Ezetimibe: Surrogate Data Mechanism of Action

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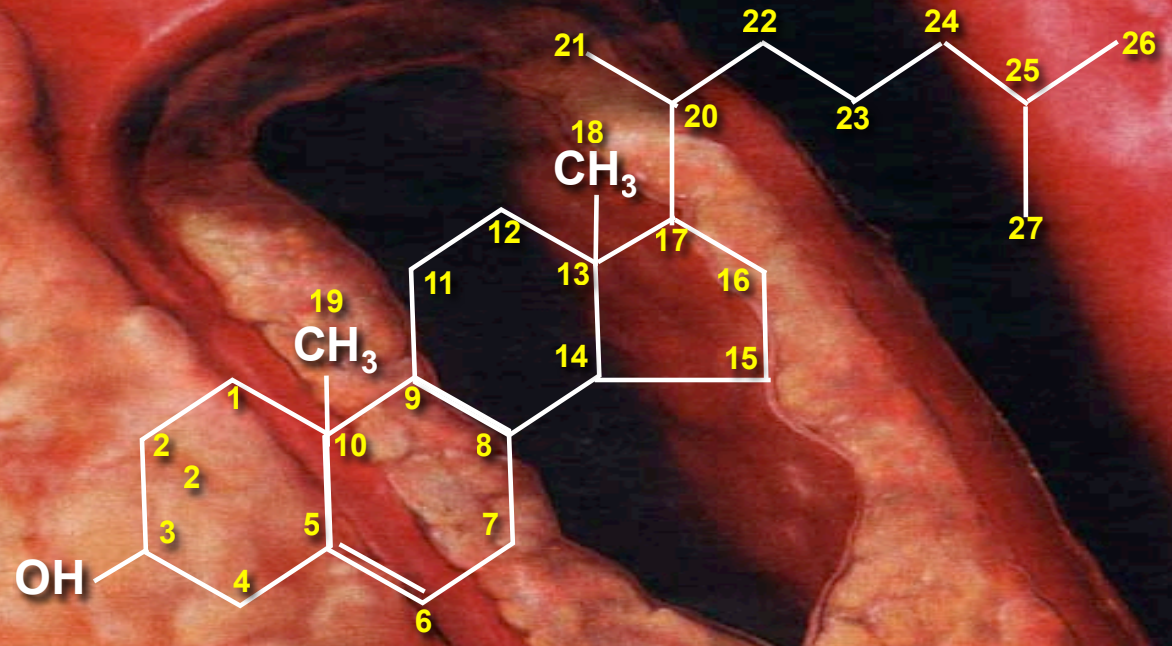
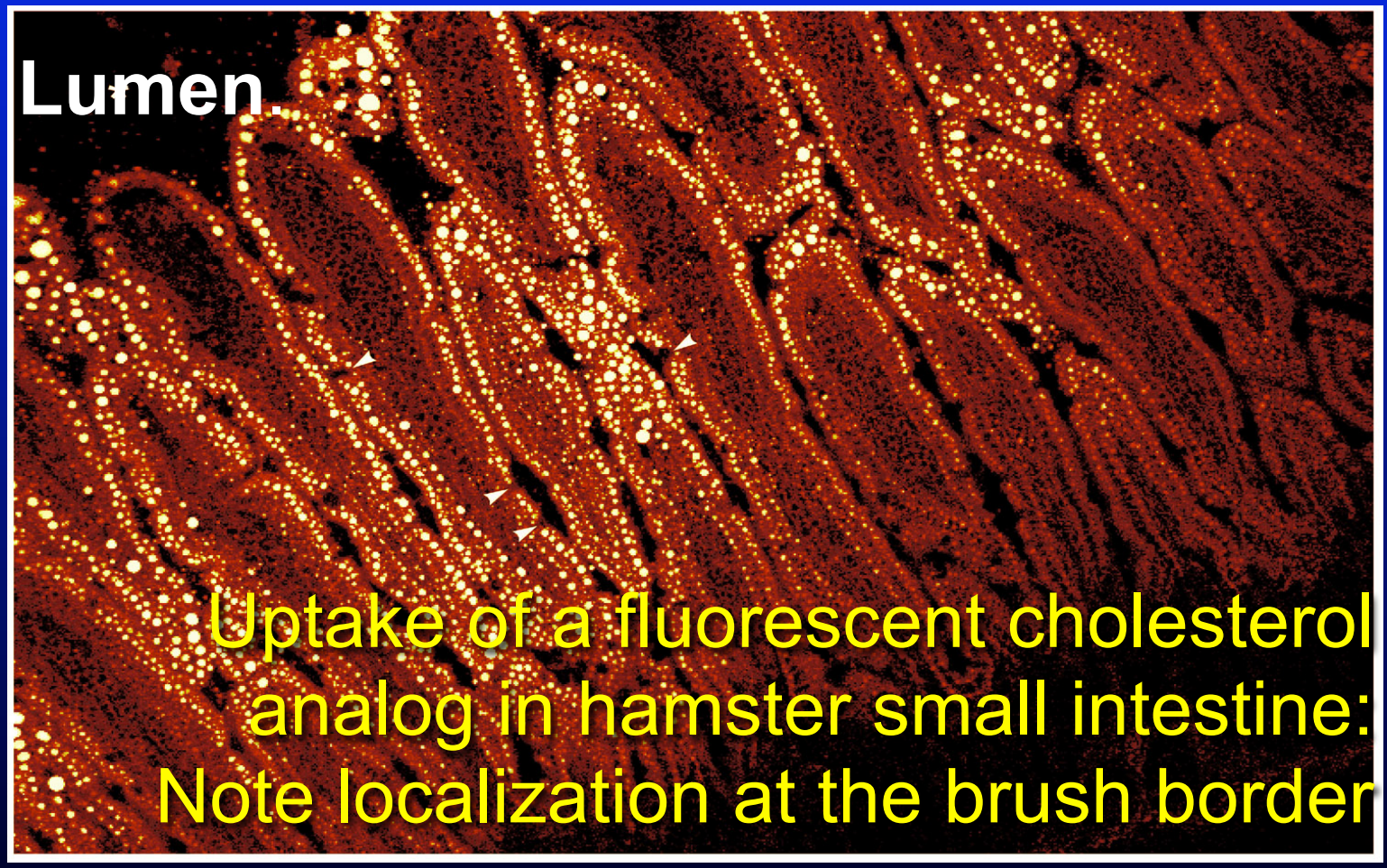
North Jersey Institute of Menopausal Lipidology Wayne, New Jersey

A sterol with 27 carbon molecules with an -OH group at the # 3 position



Cholesterol, which can be synthesized de novo or absorbed intestinally, is required by humans for cell membrane integrity and function, as well as bile acid, steroid and vitamin D production.

Cholesterol Is Absorbed Specifically by Enterocytes

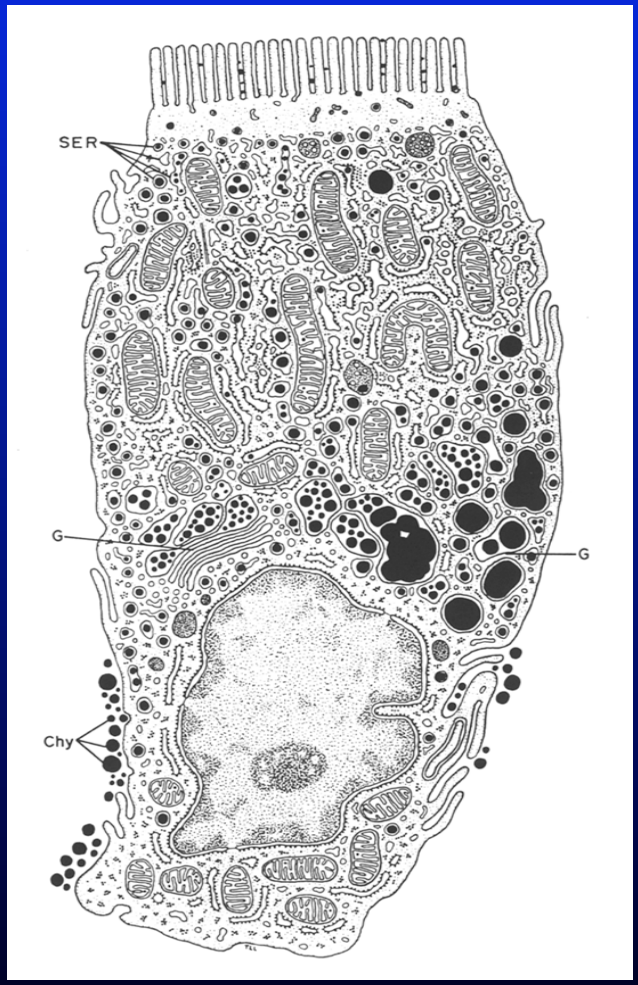


Uptake of a fluorescent cholesterol analog in hamster small intestine: Note localization at the brush border

Sparrow CP et al. J Lipid Res. 1999;40:1747–1757, with permission.

Steps of Cholesterol Absorption[1,2]

- Emulsification
- Transfer from bile acid micelle to brush border
- Transport to endoplasmic reticulum
- Esterification (ACAT)
- Incorporation into chylomicrons
- Secretion from basolateral surface
- Movement into lymph



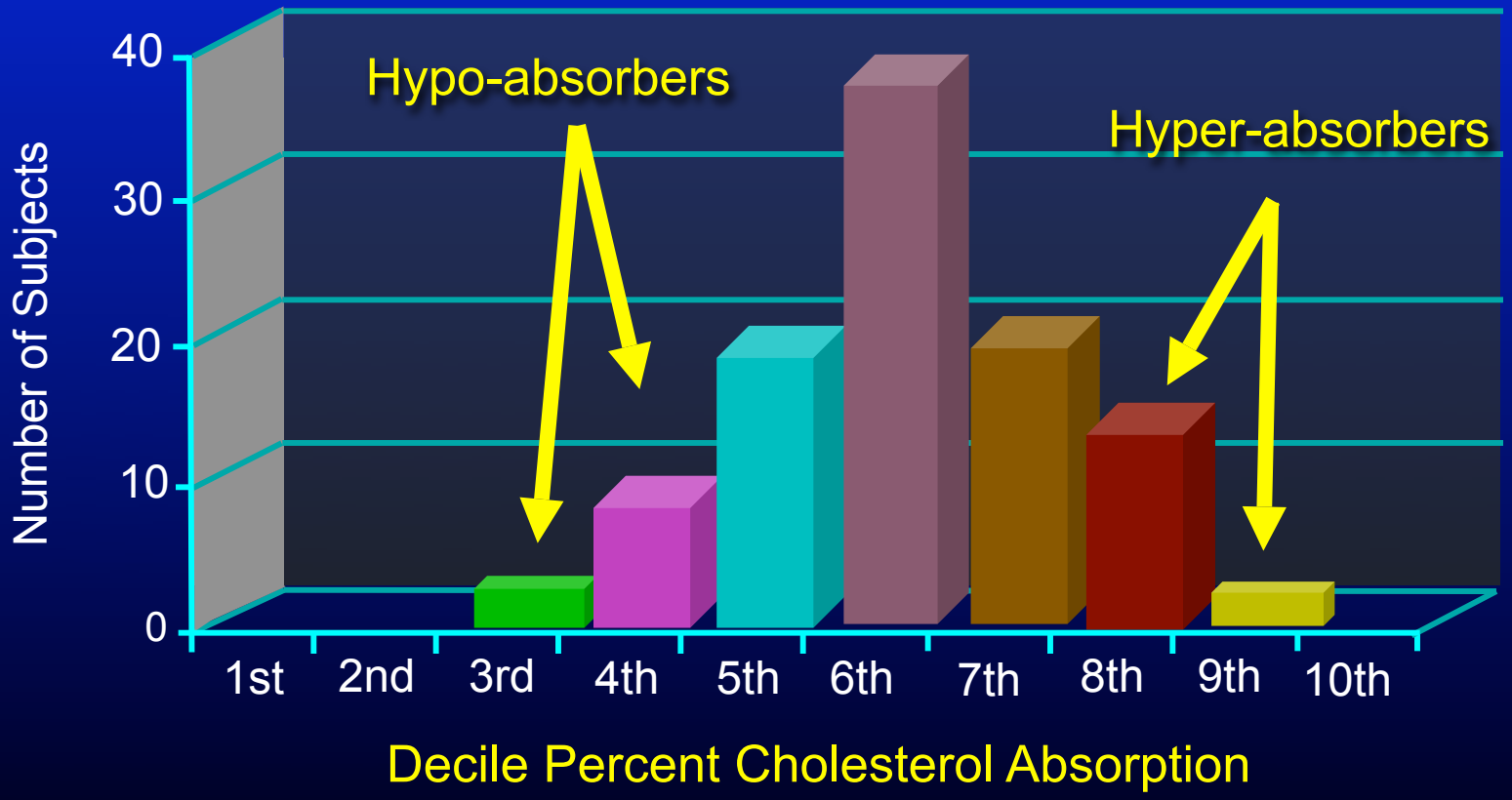
SER=Smooth endoplasmic reticulum;

G=Golgi apparatus; Chy=Chylomicra.

1. Reprinted from Lentz TL. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure,* Philadelphia, Pa: WB Saunders Co; 1971:181, with permission from Elsevier Science.
2. Hernandez M et al. *Biochim Biophys Acta.* 2000;1486:232–242.

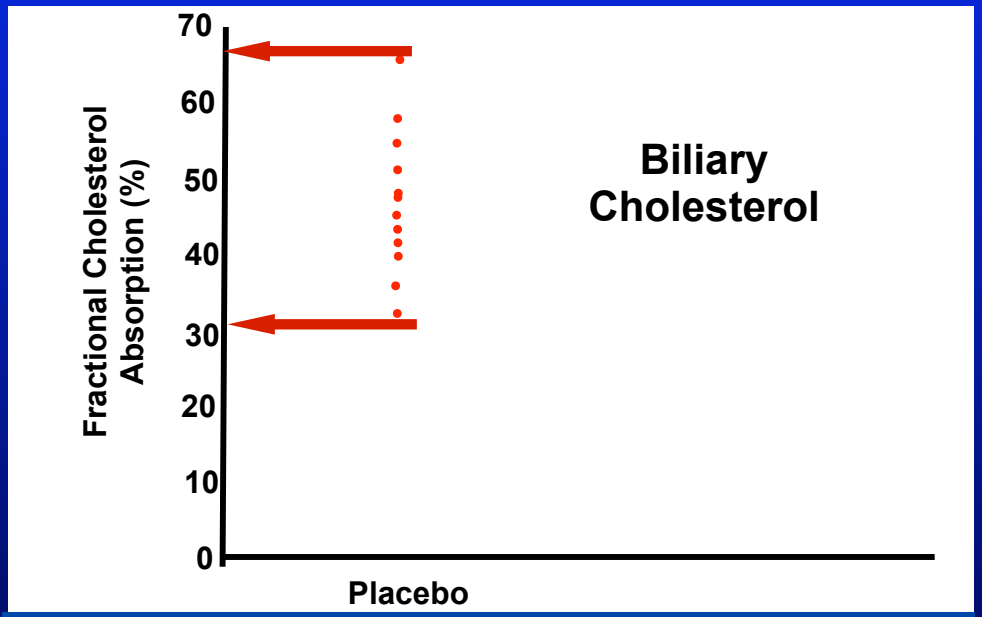
Cholesterol Absorption

Cholesterol absorption measured in 100 healthy patients using dual isotope tracer technique. The majority absorb about 55% of dietary sterols



Bosner MS et al. J Lipid Res 1999;40:302-308

The Absorption of Cholesterol in Pure Vegetarians



Fractional cholesterol absorption in individual vegetarians during the placebo phase

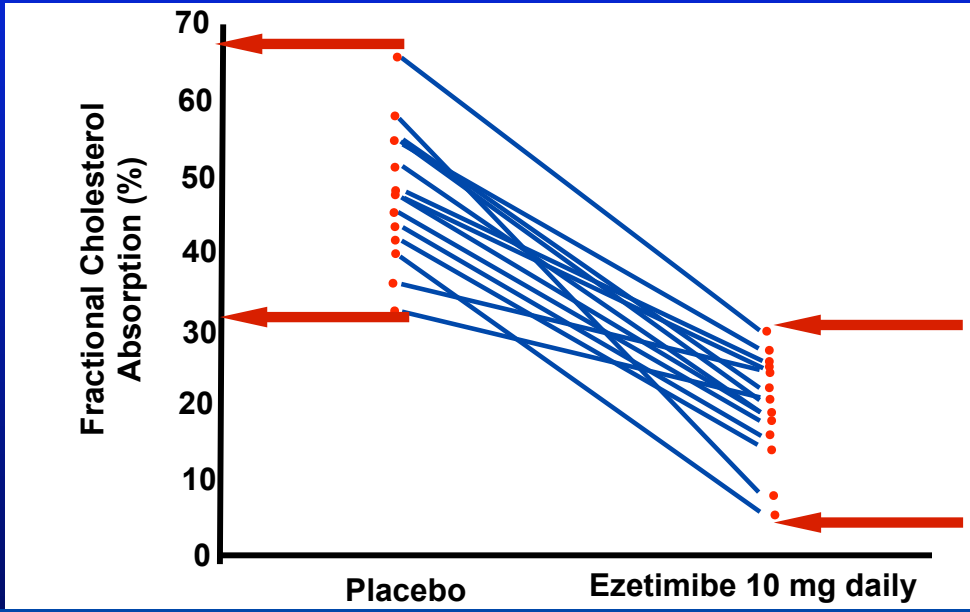
Clarenbachet JJ al. J. Lipid Res. 2006. 47: 2820–2824.

PPAR Delta and NPC1L1 Protein

- In addition, enhanced fecal neutral sterol loss as a consequence of impaired intestinal cholesterol absorption upon PPARΔ activation, which in effect increases RCT, can be considered a beneficial action.
- Indeed, studies have shown a 20% reduction of LDL levels in hypercholesterolemic humans and prevention of atherosclerosis development in *Apolipoprotein E*/ mice upon inhibition of cholesterol absorption by ezetimibe.
- Our results suggest that reduction of cholesterol absorption upon treatment with the PPARΔ agonist GW610742 is, at least in part, mediated by reduced intestinal expression of *Npc1l1*, a proposed target of ezetimibe.
- Interestingly, ezetimibe was also shown to increase plasma

Van der Vern et al. L Lip Res 2005;46: 526-534

The Lipid Lowering Effect of Ezetimibe in Pure Vegetarians



Fractional cholesterol absorption in individual vegetarians during the placebo phase and treatment with ezetimibe.

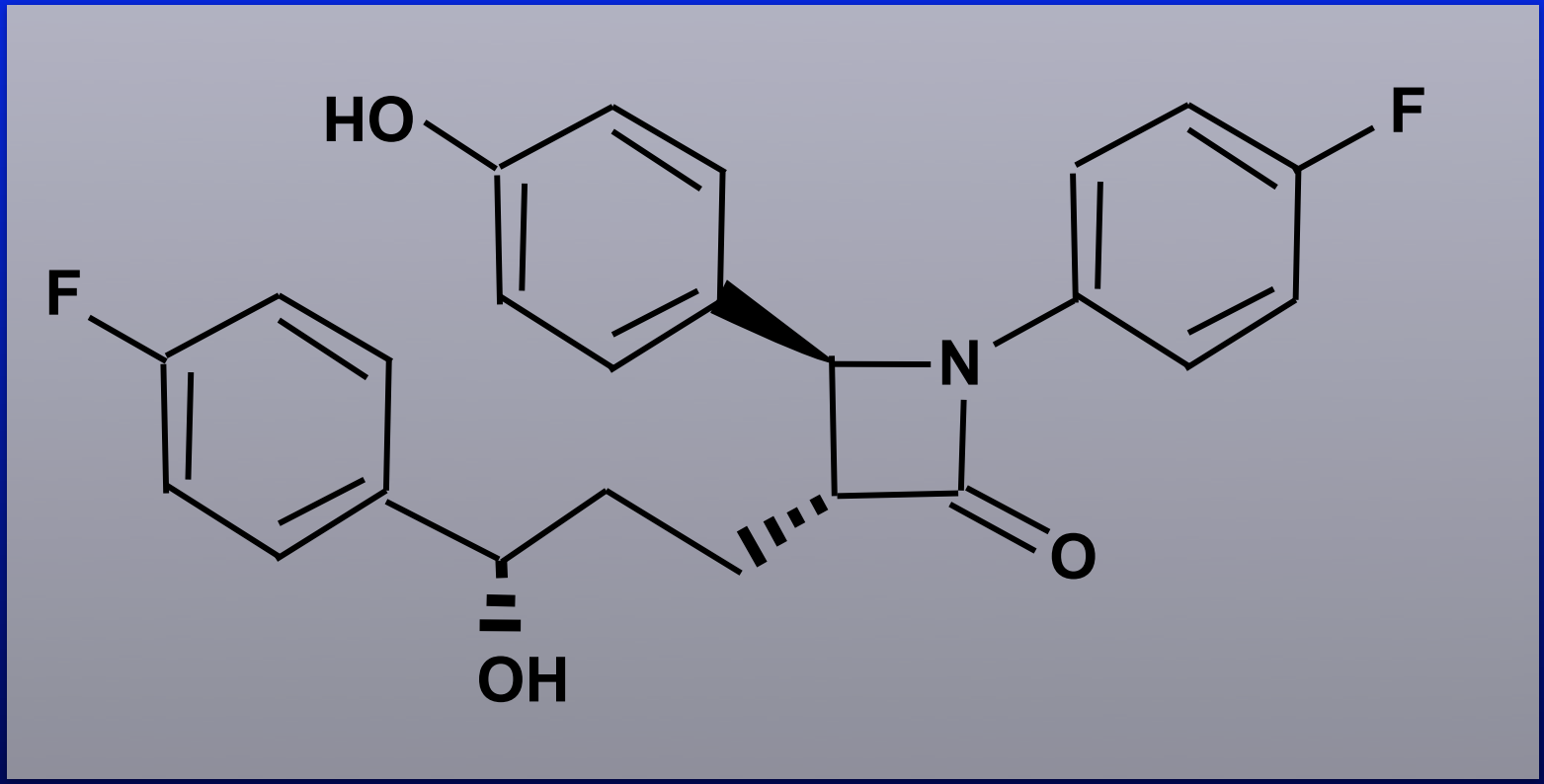
The lipid-lowering effect of ezetimibe in pure vegetarians can be attributed **almost exclusively** to the inhibition of intestinal absorption of cholesterol that originates from biliary secretion.

Ezetimibe treatment led to a significant reduction of plasma plant sterol levels compared with placebo treatment. In fact, this effect was more pronounced than the effect on cholesterol.

This finding is attributable to the different absorption rates of campesterol and sitosterol compared with cholesterol, their faster biliary elimination, and the inability of the body to synthesize plant sterols

Clarenbachet JJ al. J. Lipid Res. 2006. 47: 2820–2824.

