PHYSIOLOGY OF DIABETES: PANCREAS

Exocrine (pancreatic juices) & endocrine (insulin, glucagon) functions

Islets of Langerhans

- $\alpha \rightarrow$ glucagon, GLP-1, GLP-2
- $\beta \rightarrow$ insulin, C peptide, amylin
- $\delta \rightarrow$ somatostatin
- PP $\rightarrow$ pancreatic polypeptide

Functions of pancreatic peptide hormones:

- **Insulin** $\downarrow$ plasma sugar ($\uparrow$ glucose uptake, $\downarrow$ gluconeogenesis, $\uparrow$ glycogen synthesis)
- **Glucagon** $\uparrow$ plasma sugar ($\uparrow$ gluconeogenesis, $\uparrow$ glycogen breakdown)
- **Somatostatin** $\downarrow$ insulin & $\uparrow$ glucagon (negative feedback)
- **Amylin** $\downarrow$ sugar (co-secreted with insulin, $\downarrow$ gastric emptying time, $\downarrow$ gastric secretions, $\downarrow$ glucagon secretion, smooth out rises in glucose)
- **GLP-1** $\downarrow$ plasma sugar ($\uparrow$ insulin release, $\downarrow$ glucagon release)

**Type 1:** Viral infection or chemical triggers $\rightarrow$ immune attack $\rightarrow$ loss of $\beta$ cells $\rightarrow$ $\downarrow$ insulin  
IDDM/juvenile onset  
Evidence: antigens on $\beta$ cells; chemical triggers include zinc chelators, nitrates, rodenticides

**Type 2:** Genes/exogenous stimuli $\rightarrow$ defect in signaling/metabolic pathways $\rightarrow$ insulin resistance  
NIDDM/adult onset  
Exogenous stimuli: iron overload, glucocorticoids

**Gestational DM:** pregnant moms need 4x more insulin, but if she does not produce enough to compensate for  
$\uparrow$ metabolic needs and $\uparrow$ glucose output, she may develop gestational DM

- Correlation with obesity and family history
- Fetus risks: still birth, congenital anomalies
- $\uparrow$ Risk for mom developing Type II later

Endocrinopathies

- $\downarrow$ Insulin secretion: somatostatinoma, aldosteronoma, pheochromocytoma (tumors that $\downarrow$ insulin secreted)
- $\uparrow$ Insulin resistance: acromegaly, Cushing’s syndrome, hyperthyroidism (hormone problems $\uparrow$ resistance)

$\uparrow$ [Glucose] = $\uparrow$ osmotic pressure = $\uparrow$ bp $\rightarrow$ polyuria/polydipsia to excrete glucose $\rightarrow$ $\uparrow$ protein filtration rate $\rightarrow$ $\uparrow$ pore size

**AGE: Advanced Glycosylation End-products**

- High [glucose] $\rightarrow$ nonenzymatic glycosylation of proteins $\rightarrow$ + addition products + rearrangements + more reactions $\rightarrow$ stable AGE
- AGE affects both IC and EC
  - AGE alters enzymatic or binding activity of IC proteins $\rightarrow$ damages cell
  - AGE causes abnormal interactions in EC matrix $\rightarrow$ affects tissue adhesion & recognition systems

**Trauma $\rightarrow$ stress $\rightarrow$ catecholamines + cortisol**

- $\uparrow$ Catabolism
- $\downarrow$ Insulin release (signal)
- $\uparrow$ Glucose production from liver
- $\uparrow$ Lipolysis $\rightarrow$ $\uparrow$ plasma fatty acids
- $\uparrow$ Protein & amino acid catabolism
- Results: hyperglycemia + ketosis in DM patients after surgery

**Diabetes insipidus** ("without taste," i.e. no glucose)

- Defect in AQP-2 epithelial protein in kidneys $\rightarrow$ vasopressin insensitivity $\rightarrow$ $\downarrow$ reabsorption of H$_2$O $\rightarrow$ urination

**OGTT: oral glucose tolerance test**

- Administration of glucose to determine how quickly it is cleared from the blood and homeostasis is maintained
- The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia
- Normally: 30 min peak, return to fasting level at 2 hours
- Diabetes: later peak, does not return to fasting level ($>200$mg/dL)

**Prediabetes**

- Not quite there yet, but $\uparrow$ risk of: type II, stroke, heart attacks
- Prediabetic pt has one or both of the following:
IFG: impaired fasting glucose levels  (FBG > 100-1250mg/L)
IGT: impaired glucose tolerance  (After OGT, glucose 1400-2000mg/L)

Extreme diabetic conditions
- >1800mg/L → glucosuria
- >1400mg/L → ketoacidosis
- >6000mg/L → HHNC: hyperglycemic hyperosmolar nonketotic coma

Commonly measured analytes
- Blood: good marker, easily measure, little interference, good correlation
- Urine: needs glucose >1800mg/L to show (>3000mg/L for diabetics), ↑glucosuria in pregnancy, rickets, osteomalacia

Measuring glucose
- Electrochemical glucose monitors
  - glucose oxidase
  - Glucose → hydrogen peroxide → O₂
  - Pt (oxidation)
  - Amount of current proportional to amount of glucose oxidized
- Photometric glucose monitors
  - Measures color change
  - Test strip embedded with glucose oxidase + peroxidase + dye
  - Glucose → hydrogen peroxide → Dye oxidized → color change
  - When test strip instereti into meter, color change measured and converted to equivalent glucose level
- Hb A₁C
  - Glucose + hemoglobin → Hb A₁C
  - Hb A₁C measures average blood glucose level in past 4-6 weeks
  - Amount of elevation directly proportional to degree of hyperglycemia
  - Assay methods (e.g. chromatography, electrophoresis) may be used to quantify levels of glycosylated protein

BIOCHEMISTRY OF DIABETES: METABOLISM

GLUCOSE METABOLISM!

