Translational Science of the LDL Receptor
AN NLA CORE CURRICULUM INTENSIVE PROGRAM
History of Cholesterol and the LDL Receptor

National Lipid Association
Masters Course
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Antonio M. Gotto, Jr., MD, DPhil
Presenter Disclosure Information

Antonio M. Gotto, Jr., MD, DPhil

The following relationships exist related to this presentation:

<table>
<thead>
<tr>
<th>Category</th>
<th>Name of Commercial Interest</th>
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<tr>
<td>Advisory Board</td>
<td>Vatera Capital</td>
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<td>Board of Directors</td>
<td>Arisaph Pharmaceuticals, Esperion Therapeutics</td>
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<td>Data Safety Monitoring Board</td>
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Development of the LDL Receptor Concept

<1815  Cholesterol identified as a specific compound

1910  Human atherosclerotic plaques contain cholesterol

1913  High cholesterol diet causes atherosclerosis in rabbits

1933  Feedback inhibition of cholesterol synthesis demonstrated

1938  Familial hypercholesterolemia described

1954  Elevated LDL identified as primary focus of FH

1959  Cholesterol biosynthetic pathway elucidated

1959  FH exists in both heterozygous and homozygous forms.

1974  Cellular cholesterol control systems defined

1976  LDL receptor concept defined

1976  Statin effect described in fibroblasts, animals and in FH (1979-80)
Cholesterol Discovery

M.E. Chevreul
French chemist discovered cholesterol in 1815.
Isolated and chemically purified from bile.
Human Atherosclerotic Plaques Contain Cholesterol

Adolf Windaus Circa 1910:
First structural description of cholesterol and identification of its presence in bile and tissues.

Windaus was awarded the 1928 Nobel Prize in Chemistry for studies on:

“Constitution of sterols and their connection with other substances appearing in nature.”

From-Goldstein _Brown Scientific American 1984. Photomicrograph by L. Maximilian Buja UTSW.
High Cholesterol Diet Causes Atherosclerosis in Rabbits

Nikolai Anitchkov (1885-1964)

- Demonstrated that high cholesterol diet was responsible for cholesterol in atherosclerotic lesions in the rabbit arteries. The degree of atheromatous involvement was related to the amount of cholesterol uptake (1913).
- Anitchkov was also the first to describe the foam cell, the lipid-laden macrophage in arterial lesions, which he called the “cholesterinesterphagozyten.”

Cholesterol as a Molecule of Interest
Molecular Structure of Cholesterol

Nobel Prize for 1927
for structural work on cholesterol
and bile acids.

Heinrich Wieland, PhD
Cholesterol Synthesis

George Popjak  John Cornforth  Konrad Bloch  Feodor Lynen
Audience Question

• How is the synthesis of mevalonic acid related to the clearance of LDL cholesterol?
Cholesterol Synthesis

Acetate $\rightarrow$ Acetoacetate $\rightarrow$ CoA

HMG synthase

HMG CoA $\rightarrow$ HMG CoA reductase

Mevalonate

Dimethylallyl-PP

Isopentyl-pyrophosphate

Condensation

Farnesyl-pyrophosphate

Isoprenoids

Squalene synthase

Squalene $\rightarrow$ Lanosterol

19–20 reactions including Desmosterol, Lathosterol

4 ringed structure

Cholesterol

Isopentenyl Pyrophosphate

The Rate Limiting Step in Cholesterol Synthesis: HMG Co-A Reductase

Fig. 1. HMG-CoA reductase reaction.


Xanthomas and Blood Cholesterol
Familial Occurrence:

1. Mueller C: Xanthomata, hypercholesterolemia, angina pectoris.

   Arch Intern Med. 1939;64:675.

Mueller C: Angina pectoris in hereditary xanthomatosis.

Fig. 1 (case 13).—Xanthelasma of the eyelids.

Fig. 2 (case 17, family 5).—Xanthoma tuberosum. Besides the nodes on the elbows, fingers and knees, there were deposits also on the achilles tendons and xanthelasma of the eyelids.
Mueller C:
Angina pectoris in hereditary xanthomatosis.

