THE ROLE OF GLUCAGON IN FASTING AND POSTPRANDIAL HYPERGLYCEMIA IN DIABETES MELLITUS: AN UPDATE

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MILESTONES IN THE DISCOVERY AND RENAISSANCE OF GLUCAGON

1922 Banting and Best administer pancreatic extract to diabetic dogs and observe decreased blood glucose; a transient increase in blood glucose is noted

1923 Hyperglycemic substance in pancreatic extract named "glucagon"

1923 Banting and Macleod awarded Nobel Prize for discovery of insulin treatment, establishing its primary role in diabetes

1925 Sutherland and DeDuve define the alpha cells

1948 Unger recognizes glucagon's role as counterregulatory partner to insulin in Banting Lecture

1957 Bromer identifies amino acid sequence, paving the way for commercially available glucagon

1959 Unger develops glucagon radioimmunoassay

1968 Unger identifies 2 distinct glucagon-like peptides

1968 Valverde and Unger identify glucagon-like peptide-1 (GLP-1)

1975 Unger receives 2014 Rolf Luft Award from Karolinska Institutet for work on glucagon

1975 International Symposium focuses on glucagon's role in diabetes

1981 DeFronzo includes alpha cell as part of "Ominous Octet"

2008 DeFronzo includes glucagon as part of "Ominous Octet"

2014 Unger receives 2014 Rolf Luft Award from Karolinska Institutet for work on glucagon

GIP=glucose-dependent insulinotropic polypeptide; GLP-1=glucagon-like peptide-1.

PHYSIOLOGIC OCTET OF GLUCAGON ACTION: SOME GOOD AND SOME BAD
Campbell & Drucker, Nature Rev Endocrinal, April, 2015

REGULATION OF GLUCAGON SECRETION
GLUCAGON – AACE-NASHVILLE-2015

PROGLUCAGON-DERIVED PEPTIDES: TISSUE-SPECIFIC POST TRANSLATIONAL PROCESSING

Amino acid residue:

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<th></th>
<th>1</th>
<th>30</th>
<th>64</th>
<th>69</th>
<th>78</th>
<th>107/111</th>
<th>123</th>
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<tr>
<td>GRPP</td>
<td></td>
<td>Glucagon</td>
<td>IP-1</td>
<td>GLP-1</td>
<td>IP-2</td>
<td>GLP-2</td>
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Glicentin

MPGF

Oxyntomodulin

PROHORMONE CONVERTASE 1/3

PROHORMONE CONVERTASE 2

Pancreas

Glucagon

Glucagon

GI Tract

Glucagon

Brain

Oxyntomodulin

Miniglucagon

GLP-1

GLP-2

MPGF

ALPHA CELLS AND BETTA CELLS SHARE MANY SIMILARITIES

Sandoval & D’Alessio Physiol Rev 95:513-548, 2015

β-Cell

Glucose ➔ Glycolysis ➔ AcCoA ➔ ATP/ADP ➔ TCA ➔ ATP/ADP ➔ Glucagon ➔ Exocytosis

α-Cell

Glucose ➔ Glycolysis ➔ AcCoA ➔ ATP/ADP ➔ TCA ➔ ATP/ADP ➔ Insulin ➔ Exocytosis

OXYNTOMODULIN

- Inhibits gastric acid secretion
- Modulates hydromineral transport in the small intestine
- Promotes weight loss in humans by:
  1. inhibiting hunger center in arcuate nucleus of hypothalamus
  2. increasing energy expenditure
  3. blocking the orexigenic effect of ghrelin


MINIGLUCAGON

- Formed from glucagon by cleavage at N-terminus of basic residues by aminopeptidase
- ~5% of glucagon stores in the alpha granules are converted to miniglucagon
- At sub-picomolar concentrations, miniglucagon inhibits glucose-, sulfonylurea-, glucagon-, and GLP-1-stimulated insulin secretion by blocking calcium secretion
- Physiologic significance in man remains to be determined

Dalle S et al, Diabetes 51:406-12, 2002
MOLECULAR REGULATION OF GLUCAGON BIOSYNTHESIS AND SECRETION


α Cell Differentiation

Glucagon Biosynthesis

GLUCAGON

Neuro D1/B2 cMaf MafB

Glucagon Secretion

Pax6/4

Pax 6 - rats
Pax4 - mammals

PAX controls PC2 gene transcription (proglucagon glucagon) through Neuro D1/B2 and cMaf and directly/indirectly activates the glucagon gene

