GLP-1 e Sistema Cardiovascolare: Fisiologia

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Diapositiva preparata da FRANCESCO GIORGINO e ceduta alla Società Italiana di Diabetologia.

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Disclosures

- **Advisory Boards**: AstraZeneca/BMS; Eli Lilly; Roche Diagnostics; Takeda
- **Consultant**: AstraZeneca/BMS; Boehringer Ingelheim; Lifescan; Merck; Sharp & Dohme; Novo Nordisk; Sanofi
- **Research Support**: AstraZeneca/BMS; Eli Lilly; Lifescan; Sanofi; Takeda
GLP-1 RA and Vascular Outcomes in Type 2 Diabetes - 2016

HbA1c

MACE
- CV Mortality
- Non-fatal MI
- Non-fatal Stroke

Renal Outcomes
(AER, Nephropathy)

GLP-1 RA
- Lixisenatide
- Liraglutide
- Semaglutide
Primary Endpoint and Its Individual Components in ELIXA, LEADER, SUSTAIN-6 and EXSCEL

- **Primary composite MACE**: 0.81 (ns)
- **Cardiovascular mortality**: 0.02 (ns)
- **Myocardial infarction**: 0.01 (ns)
- **Stroke**: 0.04 (ns)
- **Unstable angina**: ns

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ELIXA

- **Hazard ratio (95% CI)**: 1.61, 1.41, 1.21, 1.00, 0.80, 0.60, 0.40
- **P value**: 0.01, 0.007, 0.046, ns

LEADER

- **Hazard ratio (95% CI)**: 1.61, 1.41, 1.21, 1.00, 0.80, 0.60, 0.40
- **P value**: 0.01, 0.007, 0.046, ns

SUSTAIN-6

- **Hazard ratio (95% CI)**: 1.61, 1.41, 1.21, 1.00, 0.80, 0.60, 0.40
- **P value**: 0.02, ns

EXSCEL

- **Hazard ratio (95% CI)**: 1.61, 1.41, 1.21, 1.00, 0.80, 0.60, 0.40
- **P value**: 0.06, ns

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CI, confidence interval; MACE, major adverse cardiovascular event; ns, not significant.
Potential Factors in CV Outcomes Trials with GLP-1 RAs

Baseline characteristics of study population (age, diabetes duration, HbA1c, % CV disease, etc.)

Study setting and follow-up

Drugs allowed in “usual care” arm

Discontinuation rate

GLP-1 RA vs. “Usual Care”

Reductions in HbA1c, blood pressure, lipids

Reduction in body weight

Reduction in hypoglycemia

Direct effects on CV system (anti-inflammatory, anti-atherosclerotic) and other targets

CV Benefit

CV Mortality

Non-fatal MI

Non-fatal Stroke

Unstable Angina

↓ All-cause Mortality

CV, cardiovascular; GLP-1 RA, glucagon-like peptide-1 receptor agonist; HbA1c, glycated haemoglobin; HF, heart failure; SGLT2-Is, sodium glucose transporter-2 inhibitors.
Glucagon-Like Peptide-1 (GLP-1) Secretion

GIP, glucose-dependent insulinotropic polypeptide; DPP-4, dipeptidyl peptidase-4
Adapted from Baggio et al., Gastroenterology, 2007
GLP-1 Derives from Proglucagon

Proglucagon gene
Proglucagon mRNA
Proglucagon protein
Tissue-specific posttranslational processing

GRPP: Glicentin-related polypeptide; GLUC: Glucagon; IP-1: intervening peptide-1; MPGF: proglucagon fragment; OXM: oxyntomodulin; IP-2: intervening peptide-2
Multiple Forms of GLP-1 Are Secreted in vivo

- Multiple forms of GLP-1 are secreted in vivo, including GLP-1(1-37) and GLP-1(1-36)NH₂, which are thought to be inactive, and GLP-1(7-37) and GLP-1(7-36)NH₂, which are biologically active.

- GLP-1(7-37) and GLP-1(7-36)NH₂ are produced from their full-length precursors by the action of prohormone convertase (PC)1/3 and appear to be equipotent in their ability to stimulate insulin secretion.

