The Role of Hepatic Endothelial Lipase in the Metabolism of VLDL

W. Virgil Brown MD
Disclosures

Received payment for:

• **Consulting:**
  • Akcea, Aegerion, Esperion, Kowa, Merck.

• **Data and Safety Monitoring Committee Service:**
  Amgen, Genzyme, Sanofi, Bristol Myers-Squibb and Esperion.

• **Honoraria for talks:**
  Merck, Kowa, Med Learning Group
Liver

**VLDL-Rem**

Hepatic triglyceride lipase and apoCIII

**Metabolism of VLDL**

apo B-100

Nascent VLDL

apo E

Mature VLDL

apo C-II, C-III

apo E

apo C-III

Cholesterol esters

HDL

LDL

VLDL Remnant

Lipoprotein LIPASE

apo E

apo C-II, C-III

Phospholipids

Fatty acids

HDL
TG rich LP Remnant Generation

- **1962- Nestel, Havel, and Bezman** (JCI v41 p1915) Hepatectomized dogs chylomicron most triglyceride was cleared but cholesteryl ester remained in the blood in TG rich particles.

- **1970- Redgrave, TR** (JCI v49 p465) Using $^{14}$C triglyceride and $^3$H cholesterol labeled chylomicrons hepatectomized rats cleared most TG in 10 minutes but $^3$H cholesterol remained in plasma as smaller particles referred to as “remnants”. Remnants were isolated from these animals and were cleared in 10 minutes in intact rats.
LPL activity did not explain the properties of Lipase activities released by intravenous heparin

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Journal/Title</th>
<th>Year/Volume</th>
<th>Page/Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAHN (Post Heparin Clearing Factor)</td>
<td>Science, 98, 19 (1943).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Korn</td>
<td>Methods Biochem. Anal. (1959); 7:145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shore, Shore</td>
<td>Am. J. Physiol. (1961); 201:915</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fredrickson, Ono, Davis</td>
<td>J. Lipid Res. (1963); 4:24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fielding -</td>
<td>Biochim. Biophys. Acta (1970); 210: 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vogel, Brunzell, Bierman</td>
<td>Lipids, (1971); 11 ;805</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lipase Activity in Post Heparin Plasma different from Lipoprotein Lipase

1. Different substrate specificity: monoglycerides, phospholipids, short chain TG, etc.

2. Different optimum assay conditions, pH, salt concentration.

2. LPL in many organs: adipose, muscle, kidney, spleen, brain, etc. - not liver.

3. HTGL only in liver of rat, pig, dog, and other species.
Endothelial Lipases
Bound by proteoglycans - Released by heparin

LIPOPROTEIN LIPASE - 50,000 D (443 AA)
Existing as a homodimer on the endothelial surface of muscle (skeletal and Heart), adipose tissue, kidney, breast etc.

Accessory proteins:
apoCII, Activates LPL as component of lipoprotein
apoA5 Activates LPL as component of endothelial complex.
LMF Lipase modulating factor.
GPIHBP1 Glycoprotein inositol anchored –
ANGPTL-4 Angiopoiten like-4.

Hepatic Triglyceride Lipase 53,000 D (463 AA)
Existing as a monomer on liver endothelial cells and hepatocytes (?)
Hepatic Lipase and the Interplay of ApoCIII

- Isolation and characterization of HTGL and apoCIII
- Biochemical studies (in vitro)
- Functional studies in genetics (human deficiency) of HTGL and of apoCIII
- Interventions that inhibit Hepatic Lipase activity
- Interventions that reduce apoCIII concentration
Fig. 5. Sephadex G-100 chromatography of the Tris-HCl-soluble protein fraction of VLDL apolipoprotein. Immunochemical reactivity is indicated as in Fig. 3. The elution volume of chymotrypsinogen (mol wt 25,000) is marked by the arrow. The exclusion volume as determined by absorbance on the right ordinate with dextran blue is indicated by □□□□□□. The protein concentration is on the left ordinate and is indicated by ●●●●●.

