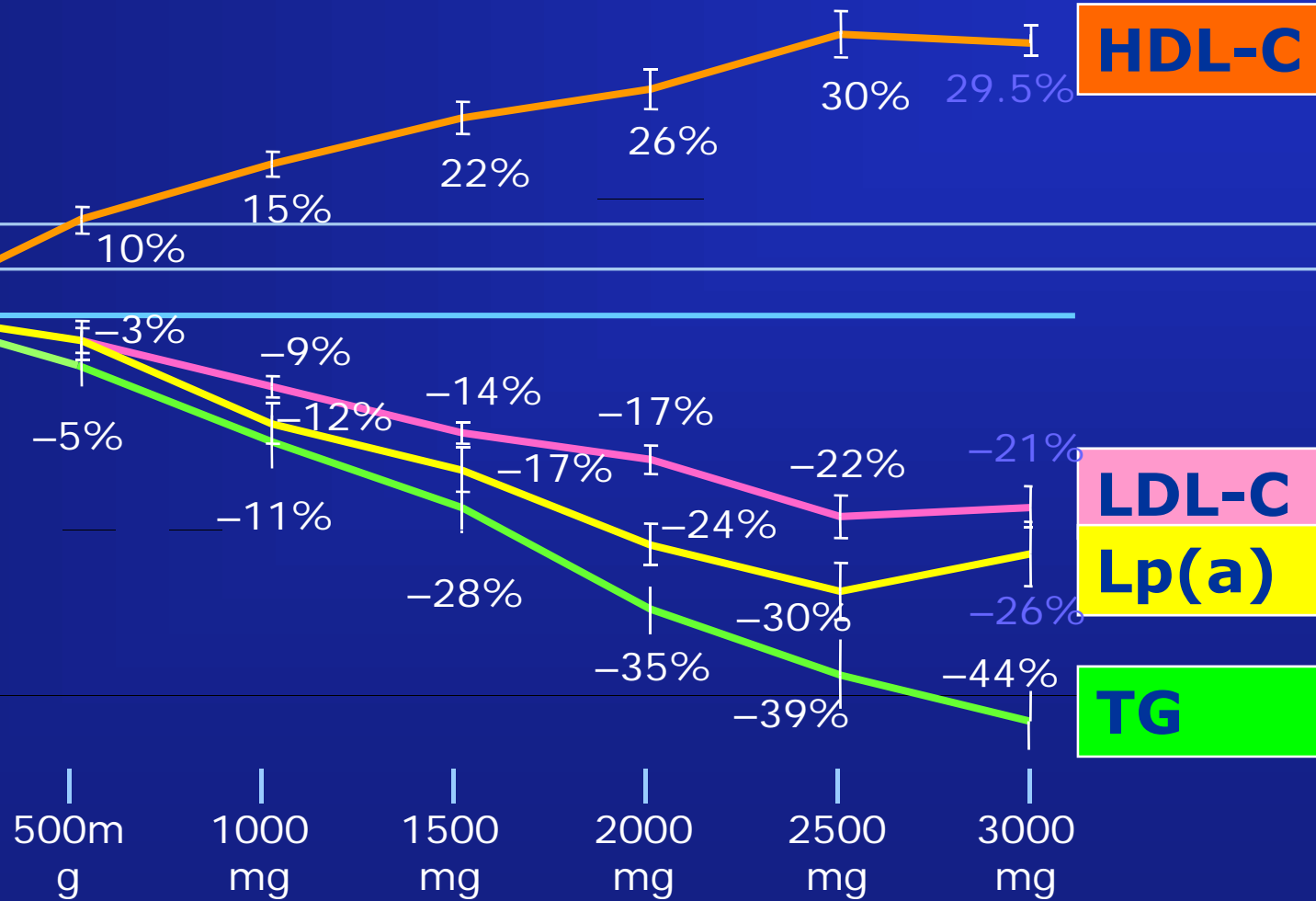
# Niacin - Mechanisms for lipid effects ?



