Lipoprotein Effects of Incretin Hormones: Implications for Clinical Practice

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Disclosure Information

The following relationships exist related to this presentation:

- **National Institutes of Health**
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- **Novartis Pharmaceuticals**: Research support
- **Abbott**: Consultant
- **Glaxo Smith Kline**: DSMB

No off label use of clinically approved agents will be discussed in this presentation.
Patients With Type 2 Diabetes May Spend 12 Hours Per Day in the Postprandial State

- Duration of Postprandial State
  - Breaks
    - Breakfast
    - Lunch
    - Dinner
  - Intervals
    - 8 AM
    - 11 AM
    - 2 PM
    - 5 PM

Relationship Between Post-Prandial State and CV Disease

**Post-Prandial Lipemia**

- CM and CM remnants induce atherosclerosis (1)
- RLP-C correlates with CIMT (2)
- Higher fasting RLP-C predicts CAD events (Honolulu Heart Study) (3)
- RLP-C predicts CAD incidence independent of TC/HDL-C/LDL-C (Honolulu HS) (4)
- In Type II DM contribution by both endogenous (VLDL) and CM to RLP depending on degree and duration of Type II DM (5)

2. Karpe et al. RLPs are related to IMT of carotid artery independent of LDL-C and plasma TG. J Lipid Res 2001; 42; 17-21

**Post-Prandial Hyperglycemia**

- DECODE reported that 2 h glucose concentrations after OGTT are better predictors of CV events and all-cause mortality than fasting blood glucose
- The Framingham Offspring Study, 2-h blood glucose predicted CV events better than A1C
- A meta-analysis of 38 prospective studies in non-diabetics confirmed a strong association between 2-h blood glucose with fatal and nonfatal cardiovascular events

**1964:** Plasma insulin higher with oral than IV glucose

**1969:** A factor from porcine intestine by Brown et al. termed GIP

### Incretin Factors (%)

**Oral glucose load**

- **Insulin (mU/l)**
  - 0
  - 20
  - 40
  - 60
  - 80

**Intravenous glucose infusion**

- **Insulin (mU/l)**
  - 0
  - 20
  - 40
  - 60

**Time (min)**

Control subjects (n=8)

- **Insulin (mU/l)**
  - 0
  - 20
  - 40
  - 60
  - 80

People with Type 2 diabetes (n=14)

- **Insulin (mU/l)**
  - 0
  - 20
  - 40
  - 60
  - 80

- **Contributions of Incretin Factors (%)**
  - 0
  - 20
  - 40
  - 60
  - 80


**Incretins/DPP4 : A Time Line**

- GLP-1 discovered as incretin and role in ↑ Insulin in 1987 (1,2)
- Exendin-4 isolated from Gila monster by John Eng 1990’s (3)
- 39 AA peptide >50% homology with native GLP-1 (3)
- DPP4 (CD26) discovered in 1960s as aminopeptidase. Incretin effect recognized 1993 (4,5)

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**GLP-1(7–36)**

```
HAEGETFTSVDVSSYLEGQAAAKEFIAWLVKG
HGEGETFTSDSLKQMEEEAVRLFIEWLKNGGPSSGAPP
```

Exendin-4

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GLP-1 and GIP Are the Two Major Incretins

- Produced by L cells in the distal gut (ileum, colon)
- Produced by K cells in the proximal gut
- Half life 1 minute
- Half life 10 minutes
- Suppresses glucagon secretion
- Stimulates glucagon secretion
- Inhibition of gastric emptying; ↓ food intake/wt
- Minimal effect on gastric emptying;
- Enhance b-cell proliferation/survival
- Enhances b-cell proliferation/survival
- Reduces Apo 48, CM and VLDL Production
- Increases Lipoprotein Production
- Insulinotropic Activity in Type II DM Preserved
- Insulinotropic Activity in Type II DM/IR Attenuated

GLP, glucagon-like peptide; GIP, glucose-dependent insulinothropic polypeptide.

<table>
<thead>
<tr>
<th>GLP-1</th>
<th>GIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Produced by L cells in the distal gut (ileum, colon)</td>
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GLP-1R-Dependent Signal Transduction Pathways in the Cardiomyocyte and Endothelial Cell

DPP4 consists of a short cytoplasmic domain (AA1-6), a transmembrane domain (AA7-29), and an extracellular domain (AA30-766). Transmembrane domain anchors DPP4 on the cell surface. The extracellular domain is responsible for the catalytic activity of DPP4, the binding of ADA and ECM components such as fibronectin and collagen.

