Nuevos horizontes en la elevación del C-HDL y Riesgo Cardiovascular:

Colesterol HDL y riesgo cardiovascular
¿Dónde estamos?

Lina Badimon
Barcelona Cardiovascular Research Center,
CSIC-ICCC, IIB-Sant Pau - Hospital Sant Pau, UAB Barcelona, Spain

SEC-2011
Colesterol HDL y riesgo cardiovascular
¿Dónde estamos?

1. IMPACTO DE LAS LDL

2. ESTATINAS Y UMBRAL DE BENEFICIO

3. IMPACTO DE LAS HDL

4. TRANSPORTE REVERSO DE COLESTEROL

5. HDL: CANTIDAD Y/O CALIDAD

6. TRATAMIENTO
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Atherothrombosis: A Generalized and Progressive Process

- Unstable angina
- MI
- Ischemic stroke/TIA
- Critical leg ischemia
- Intermitent claudication
- CV death

Atherosclerosis:

- Endothelial dysfunction
- Thrombosis
- LDL-CHOLESTEROL
- HDL-CHOLESTEROL
- Atherosclerosis
- Stable angina/Intermitent claudication

ACS
- Unstable angina
- MI
- Ischemic stroke/TIA
- Critical leg ischemia
- Intermitent claudication
- CV death
Major Cardiovascular Risk Factors

**Modifiable Risk Factors**
- Lipid deposition
- Inflammation
- Shear Rate
- Diet
- Smoking
- Exercise
- Alcohol

**Non-Modifiable Risk Factors**
- Age (menopause)
- Gender
- Genes/Family History

**Metabolic Syndrome**
- Hypertension
- Diabetes mellitus
- Obesity
  - (Small LDL, PAI-1, Microalbuminuria)

**Lipids**
- ↑LDL
- ↓HDL
- ↑TGL
- ↑HDL

**Other Factors**
- Metabolic Syndrome (Small LDL, PAI-1, Microalbuminuria)
- Non-Modifiable Risk factors
LDL-C Levels versus Events in Landmark Statin Trials

% with CHD event

LDL-C, mmol/L (mg/dL)

S = statin treated
P = placebo treated

*Extrapolated to 5 years
LDL-C and change in percent atheroma volume (IVUS)†

- ASTEROID and REVERSAL investigated active statin treatment; A-PLUS, ACTIVATE AND CAMELOT investigated non-statin therapies but included placebo arms who received background statin therapy (62%, 80% and 84% respectively).

*Median change in PAV from ASTEROID and REVERSAL; LS mean change in PAV from A-PLUS, ACTIVATE AND CAMELOT

### INTER-HEART Study: Risk Factors for MI

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio adjusted for all other risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO-B/APO-A1 (Quintile 5 vs 1)</td>
<td>3.25 (2.81 - 3.76)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>2.87 (2.58 - 3.19)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.37 (2.07 – 2.71)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.91 (1.74 – 2.10)</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>1.62 (1.45 – 1.80)</td>
</tr>
<tr>
<td>Psychosocial stress</td>
<td>2.67 (2.21 – 3.22)</td>
</tr>
<tr>
<td>Daily vegetables/fruit</td>
<td>0.70 (0.62 – 0.79)</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.86 (0.76 – 0.97)</td>
</tr>
<tr>
<td>Alcohol Usage</td>
<td>0.91 (0.82 – 1.02)</td>
</tr>
</tbody>
</table>

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## Limitations of Statin Monotherapy on CHD Events

