Lipid Pathophysiology and Monogenic Lipid Disorders

Endocrine University March 5, 2019

Vinaya Simha, M.D.
I KEEP six honest serving-men
They taught me all I knew;
Their names are What and Why and When
And How and Where and Who.
I send them over land and sea,
I send them east and west;
But after they have worked for me,
I give them all a rest.

Rudyard Kipling
The Lipid Book

- What are Lipids?
- Why are they important?
- When do they become harmful?
- How can we overcome this harm?
- Where is the evidence to guide us?
- Who are the people that need treatment?
The Lipid Book

• What are Lipids?
• Why are they important?
• When do they become harmful?
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• Who are the people that need treatment?
**Lipids**: Heterogeneous group of compounds which are relatively insoluble in water

- **Simple lipids**: esters of fatty acids with various alcohols
  - Fats (oils if liquid): esters of fatty acids with glycerol
  - Waxes: esters with more complex alcohols

- **Complex lipids**: contain additional groups
  - Phospholipids (glycero- and sphingophospholipids)
  - Glycolipids (glycosphingolipids)
  - Sulfolipids, amino lipids (lipoproteins)

- **Precursor and derived lipids**:
  - fatty acids, glycerol, other alcohols,
  - ketone bodies, fat-soluble vitamins, hormones
Structure of common lipids

A: Glycerol

B: Cholesterol
Structure of Fatty Acids

16:0 (palmitic)  
 cis-18:1 ω-6 (oleic) 
 trans-18:1 ω-6 (elaidic) 
 18:2 ω-6 (linoleic) 
 18:3 ω-3 (alpha linolenic) 
 20:5 ω-3 (EPA)
Nomenclature of fatty acids

- Saturation: saturated, unsaturated
  - TAG w C12:0 solid at 37°C
  - TAG w 18:2 liquid at 0°C
- How many double bonds?
  - monounsaturated…, polyunsaturated…
  - Monoenoic, Dieneoic, Trienoic, Tetraenoic, Pentaenoic, Hexaenoic acids
- Where are the double bonds?
  - Alpha vs. Omega
- Chain length: short, medium (6-12), long
The Lipid Book

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• How can we overcome this harm?
• Where is the evidence to guide us?
• Who are the people that need treatment?
Physiological role of lipids

- Energy supply and storage
- Transport fat soluble substances
- Thermal insulation
- Electrical insulation
- Integral components of cells:
  - Cell membrane, mitochondria, myelin etc.
- Cholesterol:
  - Cell membrane
  - Synthesis of bile acids, adrenocortical hormones, sex hormones, D vitamins
The Lipid Book

• What are Lipids?
• Why are they important?
• When do they become harmful?
• How can we overcome this harm?
• Where is the evidence to guide us?
• Who are the people that need treatment?
Lipid transport: purpose, pathways and perils
Lipid Transport: Outline

• Structure of Lipoproteins

• Transport of exogenous lipids
  • Generation and metabolism of chylomicrons

• Transport of endogenous lipids
  • Generation and metabolism of VLDL/LDL

• Reverse Cholesterol transport
  • Generation and metabolism of HDL particles

• Regulation of LDL uptake and metabolism
Lipoproteins

- Complex of lipids and proteins which help to solubilize and transport lipids
- Non polar lipids at the core (TG, CE)
- Amphipathic lipids and proteins form the shell
- Varying size and density depending on composition
<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Density (g/mL)</th>
<th>Sources</th>
<th>Lipids and (Apolipoproteins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>&lt;0.95</td>
<td>Intestine</td>
<td>Tg (A-I, -II, B-48, C-II, -III, E)</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.95-1.006</td>
<td>Liver</td>
<td>Tg (B100, C-I, -II, -III)</td>
</tr>
<tr>
<td>IDL</td>
<td>1.006-1.019</td>
<td>Catabolism of VLDL</td>
<td>Tg, C (B-100, E)</td>
</tr>
<tr>
<td>LDL</td>
<td>1.019-1.063</td>
<td>Catabolism of IDL</td>
<td>C (B-100)</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.21</td>
<td>Liver, intestine, other</td>
<td>PL, C (A-I, -II, -IV, C-I, -II, -III, D, E)</td>
</tr>
</tbody>
</table>
Roles of apoproteins