Studies of the Protein in Human Plasma
Very Low Density Lipoproteins
Brown, Levy and Fredrickson JBC 1969; 244:5687
Studies of the Protein in Human Plasma
Very Low Density Lipoproteins
Brown, Levy and Fredrickson   JBC 1969; 244:5687
# SUMMARY OF APOLIPOPROTEINS AND FUNCTIONS—PART II

## The Plasma Apolipoproteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Lipoprotein</th>
<th>Molecular Weight</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>apo-CI</td>
<td>HDL, chylomicrons, VLDL</td>
<td>6,000</td>
<td>Activator of LCAT</td>
</tr>
<tr>
<td>apo-CII</td>
<td>HDL, chylomicrons, VLDL</td>
<td><strong>9,000</strong></td>
<td>Activator of lipoprotein lipase</td>
</tr>
<tr>
<td>apo-CIII</td>
<td>HDL, chylomicrons, VLDL</td>
<td><strong>9,000</strong></td>
<td>Stabilizes surface, Provides negative charge</td>
</tr>
<tr>
<td>apo-D</td>
<td>HDL, chylomicrons*</td>
<td>21,000</td>
<td>Cholesterol ester exchange</td>
</tr>
<tr>
<td>apo-E</td>
<td>HDL, VLDL, chylomicrons*</td>
<td>34,000</td>
<td>Binds to receptor on cell membrane of liver (E and BE) and macrophage</td>
</tr>
</tbody>
</table>

*Only in nascent chylomicrons*
Effect of Apolipoprotein CII in Lipase Activity

Effect of Apolipoprotein CIII in Lipase Activity

Apolipoprotein CIII
Fig. 2. Relationship between total plasma triglyceride and apoC-III concentration as determined by radioimmunoassay in sera from a group of 176 middle-aged adult males ($r = 0.863$, $P < 0.001$).

Schonfeld, G.; George, P.K.; Miller, J.; Witztum, J.; Metabolism, 1979, (28) 1001-1010.
Enzyme Purification

I. Lipoprotein Lipase

II. Hepatic Triglyceride Lipase
The Purification of a Lipoprotein Lipase from Bovine Skim Milk


Elution of milk lipase from a heparin affinity column by increasing NaCl gradient. Arrows indicate peak enzyme activity and polyacrylamide gels document purity of the enzyme protein.
Purified Lipoprotein Lipase activity:
Inhibited by apolipoproteins CI, CII and CIII.

Assayed in the presence of increasing amounts of human apolipoproteins added to the triglyceride substrate.
Human Hepatic Lipase
Purified from Plasma
Enholm, Shaw, Greten and Brown  JBC 1975. 250:6756.

Isolated via two step process with heparin sepharose chromatography (A) and Con A Chromatography. Polyacrylamide gels after step 1 (A) and step 2 (B).

TG lipase activity  ○  PL lipase activity ▲
Human Hepatic Lipase Purified from Plasma


Localization of HTGL in situ in Rat Liver

Flourescent labeled anti-HTGL injected intravenously localizes in the capillaries of the sinusoids of liver.

Electron micrograph of ferritin labeled anti-rabbit antibody after perfusion of anti-HTGL antibody.

Functionality of Hepatic Triglyceride Lipase
HTGL activity vs TG rich Lipoproteins

HTGL is most active with triolein containing no apolipoproteins.

With lipoprotein triglycerides:
Least active with chylomicrons.

More active with VLDL.

Even more active with remnant sized LPs.

LPL is most active with chylomicrons and VLDL is only modestly elevated in patients with LPL deficiency.

Figure 3. Activity of partially-purified hepatic lipase against the triglyceride of synthetic and lipoprotein substrates (1 mmol/l TG). Values are expressed relative to the activity against triolein = 100%.
Inhibition HTGL by Antibody in Rat


Plasma concentrations before (Con) and four hours after antibody infusion.

(Values are average of six animals)
Inhibition of HTGL with Specific Antibody in Cynomolgus Monkey

A.
Complete block of HTGL activity slowed conversion of VLDL–remnant apoB to IDL and markedly slowed IDL conversion to LDL.

B.
Non-immune IgG had no effect
Inhibition of HTGL with Antibody in Cynomolgus Monkeys

ANALYTICAL ULTRACENTRIFUGATION

Difference plots with the lipoprotein mass at baseline subtracted from the lipoprotein mass after 3 hours of HTGL inhibition by antibody infusion.