<table>
<thead>
<tr>
<th>Trial</th>
<th>Drug</th>
<th>N</th>
<th>Control Group</th>
<th>Statin Group</th>
<th>Risk Reduction, %†</th>
<th>Events not Avoided, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4S WOSCOPS</td>
<td>Simvastatin, Pravastatin, Pravastatin</td>
<td>30,817</td>
<td>2,042</td>
<td>1,490</td>
<td>26</td>
<td>74</td>
</tr>
<tr>
<td>CARE</td>
<td>Pravastatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFCAPS</td>
<td>Lovastatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIPID</td>
<td>Pravastatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPS</td>
<td>Simvastatin</td>
<td>20,586</td>
<td>1,212</td>
<td>898</td>
<td>26</td>
<td>74</td>
</tr>
<tr>
<td>PROSPER</td>
<td>Pravastatin</td>
<td>5,804</td>
<td>356</td>
<td>292</td>
<td>19</td>
<td>81</td>
</tr>
<tr>
<td>ASCOT-LLA</td>
<td>Atorvastatin</td>
<td>10,305</td>
<td>154</td>
<td>100</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>67,462</td>
<td>3,764</td>
<td>2,780</td>
<td>27</td>
<td>73</td>
</tr>
</tbody>
</table>

* Nonfatal MI and CHD death; AFCAPS also included unstable angina
† Weighted average

RESIDUAL RISK after LDL lowering
<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Controls (n = 601)</th>
<th>Cases (n = 321)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoking</td>
<td>29%</td>
<td>67%*</td>
</tr>
<tr>
<td>HDL-C &lt; 35 mg/dL</td>
<td>19%</td>
<td>57%*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21%</td>
<td>41%*</td>
</tr>
<tr>
<td>LDL-C $\geq$ 160 mg/dL</td>
<td>26%</td>
<td>34%*</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1%</td>
<td>12%*</td>
</tr>
</tbody>
</table>

*Significantly different from controls ($P < 0.001$)
“On-treatment” HDL-C Predicts Cardiovascular Events: TNT

Major Cardiovascular Events

On treatment HDL-C (mg/dL)
- <40
- >40-50
- >50-60
- >60

Atorva 10
Mean LDL-C 99 mg/dL

Atorva 80
Mean LDL-C 73 mg/dL

CV events and HDL despite statins

Residual high risk for CV events despite statin therapy among patients with low HDL-C levels

HPS Collaborative Group, Lancet 2002; 360: 7
Sacks et al, Circulation 2000; 102: 1893

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Low HDL-C is a Risk Factor for CHD Even When LDL-C Levels are Well Controlled

Low HDL, even in statin-treated patients, associated with increased CVD risk

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Statin-treated patients, median events (95% CI)</th>
<th>Control participants, median events (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI*</td>
<td>7.1 (6.8–7.3)</td>
<td>8.3 (8.1–8.5)</td>
<td>0.57</td>
</tr>
<tr>
<td>Cardiovascular disease death</td>
<td>4.1 (3.5–4.5)</td>
<td>5.4 (4.8–5.8)</td>
<td>0.21</td>
</tr>
<tr>
<td>Coronary heart disease death</td>
<td>2.6 (2.5–2.7)</td>
<td>2.3 (2.2–2.4)</td>
<td>0.31</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>9.1 (8.0–10.1)</td>
<td>9.5 (8.5–10.5)</td>
<td>0.36</td>
</tr>
<tr>
<td>All-cause death</td>
<td>2.9 (2.2–3.4)</td>
<td>2.6 (1.7–3.1)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*After adjustment for LDL-cholesterol levels and age, a 10-mg/dL decrease in HDL-cholesterol levels was associated with 7.1 more MIs per 1000 patient-years in statin-treated patients and 8.3 MIs per 1000 patient-years among healthy controls.

54.6% of CAD hospitalizations have low HDL levels (<40mg/dl) independently of LDL levels

(Fonarow ACC 2007)
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REVERSE CHOLESTEROL TRANSPORT (RCT)

- MULTISTEP PROCESS RESULTING IN THE NET MOVEMENT OF CHOLESTEROL FROM THE PERIPHERAL TISSUES BACK TO THE LIVER

- CHOLESTEROL FROM NON-HEPATIC PERIPHERAL TISSUES IS TRANSFERRED TO **HDL** BY THE **ABCA1** (ATP-binding cassette transporter) - - **APO A-1** ACTS AS AN ACCEPTOR, AND THE PHOSPHOLIPIDS OF **HDL** ACT AS A RESERVOIR FOR THE MOBILIZED CHOLESTEROL