ALPHA CELLS INTEGRATE SIGNALS FROM MULTIPLE SOURCES DURING HYPOGLYCEMIA

Blood Glucose

Insulin/Zinc (Paracrine Signaling)

Glucose Sensing

Insulin Secretion

Delta Cell (Tonic Inhibition From SST)

Alpha Cell (Intrinsic Regulation)

Gluconeogenesis

Glycogenolysis

CNS=central nervous system; SST=somatostatin.
HIERARCHY OF DEFENSE AGAINST HYPOGLYCEMIA IN DIABETES

(1) Decrease in insulin secretion by β-cell

(2) Increase in glucagon secretion by α-cell

(3) Absent glucagon, an increase in epinephrine secretion by the adrenal gland

All of the above are impaired in long-standing, poorly controlled T1DM and T2DM patients


ALPHA CELLS INTEGRATE SIGNALS FROM MULTIPLE SOURCES DURING HYPERGLYCEMIA
Early understanding of islet morphology was based on murine data, but availability of human islets showed marked differences.\(^1,2\)

**Mouse islets**
- Alpha and delta cells surround a core of beta cells.\(^1,2\)
- Beta cells account for 75% of islet cells.\(^2\)

**Human islets**
- Alpha, beta, and delta cells are intermingled throughout islet.\(^1,2\)
- Fewer beta cells, more alpha and delta cells.\(^2\)

Cytoarchitecture of human islet suggests paracrine interactions.\(^1\)

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**REGULATION OF GLUCAGON SECRETION**

- **Glucose** - hypoglycemia stimulates and hyperglycemia inhibits glucagon secretion
- **Insulin** - inhibits glucagon secretion; conversely ↓ beta (cell mass ↓ insulin secretion) and deletion of insulin receptor from alpha cell → ↑ glucagon
- **Zinc and Glutamate** - released by beta cell and inhibit glucagon secretion (importance unknown)
- **Somatostatin** - tonic inhibition of glucagon secretion

---

**REGULATION OF GLUCAGON SECRETION**

- **Glucose**: hypoglycemia stimulates and hyperglycemia inhibits glucagon secretion

- **Insulin**: inhibits glucagon secretion; conversely; ↓ insulin secretion (↓ beta cell mass) and deletion of insulin receptor from alpha cell glucagon ↑

- **Glucagon-like Peptide-1**: inhibits glucagon secretion; controversial whether GLP-1 receptors are present on alpha cells

- **Somatostatin**: tonic inhibition of glucagon secretion

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**REGULATION OF GLUCAGON SECRETION**

- **Autonomic Nervous System**: SNS, PSNS, epinephrine released by adrenal gland, and brain response to hypoglycemia stimulation of glucagon secretion

- **Glucagon-like Peptide-1**: inhibits glucagon secretion; controversial whether GLP-1 receptors are present on alpha cells

- **Amino Acids**: increase glucagon secretion
REGULATION OF GLUCAGON SECRETION

- **Autonomic Nervous System:** 
  - SNS, PSNS, epinephrine released by adrenal gland, and brain response to hypoglycemia ➔ stimulation of glucagon secretion

- **Zinc and Glutamate** 
  - released by beta cell and inhibit glucagon secretion (importance unknown)

- **Amino Acids** 
  - increase glucagon secretion

GLUCAGON, ALPHA CELLS, AND INSULIN SECRETION

- **Glucagon potentiates insulin secretion through paracrine and cell-to-cell interactions**

- Glucagon receptor is expressed on beta cells and stimulates insulin secretion in vivo and in vitro by increasing cAMP

- **Proconvertase 1/3 in alpha cells ➔ GLP-1 insulin secretion**

- **IL-6 receptor is expressed on alpha cells; IL-6 from adipocytes/muscle ➔ GLP-1 by alpha cell ➔ insulin secretion**

Sandoval & D'Alessio, Physiol Rev 95:513-548, 2015
GLUCAGON AND THE GLUTACAGON RECEPTOR (GCGR)

- Glucagon binding to the GCGR
  \[ \xc2\çi\x93 cAMP \]

- Richest source of GCGR is in liver and kidney, with lesser amounts in heart, adipose tissue, CNS, adrenal, and spleen

- 70-80% of glucagon is cleared by the liver and kidney with T\(\frac{1}{2}\) of 7 minutes