Ezetimibe



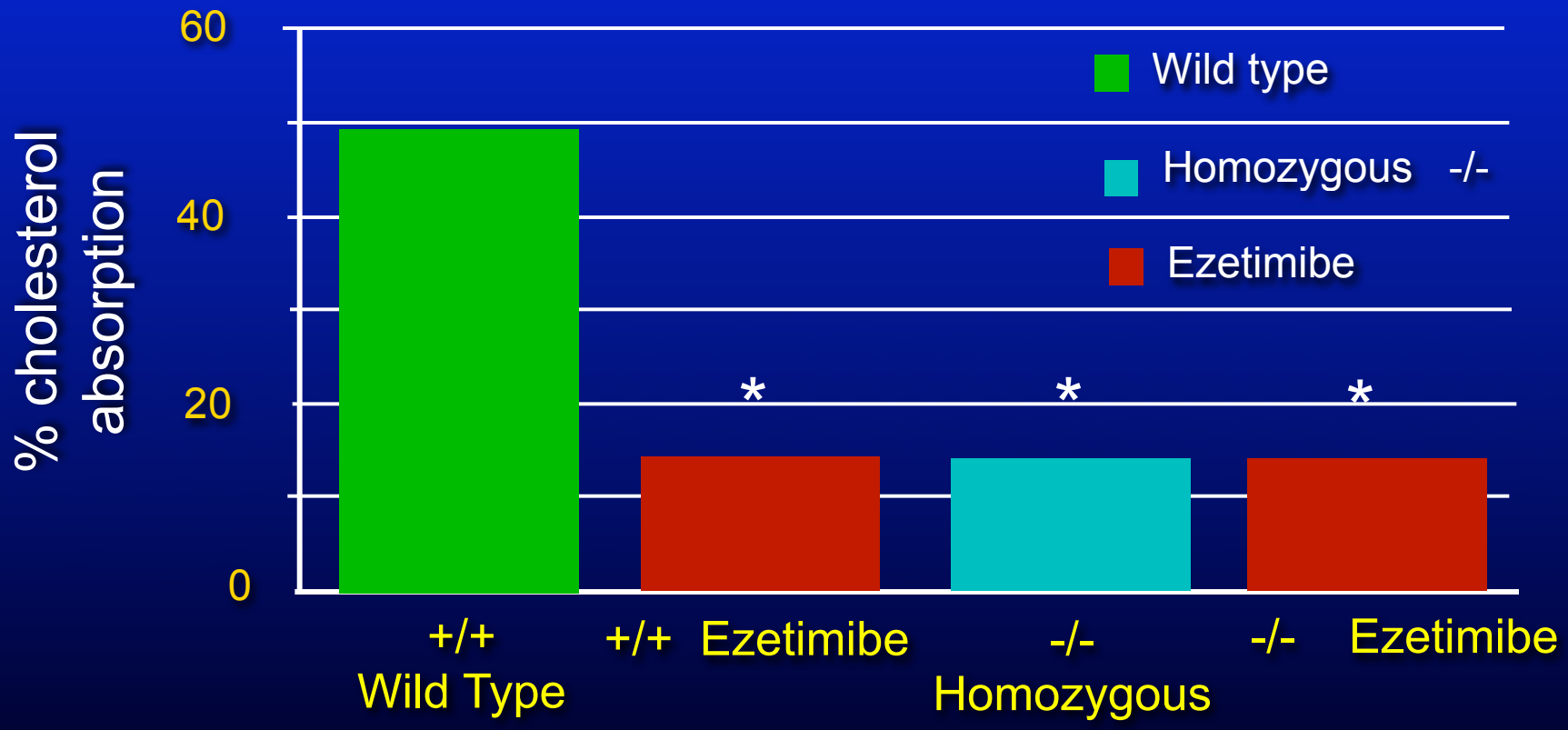
Ezetimibe is a synthetic 2-azetidinone whose full chemical name is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)- 3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone (75)

Niemann-Pick C1-like 1 Protein

- NPC1L1 is a polytopic transmembrane protein is a critical player in in sterol absorption and is expressed along the brush border of enterocytes and the hepatobiliary interface
- It contains about 1300 residues with 13 predicted transmembrane domains, the 3rd to the 7th of which are sterol sensing

Ge L, Wang J et al. Cell Metab 2008;7:508-519

Cholesterol Absorption in NPC1L1 Knockout Mice: Mechanism of Ezetimibe



NPC1L1 = Niemann-Pick C1 Like 1 Protein

Altmann SW et al. Science 2004;303:1201-1204

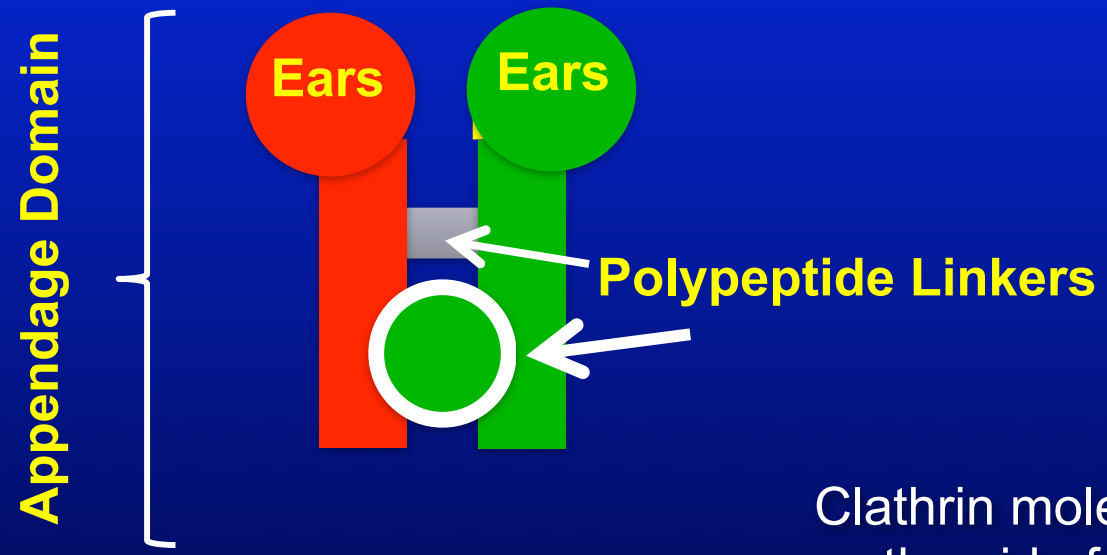
AP2 Adaptor Complex

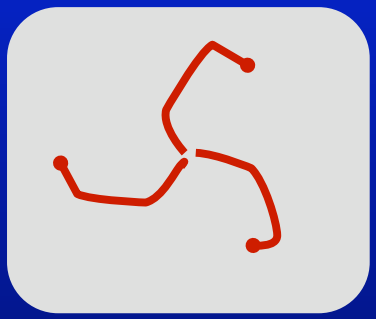
- The **AP2 adaptor** complex works on the plasma membrane to internalize cargo in clathrin-mediated endocytosis.
- It is a stable complex of four proteins which give rise to a structure that has a core domain and two appendage domains attached to the core domain by polypeptide linkers. These appendage domains are sometimes called ears.

- The core domain binds to the membrane and to cargo destined for internalization. The alpha and beta appendage domains bind to accessory proteins and to clathrin. Their interactions allow the temporal and spatial regulation of the assembly of clathrin coated vesicles and their endocytosis.

Ge L, Wang J et al. Cell Metab 2008;7:508-519

AP2 Adaptor Complex & Clathrin





Clathrin molecules are recruited with the aid of adaptor proteins to a membrane segment that is destined to be incorporated into a vesicle.

http://en.wikipedia.org/wiki/AP2_adaptors

Cholesterol Synthesis vs. Absorption

- Almost every kind of mammalian cell is capable of synthesizing cholesterol; however, de novo cholesterol synthesis is an energy consuming process.
- It costs about 18 ATP, 27 NADPH, and 11 O_2 to generate a molecule of cholesterol from acetyl-CoA (a 37 step process). Therefore, mammals obtain significant amounts of cholesterol from diet.
- Both animal sterol (cholesterol) and plant sterols are present in the intestinal lumen. Despite the structural similarity, cholesterol and plant sterols differ in the nature of their side chains, and the functions of cholesterol cannot be completely replaced by plant sterols.
- In fact, humans and animals selectively absorb cholesterol from diet.

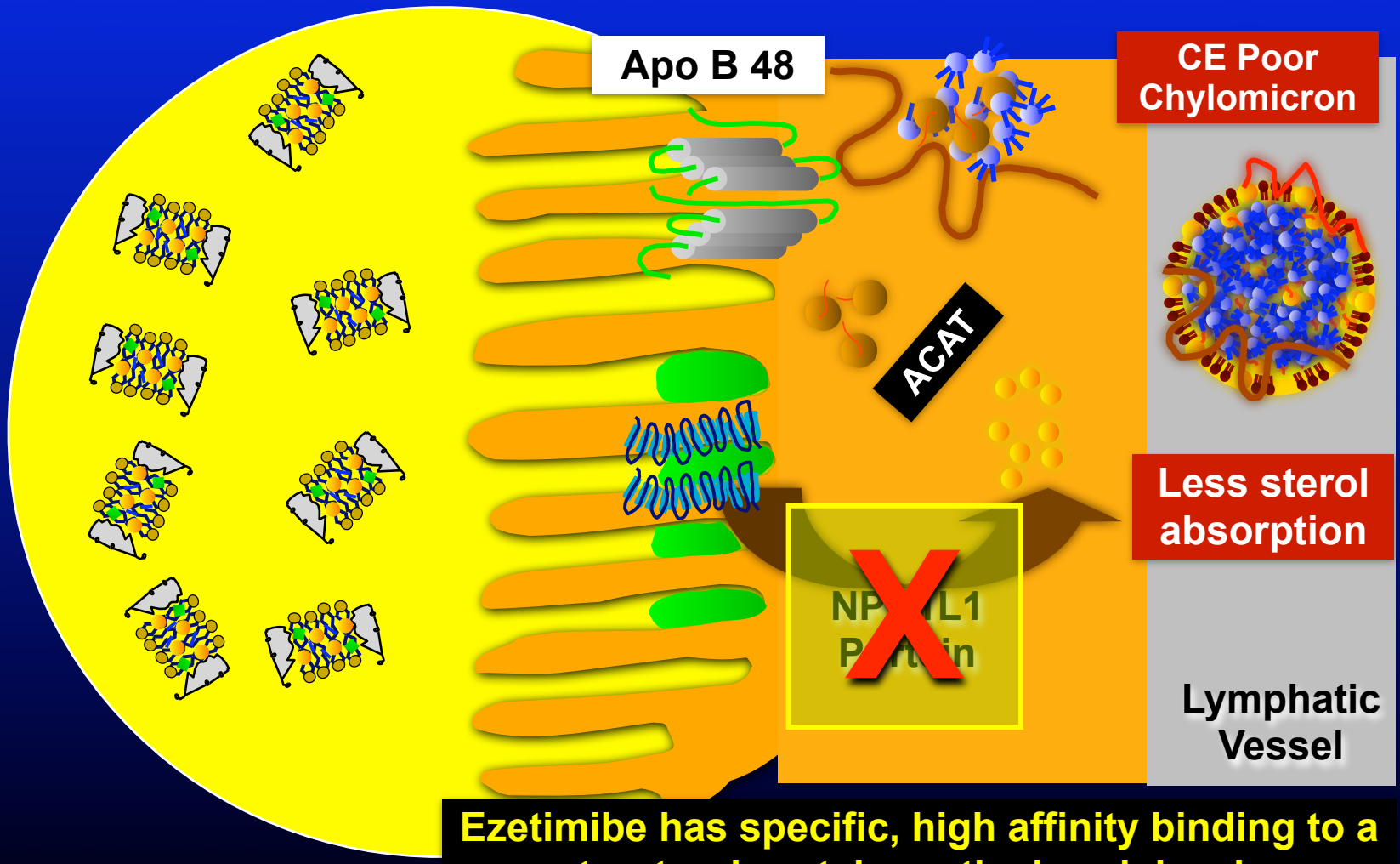
Ge L, Wang J et al. Cell Metab 2008;7:508-519

Niemann-Pick C1-like 1 Protein

- NPC1L1 mediates cellular cholesterol uptake through vesicular endocytosis, The endocytosis of NPC1L1 is dependent on microfilaments and the clathrin/AP2 complex.
- The NPC1L1 recycles between ERC and PM:
  - Depletion of cholesterol causes the transport of NPC1L1 from ERC to PM, whereas replenishment of cholesterol results in the transportation of NPC1L1 from PM to ERC. Meanwhile, cholesterol is internalized together with NPC1L1.

Ge L, Wang J et al. Cell Metab 2008;7:508-519

Ezetimibe: Mechanism of Action
Sterol Absorption Inhibitor

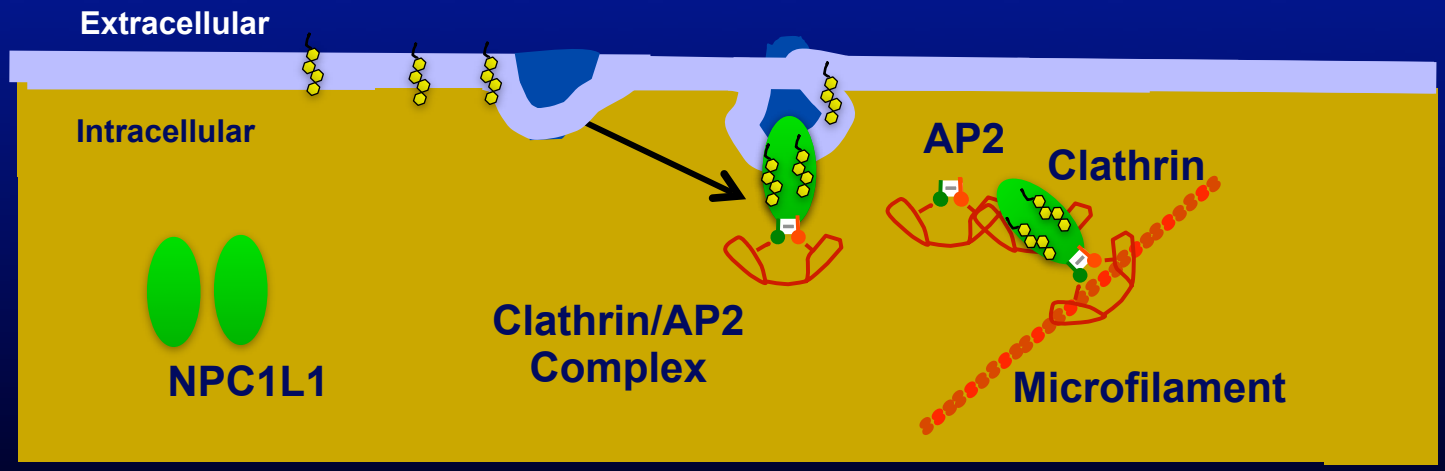


Ezetimibe has specific, high affinity binding to a structural protein on the brush border

Turley S. et al. Curr Opin Lipidol 2003;14:233-240

NPC1L1 Mediated Sterol Absorption

- NPC1L1 mediates cellular cholesterol uptake through vesicular endocytosis, The endocytosis of NPC1L1 is dependent on microfilaments and the clathrin/AP2 complex.



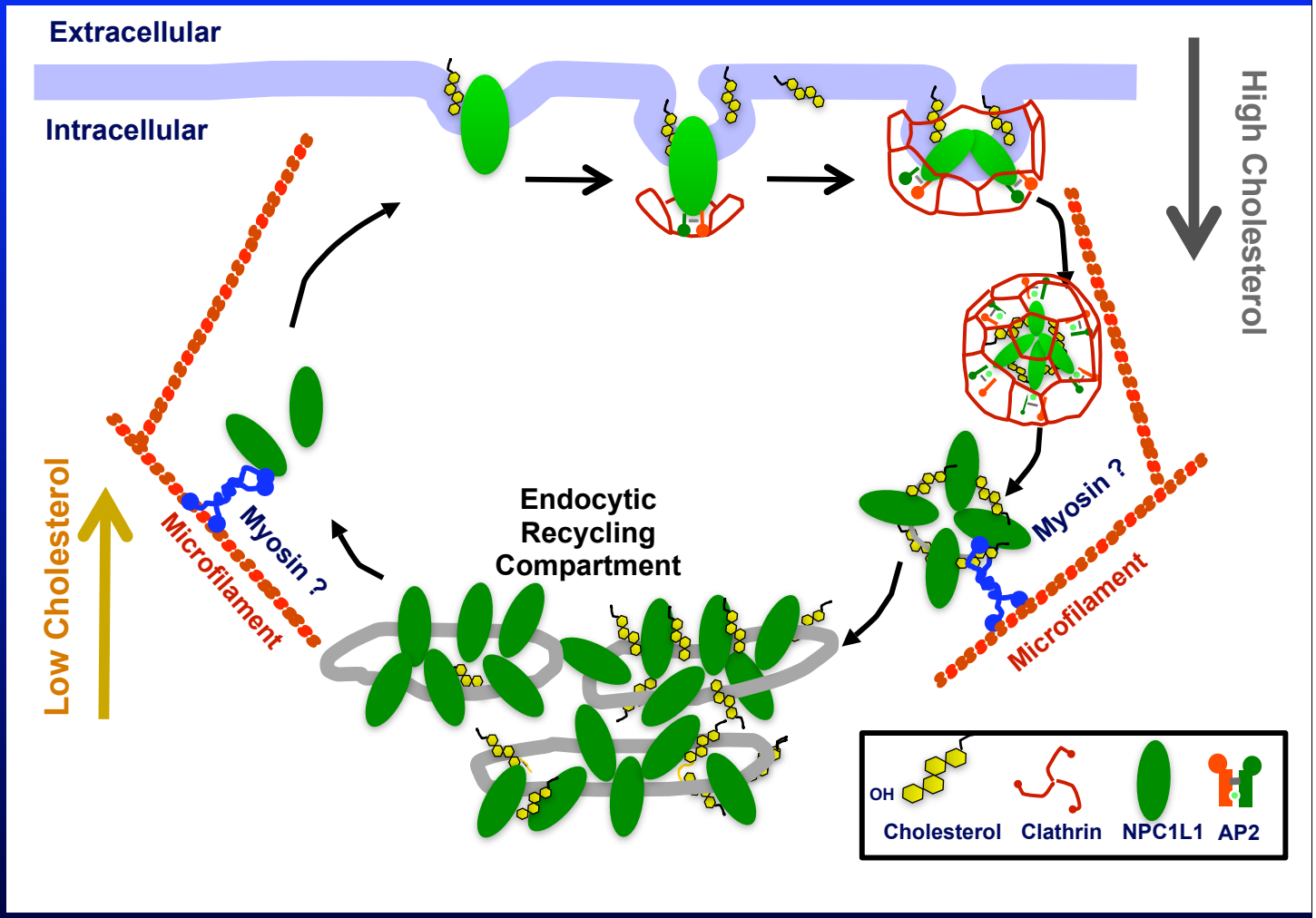
Ge L, Wang J et al. Cell Metab 2008;7:508-519

NPC1L1 Mediated Internalization of Cholesterol

NPC1L1 protein recycles between the plasma membrane (PM) and endocytic recycling compartment (ERC).

When the extracellular cholesterol concentration is high, cholesterol is incorporated into the PM and is sensed by cell surface-localized NPC1L1.

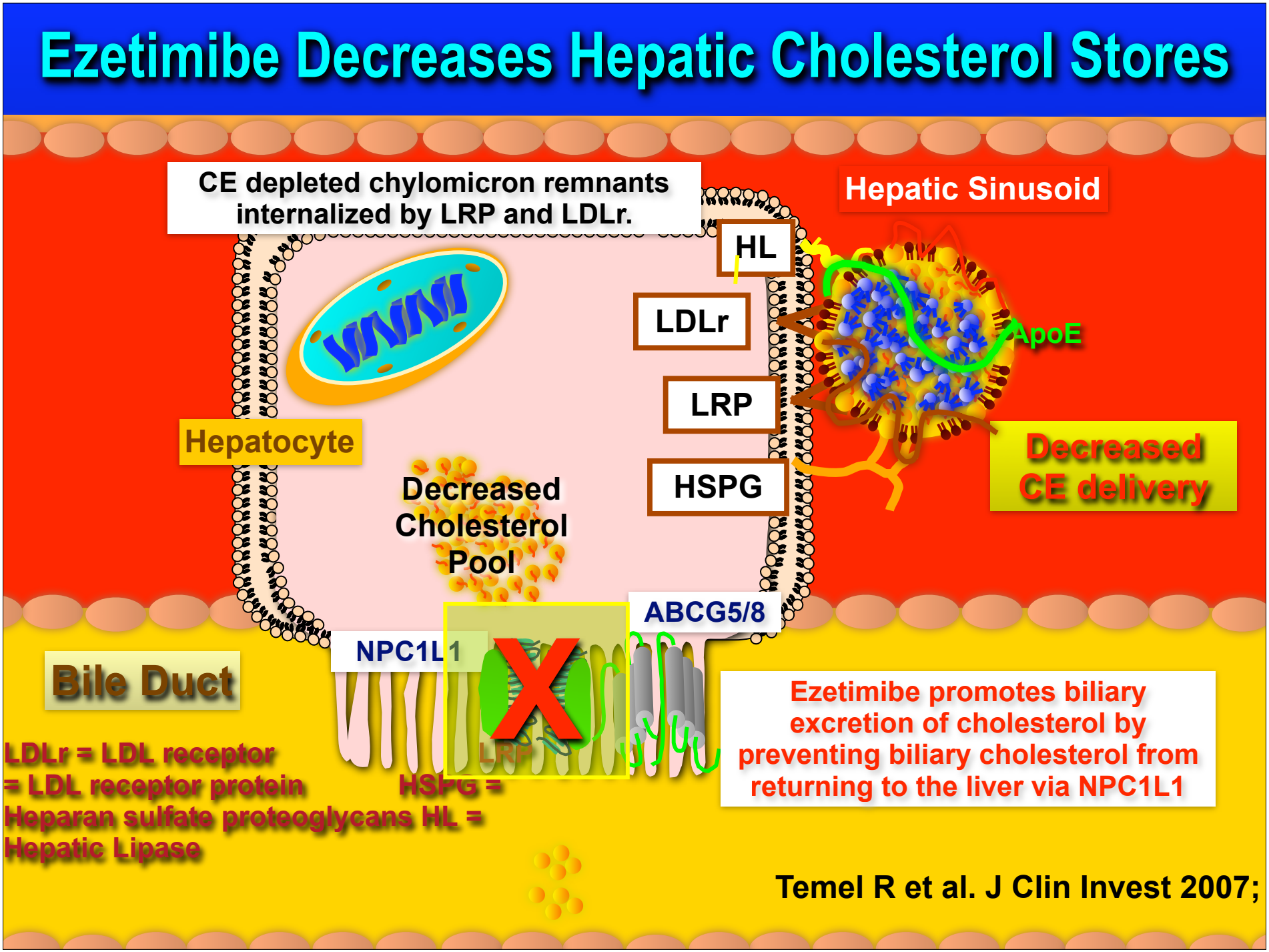
NPC1L1 and cholesterol are then internalized together through clathrin/AP2-mediated endocytosis and transported along microfilaments to the ERC in vesicles.



The ERC is where massive amounts of cholesterol and NPC1L1 are stored. of NPC1L1 and eventually decreasing cholesterol absorption. When the intracellular cholesterol level is low, ERC-localized NPC1L1 moves back to the PM along microfilaments in order to absorb cholesterol.