Dominant Inheritance Pattern
Hypercholesterolemic

Aorta from 67 year old woman
With xanthomata and angina
for 10 years prior to death.
Total cholesterol 267 mg/dL
Genetic Nature of Familial Hypercholesterolemia
In 1949 John Gofman, a biophysicist at the University of California at Berkeley, and colleagues began the studies that used the newly developed ultracentrifuge to separate plasma lipoproteins by flotation.

Gofman et al. described the atherogenic potential of the LDL particle and showed that in patients with FH, the cholesterol elevation was all in the LDL and IDL fractions.
Hypercholesterolemia Becomes Elevated LDL in Ultracentrifuge

Xanthoma Tendinosum. A group of eighteen patients with xanthomatomous lesions involving the tendons.

Clinical and lipoprotein data reported.

Some of these patients noted that lesions had been present since childhood.

KHACHADURIAN, A. K.,

KHACHADURIAN, A. K.,

KHACHADURIAN, A. K. AND UTHMAN, S. M.,
Experiences with the homozygous cases of familial hypercholesterolemia: A report of 52 cases, NW. Metabol., 20 (1973) 132.

KHACHADURIAN, A. K. AND KAWAHARA, F. S.,

KHACHADURIAN A. K., LIPSON M., AND KAWAHARA F. S.
Diagnosis of familial hypercholesterolemia by measurement of sterol synthesis in cultured skin fibroblasts. Artherosclerosis, 21 (1975) 235-244
KHACHADURIAN, A. K.,
The inheritance of essential familial hypercholesterolemia.

Found families with 52 affected relatives.
Demonstrated xanthomata occurring in heterozygotes.
Marriage of heterozygotes generated offspring with severe elevations of cholesterol, moderate elevations and normal levels compatible with the inheritance as an autosomal co-dominant or a homozygote.

Amer. J. Med., 37 (1964) 402
LDL Physiology in FH Versus Normal
The Kinetics of LDL Clearance in FH Heterozygotes is Slower than Normal.

$^{125}$I ApoB decay curves

ApoB kinetic parameters

<table>
<thead>
<tr>
<th>apoB</th>
<th>1/2 life</th>
<th>FCR</th>
<th>Synthetic rate</th>
<th>% IV</th>
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<tbody>
<tr>
<td></td>
<td>days</td>
<td></td>
<td>µg/kg/day</td>
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Studies of Cellular Cholesterol Metabolism
Normal Serum Inhibits Cholesterol Synthesis in Normal Fibroblasts but Much Less in homo-FH

Titration of cell cultures with increasing lipoprotein concentration in culture medium.

Incorporation of 14C acetate is markedly inhibited in normal cells but less so in homo-FH cells.

KHACHADURIAN A. K., LIPSON M., AND KAWAHARA F. S. Diagnosis of familial hypercholesterolemia by measurement of sterol synthesis in cultured skin fibroblasts. *Atherosclerosis*, 21 (1975) 235-244
Endothelial Cells Bind and Degrade $^{125}$I-LDL

Normal fibroblasts showing saturation binding followed by degradation.

Cells from FH patients show markedly decreased binding and degradation.

LDL inhibits HMG-CoA reductase in Normal cells but not in ho-FH

Pronase digestion of cell surface proteins reduces response to LDL-C in normal cells.
Major Concepts in Cellular Cholesterol Metabolism


Immuno-Electron Cryo-Microscopy Map of Apo B on LDL

A

N \[\beta\alpha_1\] \[\beta_1\] \[\alpha_2\] \[\beta_2\] \[\alpha_3\] C

Mb19 (71) Mb3, Mb11 (1022--1031) Bsol4 (2488--2543) 5E11 (3506) Bsol7 (4521--4536)

1000 2000 3000 4000

B

Front Left Back Right

Top Bottom

10nm
SREBP Pathway

http://www4.utsouthwestern.edu/moleculargenetics/pages/gold/current.html
Feedback Regulation of Cholesterol Synthesis and LDL Receptors in Cultured Cells

Normal Subjects (A)
Children with Homozygous FH (B)

(A) Normal cells obtain cholesterol from two sources:
1. endogenous synthesis and
2. receptor-mediated uptake and lysosomal hydrolysis of LDL.