Goldberg, Le, Paterniti, Ginsberg, Lindgren, and Brown
J Clin Invest. 1982; 70: 1184-1192
Genetically Deficient HTGL

Particles separated by size on gel filtration.

NORMAL
Cholesterol and Triglycerides before and after IV heparin

<table>
<thead>
<tr>
<th></th>
<th>E3/E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>114 mg/dL</td>
</tr>
<tr>
<td>Chol</td>
<td>164 mg/dL</td>
</tr>
<tr>
<td>HDL-C</td>
<td>35 mg/dL</td>
</tr>
</tbody>
</table>

HTGL deficient #1
Triglycerides before and after IV heparin

<table>
<thead>
<tr>
<th></th>
<th>E3/E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>330 mg/dL</td>
</tr>
<tr>
<td>Chol</td>
<td>466 mg/dL</td>
</tr>
<tr>
<td>HDL-C</td>
<td>59 mg/dL</td>
</tr>
</tbody>
</table>

HTGL deficient #2
Triglycerides before and after IV heparin

<table>
<thead>
<tr>
<th></th>
<th>E3/E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>1128 mg/dL</td>
</tr>
<tr>
<td>Chol</td>
<td>375 mg/dL</td>
</tr>
<tr>
<td>HDL-C</td>
<td>35 mg/dL</td>
</tr>
</tbody>
</table>
Hepatic lipase is important in: VLDL remnant clearance and in generation of LDL.

Question:
How does apoCIII and other apolipoproteins interact with the HTGL and LPL?
HTGL is inhibited by apo C1, C2 and C3.
Apo CIII is the most effective inhibitor and the most common of these apolipoproteins.

Apolipoproteins inhibit uptake of triglyceride by perfused rat liver.

Rat liver perfusion experiments with C\textsuperscript{14} labeled triolein containing added human apolipoproteins in equal mass. ApoCIII demonstrated the greatest inhibition of triglyceride uptake with or without added apoE.

*Quarfordt et al. JBC 1982; 24:14642-14647.*
Apolipoprotein C-III

- Inhibits catabolism of triglyceride rich lipoproteins.
- Is it inhibiting lipid removal by its action on lipases or by inhibiting the uptake and degradation of the whole particle?
Lipase Apolipoprotein Interaction

- Apolipoproteins are removed by Lipase Action.
- Is there specificity with regard to apolipoprotein removal from VLDL by LPL versus HTGL?
- Does LPL remove apoCIII equally efficiently as removal of apoE?
Changes in Lipoprotein lipids and apolipoproteins by release of LPL and HTGL with heparin injection

44 YO Man:

TC - 196 mg/dL
TG - 183 mg/dL
HDL-C - 31 mg/dL

<table>
<thead>
<tr>
<th>LIPASE ACTIVITY (μmole FFA/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTGL - 45</td>
</tr>
<tr>
<td>LPL - 11</td>
</tr>
</tbody>
</table>

Rubenstein et al. JCI 1985;75:710-721
Adding purified LPL versus HTGL
Changing apoCIII Content of Remnants

Gel filtration of normal human plasma (fasting)

Adding LPL Activity to Normal Plasma.
apoCIII moves into HDL

Adding HTGL Activity to Normal Plasma.
apoCIII is not changed

Purified LPL and HTGL added to Normal Human Plasma
Lipoproteins in Humans with Lipoprotein Lipase Deficiency
Effects of Post Heparin Lipolytic Activity in Lipoprotein Lipase Deficiency

Baseline values

Post Heparin effect of HTGL release on apoE (above) and apoCIII below.

ApoE and ApoCIII in LPL Deficiency

LPL and HTGL release by heparin in normal persons moves both apoCIII and apoE from VLDL to HDL.

When HTGL is released by heparin in the absence of LPL, apoE moves to HDL but apo CIII remains on VLDL or chylomicrons.