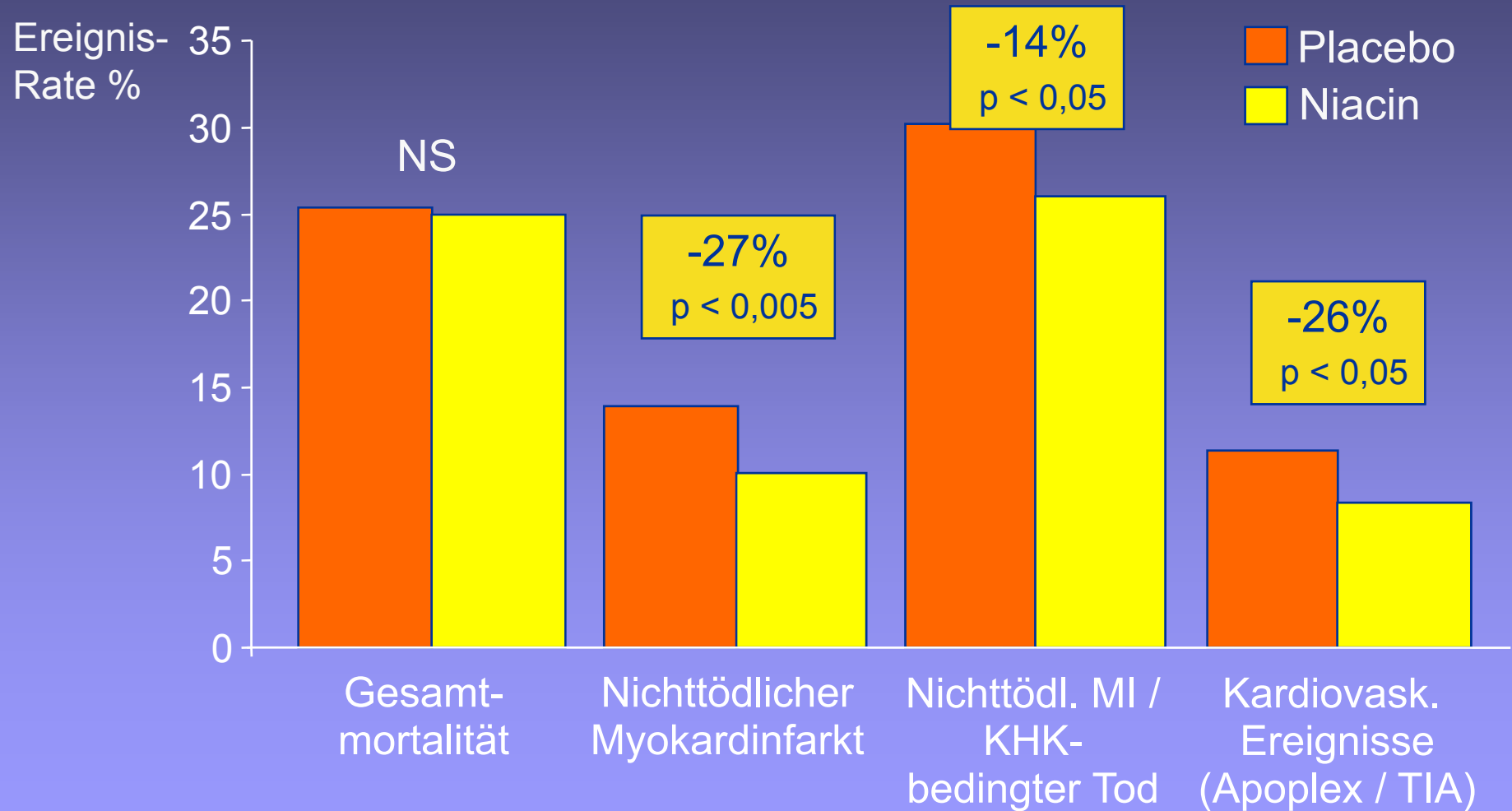
“broad-spectrum lipid drug”

# Effect of niacin on lipid profile

Change from Baseline



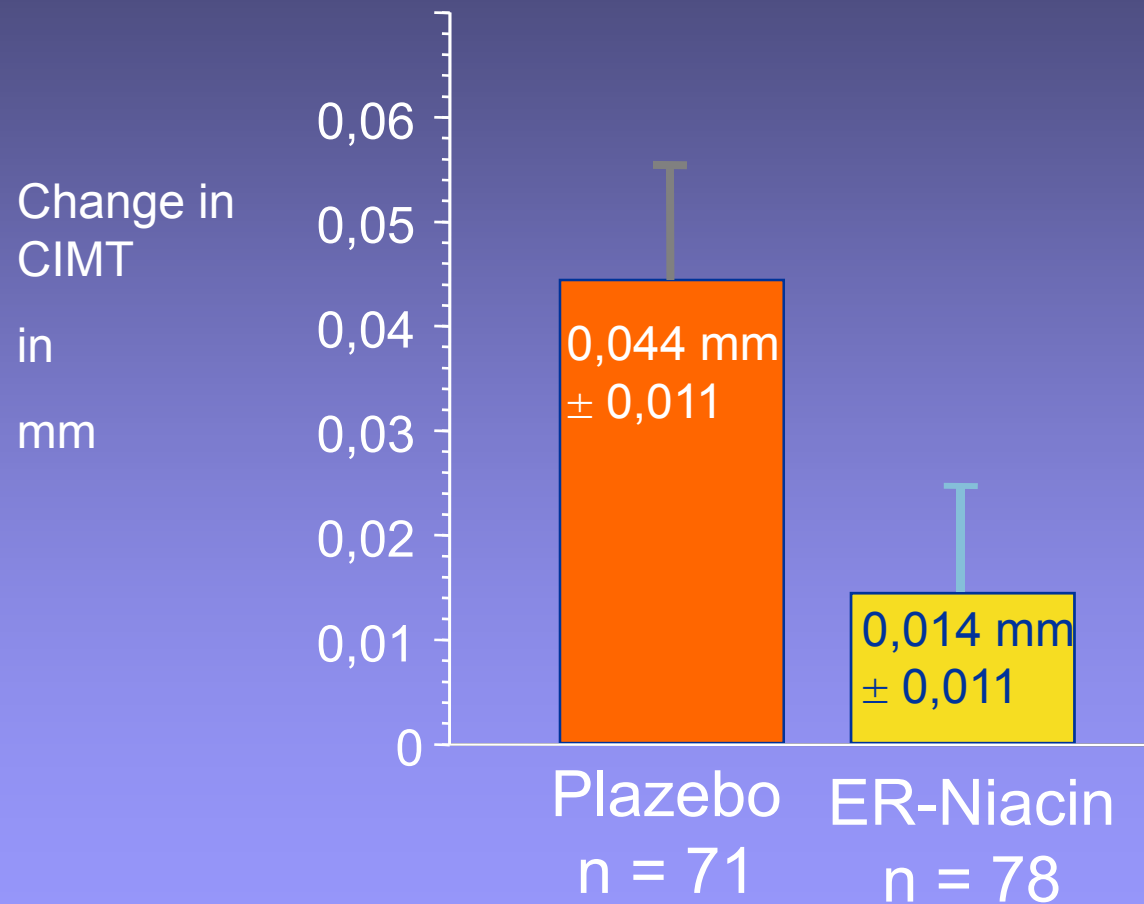
# Niacin - clinical endpoint studies



# Niacin and Imaging studies: ARBITER 2

	Placebo n = 80	Niacin n = 87
Statin-Dose (Mean)	36 ± 22 mg	34 ± 19 mg
Beta-blocker	79%	79%
ACE-Inhibitor	58%	62%
ASS	85%	86%
Vitamin E*	18%	25%
Vitamin C*	10%	12%

