The functionality of HDL in atherosclerosis and beyond

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No conflict of interest to disclose
HDL has been an emerging target in the prevention and treatment of coronary heart disease
Classical view: Elevated HDL cholesterol levels correlate with reduced risk to develop CHD

• According to the classical view, increased HDL levels correlate with atheroprotection:
  • Men ≥ 40 mg/dl
  • Women ≥ 50 mg/dl


• *Arteriosclerosis.* 1988; 8:737-41
Niacin
CETP inhibitors
ApoA-I Milano
ApoA-I mimetic peptides
Inducers of apoA-I expression
EL inhibitors
Delipidated HDL
Most experimental drugs focus on raising HDL-cholesterol levels.....

...but is this the right thing to do?...
Niacin

- **AIM-HIGH trial** (http://www.aimhigh-heart.com/overview.aspx)

**Hypothesis:** patients with residual atherogenic dyslipidemia (low high-density lipoprotein cholesterol [HDL-C] and high triglycerides) will benefit from addition of niacin to statin regimens

**Results:** NIH stops clinical trial on combination cholesterol treatment-Lack of efficacy in reducing cardiovascular events prompts decision (http://www.nih.gov/news/health/may2011/nhlbi-26.htm)

(Poorly designed trial!! – it may have led to faulty conclusions)

CETP inhibitors

ILLUMINATE trial (Torcetrapib)

dal-PLAQUE and dal-VESSEL trials (Dalcetrapib)

ACCELERATE trial (Evacetrapib)
Nicholls SJ, Brewer HB, Kastelein JJ, Krueger KA, Wang MD, Shao M et al.. Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol: a randomized controlled trial. JAMA 2011;306:2099-109.

REVEAL trial (Anacetrapib)

SNPs associated with increased HDL-C levels may not be used as surrogate markers of atheroprotection.
Modern view: HDL quality does matter!


No-atheroprotection

- Reduced RCT
- Reduced PON
- Reduced affinity for HPETE and HPODE
- Increased $\alpha$3 HDL
- Reduced $\alpha$1, pre-$\alpha$1, pre-$\alpha$2 and pre-$\alpha$3 HDL
- Reduced 17$\beta$-Estradiol
- Failure to recruit endothelial progenitor cells at the site of vascular injury

Atheroprotection

- Enhanced RCT
- Increased PON
- Increased affinity for HPETE and HPODE
- Reduced $\alpha$3 HDL
- Increased $\alpha$1, pre-$\alpha$1, pre-$\alpha$2 and pre-$\alpha$3 HDL
- Increased 17$\beta$-Estradiol
- Enhanced recruitment of endothelial progenitor cells at the site of vascular injury
What do we really know about HDL?
HDL is not just a cholesterol...
HDL comes in various sizes and shapes...

The main protein component of HDL is apolipoprotein A-I

Intestine

Liver

Intestine

Lipid free apoA-I

SR-BI

HL, EL

HDL-2

HL, EL

Preβ-HDL

Minimally lipidated apoA-I

ACBG1?

ABCA1

(PL, free chol efflux from tissues)

Minimally lipidated apoA-I

ABCA1?

LCAT

ABCG1

HDL-3

TRL

TG

CE

CETP

HDL

HDL-3

HDL-2

HDL-1

HDL-0

HDL-3

HDL-2

HDL-1

HDL-0

HDL-3

HDL-2

HDL-1

HDL-0
HDL performs a number of functions in relation to disease

• Reverse cholesterol transport

• Antioxidant

• Antinflammatory

• Regulation of plasma triglyceride levels

• NO production (eNOs stimulation)

Karavia et al., 2014, Expert Review of Cardiovascular Therapy
...We need to learn much more about HDL...
Is HDL a single particle?

• Are other apolipoproteins capable of promoting the de novo biogenesis of HDL particles independently of ApoA-I?