Rubenstein et al. JCI 1985;75:710-721
Lipoproteins in Humans with Hepatic Triglyceride Lipase Deficiency
Heparin released lipase effects in Normal versus HTGL deficient

### LPL deficient
- TG: 630 mg/dL
- Chol: 124 mg/dL
- HDL-C: 12 mg/dL
- Apo E moves to HDL but not apoCIII

### HTGL deficient # 1
- TG: 330 mg/dL
- Chol: 466 mg/dL
- HDL-C: 59 mg/dL

### HTGL deficient # 2
- TG: 1128 mg/dL
- Chol: 375 mg/dL
- HDL-C: 35 mg/dL

Active LPL moves apoCIII to HDL but not apoE.
Genetic Deficiency of apoCIII
Does altering the content of apoCIII alter the metabolism of triglyceride rich lipoproteins?

- ApoCIII transgenic mice develop severe hypertriglyceridemia with a gene dose response.
- ApoCIII KO mice have profound reductions in plasma triglycerides with clearance rates 4 - 5 X normal.
- Familial apoCIII deficiency is associated with TG concentrations in the 20 to 50 Mg/dl range and with fractional clearance rates of plasma TG 4 - 5X normal.
Triglyceride Kinetics in apoCIII and apoAI Deficiency

Note the rapid clearance of VLDL triglycerides in both patients compared to the normal control. (Endogenous injection of $[^3H]$ glycerol)
In the study of two young women with apoCIII deficiency there was very rapid clearance of VLDL and IDL triglycerides and apoB.

**LDL apoB conversion rates** from VLDL were the same as women of similar age but with desirable lipoprotein concentrations.

This strongly indicates that the remnant particles from VLDL hydrolysis are not being directly cleared in apoCIII deficiency.
Degradation of Very Low Density Lipoprotein in Blood Plasma

Liver

Apo B-100

VLDL

LPL

Cell surface receptors

Liver Cell

RLP

HTGL

FREE FATTY ACIDS
MONOGLYCERIDES
LYSOLECITHIN

LTGL

Liver Cell

Muscles

Adipose

HTGL

TG Depleted
Degradation of Very Low Density Lipoprotein in Blood Plasma

Liver

Apo B-100

VLDL

LPL

Cell surface receptors

Liver Cell

RTL

HTGL

Particle number depleted

MUSCLE

ADIPOSE
If the major effect of enhanced apoCIII on remnants is to inhibit HTGL, the reduction of apoCIII and enhanced action of HTGL should be the increased conversion of remnants to LDL and increase in LDL apoB concentration.
Pharmacological suppression of apoCIII with IsisApoC3Rx, an antisense oligonucleotide
ASO treatment of Hypertriglyceridermia.

1. RDBCT - placebo versus weekly doses of **ISIS 304801**
2. Triglycerides at baseline: 200 - 1400 mg/dL.
3. Fifty seven patients randomized to 4 groups: placebo, 100, 200, 300 mg/wk.

Change in Triglycerides

Change in HDL-C

The LPL Independent Pathway

Figure 2. Plasma Triglyceride Metabolism and the Role of APOC3.

ASO Therapy and Percentage Changes in Lipoprotein Classes

![Graph showing percentage changes in lipoprotein classes after ASO therapy.]
IsisApoCIII RX in LPL Deficiency

3 subjects homozygous or compound heterozygotes (P207L or Gi88E with variants)
LDL-C change following treatment with IsisApoC3Rx in LPL Deficiency
Summary

• LPL and HTGL seem to work in sequence to remove lipids from TG rich lipoproteins.

• LPL initially and preferentially removes apoCIII and triglycerides from large particles.

• HTGL follows with high activity against smaller apoCIII depleted particles and preferentially removes apoE.

• HTGL is particularly sensitive to apoCIII inhibition and in the absence of apoCIII becomes a much more effective triglyceride lipase with larger TG rich lipoproteins.
In apoC3 deficiency, is the triglyceride reduction due to reduction of lipase inhibition or due to the increase in direct remnant particle uptake by the liver through various receptors such as the LDL-R, the VLDL-R, or LRP?

The evidence in favor of reduced inhibition on both LPL and HTGL:
1. ApoC3 inhibits both LPL and HTGL in vitro.
2. In complete apoC3 deficiency, the production rates of LDL apoB are normal and LDL-C concentrations are within normal limits (2 patients).
3. ASO inhibition of apoC3 concentrations by 80% do not change apo B concentrations and LDL particle size and cholesterol concentrations increase.