# Primary Endpoint CIMT (Carotis Intima-Media-Thickness)



△ CIMT after 12 Mon.

p-Values:

1) in Groups:

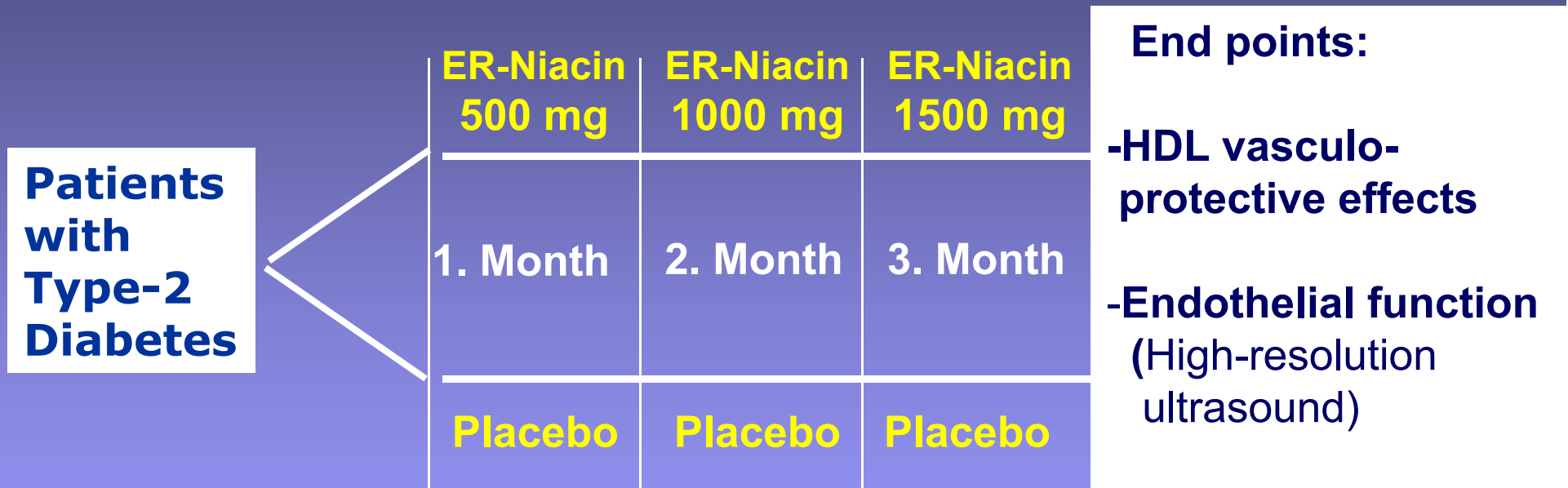
Plazebo:  $p < 0,001$

ER-Niacin:  $p = 0,23$

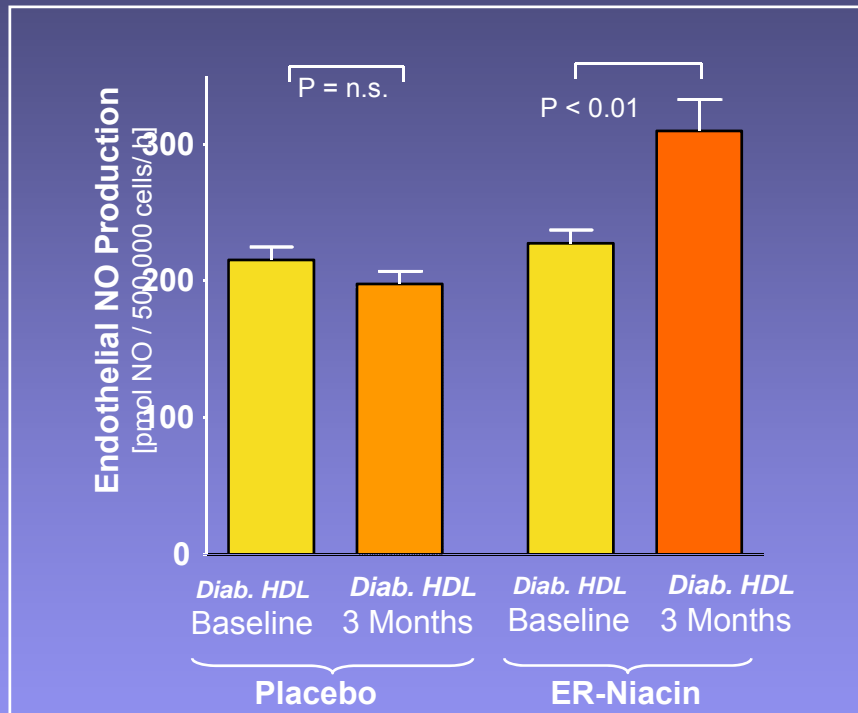
2) Between Groups:

$p = 0,048$  (ITT-Analysis for all 167 Pat.)