- In humans, the majority of GLP-1 in the circulation is GLP-1(7-36)NH₂.
GLP-1 Signaling in Beta-Cells


- **Proliferation / Inhibition of Apoptosis**
- **Differentiation of β-Cell Progenitors**

**GLP-1R**
- cAMP
- PKA
- Epac

**EGFR**
- Foxo1
- B-Raf
- MEK
- ERK

**Insulin Secretion**
- IRS-2 → PI 3-K
- PDX-1 → AKT

**Insulin Gene Expression**
- PDX-1

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# Differentiation of GLP-1 RAs: Glycemic Targets and Mechanisms of Action

**Parameters**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Short-acting (Prandial) GLP-1 receptor agonists</th>
<th>Long-acting GLP-1 receptor agonists</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exenatide (twice daily)</td>
<td>Liraglutide (once daily)</td>
</tr>
<tr>
<td></td>
<td>Lixisenatide (once daily)</td>
<td>Albiglutide (once weekly)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dulaglutide (once weekly)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exenatide LAR (once weekly)</td>
</tr>
<tr>
<td>Half-life</td>
<td>2–5 hours</td>
<td>12 hours – several days</td>
</tr>
<tr>
<td>Receptor activation</td>
<td>Intermittent</td>
<td>Continuous</td>
</tr>
</tbody>
</table>

**Effects**

- **Fasting glucose reduction**: Modest, Strong
- **Fasting insulin secretion**: No effect, Stimulation
- **Fasting glucagon secretion**: Mild reduction, Strong reduction
- **Postprandial glucose excursion**: Strong reduction, Modest reduction
- **Postprandial glucagon secretion**: Strong reduction, Mild reduction (?)
- **Postprandial insulin secretion**: Reduction, Stimulation
- **Gastric emptying**: Strong deceleration, Mild deceleration
- **HbA1c reduction**: -0.6–1.2%, -0.9–1.6%

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Meier JJ. *Nat Rev Endocrinol* 2012; 8: 728-742
Amino Acid Residues in the GLP-1 Receptor That Are Important for GLP-1, Oxyntomodulin, and Exendin-4 Binding and Signaling

Residues highlighted in red reduce function of all three peptides, those in pink selectively reduce GLP-1 only, those in yellow selectively reduce exendin-4 only, and those in green selectively reduce oxyntomodulin only.

A large number of residues are important for both GLP-1 and exendin but not oxyntomodulin, and these are highlighted in orange.

Other colors represent either enhanced function or existence of opposite effects when mutated on oxyntomodulin compared to GLP-1 and exendin-4.

Wootten et al., Cell 2016;165:1632–1643.
GLP-1 Effects on CV Risk through Direct and Indirect Actions in Multiple Organs

- Kidney: ↑ Natriuresis, ↑ Diuresis
- Heart: ↓ Postprandial lipids, ↑ Cardioprotection
- Platelets: ↓ Coagulation
- Blood vessel: ↓ Blood pressure
- Fat & other tissues: ↓ Glucagon secretion, ↑ Insulin secretion, ↑ Insulin biosynthesis, ↓ Glucose, ↓ Hypoglycaemia, ↓ Apoptosis, ↓ Inflammation
- Intestine: ↑ Glucagon secretion
- Brain: ↓ Body weight, ↓ Blood pressure, ↓ Hypoglycaemia

CV, cardiovascular; GLP-1, glucagon-like peptide-1
Drucker DJ. Cell Metab 2016;24:15–30

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Direct and Indirect Actions of GLP-1 in the Heart and Blood Vessels

GLP-1↓
- Inflammation
- Smooth muscle cell proliferation
- Platelet aggregation
GLP-1R

GLP-1↑
- Vasodilation
- Plaque stability
- Blood flow
- Smooth muscle cell proliferation
- Endothelial function
- Platelet aggregation

GLP-1R

↓ Inflammation
↑ LV function
↓ Ischaemic injury
↑ Heart rate

CV, cardiovascular; GLP-1, glucagon-like peptide-1
Drucker DJ. Cell Metab 2016;24:15–30
# Effects of GLP-1 or GLP-1 Receptor Agonists in Human Studies, with Potential Impact on Cardiovascular Function

<table>
<thead>
<tr>
<th>Effect</th>
<th>GLP-1 [7-36 amide or 7-37]</th>
<th>Liraglutide</th>
<th>Exenatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardioprotection against ischemia</td>
<td>↑ LVEF; ↑ regional wall motility</td>
<td>preserved LVEF after PCI/NSTEMI</td>
<td>↑ salvage index after STEMI; ↓ infarct size</td>
</tr>
</tbody>
</table>

ACh, acetylcholine; CPC, cardiac progenitor cell; EC, endothelial cell; HUVEC, human umbilical vein endothelial cell; IL, interleukin; LVEF, left ventricular ejection fraction; NOS, nitric oxide synthase; NSTEMI, non ST-elevated myocardial infarction; PCI, primary coronary intervention; T2D, type 2 diabetics; TNF, tumour necrosis factor.