• As an example we used apolipoprotein E (ApoE)
Gene transfer of apoE in apoA1-deficient mice

Apoa1−/− mice received 1) WT apoE4, or 2) WT apoE4 and lcat

Plasma was isolated 5 days post infection

Lipid levels were determined
Plasma was fractionated by density gradient ultracentrifugation
HDL particles were visualized by electron microscopy

ApoE promotes *de novo* biogenesis of HDL particles independently of ApoA-I

Lipid free apoE → ABCA1 (PL, free chol efflux from tissues) → Preβ-ApoE HDL → ABCG1 → LCAT → ApoE-HDL → SR-BI → Liver

But this is the case for other apolipoproteins also such as apoCIII

The mouse studies indicated that HDL is a mixture of particles with distinct apoprotein composition and densities in the HDL region.

Do these HDL subpopulations exist in humans?

What do they mean for human physiology and disease?

Information from relevant clinical examples
Biliopancreatic diversion by *Roux en Y* with long limps (both restrictive and malabsorptive)

Significant reduction in CHD risk 10 years following the operation

Rapid weight loss results in a significant improvement in the antioxidant potential of HDL and a reduction in plasma CETP and LCAT activities.

Distribution of apolipoproteins A-I, E, CIII and CI in various HDL fractions pre and postoperatively

<table>
<thead>
<tr>
<th>Prior</th>
<th>After</th>
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35% of Patients

<table>
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<th>HDL</th>
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<td>ApoCIII</td>
<td>ApoE</td>
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Distribution of apolipoproteins A-I, E, CIII and CI in various HDL fractions pre and postoperatively

65% of Patients

Prior

After

A

B

VLDL
IDL/LDL
HDL
Lipid free proteins

Density: 1.022 1.028 1.060 1.071 1.084 1.109 1.123 1.152 d>1.21

30 kDa – 25 kDa – 17 kDa – 7 kDa –

30 kDa – 25 kDa – 17 kDa – 7 kDa –

M 1 2 3 4 5 6 7 8 9 10

ApoA-I
ApoCIII
ApoE
ApoCI
PON1

30 kDa – 25 kDa – 17 kDa – 7 kDa –

30 kDa – 25 kDa – 17 kDa – 7 kDa –

M 1 2 3 4 5 6 7 8 9 10

ApoA-I
ApoCIII
ApoE
ApoCI
PON1

Distribution of apolipoproteins A-I, E, CIII and CI in various HDL fractions in control subjects of normal BMI

A

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B

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<tr>
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<th>35% Pre</th>
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<tbody>
<tr>
<td>apoA-I</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>apoE</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>apoCIII</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>apoCI</td>
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<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PON1</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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C

Relative apoA-I/apoE ratio

Roux et Y surgical intervention influences the HDL apolipoprotein ratios

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<tr>
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<tr>
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<td>+</td>
<td>+++</td>
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<tr>
<td>apoE</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>apoC III</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
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<td>++</td>
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<tr>
<td>[apoE]</td>
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Structural characteristics of HDL in young patients of an acute myocardial infarction

• Patients were at an age \( \leq 35 \) years.

ApoA-I/apoCIII \((MI)\) ↓

ApoA-I/apoE \((MI)\) ↓

It appears that in the clinical examples studied, increased apoE and apoCIII content in HDL correlates with dysfunctional HDL and disease states.

The apolipoprotein content of HDL defines its functionality.
Can we influence HDL functionality by modulating apolipoprotein content?

Back to the study of HDL in experimental mouse models
Gene transfer of human *apoe* or *apoa1* in *apoa1* x *apoe−/−* double deficient mice

*apoe−/− x apoa1−/−* mice received:
1) WT *apoe3*, or
2) WT human *apoa1*

Plasma was isolated 5 days post infection

Lipid levels were determined
Plasma was fractionated by density gradient ultracentrifugation
HDL particles were visualized by electron microscopy
Protein expression and lipid data

A

![Graph showing protein expression and cholesterol levels over time.](A)

B

![Graph showing cholesterol levels across different fractions.](B)

C

![Diagram showing molecular weight (MW) of AdGFP and AdGFP-E3.](C)

D

![Graph showing protein expression and cholesterol levels over time.](D)