- CHOLESTEROL IS CONVERTED IN **CE** BY THE ENZYME **LCAT**

- **CE** CAN BE TRANSFERRED TO OTHER LIPOPROTEINS (such as **LDL**) AND THESE LIPOPROTEINS CAN BE TAKEN UP BY THE LIVER VIA THE **LDLR**
### HDL - Experimental Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>PLACEBO</th>
<th>TREATED</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression¹</td>
<td>—</td>
<td>38 ± 6</td>
<td>15 ± 2</td>
<td>(p&lt; 0.001)</td>
</tr>
<tr>
<td>Regression²</td>
<td>34 ± 4</td>
<td>39 ± 5</td>
<td>18 ± 4</td>
<td>(p&lt; 0.001)</td>
</tr>
</tbody>
</table>

(*) = Cholesterol diet (0.5%) — Sudan IV positive area
- Homologous HDL-VHDL preps

1 = Placebo 8 weeks (*) — Treated 8 weeks (*), HDL-VHDL 50 mgs/ once a week
   Badimon JJ, Badimon L, Fuster V. Lab Inv 60: 455, 1989
2 = Control: 8 weeks (*) — Placebo and treated: 8 and 4 weeks

These observations have been later supported by several transgenic models
Apo A-1

• The major protein component of HDL
• Chylomicrons secreted from the intestinal enterocytes contain ApoA1 which is transferred to HDL in the bloodstream

FUNCTIONS
• Interacts with cellular SR-B1 for bidirectional cholesterol fluxes
• Source of CE for triglycerides
• Interact with ABCG-1 to accept free cholesterol
• Accepts free cholesterol and phospholipids from cellular ABCA-1
• Promotes cholesterol flux to the liver for excretion
• Acts as cofactor for LCAT which is responsible for the formation of most of the plasma cholesteryl-esters
MULTIPLE BIOLOGICAL ACTIONS OF HDL ON THE VASCULAR WALL

Gonzalez-Diez M, Badimon L, Martinez-Gonzalez J. J Thromb Haemost. 2008
Vinals M, Badimon L. Arterioscler Thromb Vasc Biol 1997; 17:3481-3488
Pomerantz KB, Summers B, Hajjar D. P. Biochemistry. 1993;32:13624–13635
HDL-induced PGI2 in human smooth muscle cells
Serum from individuals on diets rich in the different fatty-acids
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HDL FUNCTIONALITY

• Low HDL-C is an independent risk factor for CAD in men and women

• HDL particles have a complex structure that has diverse protective and pro-inflammatory functions

• Diverse studies continue to investigate the role of HDL quantity and quality on cardiovascular risk reduction
HDL: Cantidad vs. Calidad

Hígado

HDL “nascent”

- características
- composición lipídica
- actividad

HDL 2 and HDL 3

Transporte reverso colesterol
- Anti-trombótica
- Anti-oxidante
- Anti-inflamatoria
- Cito-protectora

HDL3

- ↓ diámetro
- ↑ densidad
- ↓ colesterol
- ↓ fosfolípidos

HDL2

- ↑ diámetro
- ↓ densidad
- ↑ colesterol
- ↑ fosfolípidos

Cubedo J, Padro T, Badimon L. 2011
Caracterización proteoma HDL

HDL fraction

- Dialysis (24h)
- Characterization by Electrophoresis Gel
- Protein Quantification by BCA Kit

2D Electrophoresis

IEF
2D-PAGE

Fluorescent staining

Typhoon 9400

Protein identification

Mass spectrometry
- LC/MS/MS
- MALDI-TOF

Plasma EDTA

Ultracentrifugation in Density Gradient

Protein
Peptides
Trypsin digest

120 kDa

pH 4 - 7

Cubedo J, Padro T, Badimon L. 2011
HDL: Quantity vs. Quality – (1-DE Analysis)

Differential Band Pattern By 1DE

Differential protein content in HDL

Cubedo J, Padro T, Badimon L. 2011
HDL: Quantity vs. Quality – (2-DE Analysis)

1. Alfa-1-antitripsin
2. Paraoxonase-1
3. Apolipoprotein L1
4. Apolipoprotein J
5. Apolipoprotein E
6. Apolipoprotein D
7. Apolipoprotein AIV
8. Transtirhetin