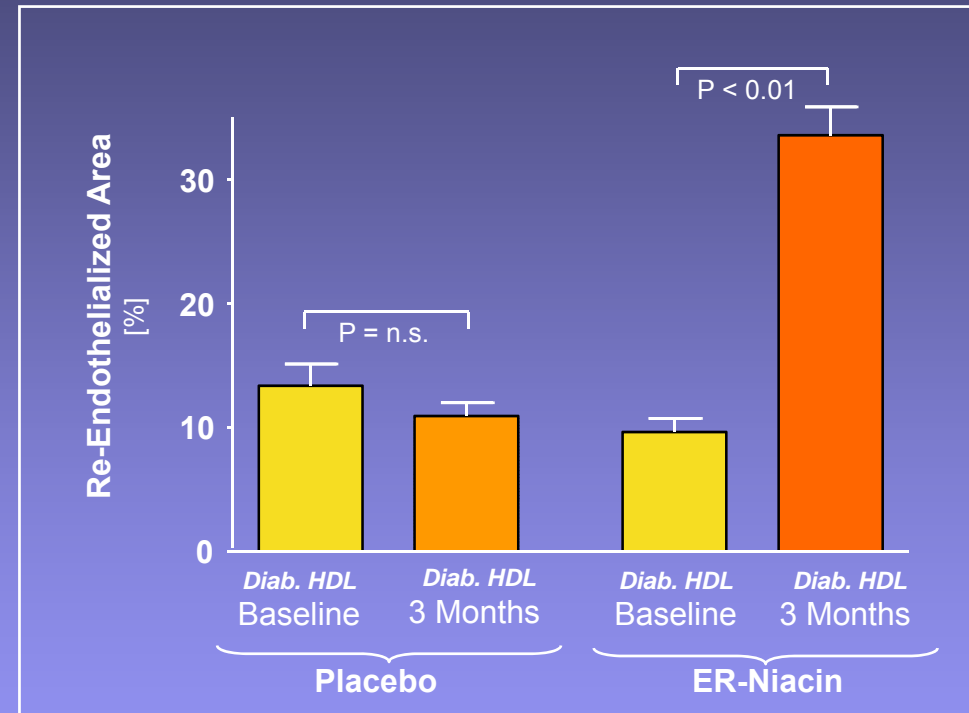
# Effect of extended-release niacin on HDL's endothelial effects in diabetes



# Effect of ER-niacin on HDL's vascular effects in diabetic patients



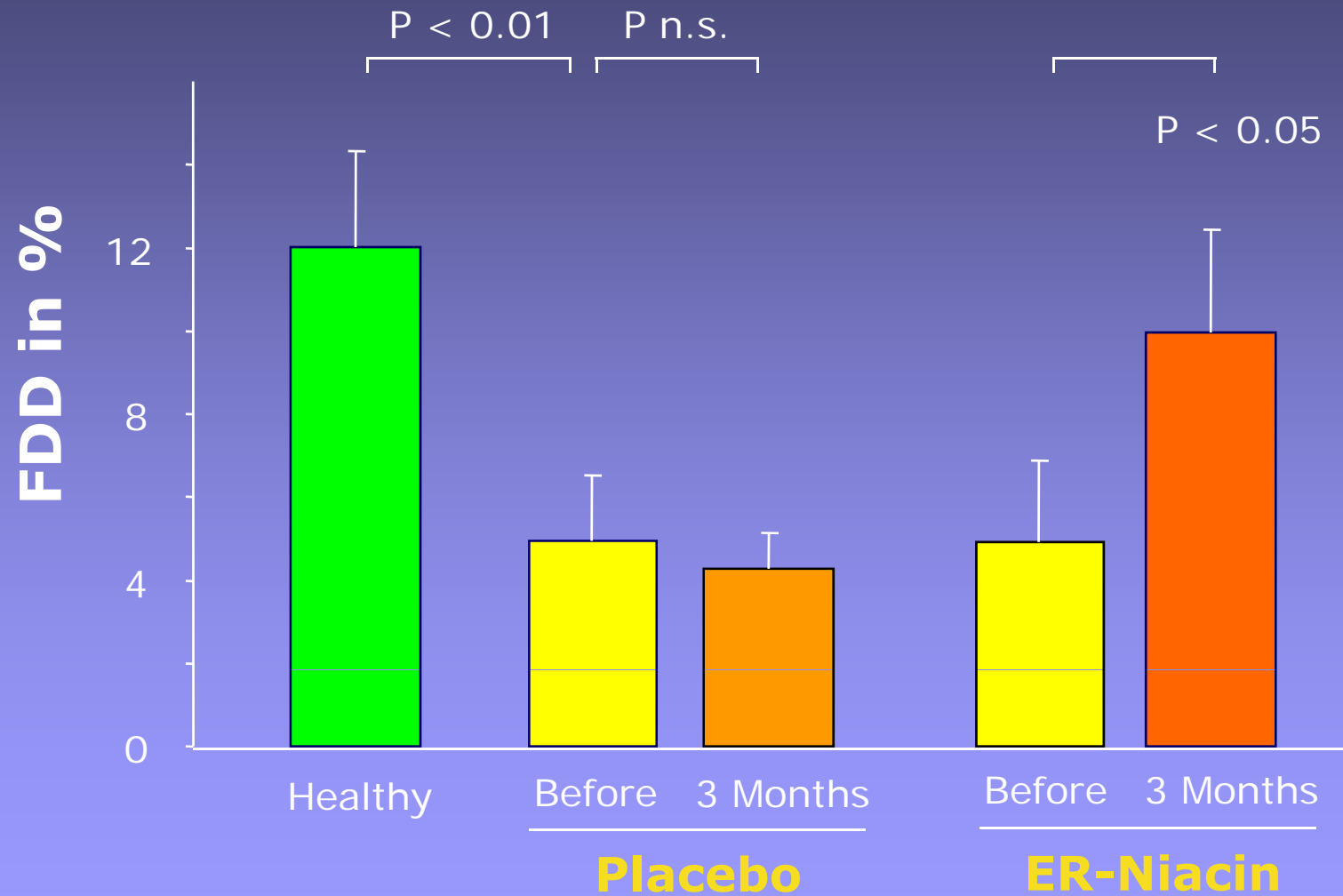
**HDL's effect  
endothelial  
NO production**



