Diabetes and Current Therapeutics

MacMillan Group Meeting
April 4, 2012
Scott Simonovich
Definition and World Prevalence

Diabetes mellitus

Metabolic disease in which abnormally high blood glucose levels result from poor production of insulin and/or inefficient use of insulin.
Definition and World Prevalence

- Incidence of diabetes increasing tremendously around the world
- Less physical activity and high caloric nutrition at low cost
- Prevalence will double in the next 20 years
- Expected to be 440 million type 2 diabetics by 2030
- Increased prevalence in children and adolescents

Abstract submissions to the 2011 World Diabetes Congress

Number of diabetics per 1000 people
Outline

- Insulin - structure, physiology, and link to hyperglycemia

inactive insulin hexamer
Outline

- Insulin - structure, physiology, and link to hyperglycemia
- Diabetes - pathophysiology and environmental causes of type 2
Outline

- Insulin - structure, physiology, and link to hyperglycemia

- Diabetes - pathophysiology and environmental causes of type 2

- Current therapeutics: Insulin analogues
  - GLP-1 agonists
  - DPP-4 inhibitors

Liraglutide

Saxagliptin
Insulin - General Overview

- Hormone produced by β-cells in pancreas (Islets of Langerhans)
Insulin - General Overview

- Hormone produced by β-cells in pancreas (Islets of Langerhans)

optical microscopy image of islet
(cell nuclei and insulin are stained)

optical microscopy image of islet
(cell nuclei, insulin, and glucagon are stained)
**Insulin - General Overview**

- Hormone produced by β-cells in pancreas (Islets of Langerhans)
- Stored in body as inactive hexamer, while active form is monomeric

C3 symmetry in hexamer
Histidine residues coordinate to central zinc ion
**Insulin - General Overview**

- Hormone produced by β-cells in pancreas (Islets of Langerhans)
- Stored in body as inactive hexamer, while active form is monomeric
- Central in regulating carbohydrate and fat metabolism
  - Promotes liver, muscle, and fat cells to take in glucose from blood for glycogen storage
  - Stops use of fat and glycogen as energy by inhibiting the release of glucagon

Glucagon ribbon structure (29 amino acids)

<table>
<thead>
<tr>
<th>Secreted by pancreas α-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promotes liver to break down glycogen</td>
</tr>
<tr>
<td>Releases glucose into bloodstream</td>
</tr>
</tbody>
</table>
Insulin - Several Metabolic Functions

cell membrane → glucose transporter-4 → insulin receptor
**Insulin - Several Metabolic Functions**

- **Cell Membrane**
- **Glucose Transporter-4**
- **Insulin Receptor**

Insulin binds to the insulin receptor, initiating a series of metabolic responses.
Insulin - Several Metabolic Functions

influx of glucose from bloodstream into cell
Insulin - Several Metabolic Functions

- Influx of glucose from bloodstream into cell
- Synthesis of glycogen from glucose
Insulin - Several Metabolic Functions

- Influx of glucose from bloodstream into cell
- Synthesis of glycogen from glucose
- Glycolysis

Cell membrane

Glucose transporter-4

Insulin receptor

Insulin

(pyruvate)
Insulin - Several Metabolic Functions

- influx of glucose from bloodstream into cell
- synthesis of glycogen from glucose
- glycolysis
- fatty acid synthesis

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Insulin - Several Metabolic Functions

- Influx of glucose from bloodstream into cell
- Synthesis of glycogen from glucose
- Glycolysis
- Fatty acid synthesis

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Insulin - Simplified Signal Transduction Pathway

insulin receptor

insulin

glycogen synthase kinase

glycogen synthase

glycogen synthase (inactive)

P_i

convert glucose to glycogen

cell membrane
**Insulin - Simplified Signal Transduction Pathway**

- **IRS-1**
- **glycogen synthase kinase**
- **glycogen synthase** (inactive)
- convert glucose to glycogen

Diagram depicts the interaction and phosphorylation processes involved in the insulin signaling pathway, highlighting key components such as insulin receptor, IRS-1, and glycogen synthase.
Insulin - Simplified Signal Transduction Pathway