Sandoval & D'Alessio, Physiol Rev 95:513-548, 2015

I. Glucagon Physiology – normal
   1. Basal
   2. Post-prandial

II. Glucagon Pathophysiology

III. Inhibitors of Glucagon Secretion
   1. Somatostatin
   2. Amylin/Pramlintide
   3. Incretins (GLP-1/GIP)

IV. Glucagon Receptor Antagonists
NORMAL GLUCOSE HOMEOSTASIS:
POSTABSORPTIVE (BASAL) STATE

NON-DIABETIC:
POSTABSORPTIVE (BASAL) STATE

GLUCAGON – AACE-NASHVILLE-2015
EFFECT OF SELECTIVE HYPOGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION IN NON-DIABETIC SUBJECTS

Baron, Diabetes 36:274, 1987

CONTRIBUTION OF BASAL GLUCAGON LEVELS TO MAINTENANCE OF HEPATIC GLUCOSE PRODUCTION IN NON-DIABETIC SUBJECTS

Baron, Diabetes 36:274, 1987
NORMAL GLUCOSE HOMEOSTASIS:
POSTPRANDIAL (FED) STATE

NON-DIABETIC: POSTPRANDIAL (FED) STATE

LIVER

GLUCAGON

PANCREAS

INSULIN

140 mg%

0.5 mg/kg-min

6 mg/kg-min
THE LIVER IS GLUCAGON’S TARGET ORGAN

- Glucagon is the primary regulator of hepatic glucose production:
  - Glycogenolysis – breakdown of stored glycogen into glucose
  - Gluconeogenesis – glucose synthesis from non-carbohydrate sources

- Glucagon’s effects on the liver:
  - Increase hepatic glucose production secondary to glycogenolysis
  - Lesser and later effect on gluconeogenesis as liver glycogen becomes depleted

BIOCHEMICAL/MOLECULAR ACTIONS OF GLUCAGON ON THE LIVER

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<thead>
<tr>
<th>Glucagon</th>
<th>Glycogen phosphorylase</th>
<th>PEPCK through CREB and Fox01</th>
<th>Glucose-6-phosphatase</th>
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<td>Glycogenolysis; Gluconeogenesis</td>
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<td>Pyruvate kinase</td>
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<th>Lipid oxidation; Ketogenesis</th>
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<td>beta oxidation of FFA</td>
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<td>Carnitine palmitoyl transferase 1</td>
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<td>Malonyl CoA</td>
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</table>


Sandoval & D’Alessio, Physiol Rev 95:513-548, 2015
Native GLP-1 Decreased Glucose Concentrations During Pancreatic Clamp

GLP-1 REGULATES GLUCOSE HOMEOSTASIS VIA EXTRA-PANCREATIC EFFECTS


THE ROLE OF GLUCAGON IN THE NORMAL REGULATION OF BASAL AND POSTPRANDIAL GLUCOSE HOMEOSTASIS
EFFECT OF PROLONGED FASTING ON PLASMA GLUCOSE, INSULIN, AND GLUCAGON CONCENTRATIONS


Glucose (mM)
Insulin (µU/ml)
Glucagon (pg/ml)

Time (hours)

EFFECT OF PROLONGED FASTING ON HGP, GLYCOGENOLYSIS, AND GLUCONEOGENESIS


Glucose Production (µmol/kg•min)

0 - 22 h
22 - 46 h
46 - 64 h

64 %
82 %
96 %
CONTRIBUTION OF GLUCONEOGENESIS TO GLUCOSE PRODUCTION DURING FASTING

Landau, JCI 98:378, 1996

EFFECT OF HYPOGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION IN DOGS

Cherrington, JCI 58:1407, 1976
EFFECT OF HYPOGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION

Cherrington, Diabetes 48:1198, 1997

EFFECT OF HYPERGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION

Cherrington, Diabetes 48:1198, 1997
EFFECT OF PHYSIOLOGIC HYPERGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION

Rizza, JCEM 48:352, 1979
DOSE RESPONSE EFFECT OF GLUCAGON ON ENDogenous GLUCOSE PRODUCTION


GLUCOSE HOMEOSTASIS IN DIABETES MELLITUS
NOCTURNAL HYPERGLYCEMIA IN TYPE 2 DIABETES IS RELATED TO EXCESS HEPATIC GLUCOSE PRODUCTION (HGP)

- Overnight HGP is increased in type 2 diabetes
  - Results in an extra 25-30 g of glucose added to the circulation every night
- Excess HGP results from increased hepatic gluconeogenesis that is related to:
  - Insulin resistance in the liver
  - Increased glucagon levels
  - Increased hepatic sensitivity to glucagon


Basal Hepatic Glucose Production After an Overnight Fast
ARE FASTING PLASMA GLUCAGON LEVELS INCREASED IN TYPE 2 DIABETIC PATIENTS?