Adapted from Nauck MA, et al., Circulation 2017;136:849-870.
Cardioprotective Effects of Exenatide in Patients With Myocardial Infarction Undergoing Primary PCI

GLP-1 RA and Cardioprotection

PBS vs Exenatide

Infarcted area (white)
Area at risk (red)
Healthy area (blue)

Vehicle Lixisenatide
GLP-1 Receptor Stimulation Preserves Primary Cortical Neurons in Rodent Models of Stroke

Li Y, PNAS, 2009
GLP-1 Mimetic Decreases Inflammatory Signaling and Atherosclerotic Lesion Area in a Murine Model

Treatment with Exendin-4 decreased inflammatory and adhesion molecules on monocytes and macrophages and reduced atherosclerotic lesion area.

*P<0.05 vs. control

CD11b, monocyte adhesion molecule; Ex4, exendin-4; MDL, MDL-12330A (a cAMP inhibitor); NF-kB, Nuclear factor kappa B.

Arakawa M et al. Diabetes. 2010;59:1030-1037
Liraglutide Reduced Obesity-induced Perturbations in Cardiac Endothelial NO Synthase and Markers of Hypertrophy and Inflammation

- Effect of 1-week treatment with liraglutide (30 µg/kg) in mice fed on 45% high-fat diet

Markers of coordinated electrical activity (Connexin-43) and regulation of coronary blood flow (eNOS)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Connexin-43</th>
<th>eNOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular diet – placebo</td>
<td>[value]</td>
<td>[value]</td>
</tr>
<tr>
<td>HFD – placebo</td>
<td>[value]</td>
<td>[value]</td>
</tr>
<tr>
<td>HFD – liraglutide</td>
<td>[value]</td>
<td>[value]</td>
</tr>
</tbody>
</table>

Markers of hypertrophy

- ANP
- BNP
- α-MHC
- β-MHC

Inflammatory markers

- TNF-α
- NFκB

*P<0.05 vs regular diet – placebo; †P<0.05 vs high-fat diet – placebo, by Tukey multiple comparison post-hoc test

eNOS, endothelial nitric oxide synthase; HFD, high-fat diet

Atherosclerosis is Associated with Reduced Levels or Dysfunction of Circulating Endothelial Progenitor Cells (EPCs)

Movement of endothelial progenitor cells in health and diabetes

**DIABETES**

- Glycation ↑
- HIF-1α ↓
- NO ↓
- SDF-1α ↓

**Hyperglycaemia**

- Oxidative stress
- MAPK overactivity

**SDF-1α/CXCR4 ↓ Apoptosis ↑ Survival ↓**

**Bone marrow**

- Mobilisation

**Target tissues/organs**

- EPCs
- Homing
- Survival

**Physiology**

AGE, advanced glycation end-product; CRP, C-reactive protein; EPC, endothelial progenitor cell; FFA, free fatty acid; HIF-1α, hypoxia-inducible factor-1α; MAPK, mitogen-activated protein kinase; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; SDF-1α, stromal cell-derived factor-1α; TG, triglyceride; TNFα, tumour necrosis factor α; VLDL, very low-density lipoprotein


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The Heart is a Self-renewing Organ

Cardiac stem cells

- c-kit
- MDR-1
- Sca-1

Endothelial cell
Cardiomyocyte
Smooth muscle cell

Cardiac disease = cardiac stem cell compartment disease?