**HDL's effect  
on endothelial  
repair**

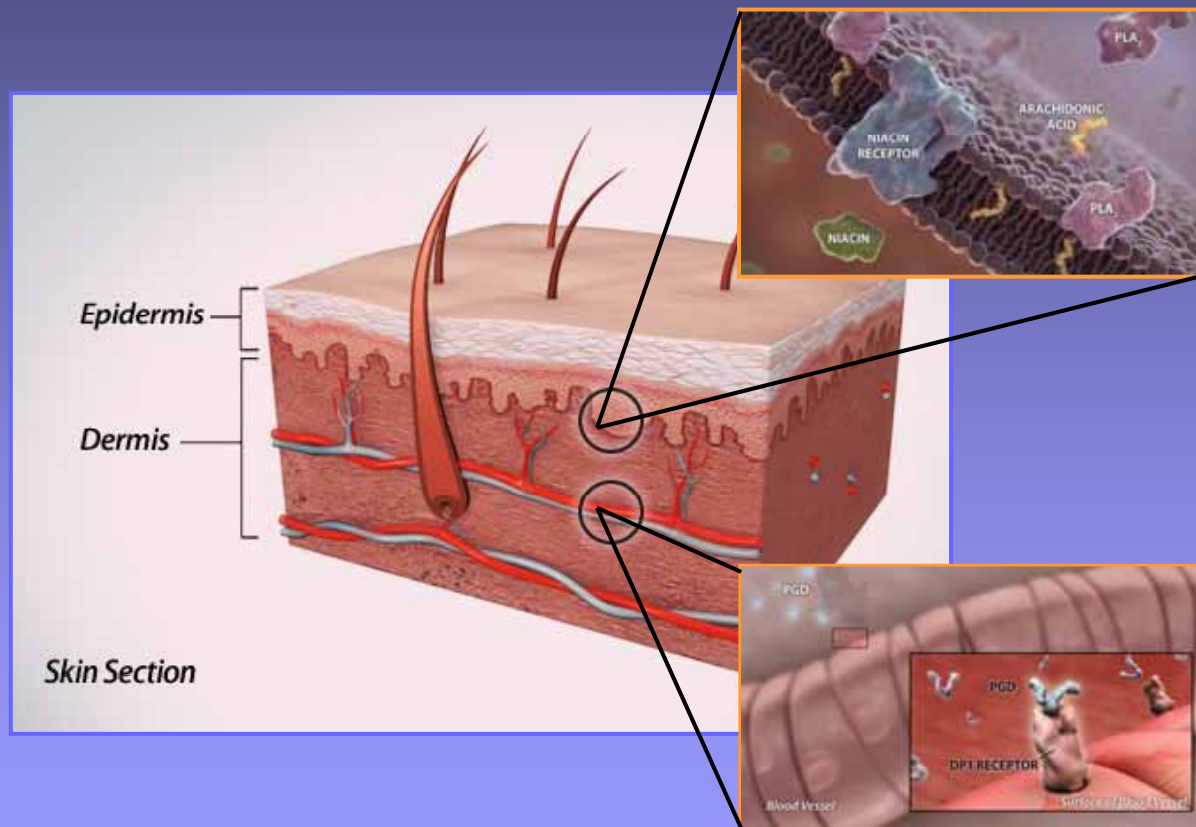
Sorrentino et al. (submitted)

# ER niacin improves endothelial function in patients with type-2 diabetes



# Niacin - discovery of "flush" receptor

## Flush: PGD<sub>2</sub>-/DP-1 Receptor dependent



1. Epidermal Langerhans Cells<sup>1</sup>
  - Nicotinic acid binds
  - PGD<sub>2</sub> is produced

2. Dermal Blood Vessels<sup>2,3</sup>
  - - PGD<sub>2</sub> binds to DP1
  - - Vasodilation results

PGD<sub>2</sub>=prostaglandin D<sub>2</sub>; DP1=prostaglandin D<sub>2</sub> receptor 1.

1. Benyó Z et al. *Mol Pharmacol*. 2006;70:1844–1849.

3. Cheng K et al. *Proc Natl Acad Sci U S A*. 2006;103:6682–6687.

# HPS2- THRIVE (Treatment of HDL to Reduce the Incidence of Vascular Events) Study Overview

- Objective:

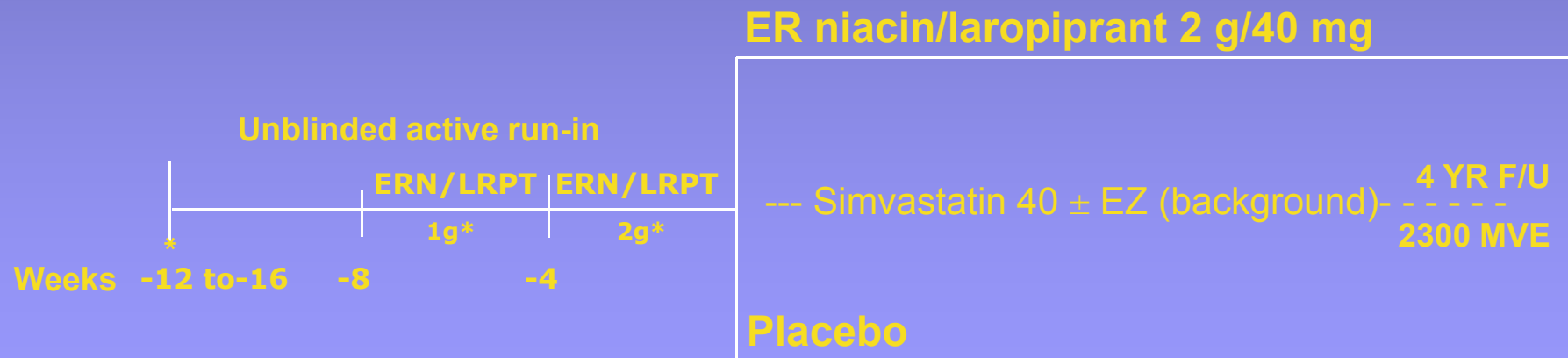
- \* To assess the effect of ER niacin/laropiprant 2 g/40 mg vs placebo on CV events, on a background of simvastatin 40 mg