Differential protein contents

Antioxidants
Cytoprotectors
Antiinflammatory
HDL: Quantity vs. Quality – (WB validation)

TTR o Transtirhetin → Acute phase reactant protein
Transport of thyroid hormones
Transport of RBP4

PON1 o Paraoxonasa-1 → Antioxidant properties
Differential HDL patterns in patients with high LDL (hFH)

18 families analyzed (3 persons in each one)
212 spots validated
96 spots identified

+/- mutation (Mut)
+/- coronary artery disease (CAD)

1. Albumin
2. LCAT
3. Alpha-1-antitrypsin
4. PON-1
5. Apo A-IV
6. Apo L-1
7. Apo J
8. Apo A-I
9. Apo E
10. Apo D
11. Apo M
12. TTR
Differential Apo L1 pattern in hFH patients

Apo L1: Apoptosis-related protein

Chain of spots of 45 kDa (high MW)

Chain of spots of 38 kDa (low MW)

Truncated form

Mut CAD vs. Mut No CAD
Effect of the pathology

Mut-CAD and Mut-NoCAD vs. Control
Effect of the mutation

Apo L1: Apoptosis-related protein
Differential LCAT patterns in hFH patients

Six differential spots

Post transductional modifications:
4 N-glicosilation spots
2 O-glicosilation spots

Mut NoCAD

Mut CAD

Reduction in basic forms

Mut CAD

Mut NoCAD

Reduction in basic forms
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Trial evidence supporting the raise of HDL

**CORONARY DRUG PROJECT (NIACIN ↑ 25% HDL)**
**LIPID RESEARCH CLINICAL TRIAL (CHOLESTERYRAMINE ↑ 3% HDL)**
**HELSDINKY HEART TRIAL (GENFIBROZIL ↑ 10% HDL)**
**VETERANS HDL INTERVENTION TRIAL (GENFIBROZIL ↑ 6% HDL)**

**Imaging/Angiographic studies**

- FATS (nicotinic acid)
- HATS (nicotinic acid)
- REVERSAL (statin)
- ASTEROID (statin)
- Apo A-I Milano (Apo A-I)
- ERASE (rHDL)
Emerging Strategies to raise HDL

Apo A-I Milano
PPAR’s Agonists
  alpha - fibrates
Non-flushing Niacin
  (Niacin-Laropiprant)
CETP Inhibitors

Apo A-I mimetics
LXR/RXR activation
SR-B1 overexpression
ABC 1 gene overexpression
ARBITER 6

At Baseline
CHO 146 mg/dl    HDL 43 mg/dl    LDL 82 mg/dl    TGL 124 mg/dl
AIM - HIGH

Atherothrombosis Intervention in Metabolic Syndrome with Low HDL-C/High Triglycerides and Impact on Global Health Outcomes

• Randomized trial on niacin vs placebo in the background of simvastatin therapy in approximately 3300 patients with cardiovascular disease, low HDL and high triglycerides
  • AIM was stopped for FUTILITY with about 2/3 of the events already occurred
  • HDL levels were higher and triglycerides lower in the niacin group with the LDL levels very low and equal in the two groups
  • There were more strokes in the niacin group

LIMITATIONS IN TRIAL DESIGN
• Mechanisms of action can not be determined from clinical trials if the agent has multiple effects
• AIM-HIGH was inadequately powered (<3500 patients)
• AIM-HIGH was not targeted at patients who would benefit the most (those with high TGL and low HDL)
Reverse Cholesterol Transport and CETP

Brewer B, NEJM 2004;350:1491
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ANTI-ATHEROTHROMBOTIC ACTIVITIES OF HDL PARTICLE COMPONENTS

CARRIER OF ACTIVE MOLECULES

Antioxidant
Antiapoptotic
Antiinflammatory

PARTICLE SIZE-ASSOCIATED FUNCTIONALITY

Normalization of EC function
Antithrombotic effects
Fibrinolysis
PGI2
NO

REVERSE CHO TRANSPORT

Badimon et al 1990-2005
Escudero et al 2003