## Incretin Based Therapies

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td><strong>GLP-1 Agonists</strong></td>
<td></td>
<td></td>
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<tr>
<td>Exenatide</td>
<td>5-10 mcg bid</td>
<td>A</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>1.2-1.8 qd</td>
<td>A</td>
</tr>
<tr>
<td>Exenatide LA</td>
<td>2 mg weekly</td>
<td>A</td>
</tr>
<tr>
<td>Taspoglutide</td>
<td>20-30 mg weekly</td>
<td>I</td>
</tr>
<tr>
<td>Albiglutide</td>
<td>30-50 mg weekly</td>
<td>I</td>
</tr>
<tr>
<td>AVA0010</td>
<td>5-30 mcg qd/bid</td>
<td>I</td>
</tr>
<tr>
<td>CJC-1134-PC</td>
<td>1.5-3.0 mg weekly</td>
<td>I</td>
</tr>
<tr>
<td>NN9535</td>
<td>0.1-1.6 mg weekly</td>
<td>I</td>
</tr>
<tr>
<td>LY2189265</td>
<td>0.25 mg weekly</td>
<td>I</td>
</tr>
<tr>
<td>LY2428757</td>
<td>0.5-17.6 mg weekly</td>
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<tr>
<th>Agent</th>
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<tr>
<td><strong>DPP-4 Inhibitors</strong></td>
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<tr>
<td>Sitagliptin</td>
<td>5-10 mcg bid</td>
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<td>Vildagliptin</td>
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<td>Linagliptin</td>
<td>20-30 mg weekly</td>
<td>A</td>
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<tr>
<td>Dutogliptin</td>
<td>30-50 mg weekly</td>
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<tr>
<td>Alogliptin</td>
<td>25 mg qd/bid</td>
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</tbody>
</table>

A=Approved
I=Investigational
Summary of pharmacological incretin action on different target tissues

- **Heart**:
  - Cardioprotection
  - Cardiac output

- **Brain**:
  - Neuroprotection
  - Appetite

- **Stomach**:
  - Gastric emptying

- **Pancreas**:
  - Insulin biosynthesis
  - Beta-cell proliferation
  - Beta-cell apoptosis

- **Liver**:
  - Insulin sensitivity
  - Glucose production
  - VLDL Production

- **Muscle**:
  - Insulin secretion
  - Glucagon secretion

- **Vasculature**:
  - Inflammation
  - Vasodilation
  - Endothelial function
  - Apo 48 production
  - CM Production

- **GLP-1**:
  - Insulin secretion
  - Glucagon secretion
Effect of DPP4i in Atherosclerosis

- Alogliptin reduces atherosclerosis and Adipose inflammation in High-Fat Fed Insulin Resistant LDLR⁻/⁻

- PKF275-055 reduces atherosclerosis in ApoE⁻/⁻

NV=Normal diet vehicle
ND=Normal diet Alogliptin
HV=High fat diet Vehicle
HD=High fat diet Alogliptin


Effects of GLP-1R Signaling on Lipoprotein Metabolism

↑ Insulin

↓ Plasma FFAs

↑ GLP-1R signaling in Gut

↓ Lipolysis

↓ Plasma FFAs

Direct

↓ Gastric emptying?

↓ TG absorption

Sympathetic enteric neurons?

?↓ Apo48

?↓ CM Biogenesis

↓ VLDL?

?↓ Competition for CM Clearance?

↓ Apo B100?

↑ FA β-oxidation?