IRS-1 $\rightarrow$ PI3K $\rightarrow$ IRS-1 $\rightarrow$ glycogen synthase

glycogen synthase kinase $\rightarrow$ glycogen synthase $\rightarrow$ convert glucose to glycogen

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Insulin - Simplified Signal Transduction Pathway

- **IRS-1**
  - Phosphorylated (P)
  - Activates PI3K

- **PI3K**
  - Converts PIP2 to PIP3

- **PIP3**
  - Activates glycogen synthase kinase

- **Glycogen synthase**
  - Converts glucose to glycogen

- **Glycogen synthase kinase**
  - Phosphorylates (P)
  - Activates glycogen synthase (inactive)

- **PIPK**
  - Converts PIP2 to PIP3

- **Insulin receptor**
  - Activates IRS-1

- **Insulin**
  - Binding activates receptor

Cell membrane

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**Insulin - Simplified Signal Transduction Pathway**

- Insulin receptor
- IRS-1
- PI3K
- PIP3
- PKB
- glycogen synthase kinase
- glycogen synthase
  - (inactive)
- PIP2
- convert glucose to glycogen
**Insulin - Simplified Signal Transduction Pathway**

- **Insulin receptor**
- **Insulin**
- **Cell membrane**

**Key Components**:
- **PI3K**
- **IRS-1**
- **PI3K**
- **PI3K**
- **PIK3**
- **PIK3**
- **PKB**
- **PKB**
- **glycogen synthase kinase**
- **glycogen synthase**
- **(inactive)**

**Pathway**:
1. Insulin binds to the **insulin receptor**.
2. The receptor phosphorylates **IRS-1**.
3. Phosphorylated IRS-1 (P₁) activates **PI3K**.
4. PI3K catalyzes the formation of **PIP3**.
5. PIP3 recruits and activates **PKB**.
6. PKB phosphorylates **glycogen synthase kinase**.
7. Phosphorylated glycogen synthase (P₁) is active.
8. Active glycogen synthase converts glucose to glycogen.
9. Phosphorylated glycogen synthase (P₁) remains inactive.

**Notes**:
- The pathway shows the interplay between insulin signaling and cellular glucose metabolism.
Insulin - Degradation

- Endocytosis of receptor-insulin complex
- Released back into bloodstream
**Insulin - Degradation**

- Liver clears during first-pass transit
- Kidney clears during systemic circulation

released back into bloodstream → Liver and kidney

endocytosis of receptor-insulin complex → insulin receptor
**Insulin - Degradation**

- Liver clears during first-pass transit
- Kidney clears during systemic circulation
- Insulin degraded within 1 hour of release into circulation
- Insulin half-life is about 4-6 minutes
What If Metabolic Homeostasis Malfunctions?

Glycosylated hemoglobin (HbA1c) - identifies average blood glucose concentration over prolonged time

- Hemoglobin undergoes non-enzymatic glycation when exposed to plasma glucose
- Process is non-reversible and shows glucose exposure during 120-day lifecycle of red blood cell
- Normal levels are 4.0 - 5.9%
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Hyperglycemia - shows that insulin is not properly regulating blood glucose levels
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Hyperglycemia - shows that insulin is not properly regulating blood glucose levels

- Pancreas β-cells have stopped producing insulin - **Type 1 Diabetes** (10% of diabetes cases)
  - Generally will be otherwise healthy
  - Autoimmune destruction of β-cells
  - Due to genetic susceptibility and environmental trigger (virus, drug, or diet)
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- Muscle and fat cells show resistance to insulin signalling- **Type 2 Diabetes** (90% of diabetes cases)
  - Impaired β-cell function
  - Interruption of insulin signal transduction pathways
  - Heavily dependant on lifestyle and environmental factors, as well as genetic susceptibility
What If Metabolic Homeostasis Malfunctions?