<table>
<thead>
<tr>
<th>A-I</th>
<th>HDL / Chylomicron</th>
<th>LCAT activator (lecithin: cholesterol acyltransferase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B48</td>
<td>Chylomicron</td>
<td>Carry cholesterol esters</td>
</tr>
<tr>
<td>B100</td>
<td>VLDL → IDL → LDL</td>
<td>Binds LDL receptor</td>
</tr>
<tr>
<td>C-II</td>
<td>Chylomicron, VLDL, IDL, HDL</td>
<td>Activates LPL</td>
</tr>
<tr>
<td>C-III</td>
<td>Chylomicron, VLDL, IDL, HDL</td>
<td>Inhibits LPL</td>
</tr>
<tr>
<td>E</td>
<td>Chylomicron remnant, VLDL, IDL</td>
<td>Binds LDL receptor</td>
</tr>
</tbody>
</table>

Structural integrity; activator or inhibitor of enzyme; and ligands for lipoprotein receptors
Transport of Exogenous lipids: 

generation and metabolism of chylomicrons
Metabolism of Chylomicrons: secretion by intestinal epithelial cells
Synthesis of chylomicrons

- **Microsomal Triglyceride Transfer Protein (MTP):** critical enzyme mediating the transfer of triglyceride to **Apo B48** (lipidation)
- Defects in MTP and Apo B48 affect chylomicron synthesis
  - Abetalipoproteinemia
  - Hypobetalipoproteinemia
- Potential drug target for lipid lowering
Metabolism of Chylomicrons:
Acquisition of ApoC in circulation and subsequent TG hydrolysis by LPL
Metabolism of Chylomicrons: peripheral TG hydrolysis by LPL in the presence of Apo CII and Apo AV
Metabolism of chylomicrons

- **Lipoprotein Lipase (LPL):** critical enzyme mediating triglyceride hydrolysis and transfer of FFA to tissue

- **Activators of LPL:**
  - Apo CII
  - Apo A V

- **Inhibitors of LPL:**
  - Apo CIII
  - ANGPTL 3/4

Diseases and Drug targets
Metabolism of Chylomicrons: Apo E mediated *hepatic uptake of remnant particles*
Uptake of chylomicron remnants

- Apo E: mediates uptake of remnant particles into the liver (ligand for hepatic receptors)
Familial Chylomicronemia Syndrome: LPL deficiency

- Rare autosomal recessive disorder due to LPL mutations
- Episodic abdominal pain and recurrent pancreatitis
- Erupptive xanthoma and lipemia retinalis
- Serum Triglycerides usually > 2000 mg/dL
- CHD not prominent feature
- Diagnosis: post heparin plasma lipolytic activity
- Treatment: Dietary fat restriction
Familial Chylomicronemia Syndrome: other causes

- Rare autosomal recessive disorder due to LPL mutations
- Episodic abdominal pain and recurrent pancreatitis
- Erruptive xanthoma and lipemia retinalis
- Serum Triglycerides usually > 2000 mg/dL
- CHD not prominent feature
- Diagnosis: post heparin plasma lipolytic activity
- Treatment: Dietary fat restriction

Apo CII deficiency

Apo A5 deficiency
Metabolism of Chylomicron remnants: Type III Dysbetalipoproteinemia

- Autosomal recessive
- Defective Apo-E mediated clearance of remnant particles
- Apo E mutations, E2E2 genotype
- Second hit: DM, obesity, alcohol
- Elevations in both serum TG and cholesterol (300-500)
- Characteristic palmar xanthomas
- Strong predisposition to premature CHD
- Treatment: secondary cause, fibrate, niacin, statin
Monogenic Hypertriglyceridemia

- Familial chylomicronemia syndrome
  - **Lipoprotein lipase deficiency**
  - Apo C II deficiency
  - Others: Apo A 5 deficiency, LMF, GPIHBP
- Familial Dysbetalipoproteinemia
  - Apo E2/E2 genotype
- Congenital lipodystrophies
  - Congenital generalized lipodystrophy
  - Familial Partial Lipodystrophy
Hypertriglyceridemia in Lipodystrophy

Rare disorders characterized by loss of body fat:
- Congenital or Acquired
- Generalized or Partial

Simha and Garg, *Curr Opn Lipidol* 2006
Hypert triglyceridemia in Lipodystrophy

Simha and Garg, *Curr Opn Lipidol* 2006
Transport of Endogenous lipids: 

*generation and metabolism of VLDL/LDL particles*
Metabolism of VLDL particles: hepatic secretion
Metabolism of VLDL particles: 
**peripheral TG hydrolysis**
Metabolism of VLDL particles: remnant particle metabolism to LDL.
LDL uptake and metabolism
10 y old girl with Coronary Artery Disease
Regulation of cholesterol synthesis: *skin fibroblasts in lipoprotein free medium*

Goldstein and Brown; *Proc Nat Acad Sci* 1973
Regulation of cholesterol synthesis: skin fibroblasts in lipoprotein free medium, changes after addition of LDL
Increased cholesterol synthesis in fibroblasts from FH patients