(B) Lacking LDL receptors, FH cells maintain normal levels of cholesterol by increasing synthesis of cholesterol, leaving excess LDL in the culture medium.

Brown and Goldstein. Cell 2015;:161-172
The Role of a Statin in Developing Receptor Concept
From Citrinin to Compactin (ML-236B)


Figure 2: Akira Endo in the Sankyo Company research laboratories as a young research scientist. From: Science Heroes.Com <http://www.scienceheroes.com/index.php?option=com_content&view=article&id=126&Itemid=135>.
Compactin as a Tool to Develop the LDL Receptor Concept


**A**

<table>
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<tr>
<th>Addition to medium</th>
<th>LDL receptor activity</th>
<th>HMG-CoA reductase activity</th>
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<tbody>
<tr>
<td></td>
<td>Cell-bound ¹³⁵⁻LDL</td>
<td>Rate of degradation of ¹³⁵⁻LDL</td>
</tr>
<tr>
<td></td>
<td>ng/mg</td>
<td>ng·5 h⁻¹·mg⁻¹</td>
</tr>
<tr>
<td>None</td>
<td>916</td>
<td>2176</td>
</tr>
<tr>
<td>Compactin, 0.8 μM</td>
<td>1500</td>
<td>3660</td>
</tr>
<tr>
<td>LDL, 50 μg of protein/ml</td>
<td>31</td>
<td>204</td>
</tr>
<tr>
<td>LDL + compactin</td>
<td>37</td>
<td>399</td>
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**B**

LDL receptor activity increases in the presence of compactin.

**C**

Cholesterol in cells increases with LDL and compactin.
Goldstein and Brown. Scientific American, 1984

HMG CoA reductase

Cholesterol in cell membranes
PCSK9 and the LDL Receptor


Fig. 2. Structural similarities between compactin and HMG-CoA.
First Published Tests of Compactin/Mevinolin


Hypolipidemic effects in monkeys of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase.


Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent.
Proc Natl Acad Sci 77, 3957-61.


Therapeutic effects of ML-236B in primary hypercholesterolemia.
Atherosclerosis 35, 259–266.
First Clinical Tests of Compactin

• Mabuchi H., Haba T., Tatami R., Miyamoto S., Sakai Y., Wakasugi T. et al. (1981)
  Effects of an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase on serum lipoprotein and ubiquinone-10 levels in patients with familial hypercholesterolemia.

  Reduction of serum cholesterol in heterozygous patients with familial hypercholesterolemia. Additive effects of compactin and cholestyramine.
The Akira Endo Award

The National Lipid Association Foundation presents the Akira Endo Award for discovery of an outstanding means of therapeutic intervention on lipid disorders.

The 2015 Akira Endo Award was given at the International Atherosclerosis Symposium to Harry R Davis for discovery of ezetimibe.
2008 Lasker-DeBakey Award for Clinical Medical Research

Akira Endo, PhD, Tokyo, Japan
Audience Question

• How is statin activity measured?
Recent Developments
Lipid Lowering Drugs that Operate Through the LDL Receptor

Approved
• Bile acid resins
• Ezetimibe
• Statins
• PCSK9 inhibitors

Experimental
• Bempedoic acid
• PCSK9 RNA interference
Cascade Testing to Identify FH Cases

Patient with a clinical diagnosis of FH

Has an FH gene mutation been identified?

Yes

Use the mutation, and not LDL cholesterol measurements, to identify affected biological relatives

No

Use specialized gender- and age- specific LDL cholesterol criteria to identify affected biological relatives

Include at least first- and second-degree (and if possible, third-degree) biological relatives

From Medscape, based on NICE algorithm
LDL Receptor (LDLR) Variants

• More than 1800 variants have been described in the LDLR gene of patients with FH

• About 50% of variants need further evidence to be considered pathogenic

• Reporting the variants with unknown pathogenicity to patients holds the risk that it might need to be withdrawn

• Investment in functional assays is crucial
Summary

• Discovery of cholesterol
• Cholesterol pathway synthesis
• LDL and Familial Hypercholesterolemia
• LDL receptor
• Discovery of statins
Discussion.