- Glucose coats red blood cells, making circulation difficult
- Promotes clotting and cholesterol buildup in blood vessels
- Eyes, kidneys, and feet are most susceptible to damage

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Insulin Resistance (Type 2 Diabetes)
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high caloric intake

(macrophages)
Insulin Resistance (Type 2 Diabetes)

(high caloric intake) → (macrophages) → inflammation → (cytokines)
Insulin Resistance (Type 2 Diabetes)

high caloric intake → inflammation → (macrophages) → JNK/API signal transduction (leads to insulin resistance) → (cytokines)
**Insulin Resistance (Type 2 Diabetes)**

Hypoxia - state in which tissue fails to acquire adequate oxygen supply

JNK/API signal transduction

(leads to insulin resistance)
Insulin Resistance (Type 2 Diabetes)

- increase in FFA
- microhypoxia
- high caloric intake
- JNK/API signal transduction

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Insulin Resistance (Type 2 Diabetes)

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- High caloric intake

ER stress leads to increase in unfolded and/or misfolded proteins

JNK/API signal transduction
(leads to insulin resistance)
Insulin Resistance (Type 2 Diabetes)

- Increase in FFA
- Microhypoxia
- ER stress
- Unfolded protein response
- High caloric intake
- JNK/API signal transduction (leads to insulin resistance)

- Halts protein translation
- Produces more molecular chaperones
- Can lead to apoptosis
Insulin Resistance (Type 2 Diabetes)

- **increase in FFA**
- **microhypoxia**
- **high caloric intake**
- **ER stress**
- **unfolded protein response**
- **cytokines**

**JNK/API signal transduction**
(leads to insulin resistance)

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Insulin Resistance (Type 2 Diabetes)

- Increase in FFA
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- Inflammation
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- Cytokines

JNK/API signal transduction
(leads to insulin resistance)
Insulin Resistance (Type 2 Diabetes)

- PI3K
- IRS-1
- PIP3
- PIP2
- PKB
- glycogen synthase kinase
- glycogen synthase

Cell membrane

Insulin receptor

Glycogen synthase kinase (inactive)

Glycogen synthase (inactive)

Convert glucose to glycogen
Insulin Resistance (Type 2 Diabetes)

- Insulin promotes tyrosine phosphorylation on IRS-1
Insulin Resistance (Type 2 Diabetes)

- Insulin promotes tyrosine phosphorylation on IRS-1
- JNK/API signal transduction promotes serine phosphorylation
Current Therapeutics for Type 2 Diabetes
**Insulin Analogues**

- Insulin used by injection or subcutaneous injection (into hypodermus)
- Unmodified insulin has undesired properties
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- Hexameric structure causes slow action after injection
- May need injection up to several hours before meal
- Misuse may lead to hypoglycemia
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Hypoglycemia - can be significantly more dangerous than hyperglycemia

- Most cells use fatty acids when glucose is scarce
- However, neurons depend almost exclusively on glucose in non-starving humans
- Neurons have very small internal stores of glycogen
- Hypoglycemia can rapidly lead to impaired CNS function, coma, or death
Insulin Analogues

- Slightly modified versions of insulin provide treatments with more convenient and safer profile
Insulin Analogues

- Slightly modified versions of insulin provide treatments with more convenient and safer profile

- Rapid-acting or short-acting analogues allow injection from 5 to 30 minutes before meal

![Insulin Analogues Diagram](image)

- insulin aspart (novo nordisk)
- insulin lispro (Eli Lilly)
**Insulin Analogues**

- Slightly modified versions of insulin provide treatments with more convenient and safer profile

- Extended release analogues have steady effect without peak or drop (18-24 hours)

insulin glargine (sanofi aventis)
Traditional Small Molecule Treatments

- Two classes of small molecules have been used to treat T2DM
- These molecules are being used less due to undesirable side effects
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- Two classes of small molecules have been used to treat T2DM
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![Sulfonylureas](attachment://sulfurylureas.png)

- Chlorpropamide
- Gliclazide
- Tolazamide
- Glimeriride
Traditional Small Molecule Treatments

- Two classes of small molecules have been used to treat T2DM
- These molecules are being used less due to undesirable side effects
Traditional Small Molecule Treatments

- Metformin (Glucophage) is first drug choice in T2DM treatment

- Primary function is reducing hepatis gluconeogenesis by 33% on average

- Minimal side effects (GI irritation, low risk of hypoglycemia)