A. AFTER REMOVAL OF LIPOPROTEINS

- Homozygote
- Normal

B. AFTER ADDITION OF LDL

- LDL
- None
- 2 μg/ml
- 20 μg/ml

Goldstein and Brown; *Proc Nat Acad Sci* 1973
Receptor mediated endocytosis of LDL particle

Goldstein and Brown *Artscler Thomb Vasc Bio* 2009
History of Discovery: The LDL Receptor

Joseph L. Goldstein and Michael S. Brown


CHOLESTEROL FEEDBACK: FROM SCHOENHEIMER’S BOTTLE TO SCAP’S MELADL

Joseph L. Goldstein and Michael S. Brown

J Lipid Research 2009; 50:S15-27
Hepatic LDLRs Play a Central Role in Cholesterol Homeostasis

Recycling of LDLRs Enables Efficient Clearance of LDL-C Particles
PCSK9 Regulates the Surface Expression of LDLRs by Targeting for Lysosomal Degradation
Genetic Variants of PCSK9 Demonstrate Its Importance in Regulating LDL-C Levels

PCSK9 Gain of Function = Less LDLRs

PCSK9 Loss of Function = More LDLRs
Alirocumab
75-150 mg
SC q 2 week

Evelocumab
140 mg
SC q 2 week

- Heterozygous Familial Hypercholesterolemia
- Established cardiovascular disease who need additional LDL-C reduction

$14,000 - $16,000/y
LDL uptake and metabolism in the liver

Garg & Simha. JCEM 92:1581-9, 2007
Genetic defects causing Hypercholesterolemia

Garg & Simha. JCEM 92:1581-9, 2007
Genetic defects causing Hypercholesterolemia:
1: FH due to LDL receptor mutations

Familial Hypercholesterolemia
- autosomal dominant
- tendon xanthomas, premature corneal arcus
- Heterozygous: Prevalence: 1:250-500
  LDLc: 200-500
  CHD in 4th-5th decades of life
- Homozygous: Prevalence: 1 in million
  LDLc: 500-800
  CHD before 2nd decade

Garg & Simha. JCEM 92:1581-9, 2007
Genetic defects causing Hypercholesterolemia:
2: FDB due to Apo B missense mutation

Familial defective apolipoprotein B
• autosomal dominant
• clinically indistinguishable from FH
• Less frequent than FH
• Premature CAD and xanthomata also less prevalent
Autosomal recessive hypercholesterolemia (ARH)

• Autosomal recessive
• Rare, clustering of cases in Sardinia
• Inactivating mutations lead to retention of LDL receptors on the surface of hepatocytes
• Clinical features similar to homozygous FH
• Lesser prevalence of premature CAD
Genetic defects causing Hypercholesterolemia:
4: Gain of function PCSK9 mutation

Proprotein convertase subtilisin-like kexin type 9 (PCSK9)
• Gain of function mutations associated with hypercholesterolemia and features similar to FH
• Loss of function variants lead to low plasma cholesterol and CAD risk

Garg & Simha. JCEM 92:1581-9, 2007
Genetic defects causing Hypercholesterolemia:
5: Sitosterolemia due to ABCG5/8 mutation

Sitosterolemia/phytosterolemia

• Autosomal recessive
• mutation in either protein leads to increased absorption and accumulation of cholesterol and plant sterols
• tendon xanthomas and premature CHD
• responds well to dietary cholesterol/phytosterol restriction

Garg & Simha. JCEM 92:1581-9, 2007
Monogenic Hypercholesterolemia

• Autosomal Dominant
  • Familial Hypercholesterolemia
    • LDL receptor mutations
    • Apo B mutation
    • PCSK9 activating mutation

• Autosomal Recessive
  • LDL receptor adaptor protein mutation
  • Sitosterolemia (ABCG5 and ABCG8)
MEDPED LDL cholesterol thresholds for diagnosis of Familial Hypercholesterolemia

<table>
<thead>
<tr>
<th>Age</th>
<th>80% Probability of disease in</th>
<th>100% probability of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General Population</td>
<td>First degree relative of FH Pt</td>
</tr>
<tr>
<td>&lt; 20 years</td>
<td>200</td>
<td>155</td>
</tr>
<tr>
<td>20 - 29 years</td>
<td>220</td>
<td>170</td>
</tr>
<tr>
<td>30 - 39 years</td>
<td>240</td>
<td>190</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>260</td>
<td>205</td>
</tr>
</tbody>
</table>

Williams RR Am J Cardiol 1993
Other criteria for diagnosis of Familial Hypercholesterolemia