Ge L, Wang J et al. Cell Metab 2008;7:508-519

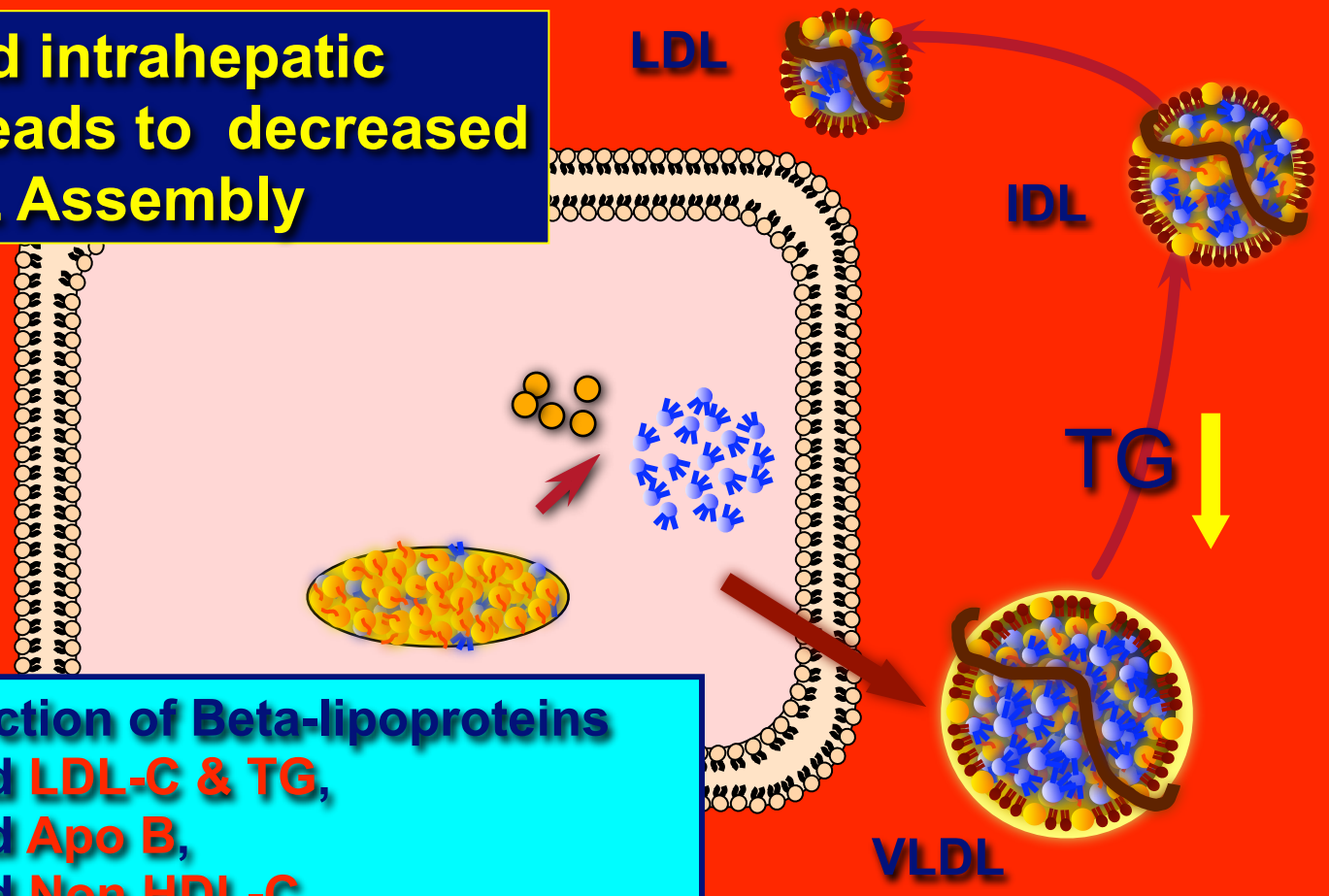
Ezetimibe Decreases Hepatic Cholesterol Stores



Temel R et al. J Clin Invest 2007;

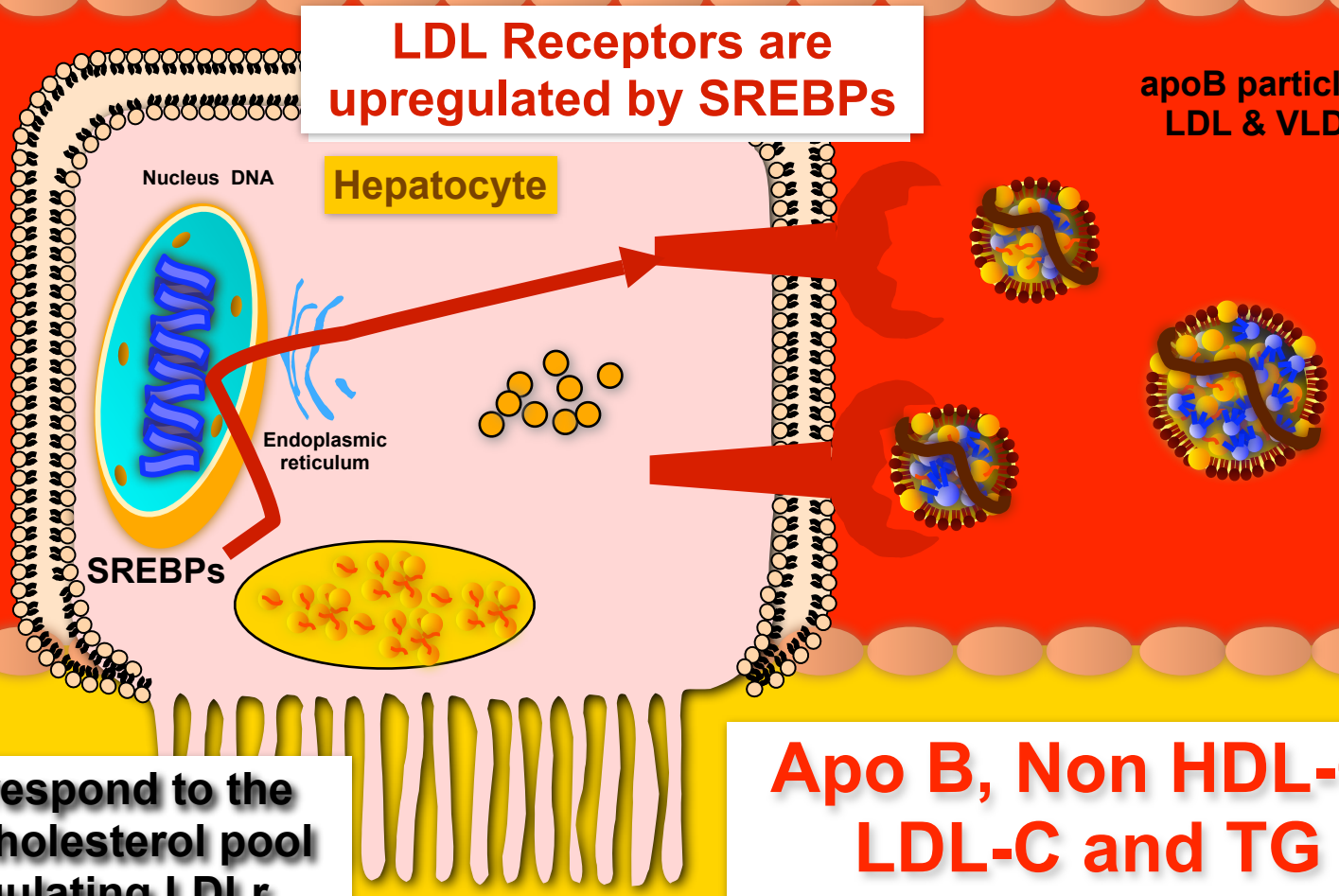
Ezetimibe and Lipoproteins

Reduced intrahepatic cholesterol leads to decreased VLDL Assembly



Reduced Production of Beta-lipoproteins
- Reduced LDL-C & TG,
- Reduced Apo B,
- Reduced Non HDL-C

Ezetimibe: Upregulates LDL Receptors

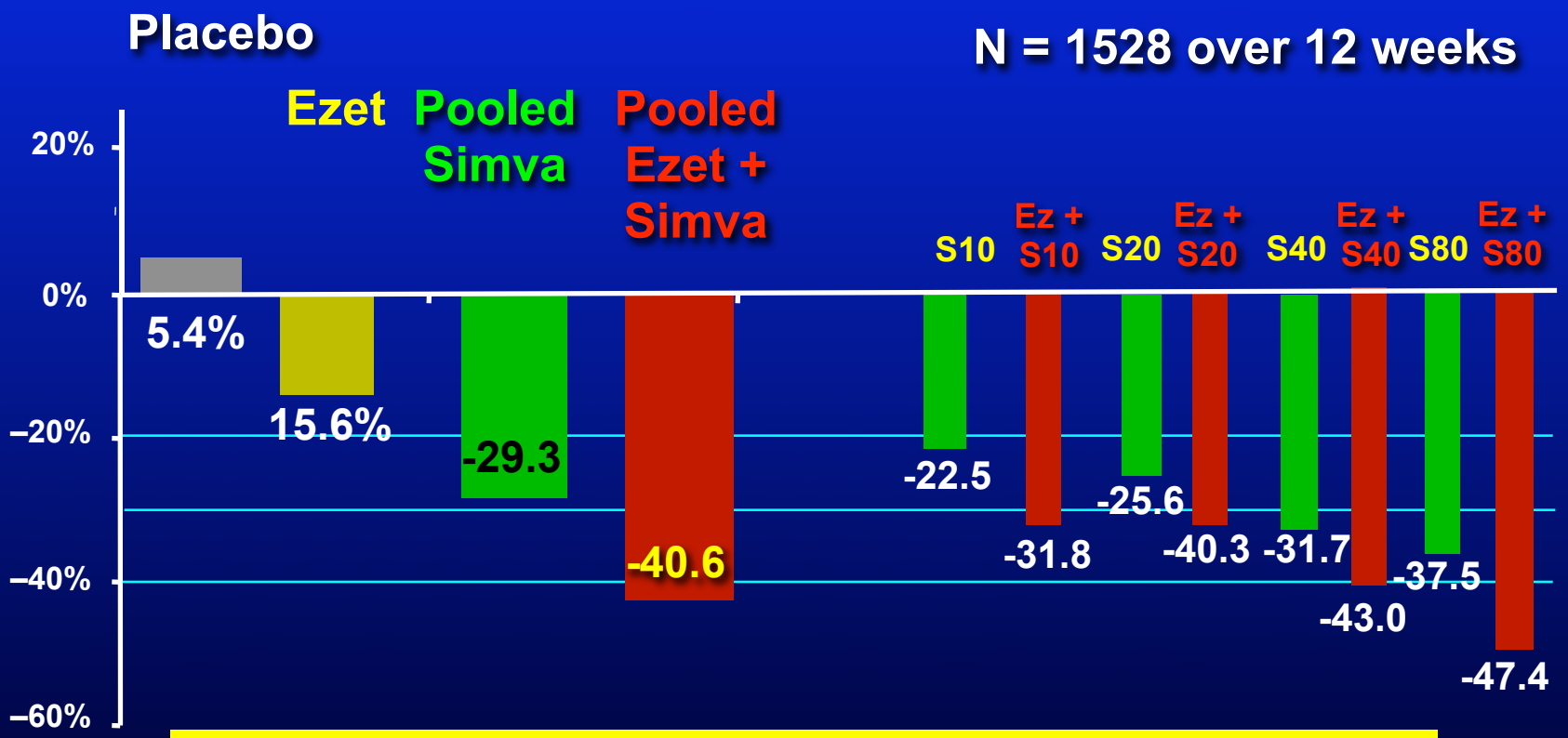


SREBPs respond to the depleted cholesterol pool by upregulating LDLr

Apo B, Non HDL-C, LDL-C and TG are lowered

SREBPs = Sterol regulatory element binding proteins

Ezetimibe + Simvastatin Effect on Remnant Lipoproteins

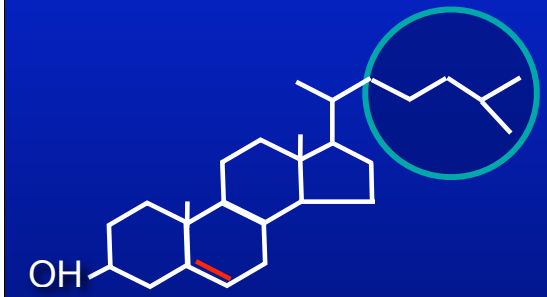


Combined administration of Eze/Simva is more effective than Simva monotherapy in reducing TG-rich lipoproteins measured by the RLP assay

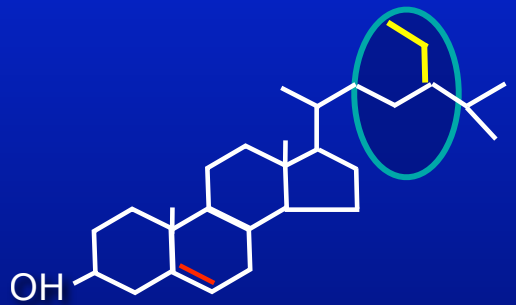
Bays H. et al. ACC Annual Meeting March New Orleans 2004

Cholesterol and Noncholesterol Sterols

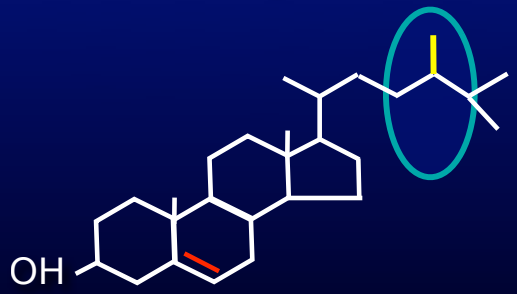
Cholesterol



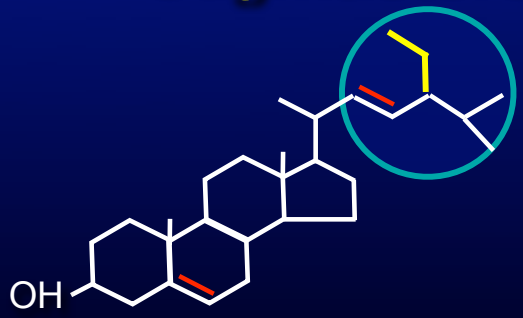
Sitosterol



Campesterol



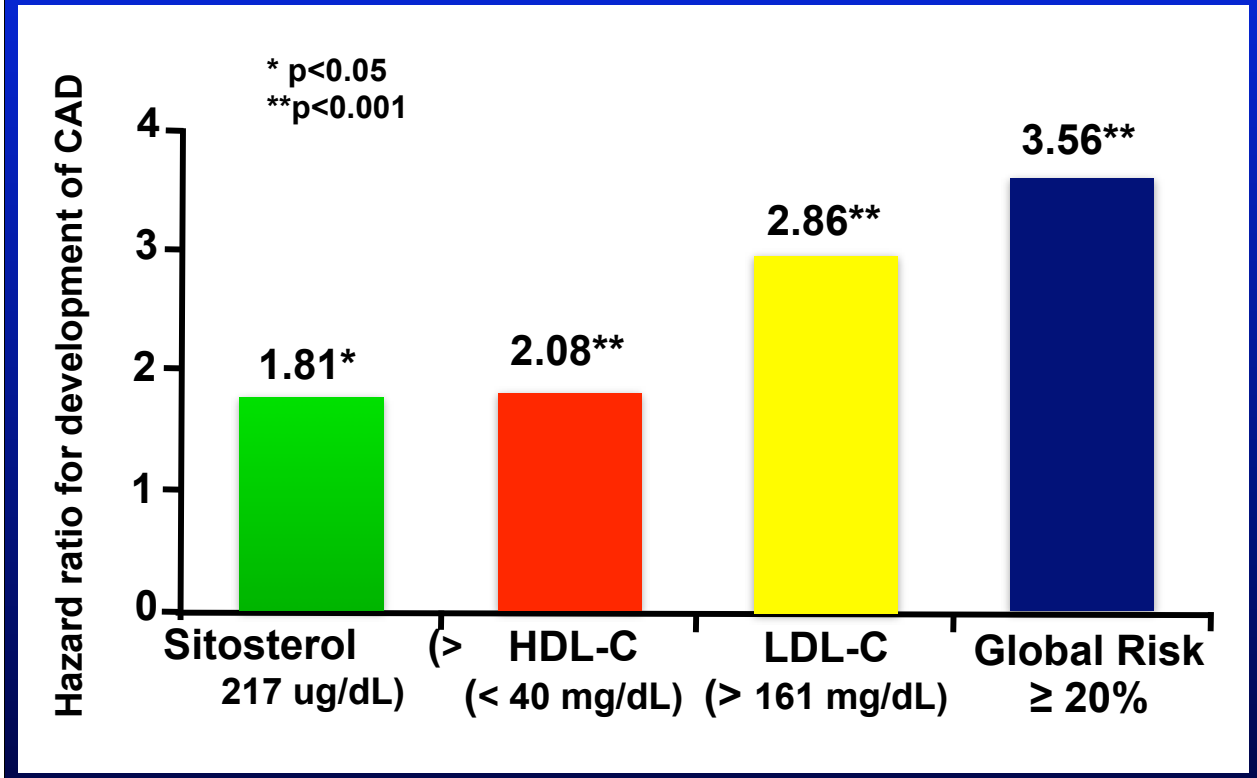
Stigmasterol



The majority of the differences are in the “R” tail with plant sterols having an extra methyl (campesterol) or ethyl (sitosterol) group at the C-24 position and different levels of desaturation

The more carbon atoms and desaturation, the less the intestinal absorption

PROspective CArdiovascular Munster Study (PROCAM): Elevated Phytosterols and CHD



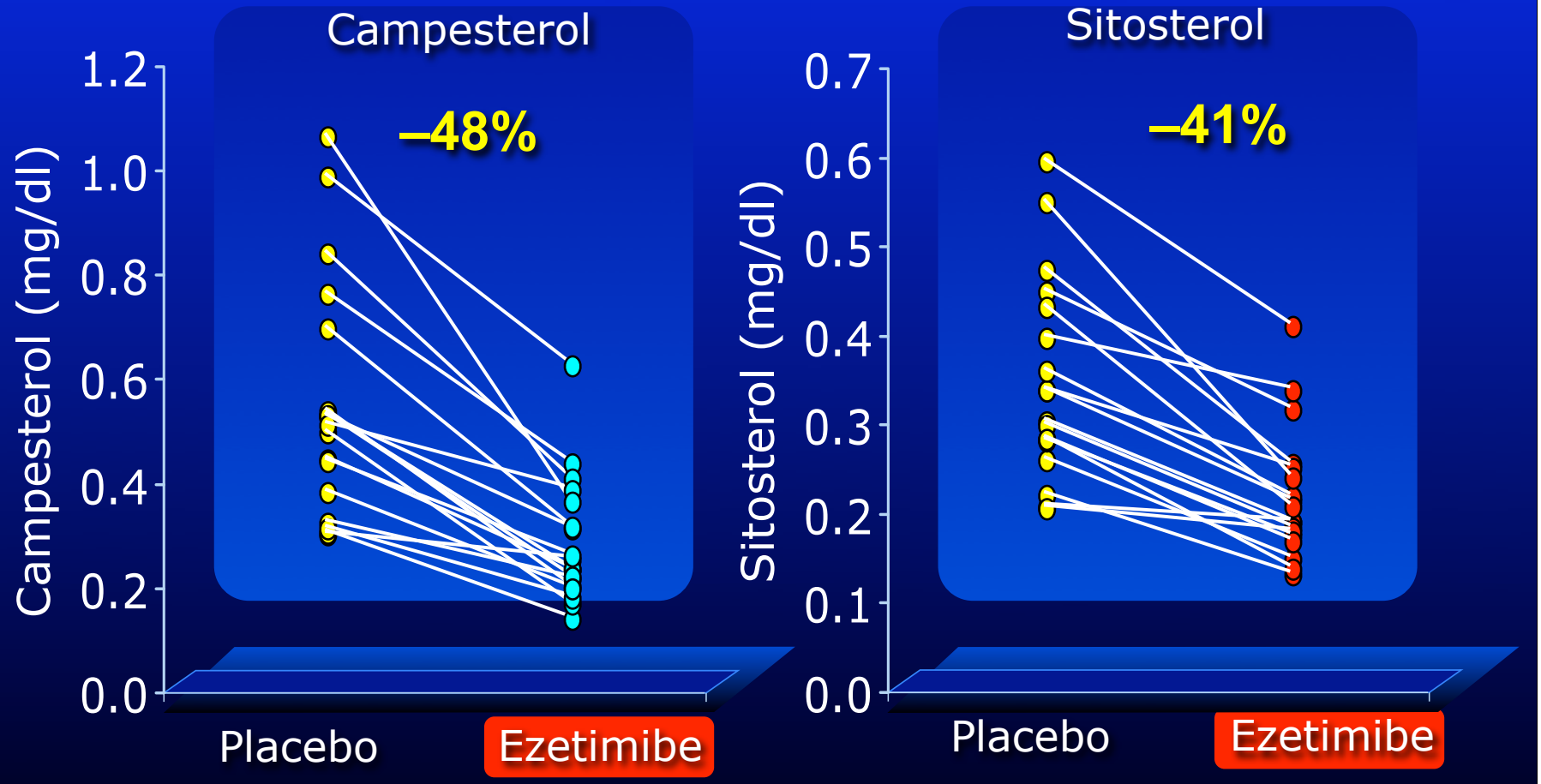
On univariate analysis, a high sitosterol concentration (>2.0) was significantly associated with a CHD risk (HR = 1.81; < 0.05) similar to that of hypertension, family CHD history, or metabolic syndrome

Of the univariate risk factors, only high LDL-C, low HDL-C and global risk > 20% (hazard ratio = 3.56) were associated with a greater relative risk of a major coronary event than elevated sitosterol.

Male Data

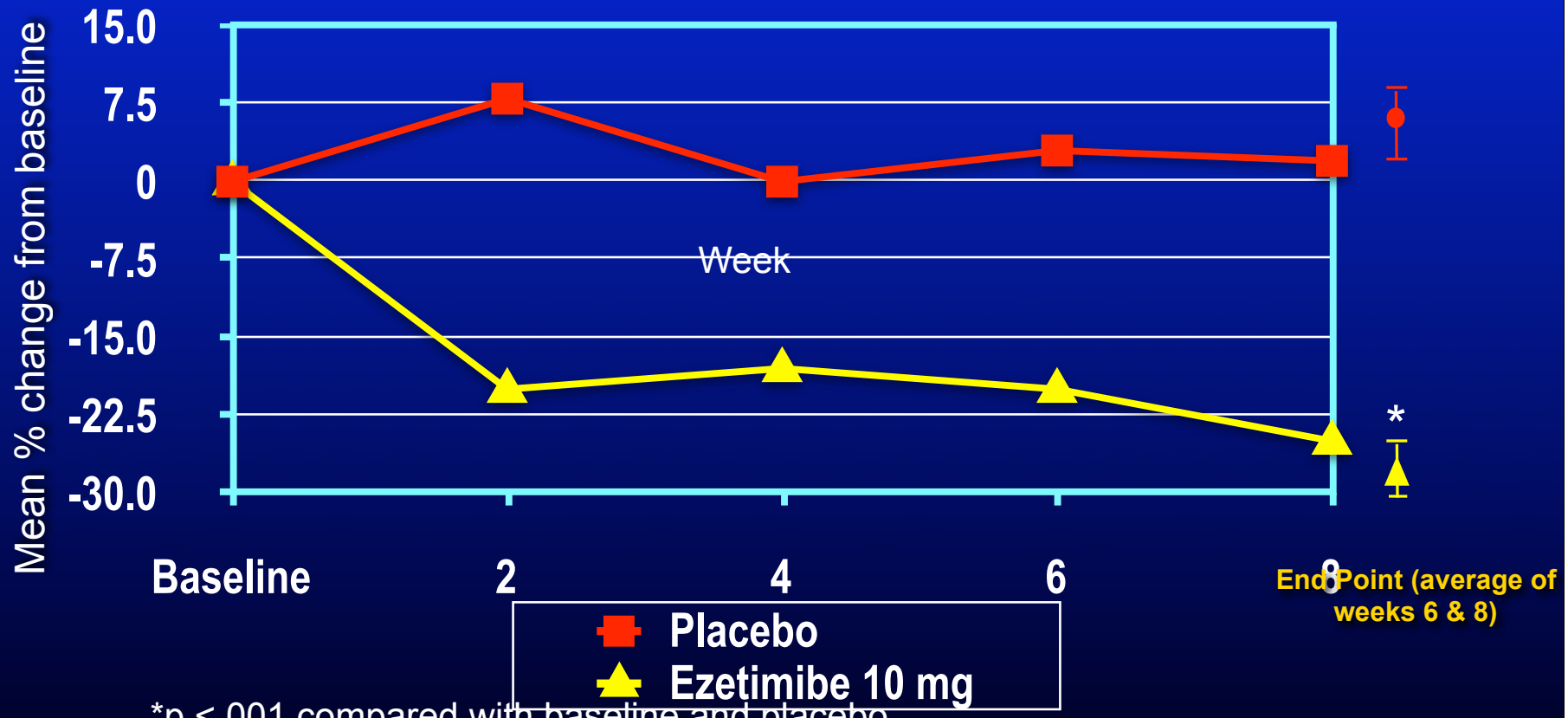
Assmann G et al. Nutrition, Metabolism & Cardiovascular Diseases (2006) 16, 13e21

Ezetimibe Lowers Phytosterols in Patients with Mild Hypercholesterolemia



Sudhop T et al. *Circulation* 2002;106:1943-1948.