The evidence for direct remnant uptake is:
1. Based mainly on VLDL triglyceride reduction on liver perfusion studies.
2. The assumption that the only lipase inhibition is LPL inhibition.
3. LDL cholesterol concentrations are slightly lower in the Amish and with some other patients with dysfunctional apoC3 gene polymorphisms but this is not consistent. Other groups with dysfunctional apoC3 have LDL within normal limits.
The Importance of Efficient Coordinated Action of:

1. Lipoprotein Lipase
2. Hepatic Lipase
3. Apo- CIII
4. Apo-CII
5. Apo-E
Triglycerides over 200 & HDL-C <40 mg/dL

Hypothesis:

• Probably polygenic factors producing dysfunction between apoCIII concentrations and HTGL activity with persistence of VLDL remnants in plasma.

• Major risk factor

• Persists after statin treatment

• Very common (10 to 20 % of many populations)

• Remnants and risk reduced when apoCIII is reduced — (fibrates and eicosapentaenoic acid).
## Prevalence of Elevated TG in US Adults

### NHANES (1999-2008)

<table>
<thead>
<tr>
<th>20+ yrs</th>
<th>➥150</th>
<th>➥200</th>
<th>➤500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>31%</td>
<td>16%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Men</td>
<td>35%</td>
<td>20%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Women</td>
<td>27%</td>
<td>13%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Heritage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican</td>
<td>35%</td>
<td>20%</td>
<td>1.4%</td>
</tr>
<tr>
<td>African</td>
<td>16%</td>
<td>8%</td>
<td>0.4%</td>
</tr>
<tr>
<td>European</td>
<td>33%</td>
<td>18%</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

Is the inhibition of Lipoprotein Lipase the major reason for elevated TG in human plasma?

**Figure 1.** Correlations between postprandial lipoprotein lipase (LPL) activity and mass vs VLDL₁-triglyceride (TG) fractional catabolic rate (FCR). Correlations between VLDL₁-TG FCR and postprandial measurements of (A) LPL activity ($r=0.08$, NS) or (B) mass ($r=0.05$, NS). $n=46$ in all correlations.
Metabolic Consequences of Hypertriglyceridermia

Nutrient flow from intestine and muscle.

Liver

VLDL

HDL

LDL

Apo A-I

Apo B-100

Insulin Resistance

↑ FFA

DGAT

Glycerol

LPL

CETP

TG

CE

HTGL

Small, dense HDL

Small, dense LDL

What is the evolutionary advantage of having apoCIII?

A protein that inhibits the clearance of TG rich lipoproteins.

A protein that leads to atherogenic remnant accumulation in plasma.

A protein that is clearly associated with the incidence of vascular disease.
Survival of TG Rich Lipoproteins

Hepatic TG lipase
Endothelial lipase
LDL receptor
LRP receptor

Chylomicron synthesis

VLDL
Dietary Cholesterol
INTESTINE
Processing of VLDL remnants

- Biliary Secretion
- VLDL receptor
- HTGL
- LRP receptor

**High FLUX**

**VLDL remnant survival**
Processing of VLDL remnants

- LRP receptor
- VLDL receptor
- HTGL

VLDL remnant to LDL

LPL

E

CIII

B-100

Low FLUX

LDL
What happened to LDL-C and apoB?

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ASO (300 mg/wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apo CIII (mg/dL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placebo</td>
<td>22.2 ± 7.7</td>
<td>22.6 ± 6.3</td>
</tr>
<tr>
<td>ISIS ASO</td>
<td>22.6 ± 6.3</td>
<td>4.4 ± 2.0</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placebo</td>
<td>523 ± 370</td>
<td>559 ± 225</td>
</tr>
<tr>
<td>ISIS ASO</td>
<td>394 ± 227</td>
<td>140 ± 36</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placebo</td>
<td>33.0 ± 7.5</td>
<td>32.8 ± 8.8</td>
</tr>
<tr>
<td>ISIS ASO</td>
<td>33.6 ± 9.6</td>
<td>48.3 ± 15.1</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placebo</td>
<td>105.1 ± 56.3</td>
<td>64.6 ± 27.7</td>
</tr>
<tr>
<td>ISIS ASO</td>
<td>102.1 ± 43.4</td>
<td>116.5 ± 53.6</td>
</tr>
</tbody>
</table>