- Patient Population:

- \* 20,000 high risk atherosclerosis patients (a) MI, (b) peripheral or cerebrovascular disease, (c) diabetes + atherosclerotic vascular disease. One third in category (c)

- Primary Study Endpoints:

- \* Major vascular events (MVE)

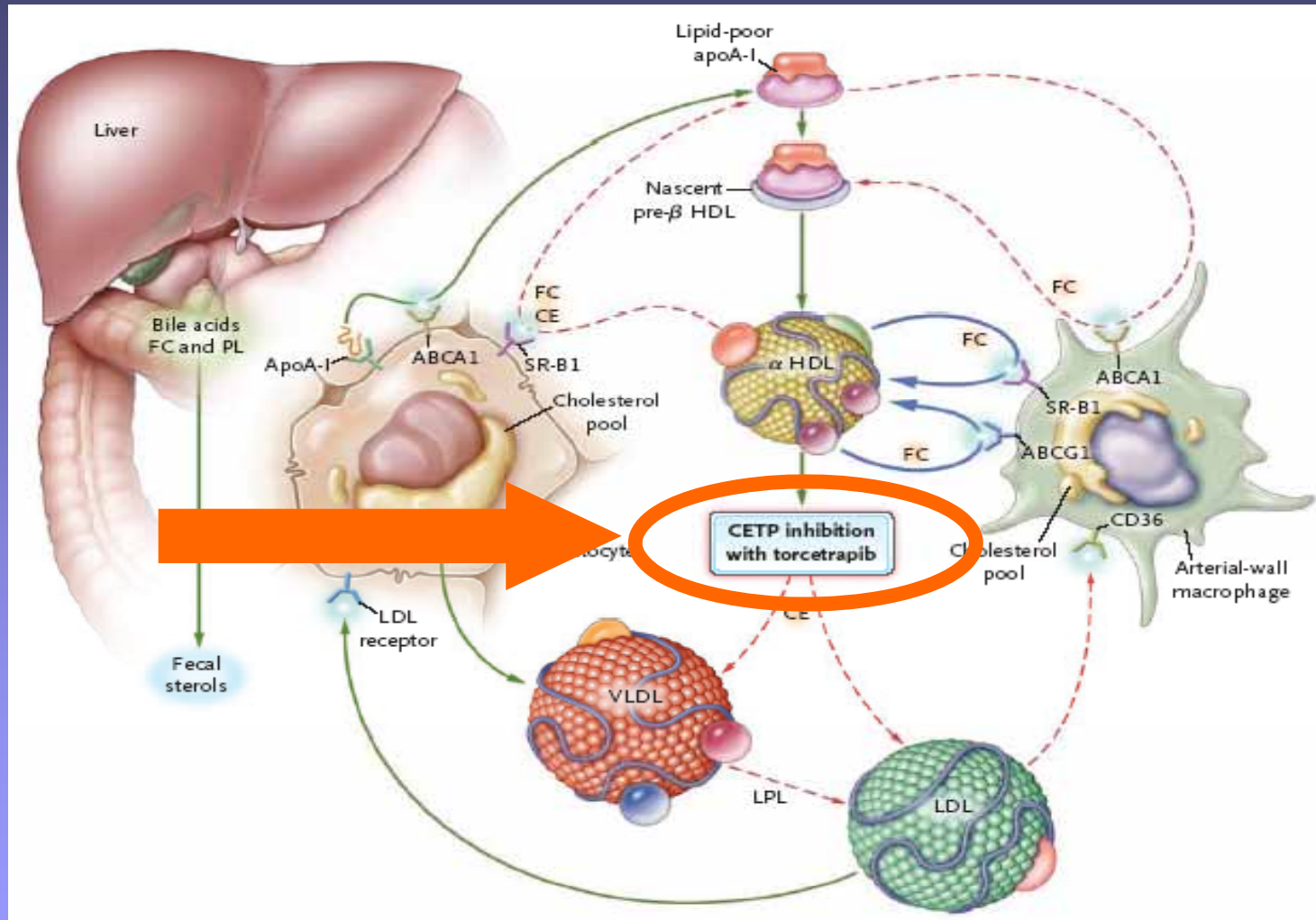


\*Patients enter on a background of either simvastatin 40 mg or ezetimibe (EZ)/simvastatin 10/40 mg. ezetimibe/simvastatin 10/40 mg initiated at week -8 if TC levels >3.5 mmol/L (LDL-C 76 mg/dL)