Ezetimibe Inhibits Phytosterol Absorption in Patients with Sitosterolemia

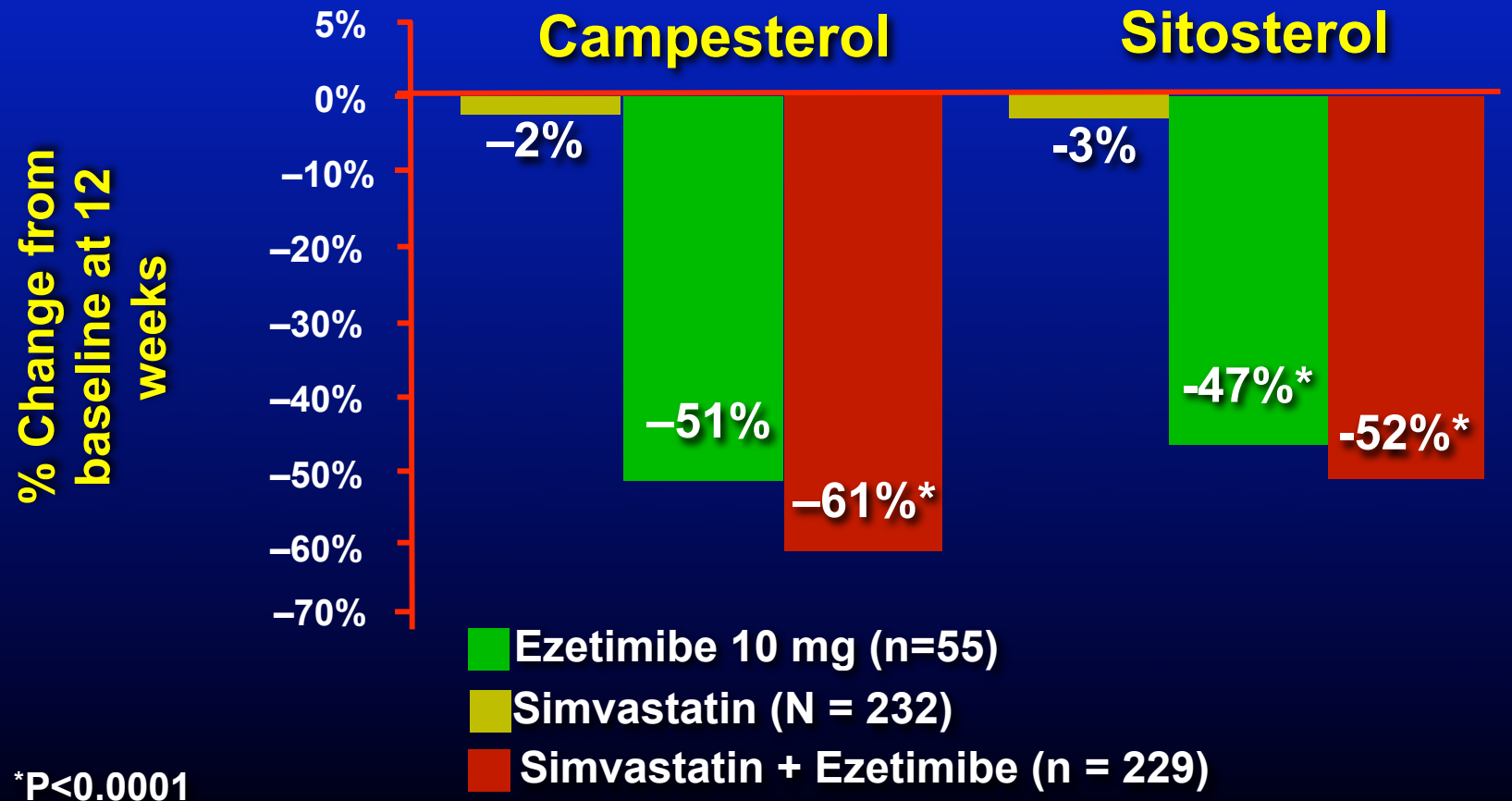


*p <.001 compared with baseline and placebo

Salen G, et al. Circulation 2004;109:966-971

Effect of Statin and Ezetimibe on Noncholesterol Sterol Levels

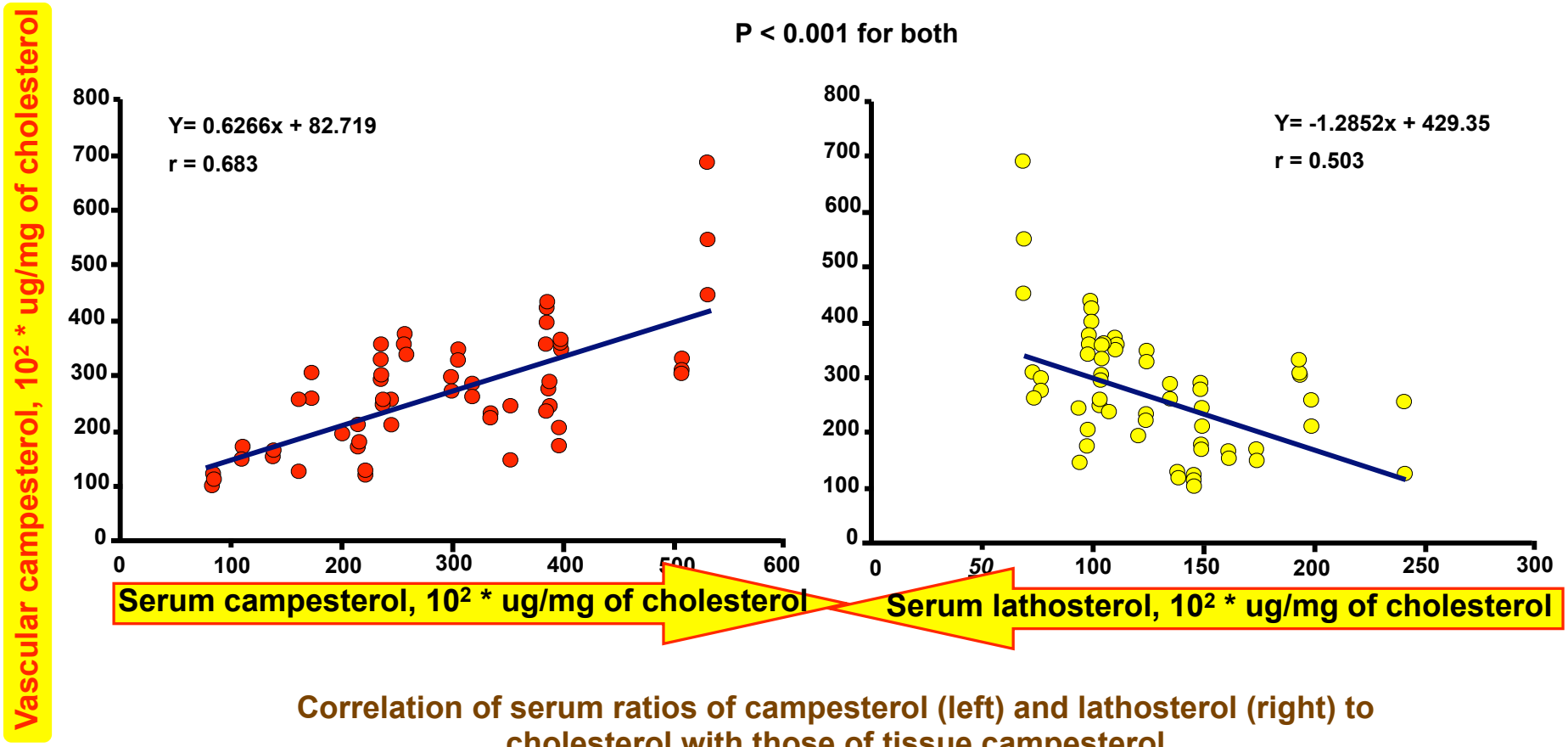
Patients with primary hypercholesterolemia



*P<0.0001

Assmann G et al. J Am Coll Card 2004;43 #5 (suppl A) Abstract 1008-183

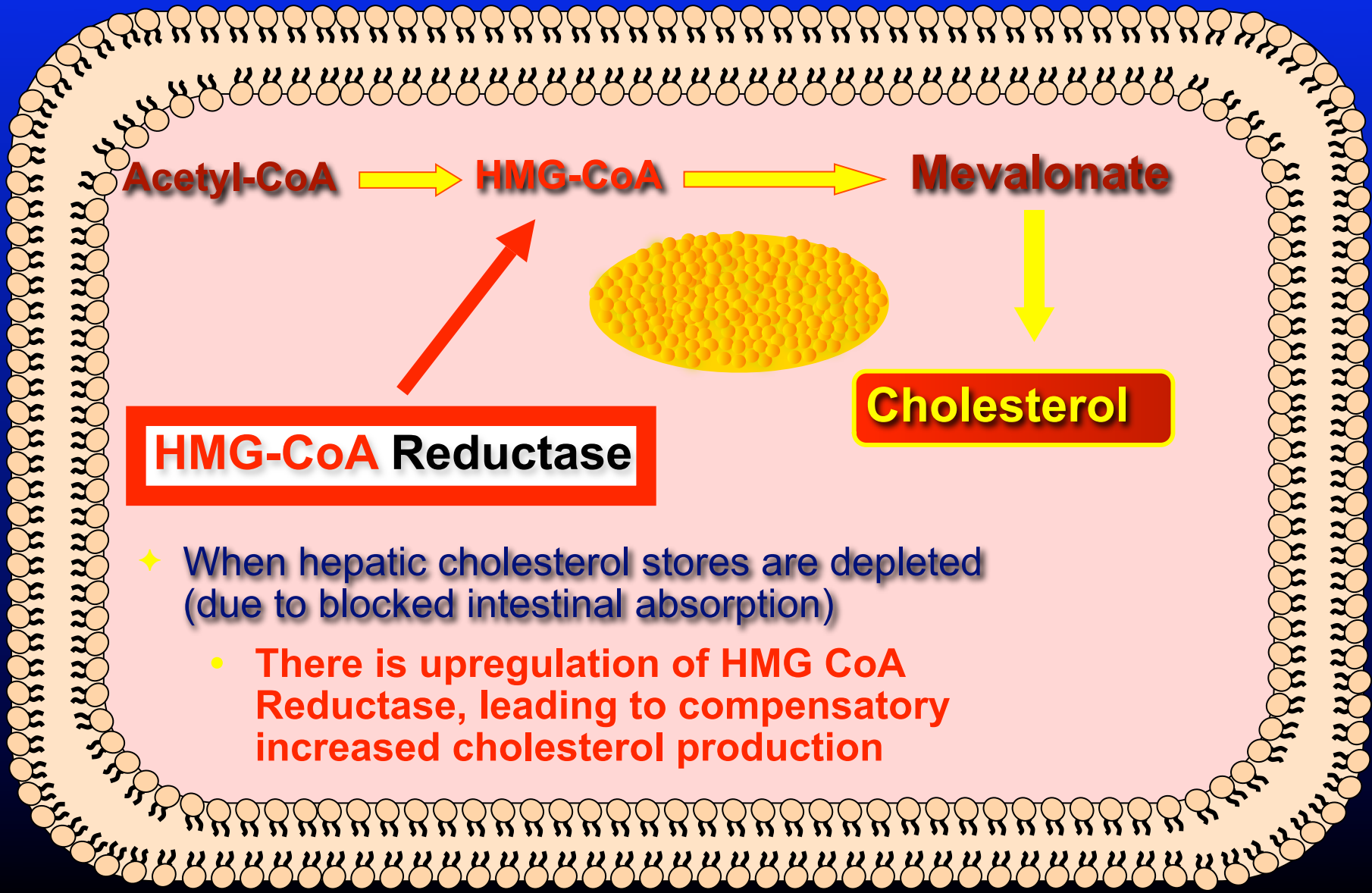
Plant Sterols in Serum and Plaque of Carotid Endarterectomy Patients



Correlation of serum ratios of campesterol (left) and lathosterol (right) to cholesterol with those of tissue campesterol

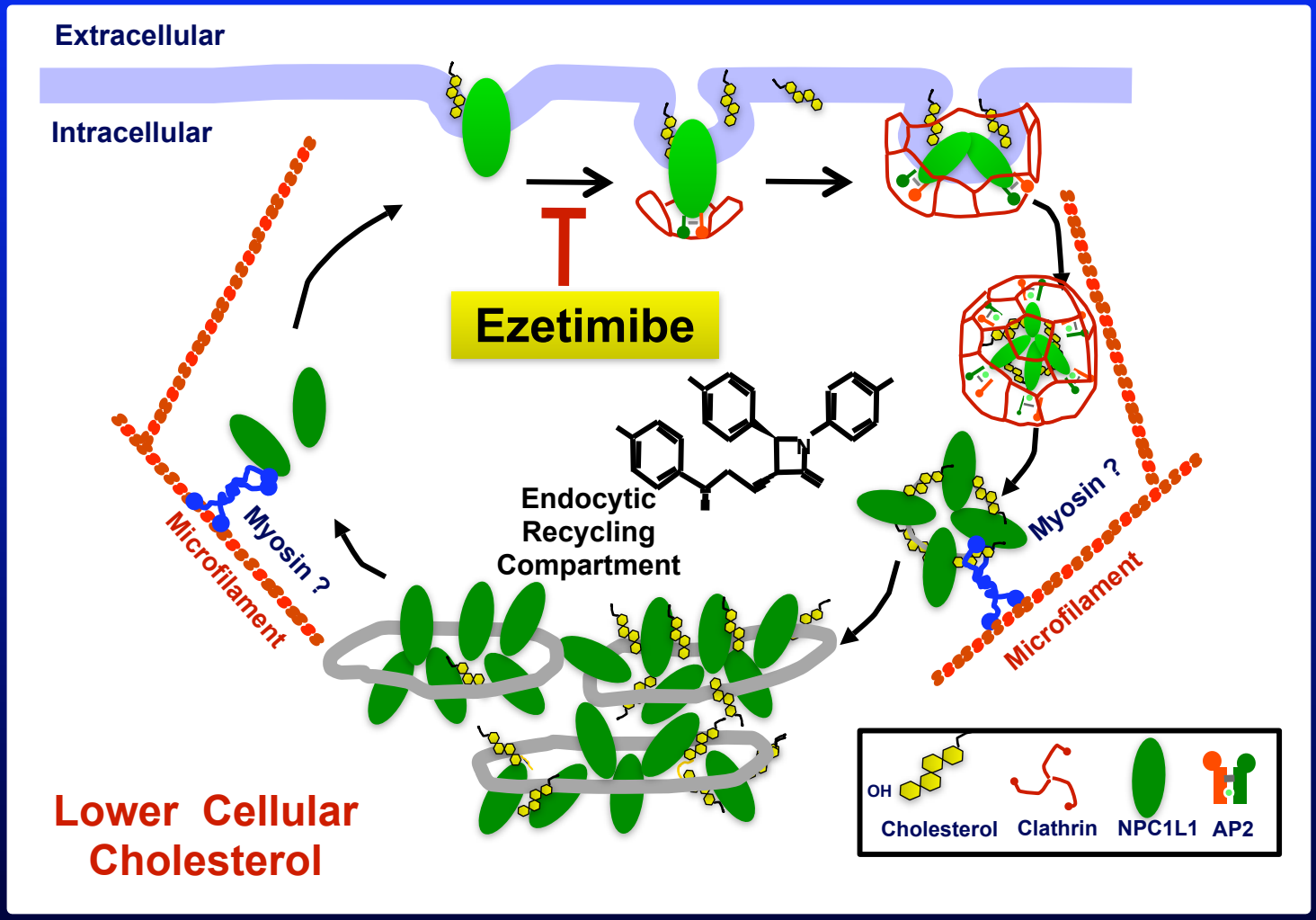
Miettinen, T et al. J Am Coll Cardiol 2005;45:1794-1801

Ezetimibe and HMG CoA Reductase



- When hepatic cholesterol stores are depleted (due to blocked intestinal absorption)
  - There is upregulation of HMG CoA Reductase, leading to compensatory increased cholesterol production

Ezetimibe Mechanism of Action

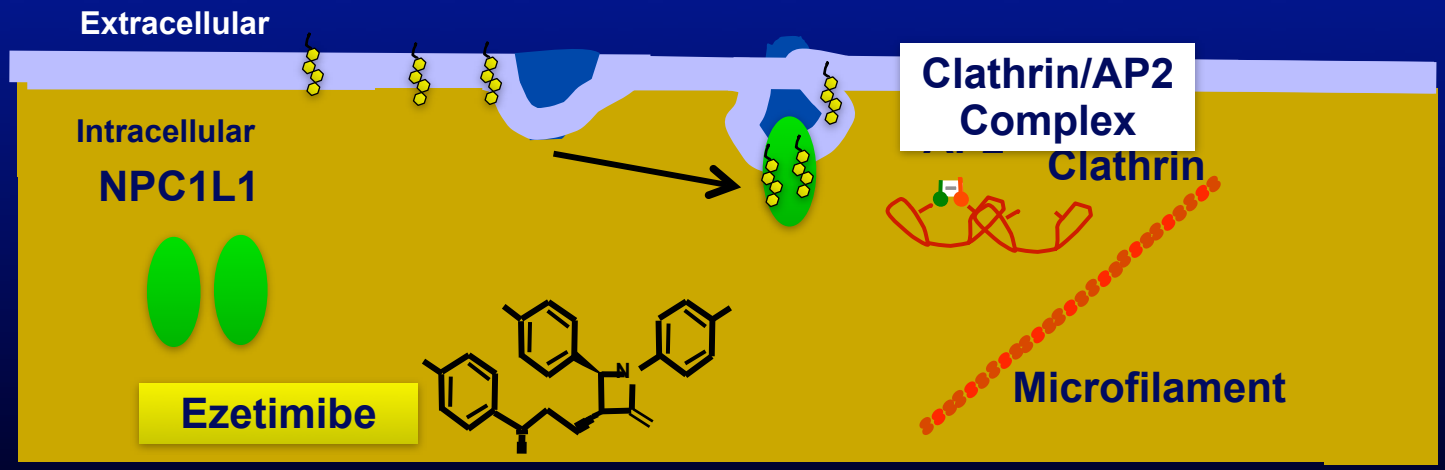


Ezetimibe prevents NPC1L1 from entering the AP2-mediated clathrin-coated vesicles, thus inhibiting the endocytosis

Ge L, Wang J et al. Cell Metab 2008;7:508-519

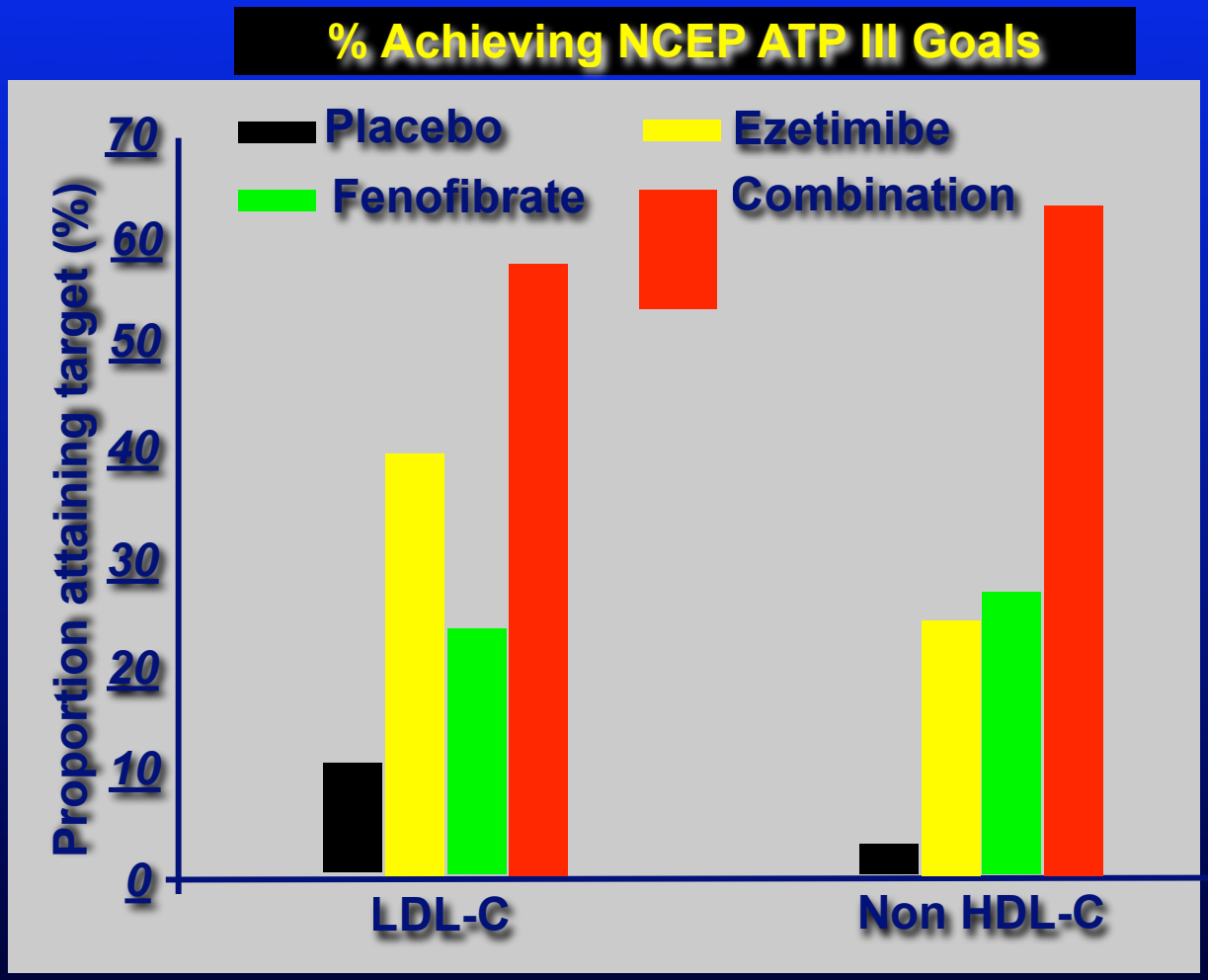
Ezetimibe Blocks NPC1L1 Mediated Sterol Absorption

- Ezetimibe prevents NPC1L1 from entering the AP2-mediated clathrin-coated vesicles, thus inhibiting the endocytosis



Ge L, Wang J et al. Cell Metab 2008;7:508-519

Ezetimibe – Fenofibrate Study



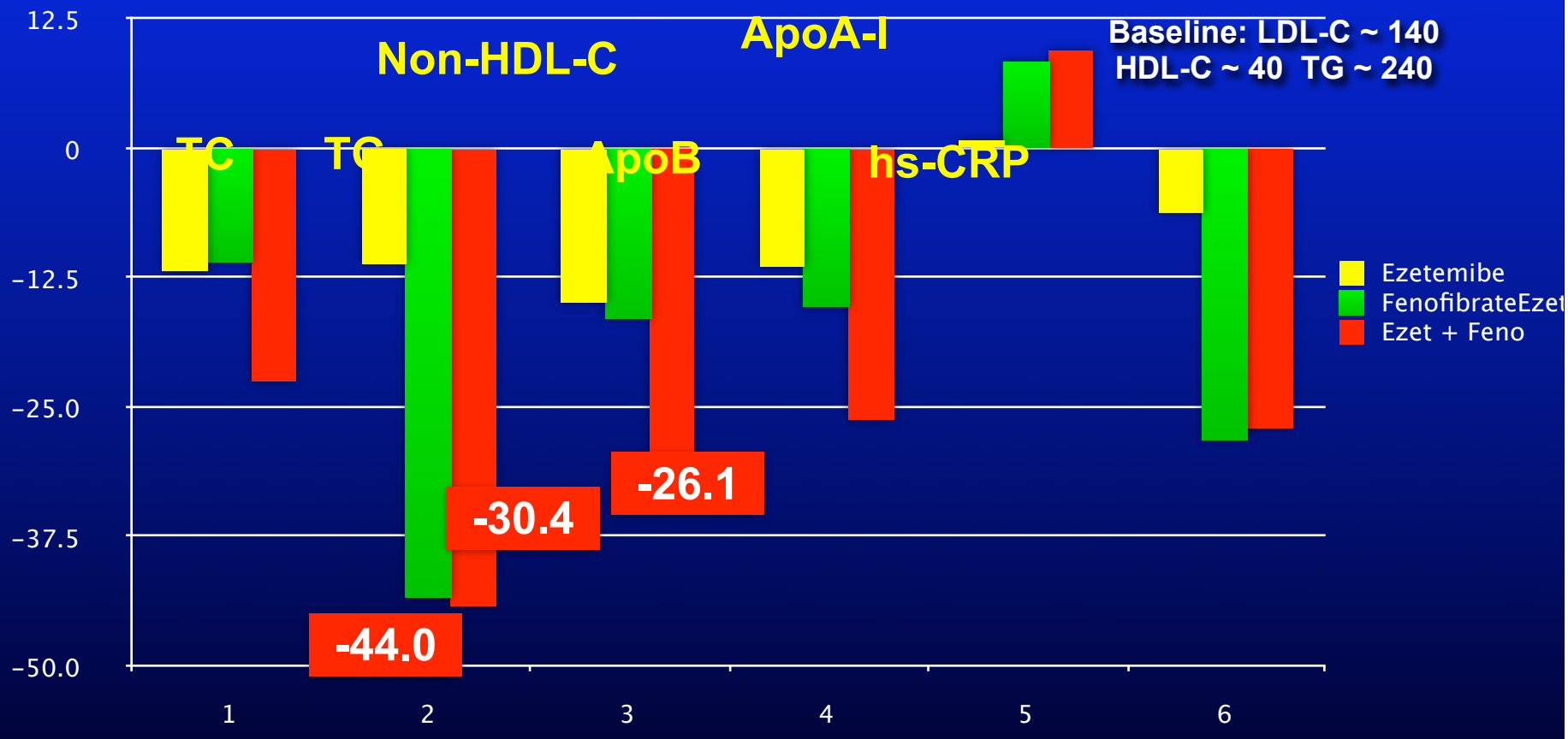
More than 62% of patients **shifted to the larger, more buoyant** LDL pattern from the smaller, more dense pattern with coadministration, and FENO alone treatments.