WHAT ROLE DOES INCREASED FASTING PLASMA GLUCAGON CONC PLAY IN IMPAIRED GLUCOSE HOMEOSTASIS IN T2DM?

WHAT IS THE EVIDENCE THAT GLUCAGON PLAYS A ROLE IN THE DYSREGULATION OF GLUCOSE HOMEOSTASIS IN T2DM?
EVIDENCE THAT GLUCAGON PLAYS A CENTRAL ROLE IN THE DEVELOPMENT OF HYPERGLYCEMIA

(1) Hypergucagonemia is present in all types of poorly controlled diabetes

(2) Glucagon increases hepatic glucose production and ketogenesis

(3) Beta cell destruction in glucagon receptor null mice does not cause diabetes

(4) Perfusion of the normal pancreas with anti-glucagon antibodies reduces hyperglycemia

Under & Orci, PNAS 107:16009-12, 2010

BASAL HEPATIC GLUCOSE PRODUCTION (HGP) IN T2DM: RELATIONSHIP WITH FASTING PLASMA GLUCOSE (FPG)

DeFronzo et al, Metabolism 38:387-395, 1989
ROLE OF GLUCAGON IN FASTING HYPERGLYCEMIA IN TYPE 2 DIABETIC INDIVIDUALS

FASTING PLASMA GLUCAGON CONCENTRATION IN TYPE 2 DIABETIC SUBJECTS (n=104)

Matsuda & DeFronzo, Metabolism, 51:1111-19, 2002

CON

T2DM

p<0.001
ALPHA AND BETA CELL MASS IN TYPE 2 DIABETIC AND NON-DIABETIC SUBJECTS

Henquin & Rahier, Diabetologia 54:1720-25, 2011

HYPERGLUCAGONEMIA IN TYPE 2 DIABETES: IS IT RELATED TO INCREASED ALPHA-CELL MASS OR DECREASED BETA-CELL MASS?

Beta cells outnumber alpha cells in healthy subjects.

Reduced beta-cell mass is a key feature of type 2 diabetes²

- Alpha-cell mass is not altered in type 2 diabetes¹
- As type 2 diabetes progresses, the ratio of alpha cells to beta cells increases, leading to hyperglycemia¹

INHIBITION OF BASAL GLUCAGON SECRETION IN TYPE 2 DIABETIC INDIVIDUALS

EFFECT OF SELECTIVE HYPOGLUCAGONEMIA ON HEPATIC GLUCOSE PRODUCTION IN T2DM SUBJECTS

Baron, Diabetes 36:274, 1987

<table>
<thead>
<tr>
<th>Serum Glucagon (pg/ml)</th>
<th>HGP (mg/kg-min)</th>
<th>Serum Glucose (mg/dl)</th>
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<tr>
<td>50</td>
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<td>220</td>
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<tr>
<td>100</td>
<td>70</td>
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<td>150</td>
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<th>Time (min)</th>
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<td>Serum Insulin (μU/ml)</td>
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<td>20</td>
<td>25</td>
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<tr>
<td>Serum Glucose (mg/dl)</td>
<td>220</td>
<td>240</td>
<td>260</td>
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CONTRIBUTION OF BASAL GLUCAGEN LEVELS TO THE MAINTENANCE OF BASAL HEPATIC GLUCOSE PRODUCTION IN TYPE 2 DIABETIC SUBJECTS

Baron et al, Diabetes 36:274-283, 1987

CONTRIBUTION OF GLUCAGON TO POSTPRANDIAL HYPERGlyCEMIA
DAY-LONG HYPERGLUCAGONEMIA IS PRESENT IN TYPE 2 DIABETIC SUBJECTS

Reaven, JCEM 64:106, 1987

CONTRIBUTION OF GLUCAGON TO IMPAIRED GLUCOSE HOMEOSTASIS IN DIABETIC INDIVIDUALS
Plasma Glucagon, Insulin, and Glucose Concentrations Following Glucose Ingestion in Type 2 Diabetic and Control Subjects