DECREASED POST-PRANDIAL TG, VLDL AND CM REMNANTS
Lymph flow rate in lipid-infused rats treated with or without recombinant GLP-1

Qin X et al. Am J Physiol Gastrointest Liver Physiol 2005;288:G943-G949
Is it GLP-1 or GIP That is Involved in Reducing Post-prandial Lipoprotein Levels
GLP-1 but not GIP is Involved in Lipoprotein Effects

- GLP-1 analogues decrease post-prandial TRL and apo48 levels
- GLP-1R antagonists [exendin(9–39)], prevent GLP-1 and DPP-IV induced reduction in plasma triglycerides in mice [27].
- DPP-IV-resistant GIP peptides with a d-alanine substitution at position 2 found to augment postprandial plasma TG and apoB48 levels in mice
- GLP-1 receptor -/- mice exhibit greater postprandial levels of TRL-triglyceride and apo48 vs. controls

Is Gastric Slowing Important in Incretin Mediated Reduction in Post-prandial Lipids in Humans?
GLP-1 Abolishes Post-Prandial TG and NEFA

Are the Effects Due to Secondary Improvements in Glycemic Control and Weight Loss?
DPP 4 therapy reduces postprandial intestinal TG-rich lipoprotein particles in type 2 diabetes


DPP 4 Inhibition Reduces Post-Prandial Lipoproteins

16 week double-blind placebo controlled trial in Type II DM
Mixed Meal Challenge at end of treatment (n=71)

Fasting TG reduced by 50 mg/dl in Alo and 72 mg/dl Alo/Pio
Glucagon Levels suppressed in Alo, Alo/Pio

VLDL Apo B100 ↓ with Alo but not Alo/Pio

Reduction in Post-Prandial Lipids with Single Dose of Exenatide

N=35 new onset Type II DM or IGT; DBRC cross-over study; 10 mg of Exenatide or placebo SQ followed by Mixed meal

Reduction of Apo CIII with Single Dose of Exenatide

- Pre-meal
  - Apo CIII (mg/l)
  - Exenatide: 180
  - Placebo: 180

- Post-meal
  - Apo CIII (mg/l)
  - Exenatide: 120
  - Placebo: 160

References:
- Kawakami A et al. TLR2 Mediates Apo CIII Induced monocyte activation. Circ Res 2008; 103; 1402-09
Are the Effects Independent of Insulin and Glucagon?

Are the Effects due to Reduced Production of Apo 48 or Due to Enhanced Clearance or Both?
n=15 healthy normal volunteers

A

Mixed Meal

Pancreatic clamp

9am 5pm 4am 7am (-2 hr) 9am (0 hr) 7pm (10 hr)

Tube Infusion Hormel Shake

Nasoduodenal tube

B

Plasma TG (mmol/l)

Time (hr)

-4 -2 0 2 4 6 8 10

Placebo

Exenatide

C

TRL-TG (mmol/l)

Time (hr)

-4 -2 0 2 4 6 8 10

Placebo

Exenatide

D

Plasma FFA (mmol/l)

Time (hr)

-4 -2 0 2 4 6 8 10

Placebo

Exenatide

Triglyceride-rich lipoprotein (TRL)-apolipoprotein (apoB)-100 (A) and apoB-48 (C) concentrations, and fractional catabolic rate (FCR) and production rate (PR) for TRL-apoB-100 (B) and apoB-48 (D). *P<0.05 vs Placebo.


Copyright © American Heart Association
Are the Effects due to Concomitant Effects on Hepatic GLP-1 Receptor Activation and Reduced VLDL Production?
GLP-1 receptor activation inhibits VLDL production and reverses hepatic steatosis

E3Leiden mice fed HFD for 13-18 weeks and treated for 4 weeks with CNTO3649 (2 doses) or Exendin (2 doses).


*P<0.05, **P<0.01, ***P<0.001 compared to HFD controls.
How Do GLP-1 Agonists affect VLDL Production: Effects on hepatic expression of genes involved in VLDL production, lipogenesis and homeostasis


Expression of Acox 1 and Cpt1 markedly increased in muscle: Increased FA oxidation
GLP-1 Treatment Reduces Hepatic Fat

Pioglitazone (PIO, 45 mg/day, n=10) and exenatide (EX, 10 μg SQ bid for 12 months (n = 11). Hepatic Fat by NMR

PIO+EX associated with greater decrease in hepatic fat (12.1 ± 1.7 to 4.7 ± 1.3%) and plasma TG (38%) vs. PIO alone. No change in weight in PIO+EX vs. gain in weight in PIO alone.