The Non HDL-C goal attainment was comparable across baseline TG values

Baseline: LDL-C ~ 140
HDL-C ~ 40 TG ~ 240

Farnier M, et al. Eur Heart J. 2005;26:897-905.

Ezetimibe – Fenofibrate Study



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Ezetimibe – Fenofibrate Study

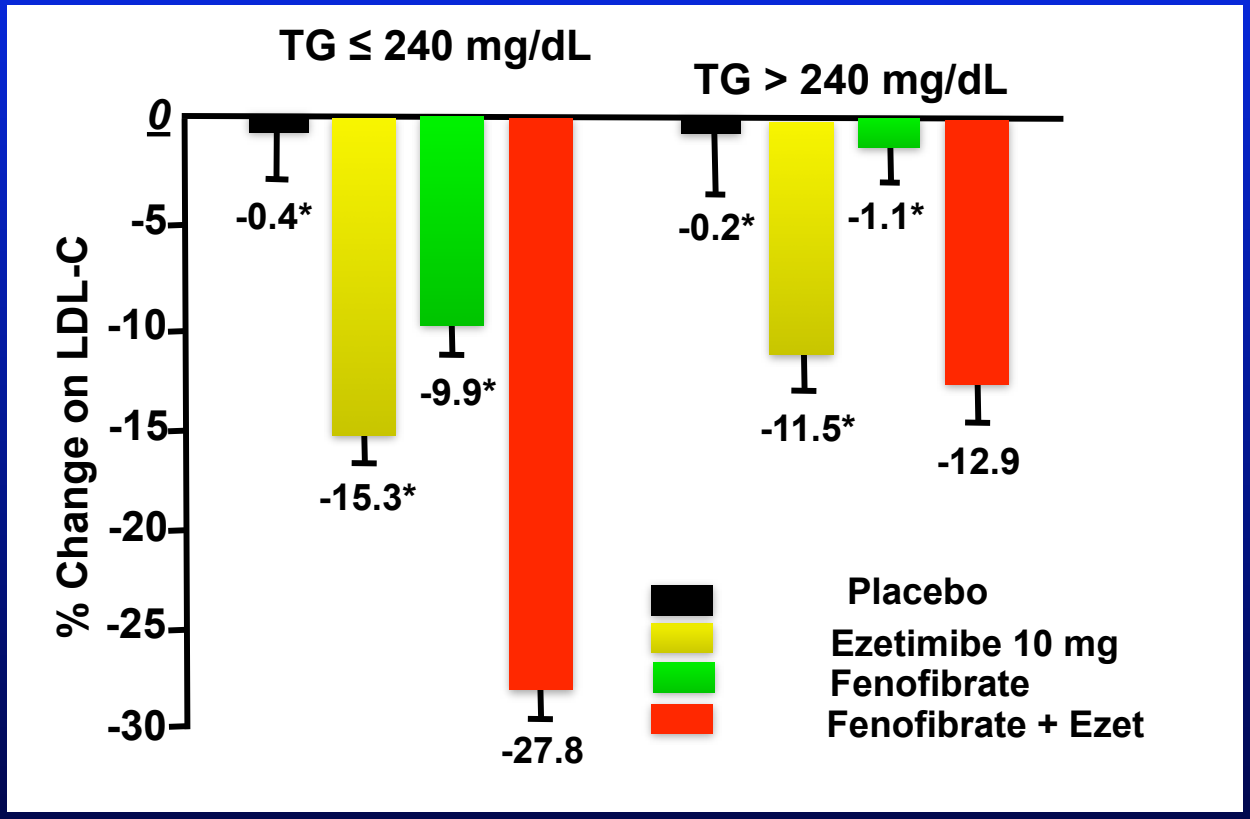
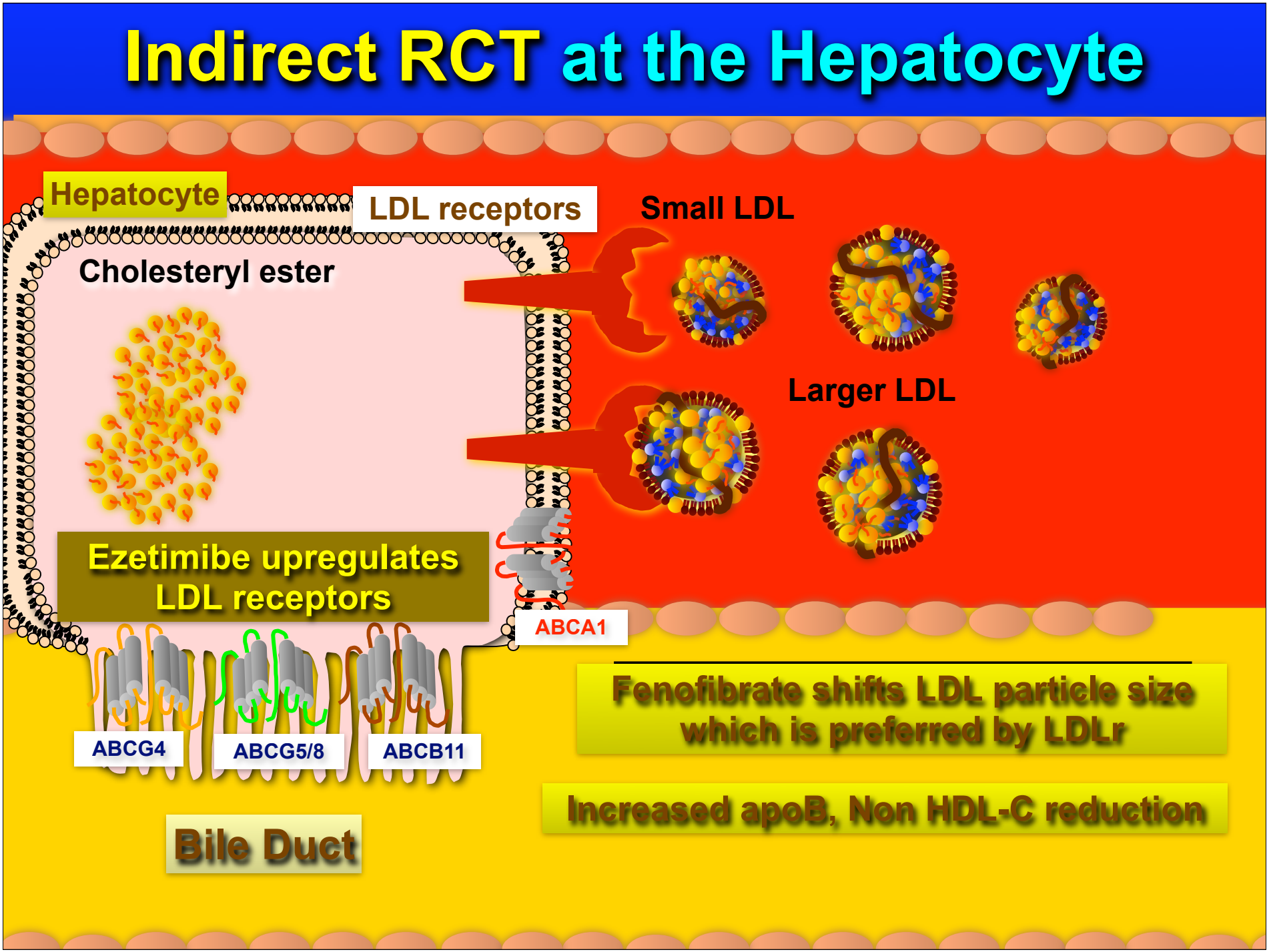


Figure 2 Least square mean per cent change (SE) in LDL-C from baseline to study endpoint for patients with baseline TG ≤ or >3.1 mmol/L (median). Significantly greater reductions in LDL-C (*$P < 0.001$ for FENO + EZE compared with FENO) were observed within both TG subgroup.

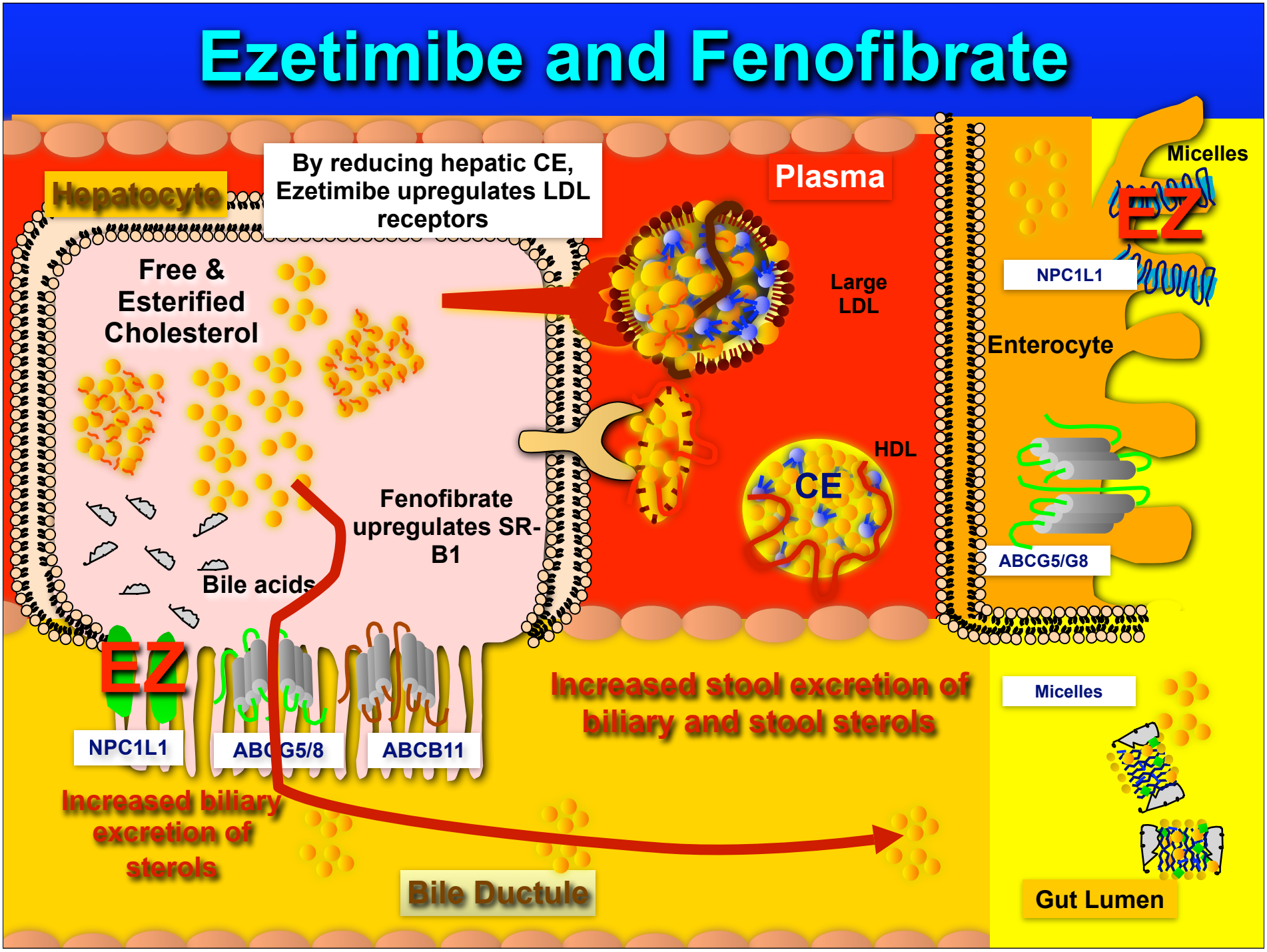
Baseline: LDL-C ~ 140
HDL-C ~ 40 TG ~ 240

Farnier M, et al. *Eur Heart J.* 2005;26:897-905.

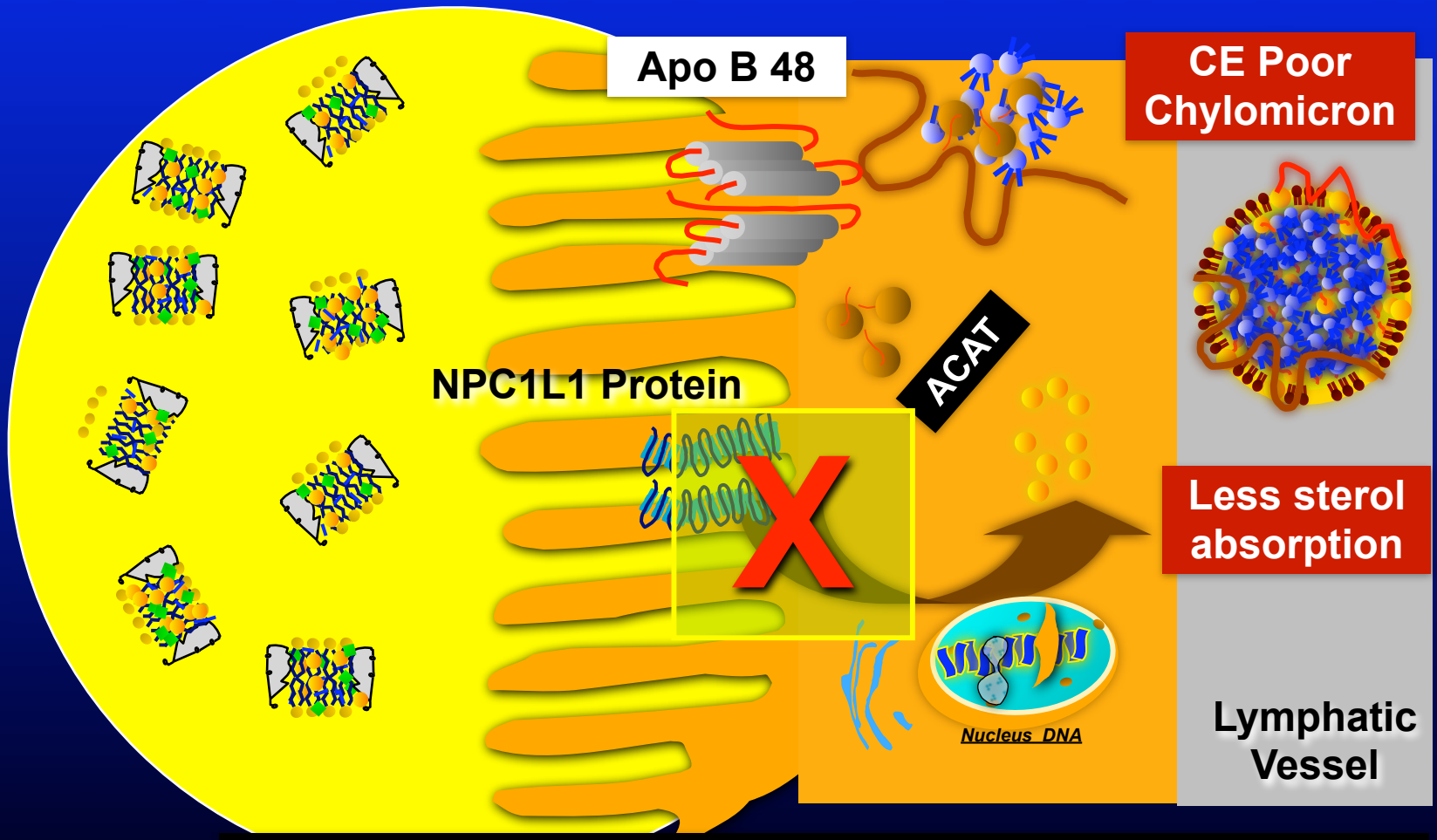
Indirect RCT at the Hepatocyte



Ezetimibe and Fenofibrate



Fenofibrate Decreases Sterol Absorption

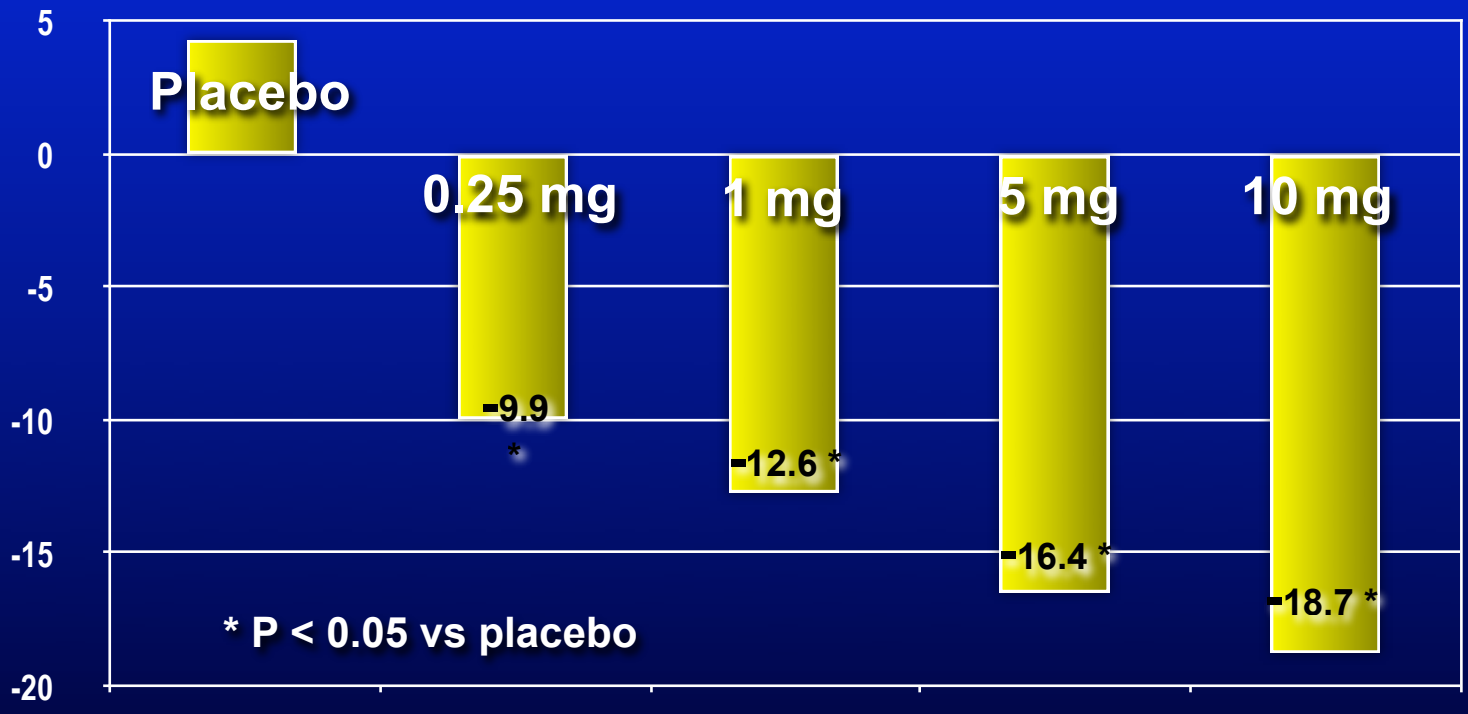


Valasek MA J Lipid Res 2007;48:2725-35

Fenofibrate, via PPARα agonism reduces expression of NPC1L1 & decreases sterol absorption

Ezetimibe Dose Response Study

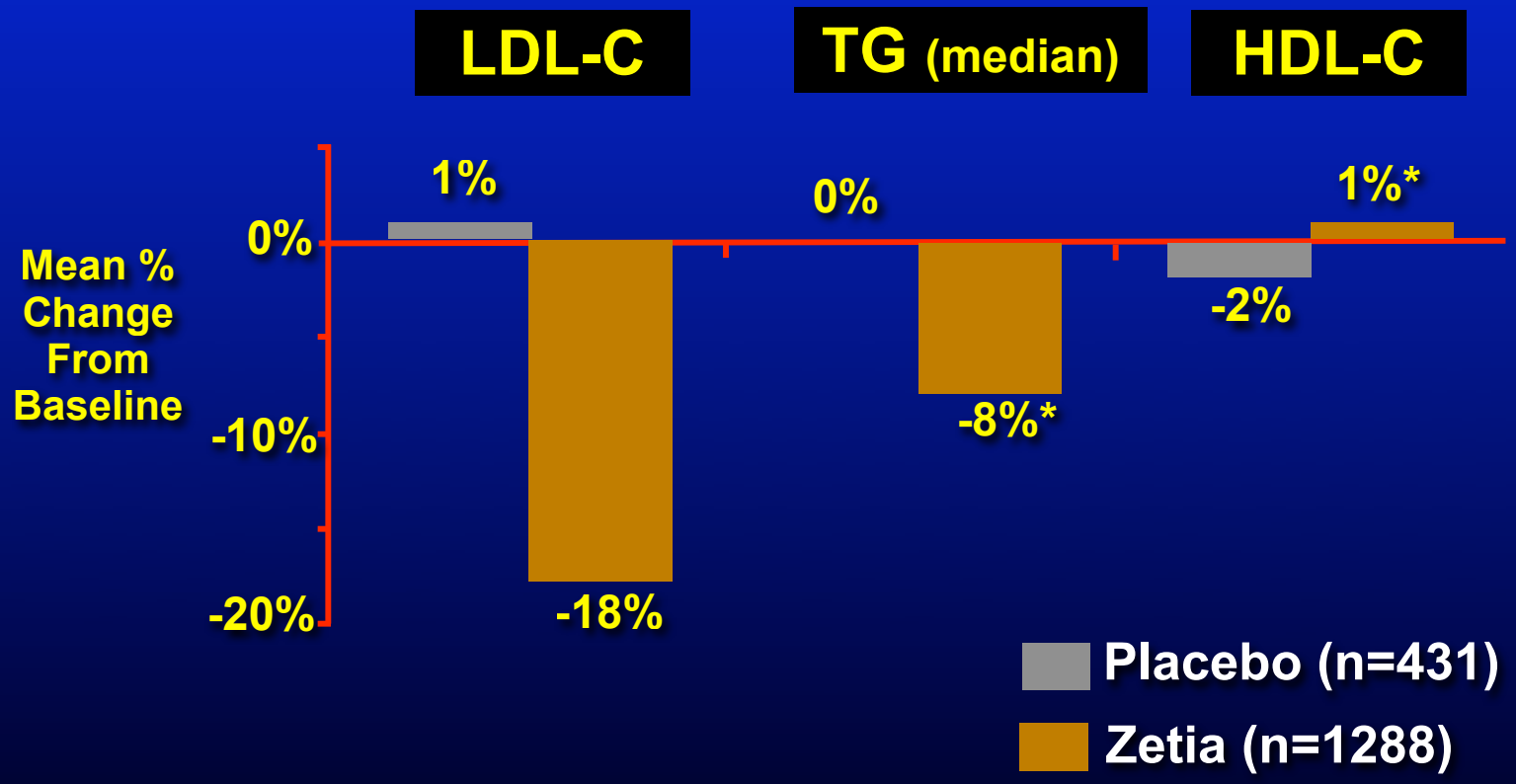
Mean % change in LDL-C from baseline at week 12