Mitrakou, Diabetes 39:1381, 1990

Hepatic Glucose Production and Whole Body Glucose Disappearance Following Glucose Ingestion

Mitrakou, Diabetes 39:1381, 1990
HEPATIC GLUCOSE OUTPUT IS ABNORMALLY INCREASED POST-MEAL IN DIABETIC SUBJECTS

- Wahren et al., *J Clin Invest*, 1976
- Frank JW et al., *Gastroenterology*, 1995

PLASMA GLUCOSE AND INSULIN CONCENTRATIONS DURING OGTT IN T2DM AND CONTROL SUBJECTS

- Ferrannini et al., *Metabolism* 37:79, 1988
ENDOGENOUS GLUCOSE PRODUCTION AND TISSUE GLUCOSE DISPOSAL FOLLOWING GLUCOSE INGESTION IN T2DM AND CONTROL SUBJECTS

Ferrannini DeFronzo et al, *Metabolism* 37:79, 1988

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<th>CON</th>
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<td>GLUCAGON VS. EGP</td>
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<tr>
<td>r=0.52</td>
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<td>p&lt;0.01</td>
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<td>p&lt;0.05</td>
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GLUCAGON – AACE-NASHVILLE-2015

PLASMA GLUCOSE AND INSULIN RESPONSES IN SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE


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<tr>
<th>Glucose (mM)</th>
<th>CON</th>
<th>IGT</th>
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<tr>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
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<td>8</td>
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<td>6</td>
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<td>4</td>
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<th>CON</th>
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<td>700</td>
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<tr>
<td>500</td>
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<td>300</td>
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<tr>
<td>100</td>
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Impaired Glucagon Suppression in IGT Subjects


Impaired Glucagon Suppression in IGT Subjects

Alpha-Cell Dysfunction Contributes to Excess Hepatic Glucose Production

Dysregulation of Glucagon Secretion during Fasting

Impaired Suppression of Glucagon Secretion after a Meal

Fasting and Postprandial Hyperglycemia in T2DM
“GLUCOTOXICITY” contributes to the elevated plasma glucagon concentration in subjects with IGT, IFG, and T2DM.

CHRONIC HYPERGLYCEMIA AND ALPHA CELL DYSFUNCTION

Jamison et al, AJP 301:E1174-83, 2011

Graph showing plasma glucose and insulin levels over the course of glucose infusion.
Fasting hyperglycemia impairs glucose-, but not insulin-mediated suppression of glucagon secretion.

Subjects: 30 NGT; 27 IGT/IFG; 32 T2DM

Methods: OGTT Euglycemic Insulin (40 mU/m²•min) Clamp
FASTING HYPERGLYCEMIA IMPAIRS GLUCOSE- BUT NOT INSULIN-MEDIATED SUPPRESSION OF GLUCAGON SECRETION: EVIDENCE FOR GLUCOTOXICITY

Abdul-Ghani & DeFronzo, JCEM 92:1778-84, 2007

NORMAL SUPPRESSION OF FASTING PLASMA GLUCAGON BY INSULIN IN SUBJECTS WITH IGT/IFG AND T2DM

Abdul-Ghani & DeFronzo, JCEM 92:1778-84, 2007
SUMMARY AND CONCLUSION

- Chronic, sustained increase in fasting plasma glucose concentration, within the non-nondiabetic range, impairs the suppression of plasma glucagon levels by hyperglycemia during an OGTT, i.e. alpha cell “glucotoxicity”
- Suppression of plasma glucagon levels by insulin is not impaired by chronically increased fasting plasma glucose levels
ABNORMAL INSULIN SECRETION AND GLUCAGON SUPPRESSION ARE CENTRAL TO TYPE 2 DIABETES PATHOGENESIS

**SUMMARY**

1. Fasting plasma glucagon levels play an important role in the maintenance of basal EGP
2. The portal vein glucagon/insulin ratio is a key determinant of basal HGP
3. Glucagon stimulates EGP in a dose-dependent fashion, by increasing glycogenolysis and gluconeogenesis
(4) Suppression of basal glucagon production is an important determinant of normal oral glucose tolerance