Figure adapted from Anversa P, et al. *Circulation* 2006;113:1451–1463.
Diabetes Mellitus Impairs Human Cardiac Stem Cell Function

Leonardini A, et al. unpublished

CSC, cardiac stem cell; LAD, left anterior descending; LVEF, left ventricular ejection fraction

Molgat AS, et al. Circulation 2014;130:S70–S76

CSC yield

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSC yield</td>
<td>1,500,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Change in LVEF (28 days post-LAD ligation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in LVEF</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Total tubule length (mm) or number of migrated cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Angiogenic capacity

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogenic capacity</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.04</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Chemotactic capacity

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotactic capacity</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.04</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>
Isolation and Characterization of Human CPC

Phenotypic Profile of Human CPC

Multilineage Differentiation Potential of Human CPC

Osteoblast
Alizarin Red

Chondroblast
Alcian Blue

Adipocyte
Oil Red O

Laviola L. et al, Endocrinology, 2012
The GLP-1 Receptor in Human CPC

Relative mRNA level

1. Marker
2. CPC
3. Heart

ASC, adipose tissue-derived stem cells; BMSC, human bone marrow stem cells; CPC, cardiac progenitor cells; CREB, cAMP-response-element binding protein; GLP-1, glucagon-like peptide-1; HUVEC, human umbilical vein endothelial cells; RTSC, human renal tubular stem cells

Mechanisms of Lipotoxicity in the Heart

**Diabetes and/or obesity**
- Increased FA delivery

**Heart failure**
- Impaired FA oxidation

**Intramyocardial lipid overload**
(oil-red-O stain for triglycerides)

- Diabetes and/or obesity
- Increased FA delivery
- Heart failure
- Impaired FA oxidation

- Intramyocardial lipid overload

- DAG, diacylglycerol; FA, fatty acid; ROS, reactive oxygen species

- ↑ Apoptosis
- ↑ Autophagy
- ↑ Senescence
- Contractile dysfunction


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DAG, diacylglycerol; FA, fatty acid; ROS, reactive oxygen species
Ceramide Production

Bickman B. et al., J Clin Invest., 2011
Exendin-4 Inhibits de Novo Production of Ceramide and Apoptosis in Human Cardiac Progenitor Cells

Leondardini A, D’Oria R et al. JCEM 2017
Exendin-4 Prevents Induction of Ceramide-Synthase 5 (CerS5) in Human Cardiac Progenitor Cells

- Palmitoyl CoA + Serine
- 3-keto-Sphinganine
- Sphinganine
- Dihydroceramide
- Ceramide

PalmitoylCoA Synthase
Serine Palmitoyltransferase
Ceramide Synthase-5
Dihydroceramide desaturase

- Relative mRNA level
- * p < 0.05 Palm vs basal
- # p < 0.05 Ex-4+Palm vs Palm

Leondardini A, D'Oria R et al. JCEM 2017
Role of Autophagy in Cell Survival

Adapted from Nishida K. et al., Circ Res. 2008
Exendin-4 Prevents Palmitate-Induced Autophagosome Formation

Monodansylcadaverine Staining

BASAL

PALMITATE

EXENDIN-4

EXENDIN-4 + PALMITATE
GLP-1R Activation Increases Human Cardiac Progenitor Cell (CPC) Survival


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Mechanisms Mediating a Beneficial Effect of GLP-1R Activation on Cardioprotection

Adapted from Nauck MA, et al., Circulation 2017;136:849-870
GLP-1 is Metabolized by the Enzyme DPP-4

GIP, glucose dependent insulinoatropic polipeptide; DPP-4, dipeptidyl peptidase-4

Adapted from Baggio et al., Gastroenterology, 2007
DPP-4 in the Cardiovascular System

- DPP-4 is widely expressed in most cells and tissues and exhibits enzymatic activity against dozens of chemokines and peptide hormones with roles in inflammation, vascular function, stem cell homing, and cell survival (Giacco et al., 2015).

- DPP-4 exhibits exopeptidase activity through its 2 principal molecular forms, a membrane-tethered 766 amino acid protein with a small intracellular tail and a soluble form that is 39 amino acids smaller, devoid of the short membrane spanning domain and intracellular tail, and yet otherwise structurally identical (Giacco et al., 2015).

- Although soluble DPP-4 exerts vascular, immune, and proinflammatory actions independent of its catalytic activity, the majority of the experimental literature has studied the importance of DPP-4-mediated peptide cleavage in the pathophysiology and treatment of cardiovascular disease.
GLP-1 RA CV Outcomes Trials - Discussion

- Selective and potent GLP-1 R activation (vs. DPP-4 inhibition)
- Mode of GLP-1 R activation (continuous vs intermittent)
- Glp-1 R signaling before, during or after CV event
- Direct anti-inflammatory, anti-atherosclerotic, anti-apoptotic/pro-survival effects
- Reduction of CV risk factors (HbA1c, weight, BP, lipids, CRP)
- Population characteristics (less vs. more advanced CVD)
- MACE component being affected