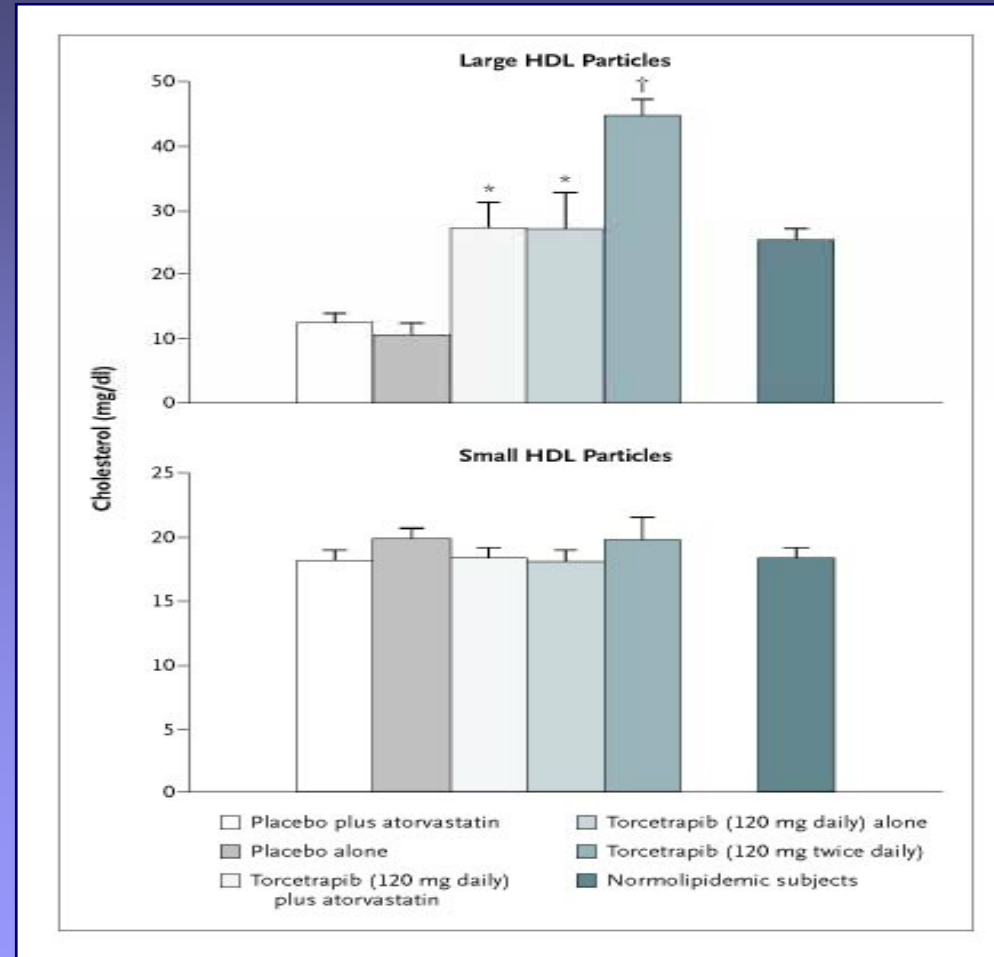
# **HDL in clinical trials - where are we now ?**

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- 4. CETP inhibitors - a painful lesson ?**

# CETP inhibition



# CETP Inhibition and Plasma Lipids



Brousseau et al.; N Engl J Med. 2004; 350(15):1505-15.

# Torcetrapib – Imaging studies

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## IVUS: ILLUSTRATE

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effect of Torcetrapib on the Progression  
of Coronary Atherosclerosis