[1]At 10 mg 68% of patients achieved ≥ 15% LDL-C reduction & 22% ≥ 25%

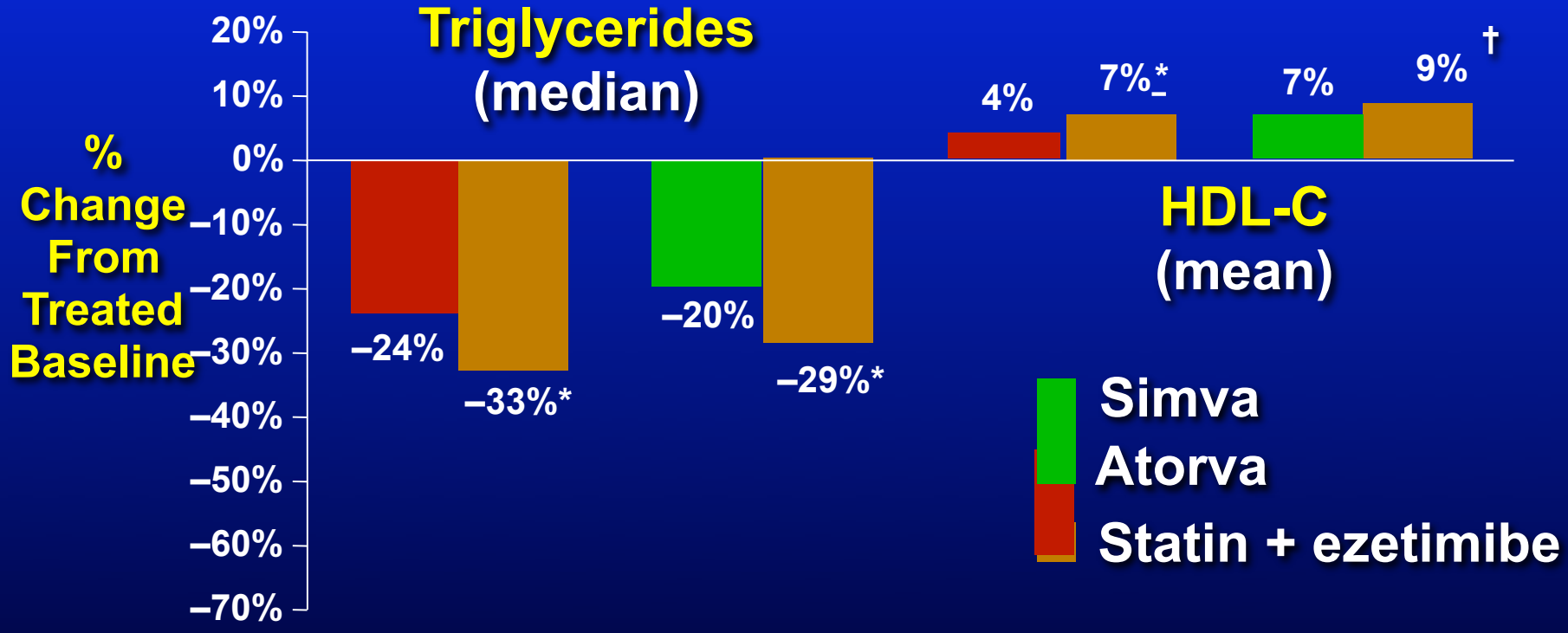
Bays, HD. et al. Clin Ther 2001;23:1209-1230

Efficacy of Ezetimibe as



Pooled data.
*P≤0.01 vs placebo.

Ezetimibe with Statin
Triglycerides and HDL-C



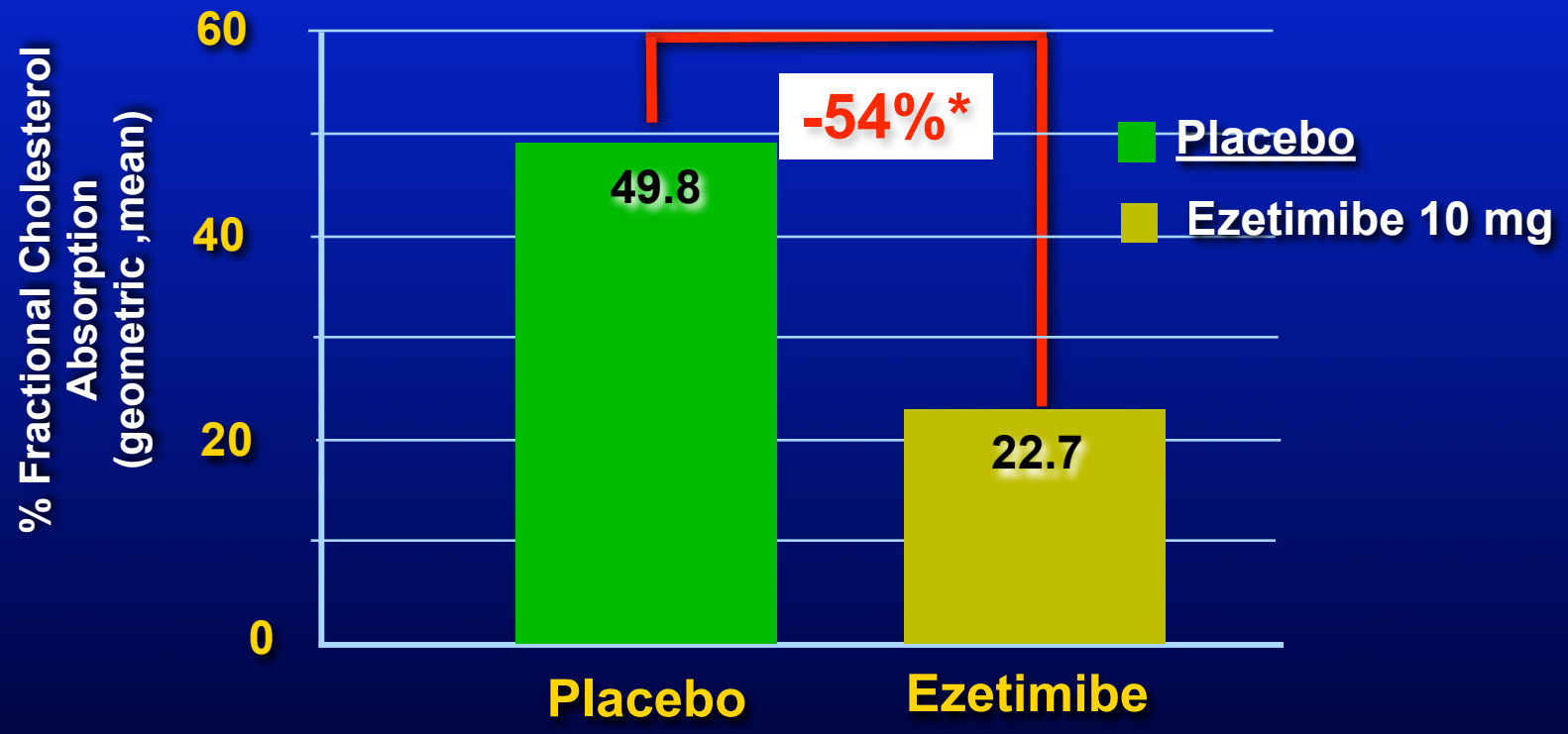
Ezetimibe **added** to Atorva or Simva

All data are pooled across doses.

*P<0.01 for ZETIA + statin vs statin alone.

†P<0.05 for ZETIA + statin vs statin alone.

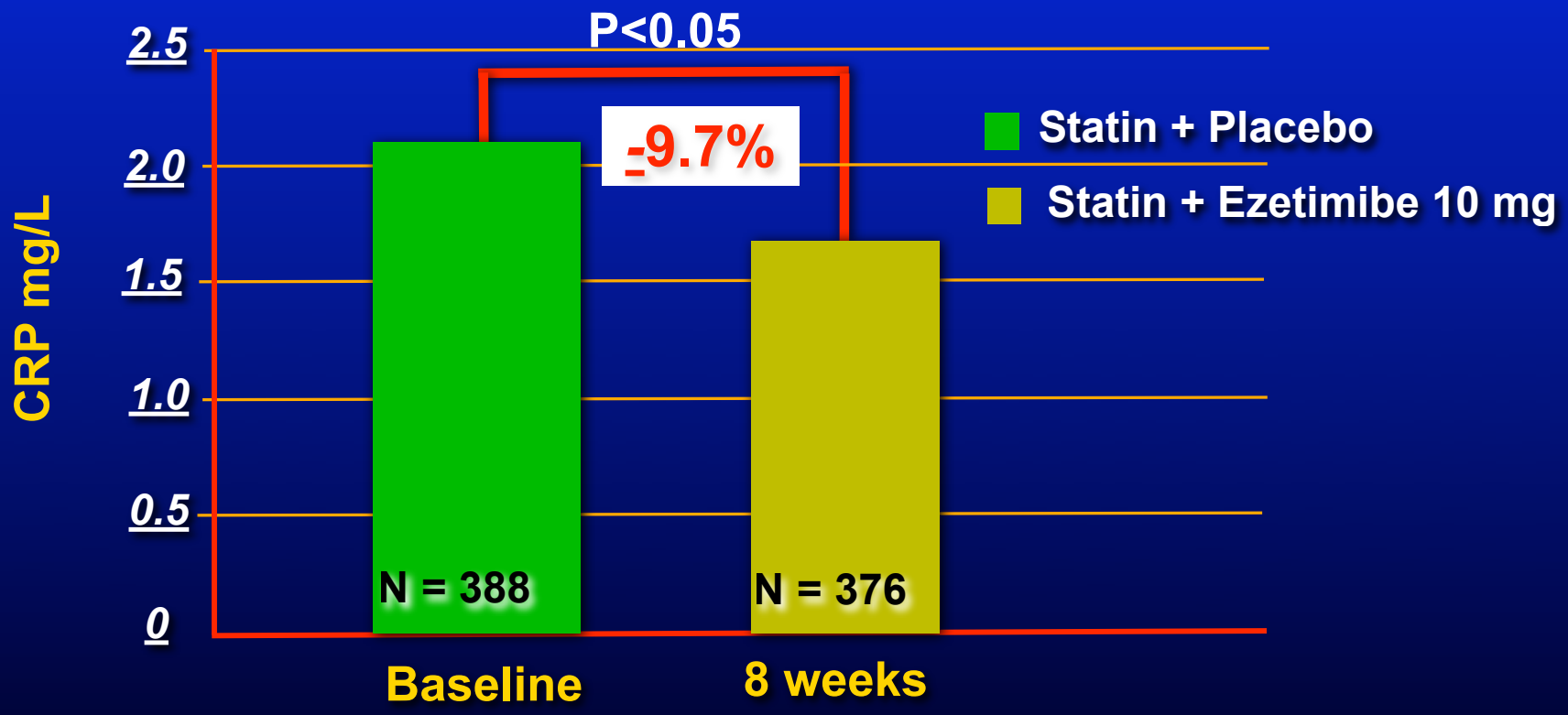
Reduction of Cholesterol Absorption in Humans by Ezetimibe



*P<0.001

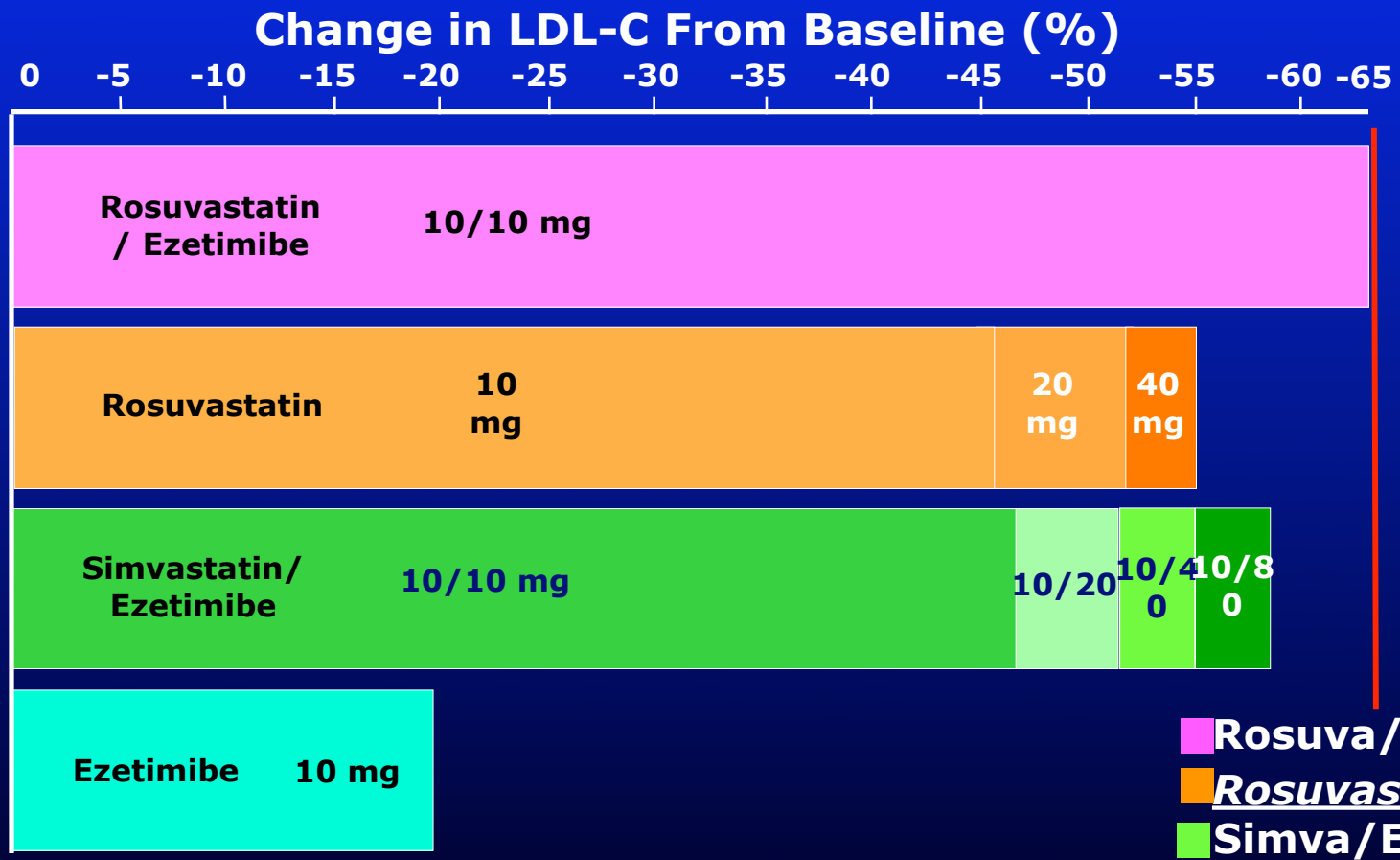
Sudhop et al. Circulation 2002;106:1943

Ezetimibe and C-Reactive protein



Gagne, C for Ezetimibe Study Group Am J Cardiol 2002;90:1084-1091

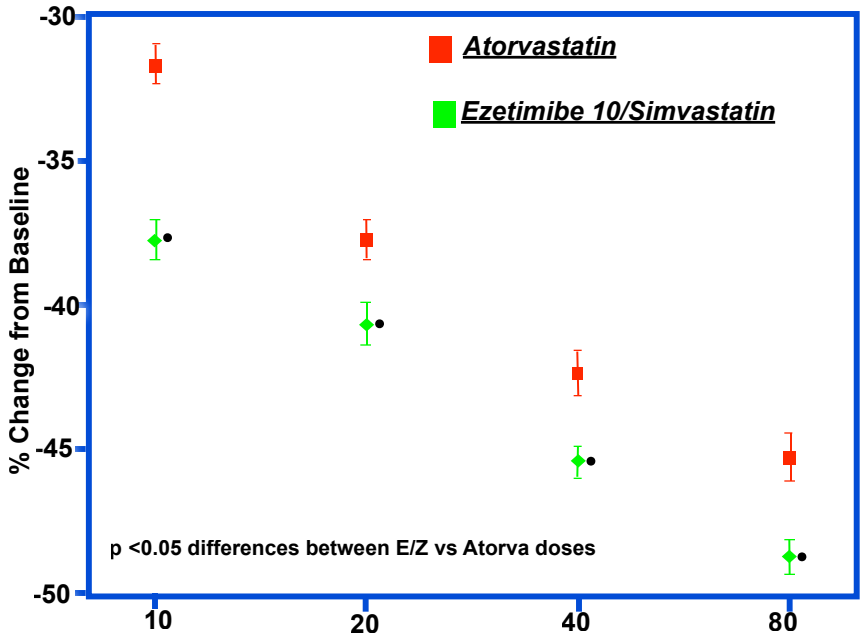
Crestor, Zetia, Vytorin



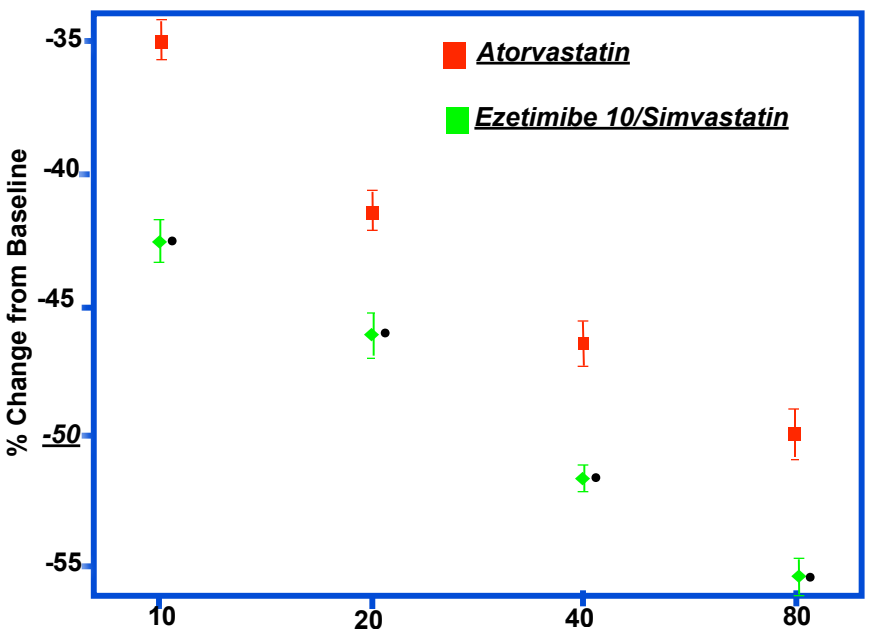
Not a Head to Head trial

Effect of Ezetimibe/Simvastatin Coadministration versus Atorvastatin

Apolipoprotein B



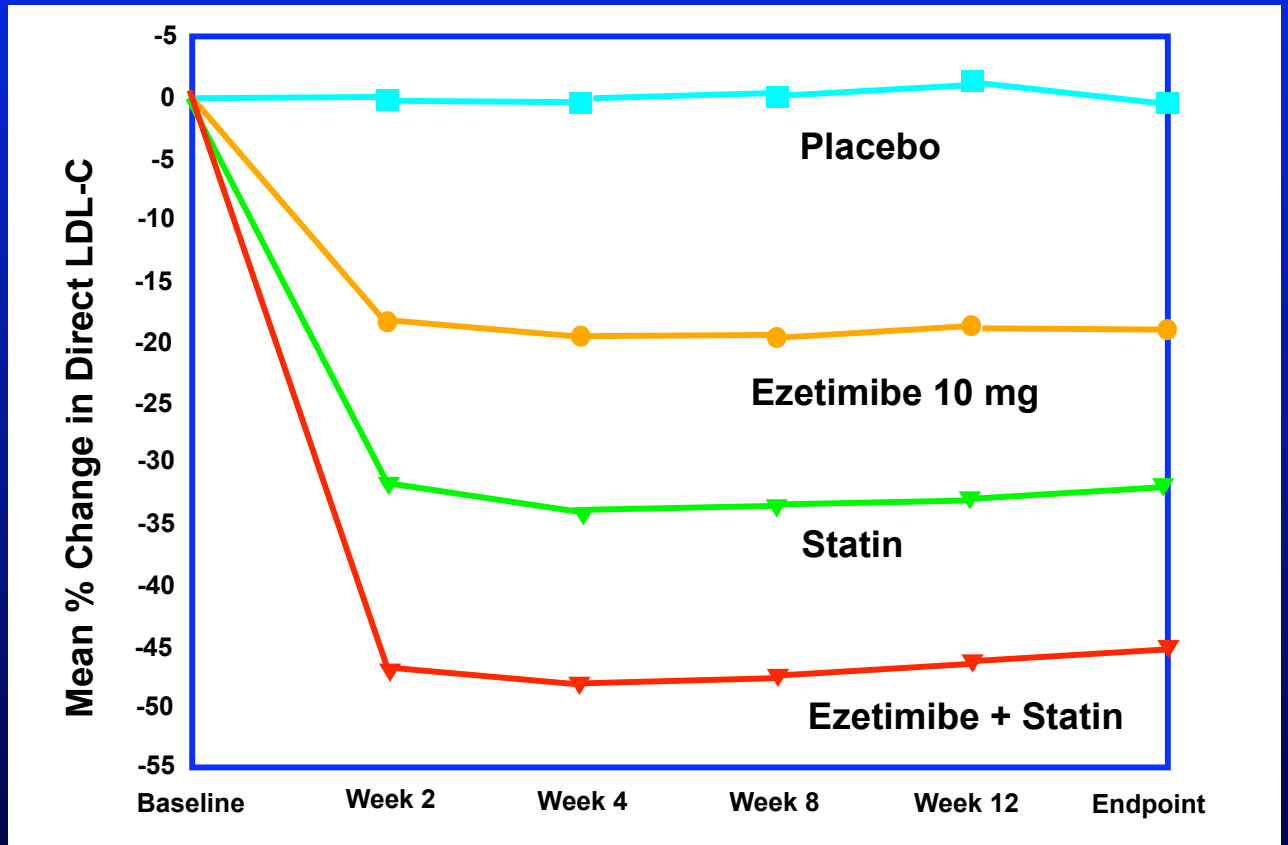
Non HDL cholesterol



Least squares adjusted mean + SE percent changes

Ballantyne C. et al Amer J Cardiol 2004;93:1487-94

Efficacy of Ezetimibe and Statins

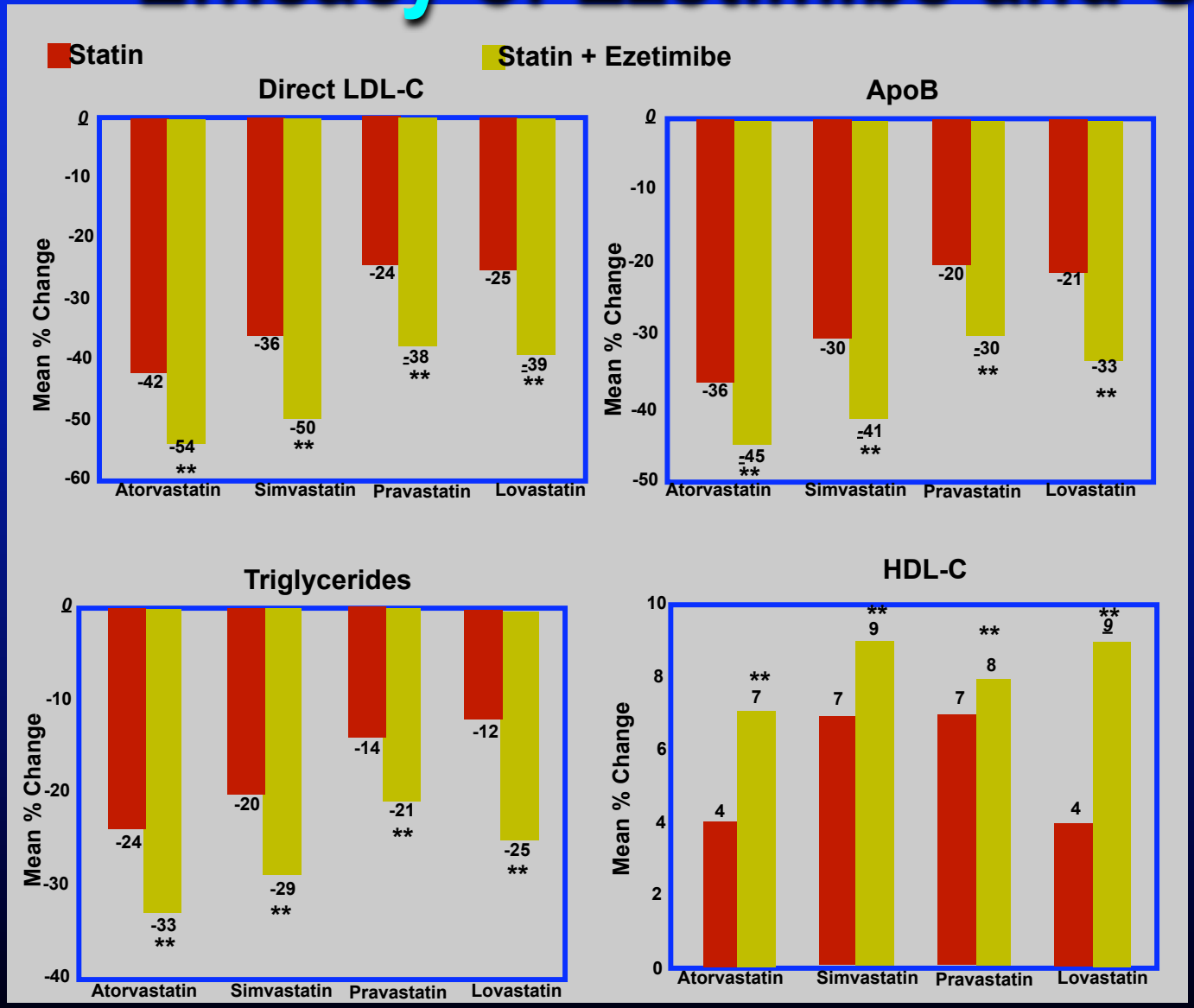


Mean % Change from baseline in direct LDL-C over time for four studies pooled

2382 patients with hyper-cholesterolemia from 4 similarly designed trails of ezetimibe and statins over 12 weeks