(5) Following mixed meal or protein/amino acid ingestion, glucagon secretion is stimulated. The resultant hyperglucagonemia contributes to impaired suppression of EGP and impaired meal tolerance in IGT, T1DM, and T2DM

EFFECT OF GLUCAGON INFUSION ON PLASMA GLUCOSE LEVELS AND HGP IN DIABETIC PATIENTS
EFFECT OF A PHYSIOLOGIC INCREMENT IN PLASMA GLUCAGON ON BASAL PLASMA GLUCOSE LEVELS IN INSULIN-WITHDRAWN (24 HOURS) TYPE 1 DIABETICS

Sherwin, NEJM 294:455, 1976

GLUCAGON INFUSION (3 ng/kg-min)

△ GLUCOSE (mg/dl)

0 10 20 30 40 50 60

0 30 60 90 120 150 180

Time (min)

CAN WE REPRODUCE THE DIABETIC STATE WITH CHRONIC GLUCAGON INFUSION?
SUSTAINED, PHYSIOLOGIC HYPERGLUCAGONEMIA CAUSES HEPATIC AND PERIPHERAL (MUSCLE) INSULIN RESISTANCE IN HEALTHY YOUNG VOLUNTEERS

**Subjects:**
14 healthy volunteers
Age = 18-38 (mean = 24) years
BMI = 24.7 ± 0.5 kg/m²

**Study Design:** Stepped euglycemic insulin clamp (0.5, 1, and 5 mU/kg.min) with 3-³H-glucose
48-hour glucagon infusion (3 ng/kg.min)
Repeat insulin clamp

Del Prato & DeFronzo, JCI 79:547-56, 1987

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FASTING PLASMA GLUCOSE, INSULIN, AND GLUCAGON CONCENTRATIONS BEFORE AND AFTER GLUCAGON INFUSION

Del Prato & DeFronzo, JCI 79:547-56, 1987

[Graph showing fasting glucose, glucagon, and insulin concentrations before and after glucagon infusion]
CHRONIC (48 HOURS) PHYSIOLOGIC HYPERGLUCAGONEMIA CAUSES PERIPHERAL (MUSCLE) AND HEPATIC INSULIN RESISTANCE IN NGT SUBJECTS

Del Prato & DeFronzo, JCI 79:547-56, 1987

ACUTE EFFECT OF BRACHIAL ARTERIAL GLUCAGON INFUSION ON FOREARM GLUCOSE UPTAKE

Schneider, Diabetologia 20:616, 1981
EFFECT OF GLUCAGON INFUSION (3 ng/kg-min) ON HEPATIC GLUCOSE PRODUCTION

Matsuda & DeFronzo, Metabolism 51:1111-19, 2002

Glucagon increases hepatic glucose output in type 2 diabetic patients by:
- impairing the suppression of hepatic glucose production
- inhibiting hepatic glycogen synthesis
ROLE OF GLUCAGON AND INSULIN IN LIVER GLYCOGEN REPLETION IN HEALTHY SUBJECTS

Roden, JCI 97:642, 1996

Hypergly Clamp(+5mM)+SRIF

Insulin + Glucagon

Legend:

- Protocol I
- Protocol II
- Protocol III
ROLE OF GLUCAGON AND INSULIN IN LIVER GLYCOGEN REPLETION IN HEALTHY SUBJECTS

Protocol I
(↑ Ins; ↓ Glug)

Protocol II
(↑ Ins; ↑ Glug)

Protocol III
(↓ Ins; ↓ Glug)

DIABETES MELLITUS: POSTABSORPTIVE (BASAL) STATE

↑Insulin

↑Glucagon

PANCREAS

LIVER

BRAIN

MUSCLE

LIVER

140 mg%

2.5 mg/kg-min
GLUCAGON RECEPTOR GENE MUTATION

Single heterozygous Gly→SER mutation in the glucagon receptor gene is

- associated with (P=0.0001)
- linked to (P<0.01)

late-onset type 2 diabetes mellitus in French and Scandinavian patients

GLUCAGON LIKE PEPTIDE-1 (GLP-1) and GLUCAGON SECRETION

NATIVE GLP-1 STIMULATES INSULIN SECRETION AND INHIBITS GLUCAGON SECRETION IN A GLUCOSE-DEPENDENT MANNER IN T2DM

MECHANISM OF ACTION OF EXENATIDE TO REDUCE POSTPRANDIAL HYPERGLYCEMIA IN T2DM SUBJECTS


Subjects

12 T2DM treated with MET (n=5), SU (n=6), or both (n=1)
Age = 44 y; BMI = 34.1 kg/m2
HbA1c = 7.5%
Diabetes duration = 6 years