Nissen et al. *N Engl J Med* 2007; 356: 1304-16

## CIMT: RADIANCE

The NEW ENGLAND JOURNAL of MEDICINE

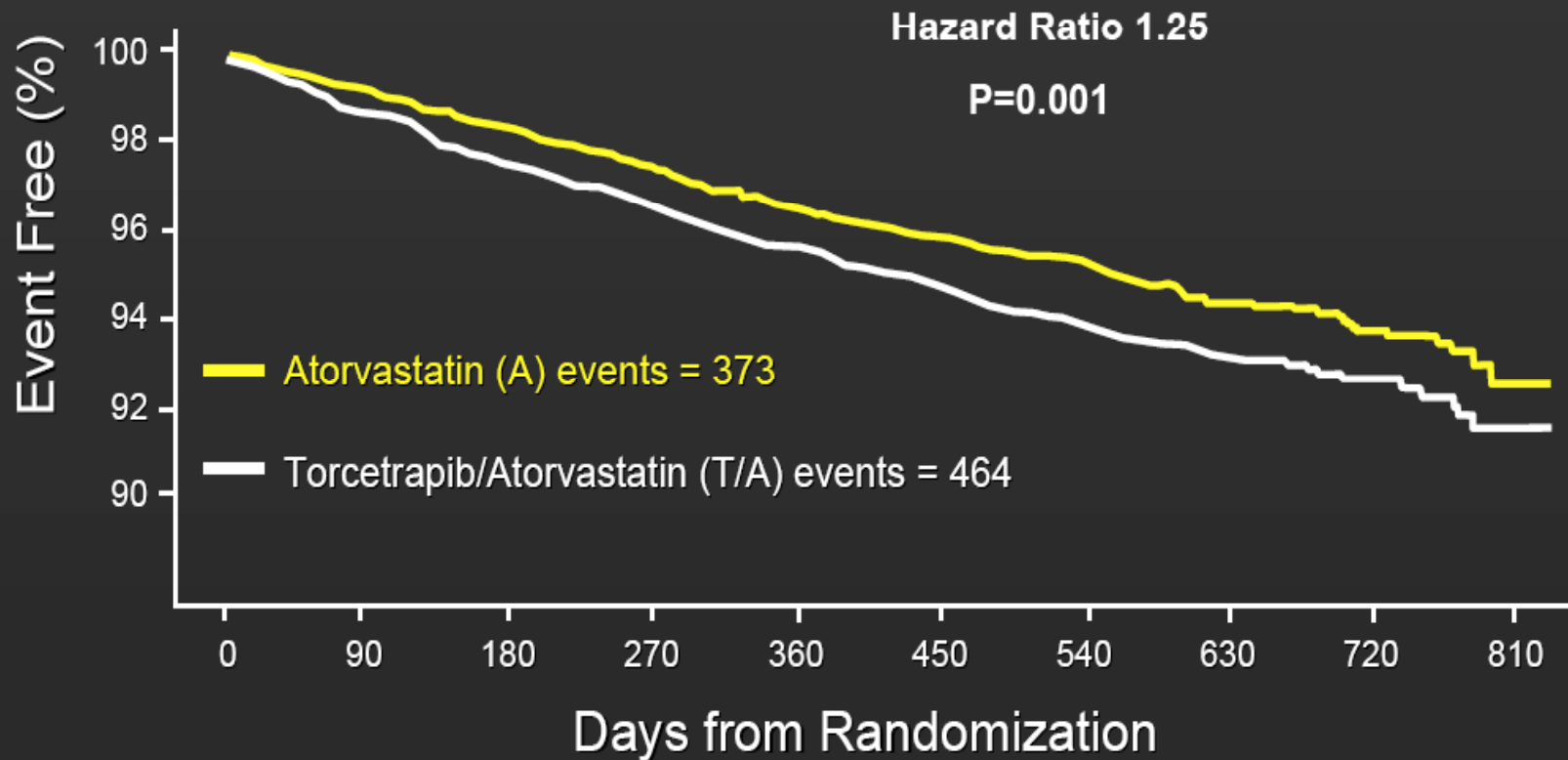
ORIGINAL ARTICLE

Effect of Torcetrapib on Carotid Atherosclerosis  
in Familial Hypercholesterolemia

Kastelein et al., *N Engl J Med* 2007; 356: 1620-30

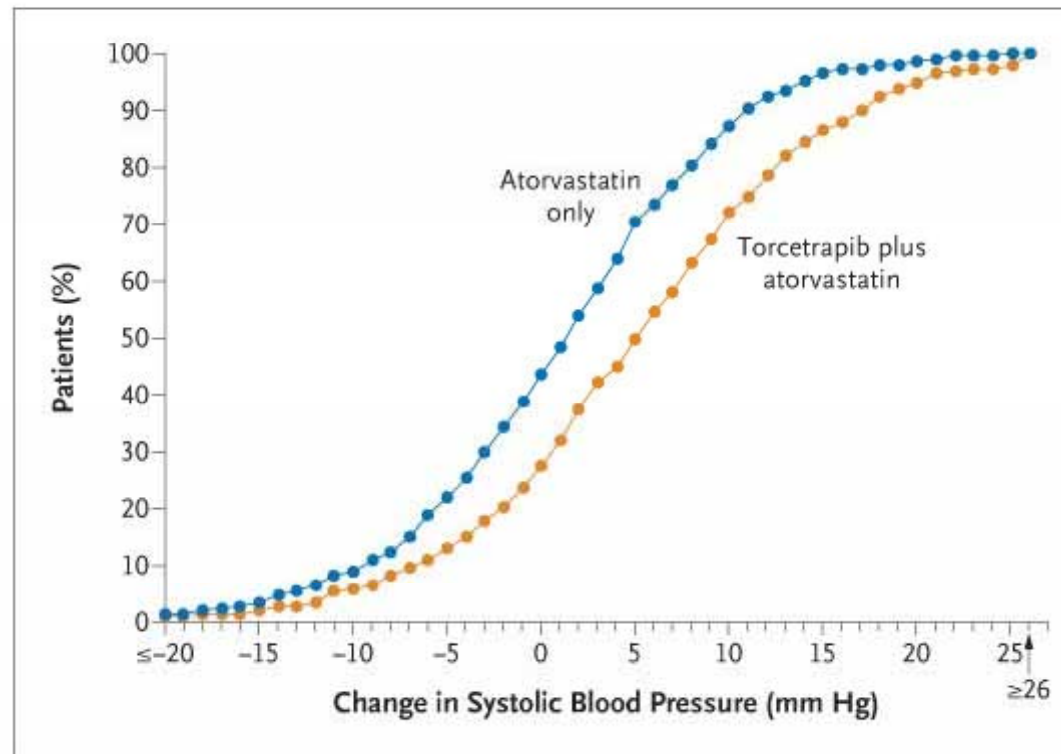
# CETP-Inhibitor - Torcetrapib - Outcome Study

## Time to First MCVE\*: Kaplan-Meier Plot



Barter et al., *N Engl J Med* 2007; 357: 2109-2122

# CETP inh. - Vasculotoxic effects of Torcetr.



**4.6 mm Hg increase in  
mean systolic blood pressure**

# CETP inh. - dal-OUTCOMES Study

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*Effect of Dalcetrapib on cardiovascular mortality and morbidity after recent acute coronary syndrome*



Dalcetrapib 600 mg

Placebo

Primary Endpoint:

Time to first event

\*KHK Tod, schwere koronare Events (nicht-letaler MI, reanimationspfl. Herzstillstand, Hospitalisierung von Biomarker-negativen ACS-Patienten mit EKG-Veränderungen), letaler/nichtletaler Schlaganfall mit atherothrombotischer Genese

THOMAS H. HUXLEY.



*T. H. Huxley*

**The deepest sin against  
the human mind is to  
believe things without  
evidence.**

Thomas H. Huxley  
(1825 - 1895)

# Summary and Conclusions

- 1. Low HDL remains associated with increased cardiovascular risk (despite intense statin therapy)**
- 2. The vascular effects of HDL are markedly different in patients with coronary disease or diabetes - HDL dysfunction may represent the true novel therapeutic target**
- 3. Clinical trials using ER-Niacin/ laropiprant or CETP inhibitors on top of statin therapy are underway**



**Thank you**