E

![Graph showing cholesterol levels across different fractions.](E)

F

![Diagram showing molecular weight (MW) of AdGFP and AdGFP-A1.](F)
Both ApoE and ApoA-I promote the formation of HDL particles
Apolipoprotein composition of APOE-HDL and APOA1-HDL
APOA1-HDL contains more polar lipids than APOE-HDL
APOA1-HDL is a better substrate for LCAT than APOE-HDL in a fluorescent in vitro LCAT assay
APOA1-HDL has a better antioxidant capacity (A) and cholesterol efflux potential than APOE-HDL (B)
Surprisingly, APOE-HDL is antinflammatory while APOA1-HDL is proinflammatory...
Conclusions

• HDL apolipoprotein composition defines its lipid composition

• HDL apolipoprotein composition defines its functionality

• APOE is prerequisite for the anti-inflammatory effects of HDL

• The antinflammatory effects of APOE-HDL are independent of APOA1.
...HDL beyond atheroprotection...
...HDL in NAFLD...
ApoA-I and LCAT are functional modulators of hepatic lipid deposition and NAFLD development

**Body Composition Analysis**

<table>
<thead>
<tr>
<th>Mice Groups</th>
<th>Wet Body Weight (g)</th>
<th>Dry Body Weight (g)</th>
<th>Body Fat (g)</th>
<th>Body Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>35.7 ± 1.5</td>
<td>25.5 ± 1.6</td>
<td>21.8 ± 2.3</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>apoA-I⁻/⁻</td>
<td>36.6 ± 0.9</td>
<td>27.4 ± 1.3</td>
<td>26.0 ± 2.4</td>
<td>9.2 ± 0.6</td>
</tr>
</tbody>
</table>

**Hematoxylin & Eosin Stain**

**Reticulin Stain**

Expression of apoA-I milano reduces significantly hepatic lipid deposition

Expression of LCAT reduces significantly hepatic lipid deposition

C57BL/6, eosin/hematoxylin, week 24

LCAT−/−, eosin/hematoxylin, week 24

LCAT−/−+AdGFP, Oil-red O, week 12

LCAT−/−+Ad-LCAT, Oil-red O, week 12

Expression of LCAT reduces significantly hepatic lipid deposition

Karavia et al., J Nutr Biochem. 2013 Mar;24(3):567-77
...HDL in insulin resistance and glucose intolerance...

- HDL cholesterol levels show inverse relation with glucose metabolism

- Deficiency in apolipoprotein A-I ablates the pharmacological effects of
  metformin on plasma glucose homeostasis and hepatic lipid deposition

  *Karavia EA et al., Eur. J. Pharmacol. 2015*
Metformin fails to improve glucose tolerance in mice lacking classical APOA1-containing HDL

Karavia EA et al., Eur. J. Pharmacol. 2015
Metformin fails to suppress hepatic gluconeogenesis in Mice lacking classical APOA1-containing HDL

Karavia EA et al., Eur. J. Pharmacol. 2015
Metformin fails to improve hepatic lipid deposition in mice lacking classical APOA1-containing HDL

Karavia EA et al., Eur. J. Pharmacol. 2015
...HDL in Bone Metabolic Disease...
HDL metabolic pathways and osteoarthritis

LCAT−/− and ApoA-1−/− mice fed WTD for 24 weeks:

• showed significant cartilage degeneration (histology and μCT studies)
• had reduced collagen type II but decreased MMP-2, -9 and -13 levels that characterize damaged cartilage and OA
• LCAT and ApoA-1 and thus HDL malfunction and/or deficiency are related to OA development in mice following high-fat diet
HDL and bone mass

ApoA-1 KO mice in comparison to WT

- Decreased bone mass (μCT analysis)
- Decreased osteoblastic function (i.e. Calcein labelling) but unaffected osteoclastic function (TRAP stain)
- Osteoblast-related molecules (Runx2, Collagen type I, Anax2) were significantly decreased, while the lipoblastic regulator (CEBP2) was strongly increased
HDL in the brain...
Brain facts:

- Free cholesterol cannot cross either the BBB or BCSFB
- Cholesterol originates from de novo biosynthesis.
- Contains almost 25% of total body cholesterol
- 70-80% of brain cholesterol constitutes structural component of myelin accomplishing an insulating role
- Cholesterol biosynthesis is regulated by mechanisms similar to those observed in peripheral organs and tissues and HMGC0AR activity is the rate limiting step
- Embryogenesis and childhood (myelinogenesis): increased brain cholesterol biosynthesis in both neurons and glial cells
- Adulthood: brain cholesterol biosynthesis only in glial cells

Constantinou C, et al., AJP Endo Metab, 2015
**Brain-Periphery cross-talk**

- **Plasma lipoproteins can not cross BBB or BCSFB**
  - spherical High-density lipoproteins (HDL)
  - low-density lipoproteins (LDL)
  - very low-density lipoproteins (VLDLs)
  - Chylomicrons (CM)

- **Only discoidal APOA1-HDL can cross the BBB and BCSFB**

- **Brain synthesizes all apolipoproteins except APOA-I**

- **Lipoprotein regulation in the brain is largely independent from the periphery**

Constantinou C, et al., AJP Endo Metab, 2015
Brain lipoproteins

Brain and CSF lipoproteins found to have a similar size and density to plasma HDL and they have been defined as ‘HDL-like particles’

Fraction 1 (major)
Fraction 2
Fraction 3
Fraction 4

13-20 nm
13-18 nm
18-22 nm
10-12 nm

Lipid content

APOA-I
APOE
APOJ
APOA-II
APOD
APOH
APOA-IV

Constantinou C, et al., AJP Endo Metab, 2015
APOA-I-containing HDL-like particles’ novel actions

Circulation

Discoidal ApoA-I / HDL

BBB

Brain

Binds APP

Normal APP process

Binds Aβ

Normal Aβ clearance

Attenuates neuroinflammation

Membrane fluidity

Promotes cholesterol efflux from glial cells to neurons

Membrane repair

COGNITION

NEURONAL GROWTH & REPAIR

Constantinou C, et al., AJP Endo Metab, 2015
HDL-like particles’ classical actions

**ACTION**
- Lipid transport
  - cholesterol synthesis → HD
  - cholesterol efflux → AD
  - HDL-like particles maturation → stroke recovery, TBI, AD
- Antioxidative activity → PD, ALS, AD
- Anti-inflammatory activity → AD, stroke recovery, dementia

**DISEASE**
- HD; Huntington’s Diseases
- PD; Parkinson’s Disease
- AD; Alzheimer’s Disease
- TBI; Traumatic Brain Injury
- ALS; Amyotrophic Lateral Sclerosis

*Constantinou C, et al., AJP Endo Metab, 2015*
Conclusions

• HDL functionality is largely dependent on HDL proteome that further determines HDL lipidome and interactions with plasma enzymes

• HDL is a multifunctional pharmacological target beyond atherosclerosis

• The lipoprotein transport system in the brain is largely independent from that in the periphery

• Discoidal HDL is the only link between brain and peripheral lipoprotein metabolism

• The role of peripheral HDL metabolism on brain physiology needs to be further evaluated

• Changes in peripheral lipoproteins (PCSK9 inhibition etc) may not influence brain lipoprotein metabolism unless they impact the levels of discoidal APOA1-HDL
Currently we think like this…

- Obesity
- Diabetes
- Hypertension
- NAFLD
- Bone Met. Diseases

High TG, Reduced HDL-C

Karavia et al., Expert Review of Cardiovascular Therapy, 2014
Known factors contributing to HDL dysfunction:

• Defective LCAT activity
• Lack of ApoA-I
• Defective ABCA1 activity
• Defective SR-BI activity
HDL pharmacology has a bright future ahead…

• For the successful design HDL based pharmaceuticals we need to further explore the exciting world of HDL, deciphering its secret code of function.

• We need to find out how to harness its properties and exploit its full therapeutic potential for the benefit of the patient.
Acknowledgements


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