Study Design

Subjects participated in three 6-hour meal tolerance tests at 2-4 week intervals
(1) IV saline (control)
(2) IV exenatide (0.025 ug/min) started 15 min before meal
(3) IV exenatide plus glucagon

Double tracer technique (1-14C-glucose orally, 3-3H-glucose IV) and acetaminophen to quantitate EGP, RaO, Rd, and gastric emptying

EFFECT OF ACUTE EXENATIDE INFUSION ON POSTPRANDIAL GLUCOSE/HORMONAL RESPONSE IN T2DM


Plasma Glucose Conc

Plasma Insulin Conc

Plasma Glucagon Conc

Plasma Triglyceride Conc

Saline Control

Exenatide

Exenatide+Glucagon

MEAL

0 120 240 360

0 100 200 300

0 100 200 300

0 60 120 180

mg/dl

pmol/L

pg/ml

(% Change from baseline)

Time (min)

Time (min)

Time (min)
EFFECT OF ACUTE EXENATIDE INFUSION ON POSTPRANDIAL GLUCOSE/HORMONAL RESPONSE IN T2DM


90% REDUCTION IN POSTPRANDIAL GLUCOSE

- Decrease in RaO (~50%)
- Decrease in EGP (~50%)
  - ↓ Plasma glucagon (~50%)
  - ↑ Plasma insulin (~50%)
SUMMARY AND CONCLUSION

● Both amylin and exenatide attenuate postprandial hyperglycemia by suppressing endogenous (hepatic) glucose production

● Inhibition of glucagon secretion accounts for approximately half of exenatide’s effect to suppress endogenous glucose production after a meal

SUPPRESSING GLUCAGON SECRETION

● Insulin

● Amylin (pramlintide)

● GLP-1 receptor agonists

● DPP-4 inhibitors
GLUCAGON RECEPTOR BLOCKADE IN HUMANS

**BAY27-9955** - blocked stimulatory effect of glucagon on HGP in non-diabetic subjects; not further developed

**MK0893** - reduced FPG in dose-response manner in T2DM (Phase II)

**LY2409021** - caused a dose-dependent reduction in FPG and PPG (Phase II)

**PF-0G291,874** - produced a dose-dependent reduction in FPG and PPG (Phase II)

**ISIS-GCGRRx** - GCGR antisense siRNA (Phase II)

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POTENTIAL CONCERNS WITH GLUCAGON RECEPTOR ANTAGONISTS

- **Alpha cell hyperplasia (ACH)**
- Profound elevation in glucagon and proglucagon-related peptides
- **Increased pancreatic weight reflecting expansion of exocrine tissue**
- Reduced lipid oxidation secondary to impaired glucagon signaling
- Impaired recovery of hepatocytes to toxic injury
**PANCREATIC ALPHA CELL HYPERPLASIA (ACH)**

- Novel human disease (Mohvash disease)
- Homozygous inactivating glucagon receptor (GCGR) mutation
- Hyperglucagonemia
- Pancreatic neuroendocrine tumors (PNETs)
- Patients present with non-specific symptoms, i.e. abdominal pain and pancreatic mass

Yu R, JCEM 99:748-56, 2014

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**EFFECT OF DAPAGLIFLOZIN ON ENDOGENOUS GLUCOSE PRODUCTION**

<table>
<thead>
<tr>
<th>Drug Ingestion</th>
<th>Day 0</th>
<th>Day 1</th>
<th>9 AM</th>
<th>1 PM</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapagliflozin</td>
<td>2.8</td>
<td>2.4</td>
<td>1.6</td>
<td>2.0</td>
<td>2.4</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.2</td>
<td>1.6</td>
<td>2.0</td>
<td>2.4</td>
<td>2.8</td>
<td>3.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* Indicates significant difference from baseline.
MODELS OF ALPHA CELL HYPERPLASIA (ACH)

- Glucagon receptor deficient (Gcgr⁻/⁻) mouse
- Liver-specific Gsα knockout (post receptor glucagon) signaling molecule in liver
- Liver-specific Gcgr knockout
- ACH occurs in wild type (WT) islets transplanted under kidney capsule of Gcgr⁻/⁻ mice. This suggests that liver releases a humoral factor that causes ACH.