- Glycemic control continues to deteriorate; metformin does not stop β-cell degradation
Current AACE and ACE Recommendations

Lower priority on thiazolidinediones due to weight gain and heart failure

Stress much lower priority on sulfonylureas due to hypoglycemia, weight gain, and short effectiveness

Increase emphasis on incretin-based therapies (GLP-1 agonists, DPP-4 inhibitors)
[stop or reverse deterioration in β-cell function]
**Current AACE and ACE Recommendations**

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[stop or reverse deterioration in $\beta$-cell function]

**Incretin Effect**

[Diagram showing the incretin effect with Healthy Patients' insulin levels before and after a glucose challenge, comparing oral glucose and isoglycemic IV glucose infusion.]
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[stop or reverse deterioration in β-cell function]

Incretin Effect
Incretin Proteins

stomach

small intestine
Incretin Proteins

- Glucagon-like peptide-1 (GLP-1)
- (incretin protein)

- Stomach
- Small intestine

GLP-1
Incretin Proteins

GLP-1 and GIP contribute 60% of insulin secretion after meal

- inhibits gastric emptying
- promotes insulin release
- inhibits glucagon release
- suppresses appetite

GLP-1

stomach

small intestine

pancreas β-cells
Incretin Proteins

GLP-1 and GIP contribute 60% of insulin secretion after meal

GLP-1 action dependent on amount of glucose ingested

promotes insulin release

inhibits glucagon release

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GLP-1

stomach

small intestine

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inhibits gastric emptying
Incretin Proteins

GLP-1 and GIP contribute 60% of insulin secretion after meal

GLP-1 action dependent on amount of glucose ingested

promotes insulin release

inhibits glucagon release

suppresses appetite

GLP-1 slowly increases mass of β-cells over time

GLP-1

inhibits gastric emptying

stomach

small intestine

pancreas β-cells

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Incretin Proteins

GLP-1

- inhibits gastric emptying
- promotes insulin release
- inhibits glucagon release
- suppresses appetite

stomach

small intestine

DPP-4

pancreas β-cells
Incretin Proteins

- Expressed on the surface of most cells
- Signal transduction, immune response, apoptosis
- Serine exopeptidase that cleaves X-proline or X-alanine from N-terminus of polypeptides

Dipeptidyl-peptidase IV (dpp-4)
**Incretin Proteins**

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Dipeptidyl-peptidase IV (dpp-4)
**Incretin Proteins**

- Enzyme responsible for GLP-1 degradation (half-life = 1-2 minutes)
- Injection of exogenous GLP-1 results in minimal effect
- Native GLP-1 is not a practical therapeutic for T2DM

- Expressed on the surface of most cells
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- Serine exopeptidase that cleaves X-proline or X-alanine from N-terminus of polypeptides

Dipeptidyl-peptidase IV (dpp-4)
Incretin Proteins

Dipeptidyl-peptidase IV (dpp-4)

Two options for incretin therapeutic strategies:

- GLP-1 receptor agonist resistant to DPP-4 cleavage (injectable polypeptide)
- DPP-4 inhibitor (orally active small molecule)
GLP-1 Receptor Agonists

**Exenatide (Amylin and Eli Lilly)**

- First GLP-1 receptor agonist
- Approved in combination with metformin and/or sulfonylureas (when monotherapy fails)
**GLP-1 Receptor Agonists**

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- Synthetic form of exendin-4 (saliva of gila monster)

Exenatide (Amylin and Eli Lilly)
**GLP-1 Receptor Agonists**

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**GLP-1 Receptor Agonists**

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  - First GLP-1 receptor agonist
  - Approved in combination with metformin and/or sulfonylureas (when monotherapy fails)
  - Synthetic form of exendin-4 (saliva of gila monster)
  - 53% amino acid sequence of human GLP-1
  - Subcutaneous injection twice daily
  - Slow release variation in progress (once weekly)
GLP-1 Receptor Agonists

Exenatide (Amylin and Eli Lilly)
GLP-1 Receptor Agonists

Exenatide (Amylin and Eli Lilly)