- Simon Broome Register Group (UK)
  - Definite FH: LDL>190 + Tendon xanthoma
  - Possible FH: LDL>190 + F/H of MI or TC>290

- Dutch Lipid Clinic Network
  - Points for LDLc, family history, xanthoma, genetic testing
  - Definite, Probable and Possible categories based on total number of points
Dutch Lipid Clinic Network Score for Familial Hypercholesterolemia

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family History</strong></td>
<td></td>
</tr>
<tr>
<td>First-degree relative with premature coronary and/or vascular disease (men ≤ 55 years, women ≤ 60 years), OR</td>
<td>1</td>
</tr>
<tr>
<td>First-degree relative with known LDL-cholesterol ≥ 95th percentile for age and sex</td>
<td></td>
</tr>
<tr>
<td>First-degree relative with tendon xanthomata and/or arcus cornealis, OR</td>
<td>2</td>
</tr>
<tr>
<td>Children aged ≤ 18 years with known LDL-cholesterol ≥ 95th percentile for age and sex</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical History</strong></td>
<td></td>
</tr>
<tr>
<td>Patient with premature coronary artery disease (age as above)</td>
<td>2</td>
</tr>
<tr>
<td>Patient with premature cerebral or peripheral vascular disease (age as above)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Physical Examination</strong></td>
<td></td>
</tr>
<tr>
<td>Tendon Xanthomas</td>
<td>6</td>
</tr>
<tr>
<td>Arcus cornealis at age ≤ 45 years</td>
<td>4</td>
</tr>
<tr>
<td>LDL Cholesterol (mmol/L) (mg/dL)</td>
<td></td>
</tr>
<tr>
<td>LDL-C ≥ 8.5 (330)</td>
<td>8</td>
</tr>
<tr>
<td>LDL-C 6.5 - 8.4 (250 - 329)</td>
<td>5</td>
</tr>
<tr>
<td>LDL-C 5.0 - 6.4 (190 - 249)</td>
<td>3</td>
</tr>
<tr>
<td>LDL-C 4.0 - 4.9 (155 - 189)</td>
<td>1</td>
</tr>
<tr>
<td>DNA Analysis – functional mutation LDLR, APOB and PCSK9</td>
<td>8</td>
</tr>
<tr>
<td><strong>Stratification</strong></td>
<td></td>
</tr>
<tr>
<td>Definite Familial Hypercholesterolemia</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Probable Familial Hypercholesterolemia</td>
<td>6-8</td>
</tr>
<tr>
<td>Possible Familial Hypercholesterolemia</td>
<td>3-5</td>
</tr>
<tr>
<td>Unlikely Familial Hypercholesterolemia</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

Total Score
### Tendon Xanthomas: Differential diagnosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Clinical Features</th>
<th>Etiology</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial Hypercholesterolemia</td>
<td>↑ Total and LDL Cholesterol, premature CHD</td>
<td>LDLR mutations</td>
<td>Statins, LDL apheresis</td>
</tr>
<tr>
<td>Sitosterolemia</td>
<td>Chol may be normal or high, CHD</td>
<td>ABCG mutations</td>
<td>Diet, Ezetimibe</td>
</tr>
<tr>
<td>Cerebrotendinous xanthomatosis</td>
<td>Neurological manifestations</td>
<td>CYP 27 mutations</td>
<td>Bile acid</td>
</tr>
</tbody>
</table>
Reverse cholesterol transport:

HDL metabolism
Reverse Cholesterol Transport- *formation of nascent HDL*
Reverse Cholesterol Transport - *transfer of free cholesterol to HDL*
Reverse Cholesterol Transport-
esterification of cholesterol by LCAT

Mature HDL
(Spherical)

Nascent HDL
(Discoidal)

Pre-β HDL
(Globular)

BC

Bile

SR-BI

LDL-C

Receptor

Liver

CE

FC

LCAT

A-I

A-I

HL, EL

A-I

ABCG1

FC & PL

ABCA1

FC & PL

Macrophage

Free Cholesterol
& Phospholipids

VLDL/LDL

Free Cholesterol
& Phospholipids

FC & PL

FC & PL

FC & PL
Reverse Cholesterol Transport- delivery of cholesterol esters to liver
Reverse Cholesterol Transport- *direct cholesterol ester delivery to liver*

Mature HDL (Spherical)

Free Cholesterol & Phospholipids

Bile

Mature HDL

Free Cholesterol & Phospholipids

Nascent HDL (Discoidal)

CETP

VLDL/LDL

Pre-β HDL (Globular)