Davidson et al. Int J Clin Prac 2004;8:746-755

Efficacy of Ezetimibe and Statins



2382 patients with hyper-cholesterolemia from 4 similarly designed trails of ezetimibe and statins over 12 weeks

Entry Lipids

LDL-C 150-260

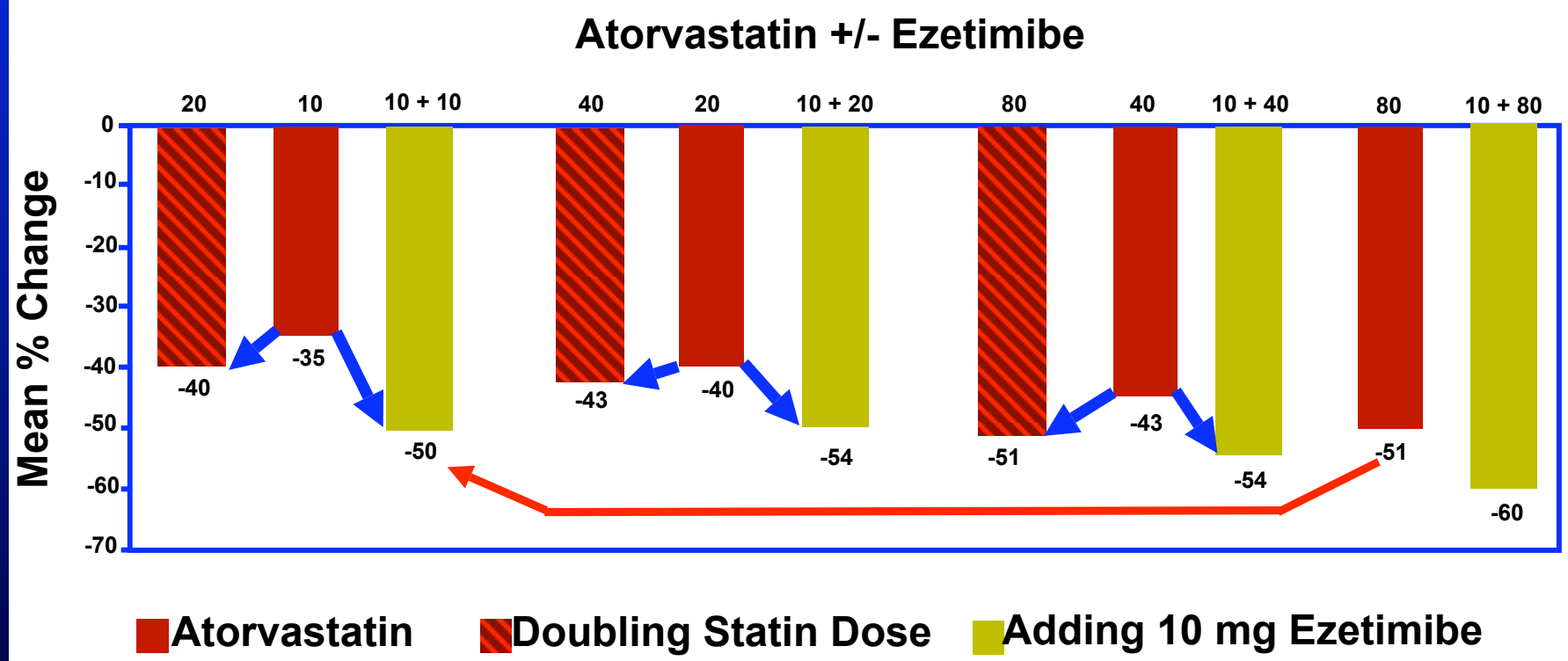
TG < 320

** p <0.01 vs statin alone

Davidson et al. Int J Clin Prac 2004;8:746-755

Efficacy of Ezetimibe and Statins

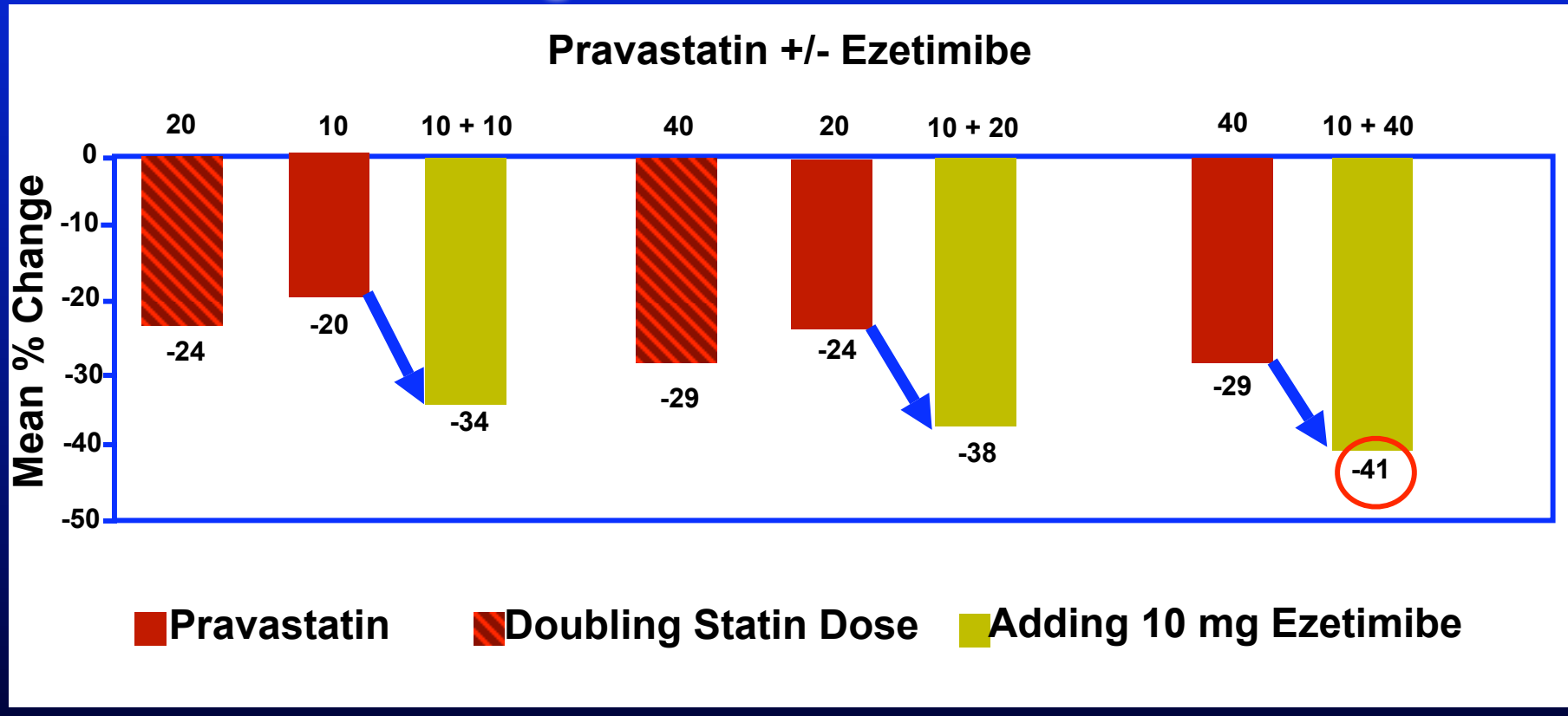
Percent Change from Baseline in Direct LDL-C



Davidson et al. Int J Clin Prac 2004;8:746-755

Efficacy of Ezetimibe and Statins

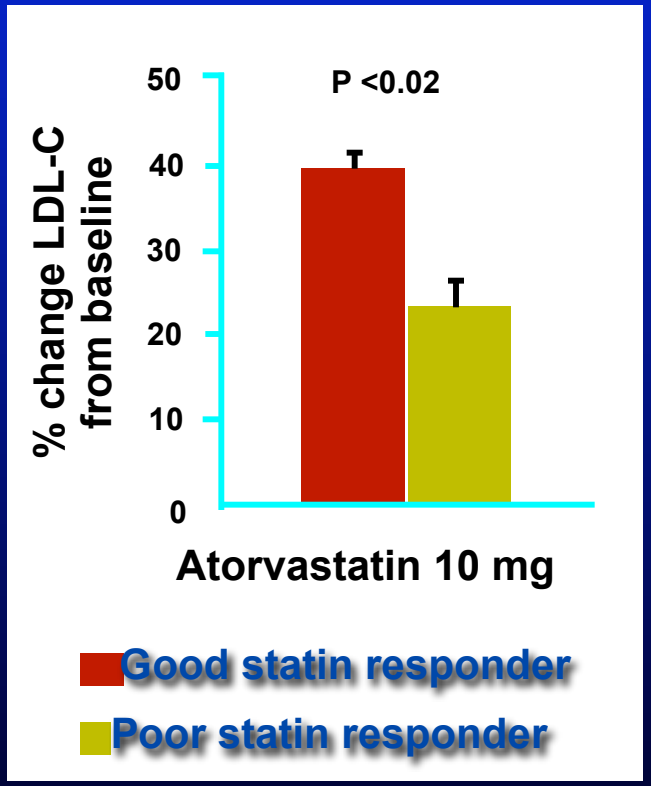
Percent Change from Baseline in Direct LDL-C



Davidson et al. Int J Clin Prac 2004;8:746-755

Variable Response to Statin Therapy in Familial Hypercholesterolemia

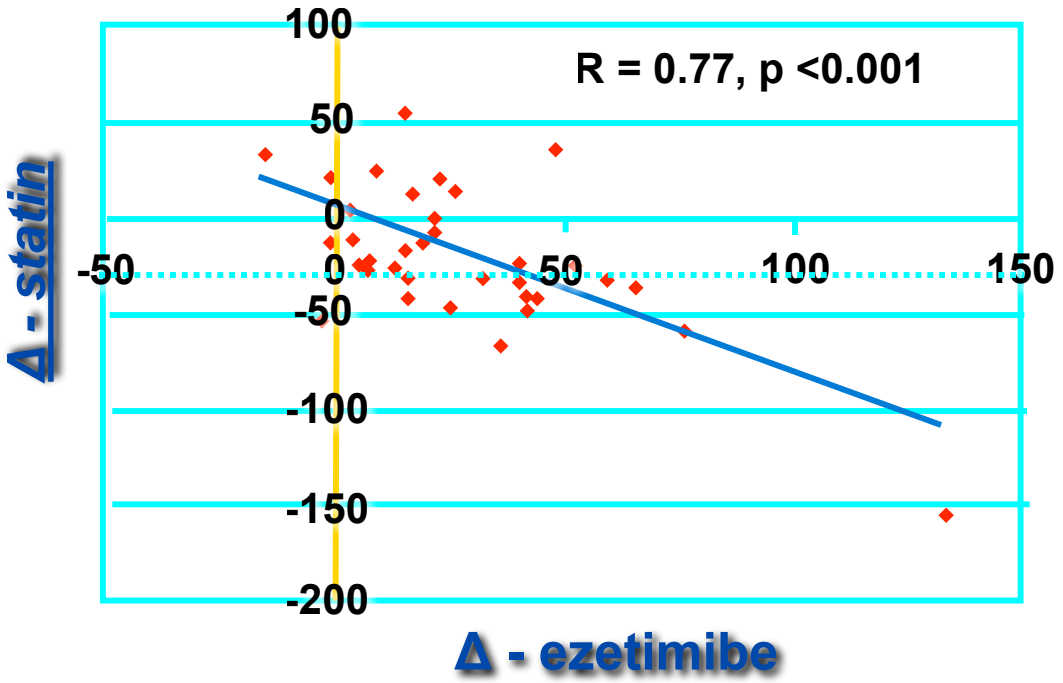
- Higher cholesterol absorption efficiency is equated with higher uptake of chylomicron remnant cholesterol by the liver
- Cholesterol hyper-absorbers had decreased levels of mevalonic acid (indicating decreased synthesis)
- In poor responders to statin therapy, a genetically determined increase in cholesterol absorption downregulates HMG CoA reductase and renders the enzyme refractory to **pharmacological inhibition**
- The E4 allele (associated with hyperabsorption) was significantly higher in the poor statin responders (75%)



O'Neill FH et al. ATVB 2001;21:832-837

LDL-C Response to Statin Predicts Ezetimibe Efficacy

Regression plot of Δ-ezetimibe (x-axis) versus Δ–statin (y axis)



Δ-ezetimibe (x-axis) versus Δ–statin (y axis) is the difference between predicted and observed changes in LDL-C

The statin **hypo-responders** are **hyper-responders** to ezetimibe

This may identify a patient population who would be **particularly responsive** to ezetimibe

Ziajka P et al. Amer J Card 2004;93:779-780

LDL-C Response to Statin Predicts Ezetimibe Efficacy

- The negative slope of the regression line demonstrates that hypo-responders to statins are hyper-responders to ezetimibe
- He variability of LDL-C response to ezetimibe was 6 to 60% (average 29%)
- 8 of 37 patients experienced a greater than 40% LDL-C reduction with ezetimibe
  - In these patients the response to the statin was < 60% of the predicted values

Ziajka P et al. Amer J Card 2004;93:779-780

The VYtorin Vs Atorvastatin (VYVA) Study

- Methods This multicenter, double-blind, 6-week parallel-group study randomized 1902 patients with LDL-C above ATP III goal to atorvastatin (10, 20, 40, or 80 mg) or to ezetimibe/simvastatin (10/10, 10/20, 10/40, or 10/80 mg). Patients were stratified by prerandomization LDL-C level.
- Results At each milligram-equivalent statin dose comparison, and averaged across doses, ezetimibe/simvastatin provided greater LDL-C reductions (47%-59%) than atorvastatin (36%-53%).

Ballantyne et al. Am Heart J. 2005;149:464-473

The VYtorin Vs Atorvastatin (VYVA) Study

- Ezetimibe/simvastatin 10/40 and 10/80 mg also provided significantly greater high-density lipoprotein cholesterol (HDL-C) increases than atorvastatin 40 and 80 mg.
- Triglyceride reductions were similar for all comparisons.
- More ezetimibe/simvastatin than atorvastatin patients with coronary heart disease (CHD) or CHD risk equivalents attained the ATP III LDL-C goal of b100 mg/dL and the optional LDL-C target of 70 mg/dL.
- C-reactive protein reductions were similar between treatment groups.
- Consecutive elevations in alanine aminotransferase and/or aspartate aminotransferase occurred in significantly more atorvastatin patients than ezetimibe/ simvastatin patients.
- No myopathy or liver-related adverse events led to study discontinuation with either drug.

Ballantyne et al. Am Heart J. 2005;149:464-473

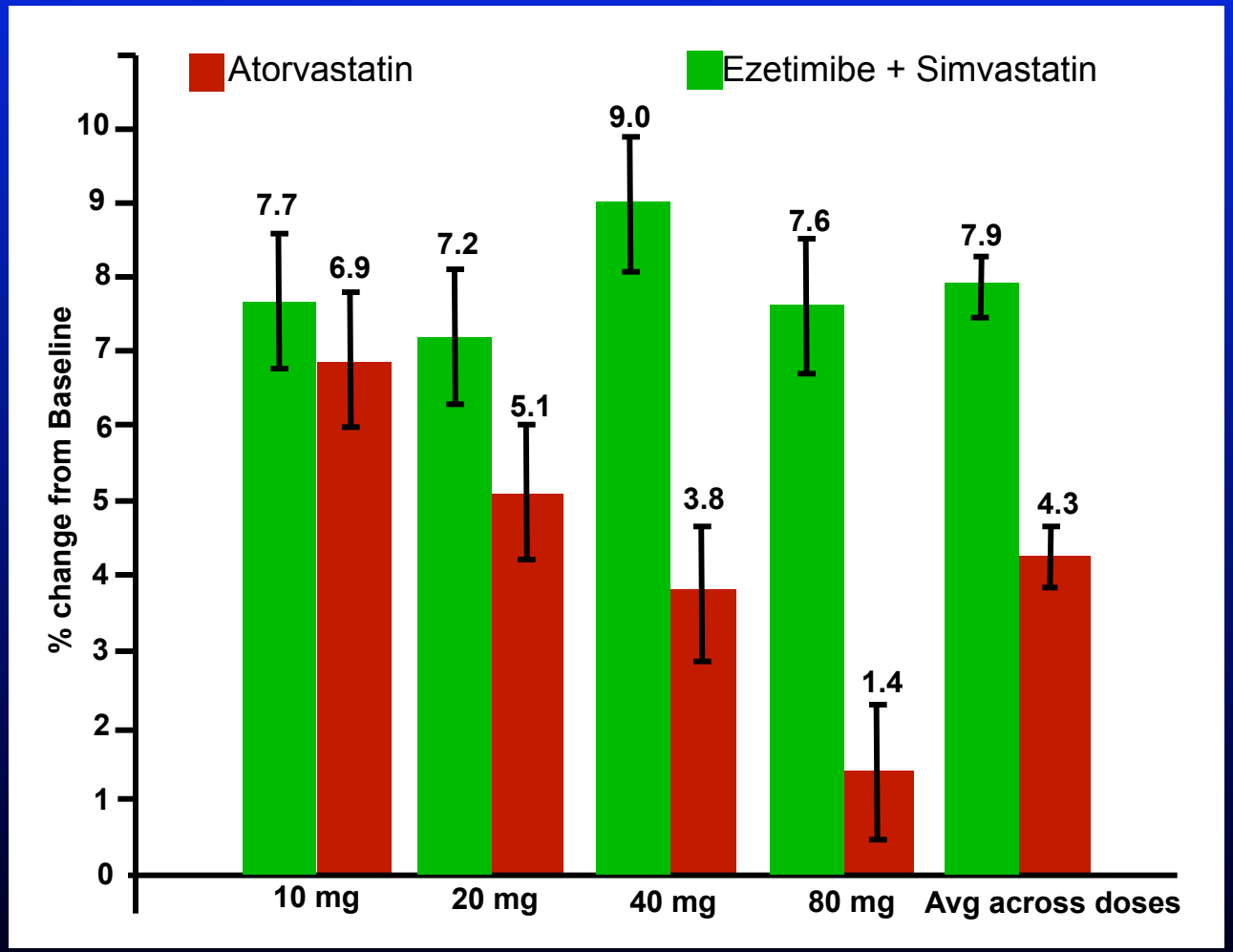
The VYtorin Vs Atorvastatin (VYVA) Study

Effect on LDL-cholesterol

Ballantyne et al. Am Heart J. 2005;149:464-473

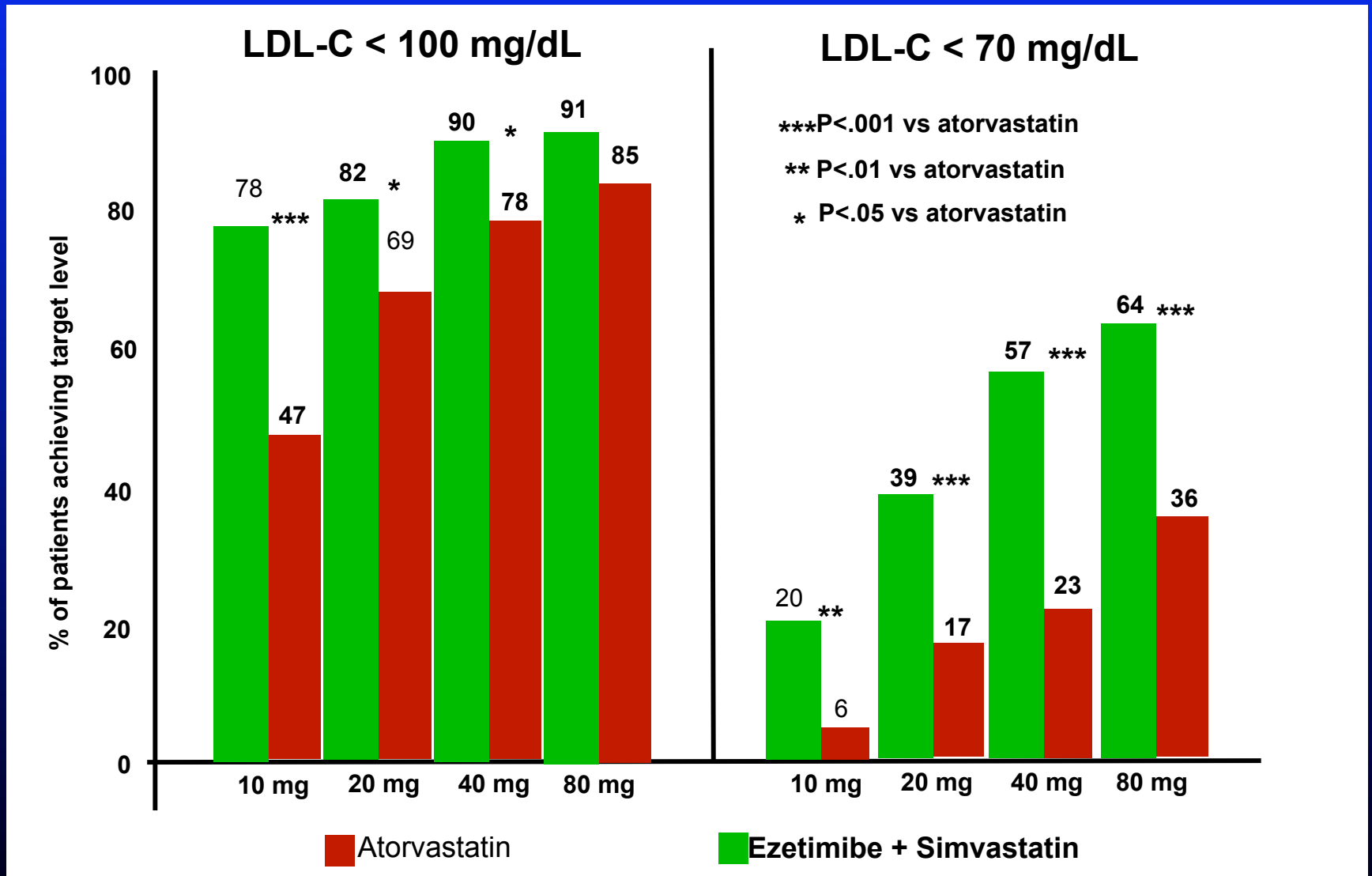
The VYtorin Vs Atorvastatin (VYVA) Study