Advantages to using Exenatide:
- 5.3 kg weight loss over 3 years
- β-cell function improves

Disadvantages to using Exenatide:
- Washout leads to decreased β-cell function
- Non-human peptide leads to antibodies
- Cases of acute pancreatitis
GLP-1 Receptor Agonists

- First human GLP-1 analogue
- Only 2 amino acid modifications
GLP-1 Receptor Agonists

- First human GLP-1 analogue
- Only 2 amino acid modifications
- Approved for once daily subcutaneous injection
- Albumin binding to fatty acid (blocks DPP-4)

Liraglutide (Novo Nordisk)
GLP-1 Receptor Agonists

Liraglutide (Novo Nordisk)

DPP-4

Native human GLP-1

albumin

Liraglutide
GLP-1 Receptor Agonists

Advantages to using Liraglutide:
- Lowers body mass and food intake
- β-cell mass and function improve
- Systolic blood pressure lowered

Disadvantages to using Liraglutide:
- GI irritation
- Antibodies produced (low amounts)
DPP-4 Inhibitors

Linagliptin (Boehringer Ingelheim)

Sitagliptin (Merck)

Vildagliptin (Novartis)

Saxagliptin (AstraZeneca and BMS)
DPP-4 Inhibitors

N-terminal β-propeller domain

C-terminal α/β-hydrolase domain

(cell surface)
DPP-4 Inhibitors

N-terminal β-propeller domain

C-terminal α/β-hydrolase domain

(cell surface)

(catalytically active as dimer)
**DPP-4 Inhibitors**

Propeller opening

N-terminal β-propeller domain

C-terminal α/β-hydrolase domain

Side opening

Active site at interface of domains (Ser, Asp, His catalytic triad)

Substrate access through propeller and side openings
DPP-4 Inhibitors

- Substrate-like inhibitors
  - Designed to mimic proline-containing peptide
  - More common than non-substrate-like inhibitors

Vildagliptin (Novartis)

Saxagliptin (AstraZeneca and BMS)
**DPP-4 Inhibitors**

- Substrate-like inhibitors
  - Designed to mimic proline-containing peptide
  - More common than non-substrate-like inhibitors
  - Can bind either covalently or non-covalently
  - Covalent more common (nitrile, boronic acid, or phosphonate)

General binding for a substrate-like inhibitor

- Vildagliptin (Novartis)
- Saxagliptin (AstraZeneca and BMS)
**DPP-4 Inhibitors**

- Covalent binding with serine to form imidate
- Slow off-rate leads to potent inhibitors
- Substrate-like inhibitors can have poor selectivity (DPP-8 and DPP-9)
- H-bonding network to primary amine
- R is generally large and lipophilic
**DPP-4 Inhibitors**

- Non-substrate-like inhibitors
  - Generally non-covalent binding
  - Aromatic ring usually occupies S1 pocket instead of pyrrolidine ring
  - Sitagliptin resulted from a search for >1000-fold selectivity for DPP-4

![Linagliptin (Boehringer Ingelheim)](image1.png)

![Sitagliptin (Merck)](image2.png)
DPP-4 Inhibitors

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    - Both DPP-4 and DPP-8 showed strong preference for proline dipeptides
    - Found $\beta$-amino acid piperazine series through SAR
    - Made piperazine into bicyclic moiety for stability

Sitagliptin (Merck)
DPP-4 Inhibitors

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![Chemical structure of DPP-4 inhibitors](image)
**DPP-4 Inhibitors**

- Non-substrate-like inhibitors
  - Xanthine-based compounds believed to have longer-lasting improvements on glucose tolerance
DPP-4 Inhibitors

- Non-substrate-like inhibitors
  - Xanthine-based compounds believed to have longer-lasting improvements on glucose tolerance
  - Different binding than other DPP-4 inhibitors
Diabetes Recap

- T2DM results from insulin resistance with impaired β-cell function

- Insulin resistance from interruption of important signal transduction pathways

- Insulin analogues, GLP1R analogs, DPP-4 inhibitors

- Avoid hypoglycemia and restore β-cell function

- Best way to avoid, reverse effects of T2DM is to practice healthy lifestyle