Effects of GLP-1R Signaling on Lipoprotein Metabolism

- **Adipocytes**: 
  - $\uparrow$ GLP-1R signaling in Gut → $\downarrow$ Lipolysis → $\downarrow$ Plasma FFAs

- **Liver**: 
  - $\downarrow$ Apo B100 → $\uparrow$ Insulin → $\downarrow$ Apo B100 → $\uparrow$ FA β-oxidation → $\downarrow$ VLDL → $\downarrow$ Competition for CM Clearance?

- **Enterocytes**: 
  - $\downarrow$ Apo48 → $\downarrow$ CM Biogenesis

Direct effects: 
- **Direct**: $\downarrow$ Gastric emptying? $\rightarrow$ $\downarrow$ TG absorption

- **VLDL**: $\downarrow$

Decreased post-prandial TG, VLDL and CM remnants.
Summary

• Incretin therapy has substantial effects on post-prandial lipids and represent an important new treatment approach to target post-prandial lipids
• The effects are rapid and involve multiple mechanisms that are independent of weight loss
• These pathways include modulation of exogenous and endogenous lipoprotein pathways and involve both production and clearance of post-prandial lipids
• The implications of these changes on CV events await definitive trials
Macrovascular Outcome Trials*

<table>
<thead>
<tr>
<th>Drug (class)</th>
<th>Landmark name</th>
<th>Study population</th>
<th>Primary outcome</th>
<th>Dosing</th>
<th>Estimated enrollment</th>
<th>Duration (years)</th>
<th>End date</th>
<th>Identifier</th>
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</thead>
<tbody>
<tr>
<td>Alogliptin</td>
<td>EXAMINE</td>
<td>T2DM with ACS (within &gt;15 to &lt; 90 days)</td>
<td>Composite†</td>
<td>6.25 mg, 12.5 mg, or 25 mg qd‡</td>
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<td>4.75</td>
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<tr>
<td>Saxagliptin</td>
<td>SAVOR-TIMI 53</td>
<td>T2DM with multiple CRFǁ</td>
<td>Composite†</td>
<td>2.5 mg or 5 mg qd</td>
<td>16,500</td>
<td>4</td>
<td>2013</td>
<td>NCT01107886</td>
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<tr>
<td>Sitagliptin</td>
<td>TECOS</td>
<td>T2DM with preexisting CVD</td>
<td>MACE§</td>
<td>50 mg or 100 mg qd‡</td>
<td>14,000</td>
<td>5</td>
<td>2014</td>
<td>NCT00790205</td>
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<tr>
<td>Linagliptin</td>
<td>CAROLINA</td>
<td>T2DM with preexisting CVD**</td>
<td>MACE§</td>
<td>5 mg qd</td>
<td>6,000</td>
<td>7.5</td>
<td>2018</td>
<td>NCT01243424</td>
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</table>

**GLP-1-Ra Outcome Trials**

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<thead>
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<th>Dosing</th>
<th>Estimated enrollment</th>
<th>Duration (years)</th>
<th>End date</th>
<th>Identifier</th>
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<tbody>
<tr>
<td>Lixisenatide</td>
<td>ELIXA</td>
<td>T2DM and ACS (within &gt;180 days)</td>
<td>MACE§</td>
<td>20 µg qd</td>
<td>6,000</td>
<td>4</td>
<td>2014</td>
<td>NCT01147250</td>
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<tr>
<td>Liraglutide</td>
<td>LEADER</td>
<td>T2DM with CVD or CRF or CKD or CHF</td>
<td>Composite†</td>
<td>1.8 mg qd</td>
<td>9,340</td>
<td>5</td>
<td>2016</td>
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<td>Exenatide</td>
<td>EXSCEL</td>
<td>T2DM</td>
<td>Composite†</td>
<td>2 mg once weekly</td>
<td>9,500</td>
<td>5.5</td>
<td>2017</td>
<td>NCT01144338</td>
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</table>

*Available at http://www.clinicaltrials.gov. Accessed April 30, 2013; †CV death, nonfatal MI, and nonfatal stroke; ‡Based on renal function; ǁAbbreviations: CRF, cardiovascular risk factors; CVD, cardiovascular disease; CKD, chronic kidney disease; CHF, chronic heart failure; §CV death, nonfatal MI, nonfatal stroke, or unstable angina requiring hospitalization; **CVD or specified diabetes end-organ damage or age ≥ 70 years or ≥ 2 RF.