Yu R. JCEM 99:748-56, 2014

IS ALPHA CELL HYPERPLASIA A PRENEOPLASTIC LESION? THE Gcgr⁻/⁻ MOUSE EXPERIENCE

<table>
<thead>
<tr>
<th>MONTH</th>
<th>PANCREAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>Alpha cells - normal</td>
</tr>
<tr>
<td>5-7</td>
<td>Markedly expanded pancreatic mass with ACH and dysplasia</td>
</tr>
<tr>
<td>10-12</td>
<td>Micro PNETs in all mice; gross PNETs in 80%</td>
</tr>
<tr>
<td>18</td>
<td>Large PNETs in all mice with local organ invasion; liver metastases in 20%</td>
</tr>
<tr>
<td>18</td>
<td>17% survival vs 80% in wild type</td>
</tr>
</tbody>
</table>

In humans with ACH, PNETs develop at middle age

Yu R, JCEM 99:748-56, 2014
SGLT2 IS EXPRESSED ON HUMAN ALPHA CELLS


- Treatment of mice with dapagliflozin increases plasma glucagon levels and hepatic gluconeogenic gene expression (G6Pase, PGC1α, PEPCK, F-1,6-BPase)

- SGLT1/2 genes are expressed in human and mouse alpha cells

- In alpha cells of diabetic humans and mice, SGLT2 expression is decreased and SGLT1 expression is increased

- Dapagliflozin (selective SGLT2 inhibitor) and siRNA-mediated knockdown in cultured human and mouse alpha cells increase glucagon mRNA and glucagon secretion

GLUCAGON ACTION IN THE BRAIN

- **Glucagon** predominantly is expressed in the brainstem and, to lesser extent, in the hypothalamus

- **Glucagon receptor** is widely expressed in the CNS

- Circulating glucagon is transported into multiple regions of the CNS

- Glucagon increases energy expenditure in humans and rodents, in part by increasing FGF-21, and by activating BAT

- Glucagon is an appetite suppressant and this forms the basis of combined GLP-1/glucagon and GLP-1/GIP/glucagon therapy for obesity

- Glucagon activates afferent neurons in portal vein to reduce food intake; blocked by vagotomy

Campbell & Drucker, Nature Rev Endo, April 2015
COMBINATION LOW-DOSE GLP-1 PLUS GLUCAGON INFUSION IN MAN

Subjects: 13 non-diabetic obese subjects
BMI = 27 ± 1.5 kg/m² (range = 24.0-32.9)
No abnormal eating behavior

Study Design: Four-way cross over with 2-day washout;
120 minute infusion of:
(1) Placebo
(2) GLP-1 at 0.4 pmol/kg.min (subanorectic dose)
(1) Glucagon at 2.8 pmol/kg.min
(2) GLP-1 plus Glucagon

At 90 minutes, a meal of known caloric value was ingested;
indirect calorimetry was performed at baseline and during meal


EFFECT OF GLUCAGON, GLP-1, GLUCAGON + GLP-1 ON PLASMA GLUCOSE AND INSULIN DURING MEAL

CHANGE IN ENERGY/FOOD INTAKE FOLLOWING GLUCAGON, GLP-1, GLUCAGON + GLP-1

Cegla J et al, Diabetes 63:3711-20, 2014

![Bar chart showing the change in energy intake and food intake following glucagon, GLP-1, and glucagon + GLP-1.](image)

EFFECT OF GLUCAGON, GLP-1, AND GLUCAGON + GLP-1 ON RESTING ENERGY EXPENDITURE

Cegla J et al, Diabetes 63:3711-20, 2014

![Bar chart showing the change in resting energy expenditure following glucagon, GLP-1, and glucagon + GLP-1.](image)
GLUCAGON REGULATION OF ENERGY HOMEOSTASIS

SUMMARY

- Glucagon plays an essential role in the maintenance of basal HGP in NGT subjects and the recovery from hypoglycemia in diabetic individuals.

- Elevated plasma glucagon levels, impaired glucagon suppression after a meal, and enhanced hepatic sensitivity to glucagon contribute to fasting and postprandial hyperglycemia in T2DM.

- Agents which inhibit glucagon secretion have an important role to play in the treatment of T2DM/T1DM patients.

- The role of glucagon in the regulation of body weight is an area that requires future investigation.