Effect on HDL-cholesterol



Ballantyne et al. Am Heart J. 2005;149:464-473

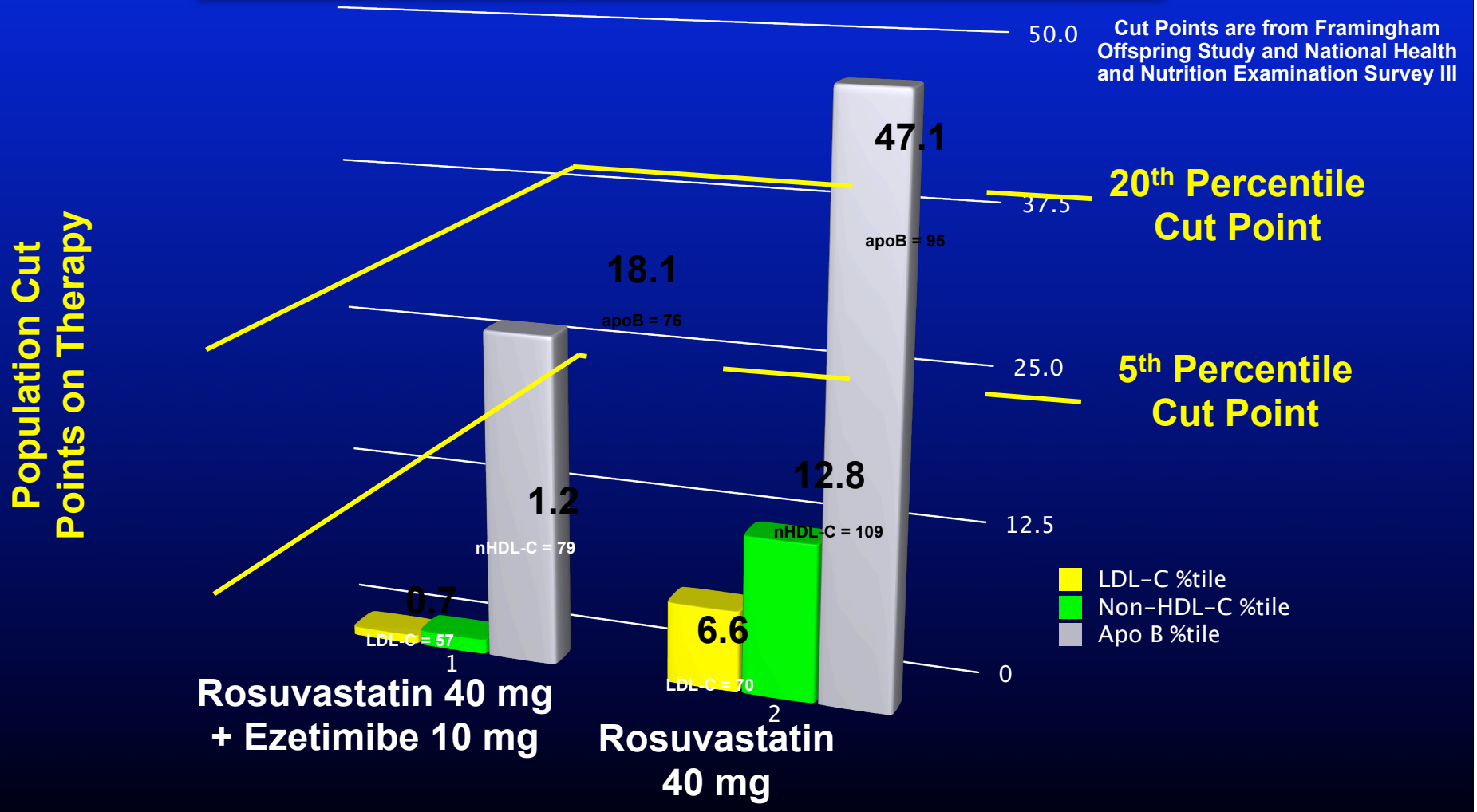
The VYtorin Vs Atorvastatin (VYVA) Study



Ballantyne et al. Am Heart J. 2005;149:464-473

Comparing LDL-C, Non-HDL-C & ApoB in the Examination of Potential Lipid-modifying effects Of Rosuvastatin in Combination with Ezetimibe versus Rosuvastatin Alone (EXPLORER) Trial

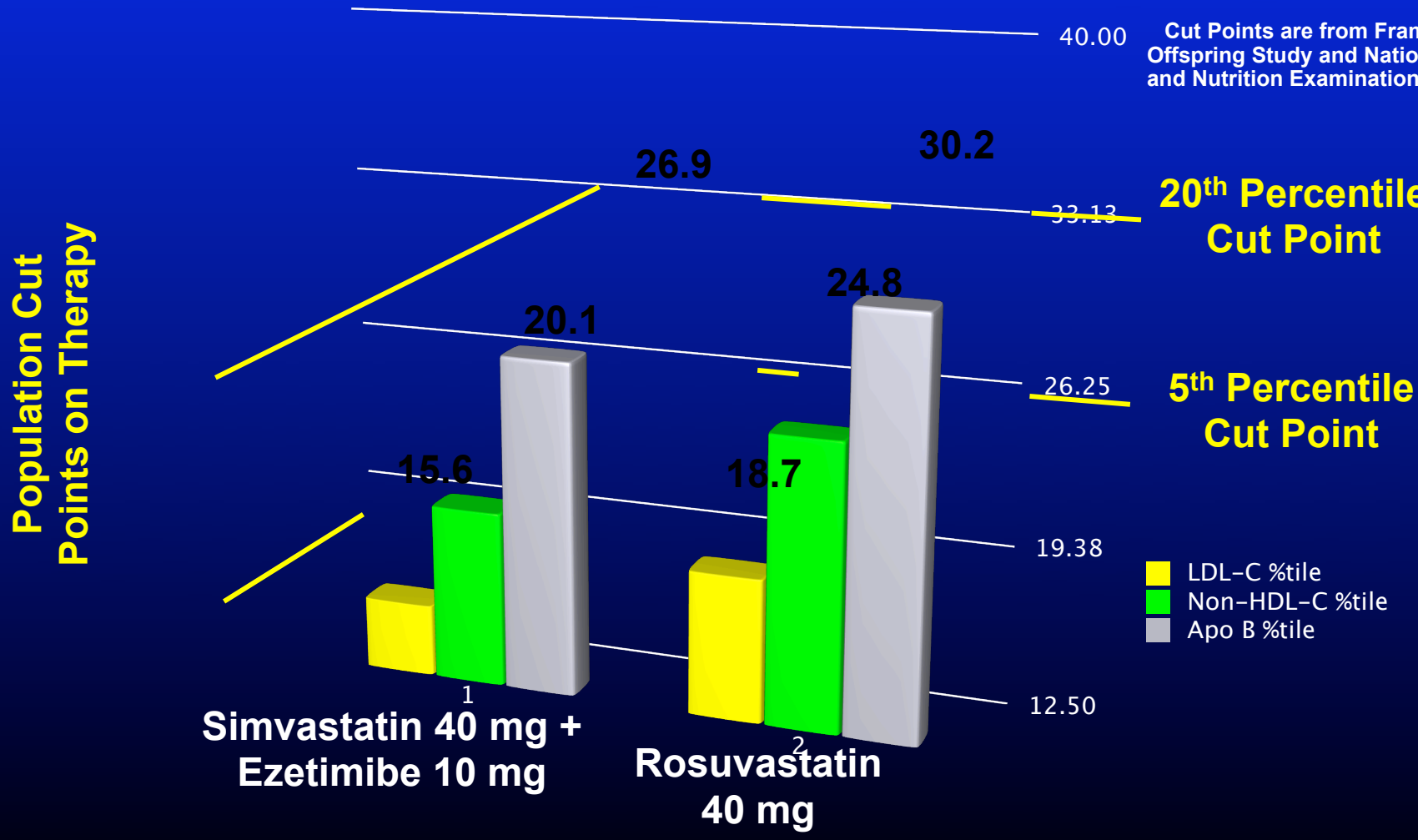
Achieving the 20th Percentile Population Cut Point



Sniderman, AD Journal of Clinical Lipidology (2008) 2, 36–42

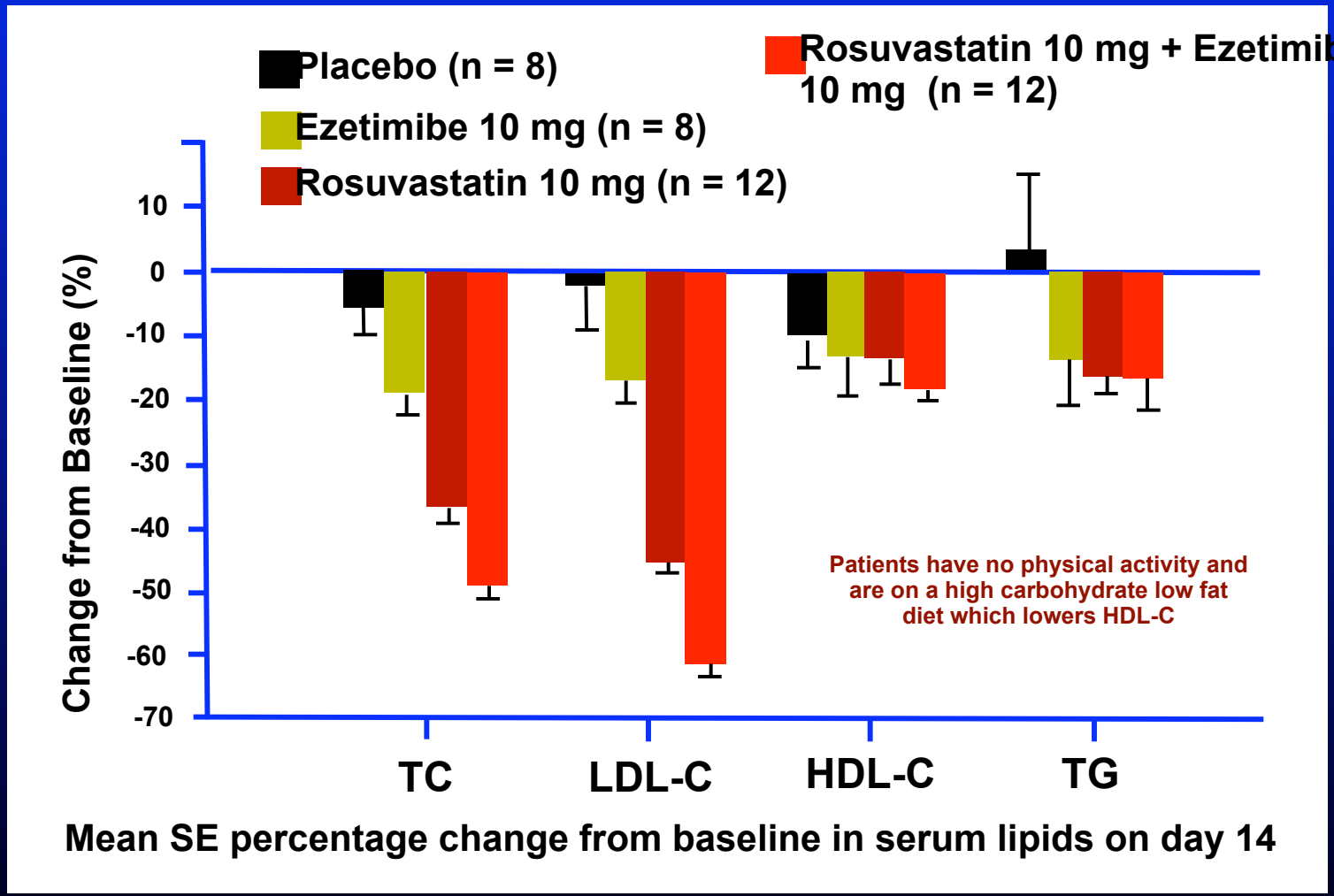
COMParative Effects on Lipid Levels of Niaspan and a Statin vs other Lipid-Modifying Therapies (COMPELL)

Achieving the 20th Percentile Population Cut Point



Sniderman, AD Journal of Clinical Lipidology (2008) 2, 36–42

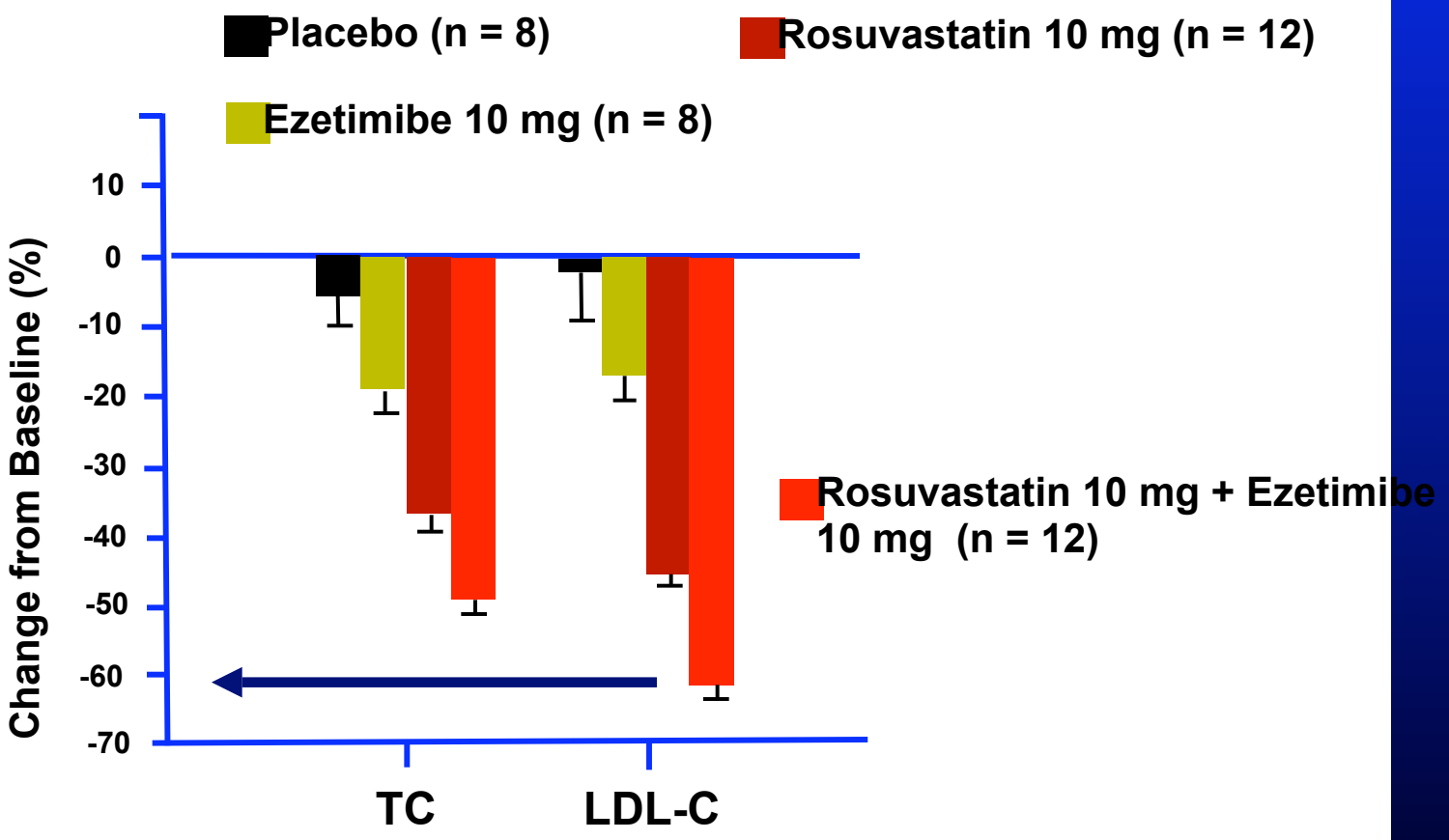
Ezetimibe – Rosuvastatin Study



Mean SE percentage change from baseline in serum lipids on day 14

Kosoglou T et al. Curr Med Res & Opin 2004;1185-1195

Ezetimibe – Rosuvastatin Study



Mean SE percentage change from baseline in serum lipids on day 14

Kosoglou T et al. Curr Med Res & Opin 2004;1185-1195

Ezetimibe – Niacin Study

- Retrospective review of medical records of 53 patients in 2 lipid clinics most of whom (81%) had ASHD, who received ezetimibe as add-on therapy to stable doses of niacin and other lipid medications. Mean percentage changes of lipoprotein cholesterol and triglyceride levels were determined.
- The niacin formulation was extended-release in 31 patients (58%), immediate-release in 17 (32%), and slow-release in 5 (9%). Most patients (75%) were also taking a statin.
- Add-on ezetimibe therapy yielded mean reductions of 18% for total cholesterol (P<0.001), 25% for low-density lipoprotein (LDL) cholesterol (P<0.001), and 17% for triglycerides (P<0.001). High-density lipoprotein (HDL) cholesterol did not change significantly (+2%).
- Only 7 patients (13%) met Adult Treatment Panel III (ATP III) LDL cholesterol goals before the addition of ezetimibe, but 24 (45%; P<0.001 compared with baseline) attained these goals after addition of ezetimibe to the therapeutic regimen.
- Ezetimibe effectiveness did not correlate with the baseline dose of niacin or the dose/efficacy of the statin used. The addition of ezetimibe to niacin-based therapy for dyslipidemia was well tolerated.

Jelesof NE et al. Endocrine Practice 2006;12:159-164

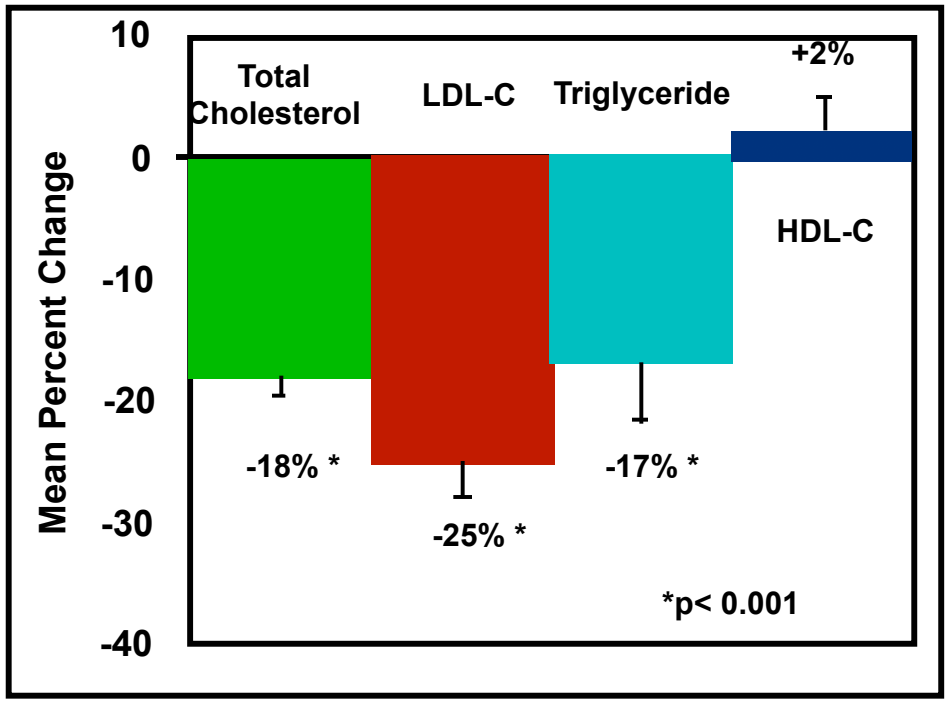
Ezetimibe – Niacin Study

The addition of ezetimibe to niacin-based regimens lowered the LDL cholesterol level by 25% and did not change the level of HDL cholesterol.

This combination can be useful in multidrug regimens for high-risk patients with dyslipidemia who are not achieving ATP III treatment goals.

Jelesof NE, Ballantyne C et al. Endocrine Practice 2006;12:159-164

Ezetimibe – Niacin Study



Mean percentage change from baseline for lipoprotein variables in 53 patients after the addition of 10 mg of Ezetimibe daily to stable dose medication regimens incorporating niacin.

Jelesof NE, Ballantyne C et al. Endocrine Practice 2006;12:159-164

The DIACOR Study Triple Therapy with a Statin, Fibrate and Ezetimibe

- Methods: 37 T2DM patients (35% female), mixed dyslipidemia with no CVD. (~age 59)
- After 12 weeks of fenofibrate 160 mg, simvastatin 20m mg or their combination, patients with an LDL-C > 100 mg/dL or TG > 150 mg/dL **were randomized to simva/feno plus placebo or ezetimibe 10 mg**.
- Followed for 6 weeks

Pearson RR et al. JACC 2006;47(Suppl) 316A Abstract 808-6

The DIACOR Study Triple Therapy with a Statin, Fibrate and Ezetimibe

- For combo + Ezetimibe
  - 23.5% vs 0% (placebo) met all 3 NCEP goals
- The likelihood of meeting all three goals was significantly increased in the combo + ezetimibe group (p=0.006)
  - There was an incremental reduction in TC (16%), LDL-C (25.2%) and VLDL-C (14%)
- No serious adversity seen

Pearson RR et al. JACC 2006;47(Suppl) 316A Abstract 808-6

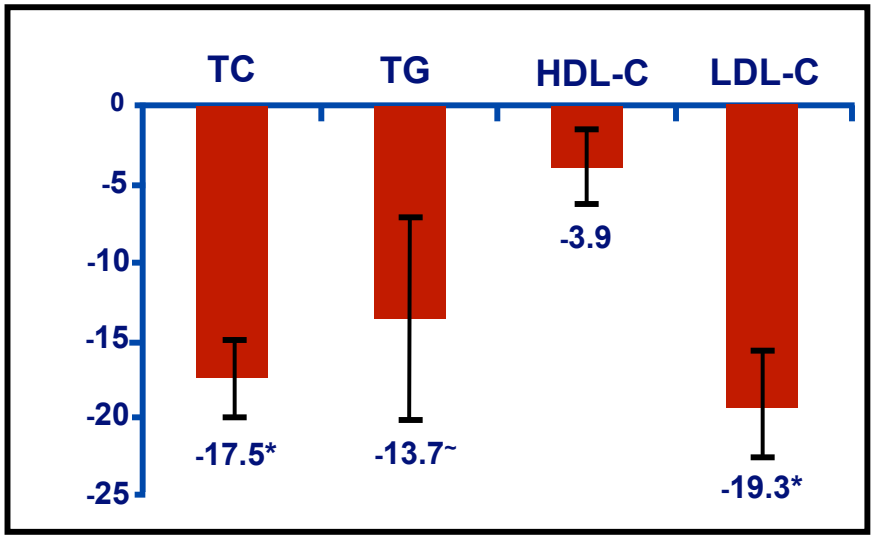
Bile Acid Resin – Ezetimibe Combination Therapy

Lipids and Transaminase Levels Before and After Addition of Ezetimibe to a BAR Regimen

Variable	Baseline on BAS	After Ezetimibe	P Value *
Total Cholesterol	259 + 44	212 + 44	<0.001
Triglycerides	162 + 86	128 + 57	0.001
LDL Cholesterol	174 + 39	138 + 42	<0.001
HDL Cholesterol	53 + 18	50 + 13	0.01
AST	30 + 12	31 + 12	NS
ALT	32 + 14	34 + 14	NS

Data are means + SDs in mg/dL for lipids and units/L for LFTs

*p Value by paired t test



Mean reduction after 107 days, %

*p<0.001 ~p=0.03

Xydakis AM et al. Am J Cardiol 2004;94:795-797