PRE-β HDL

INTERIOR OF LIVER CELL

MATURE HDL

HDL EMPTIES

APOA1

SRB1

Cholesterol

LDL-C

Receptor

BCE

Bile

CE

FC

LCAT

A-I

A-I

CE

HL, EL

Macrophage

Mature HDL

(Spherical)
Reverse Cholesterol Transport - transfer of CE to other lipoproteins

- Bile
- Liver
- SR-BI
- LDL-C Receptor
- Mature HDL (Spherical)
- LCAT
- HL, EL
- CETP
- Nascent HDL (Discoidal)
- ABCG1
- FC & PL
- ABCA1
- FC & PL
- Macrophage
- A-I
- Pre-β HDL (Globular)
- VLDL/LDL
Reverse Cholesterol Transport- *indirect* delivery of CE to liver

- **Bile**
- **Liver**
  - **CE**
  - **FC**
  - **SR-BI**
- **LDL-C Receptor**
- **Mature HDL** (Spherical)
  - **A-I**
  - **CE**
- **CETP**
- **Bile MEDIATED**
- **(Discoidal)**
- **Macrophage**
- **VLDL/LDL**
- **Free Cholesterol & Phospholipids**
- **Pre-β HDL** (Globular)
  - **CE**
  - **CETP**
  - **VLDL/LDL**
  - **HDL**
  - **CHOLESTERYL ESTER**
  - **SR-BI**
  - **LDL Receptor**

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“It’s complicated, Mister Dalton—It seems your good cholesterol is actually born-again bad cholesterol.”
Genetic disorders of HDL metabolism: Apo A1 mutations/deficiency

Apolipoprotein A1 deficiency
- mutation in Apo A1 decreases HDL formation
- typical HDL <10 mg/dL
- association with CHD not clear
Genetic disorders of HDL metabolism: *ABCA1* deficiency and Tangier’s

**Tangier disease**

- mutation in ATP binding cassette A1 (*ABCA1*; mediates efflux of cellular cholesterol and phospholipids to HDL) results in accumulation of lipid-laden macrophages
- homozygous: HDL <5 mg/dl
- heterozygous: HDL <25 mg/dl
- enlarged orange tonsils (cholesterol deposits), peripheral neuropathy, and premature CHD
Genetic disorders of HDL metabolism: 
**LCAT deficiency**

Lecithin:cholesterol acyltransferase (LCAT) deficiency
- catalyzes esterification of free cholesterol
- mutations interfere with maturation of nascent HDL particles
- homozygous: corneal opacifications (fish eye disease) and renal disease
- heterozygous: no clinical features
- unclear association with CHD
Genetic disorders of HDL metabolism: **CETP deficiency**

**Cholesteryl ester transfer protein (CETP) deficiency**
- mutations interfere with exchange of triglycerides for cholesterol esters between HDL and VLDL
- mostly in Japanese
- deficiency leads to high HDL (>100mg/dl in homozygous) not hypoalphalipoproteinemia
- heterozygous actually have increased CHD
Monogenic disorders of HDL metabolism

• Apo A1 deficiency
  • Low HDL chol, variable association with CHD

• Tangiers disease
  • ABCA1 deficiency (Orange tonsils)

• Fish eye disease
  • LCAT deficiency

• CETP deficiency
  • High HDL cholesterol
Mechanisms Relating Insulin Resistance and Dyslipidemia

Fat Cells → Liver

Insulin → IR

↑FFA
Mechanisms Relating Insulin Resistance and Dyslipidemia

Fat Cells

Liver

↑ FFA

↑ TG

↑ Apo B

↑ VLDL

IR

Insulin
Mechanisms Relating Insulin Resistance and Dyslipidemia

Fat Cells

Liver

\[ \text{Fat Cells} \rightarrow \text{Liver} \]

IR

Insulin

\[ \uparrow \text{FFA} \]

\[ \uparrow \text{TG} \]

\[ \uparrow \text{Apo B} \]

\[ \uparrow \text{VLDL} \]

\[ \text{CETP} \]

\[ \text{HDL} \]

\[ \text{(hepatic lipase)} \]

\[ \text{Apo A-1} \]

\[ \text{Kidney} \]
Mechanisms Relating Insulin Resistance and Dyslipidemia

Fat Cells

Liver

IR

Insulin

↑ FFA

↑ TG

↑ Apo B

↑ VLDL

(VLDL)

(CETP)

CE

HDL

(TG)

(Apo A-1)

Kidney

(LDL)

(SD LDL)

(SD LDL)

(lipoprotein or hepatic lipase)
Nature is nowhere accustomed more openly to display her secrets, than in cases where she shows traces of her workings apart from the beaten path.

William Harvey
Thank You
simha.aj@mayo.edu