Glucose transporters “GLUT”
- Structure: transmembrane proteins
- Mechanism: eversion
- Purpose: glucose uptake (rate limiting step)
- Types:

<table>
<thead>
<tr>
<th>TRANSPORTER</th>
<th>WHERE</th>
<th>WHAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT1</td>
<td>Everywhere</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Liver, pancreatic islets, intestine</td>
<td>Liver: remove excess glucose from blood Pancreas: regulate insulin release</td>
</tr>
<tr>
<td>GLUT3</td>
<td>Brain neurons</td>
<td>Basal glucose uptake</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Muscle, fat, heart</td>
<td>Activity increased by insulin</td>
</tr>
<tr>
<td>GLUT5</td>
<td>Intestine, testis, kidney, sperm</td>
<td>Fructose transport</td>
</tr>
</tbody>
</table>

Obesity
- Factors: sedentary lifestyle, high caloric intake
- High correlation between obesity ↔ glucose metabolism ↔ lipid metabolism
- Obesity → insulin resistance → DM → lipid metabolism upsets

LIPID METABOLISM!

Overview
- Breaking it down in the gut: TG → FA + acylglycerols
- Absorption: by the gut
- Re-synthesis & secretion: TG + lipoproteins (from lymph to blood, passing by liver and peripheral tissues)
Hydrolyzation: \( \text{TG} \xrightarrow{\text{lipases}} \text{non-esterified fatty acids (NEFA) + glycerol} \)

Storage: FA stored as TG in fat droplets

Liver action: \( \text{NEFA} \xrightarrow{\beta-oxidation} \text{acetyl CoA} \xrightarrow{TCA cox actors, oxidative phosphorylation} \text{ATP generation} \)

Glycerol \( \rightarrow \) supports gluconeogenesis

**Thrifty Genes & Fat Storage**
- Fat as preferable energy storage due to greater density (vs. glycogen)
- Natural selection for thrifty genes/traits are a survival mechanism to protect against starvation
- When food is abundant, thrifty genes chose to store calories as triglycerides/fat

**Randle Hypothesis**
- \( \uparrow \text{FA metabolism} \rightarrow \uparrow \text{acetyl CoA} + \uparrow \text{citrate} \rightarrow (-) \text{PFK & pyruvate dehydrogenase} \rightarrow \downarrow \text{glycolysis rate} \rightarrow \uparrow \text{intracellular glucose} \rightarrow (-) \text{hexokinase} \rightarrow \downarrow \text{glucose usage} \)
- Overall: \( \downarrow \text{glucose uptake, } \uparrow \text{resistance} \)
- Contrary evidence: incorrect for muscle tissue
  - \( \uparrow \text{FA} \rightarrow \uparrow \text{acyl CoA} + \uparrow \text{diacylglycerol} \rightarrow (-) \text{insulin stimulated GLUTs} \)
  - Not direct inhibition, but acyl Coa and diacylglycerol activate pathways that cause suppression of signals
  - Linked to mitochondrial defects in beta-oxidation of FA \( \rightarrow \) causes accumulation of acyl CoA \( \rightarrow \) leas to interference with insulin signals

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**ENDOCRINOLOGY OF DIABETES: HORMONES**

**Structure of insulin**
- Really long: 110 aa sequence
- Held together by S-S disulfide bridges

**Insulin maturation**
- Starts off as preproinsulin
- Cleaved in ER, where 24 aa removed from N-terminus \( \rightarrow \) proinsulin
- Proinsulin folds, forming 3 S-S bonds
- C peptide cleaved in golgi apparatus and 4 more aa removed \( \rightarrow \) insulin

**Insulin release**
- GLUT2 mediates glucose uptake in \( \beta \) cells
- Glucose metabolism in \( \beta \) cells \( \rightarrow \) ATP production \( \rightarrow \) causes K+ channels to close \( \rightarrow \) membrane depolarization \( \rightarrow \) \( \text{Ca}^{2+} \) entry into \( \beta \) cell \( \rightarrow \text{IP}_3 + \text{DAG} \rightarrow \) exocytosis of insulin stimulated
- \( \text{Ca}^{2+} \) activates CREB protein \( \rightarrow \) insulin gene expression \( \quad \text{(CREB = Ca}^{2+} \text{ responsive Element Binding)} \)
- Kinetics: \( 1^{\text{st}} \) phase=immediate bolus; \( 2^{\text{nd}} \) phase=lower levels but elongated plateau

**Insulin receptor**
- Transmembrane receptor: tyrosine kinase class
- Heterodimer: \( 2\alpha + 2\beta \) subunits
  - \( \alpha \) subunits: extracellular, binds hormone
  - \( \beta \) subunits: transmembrane, binds ATP, contains tyrosine kinase domains
- S-S disulfide bonds stabilize dimeric structure

**Signal transduction cascades**
- Insulin mediates many different metabolic pathways in the liver, muscle, & fat; but overall \( \uparrow \text{cellular respiration} \)
- Aspects of the cascade: autophosphorylation of tyrosine kinase, \( \uparrow \text{glucose transporters, GLUT4 in peripheral tissue} \)
- Interruption of insulin signals
  - Apparent starvation: X insulin \( \rightarrow \downarrow \text{glucose entry into peripheral tissues} \rightarrow \text{energy starved} \)
  - \( \uparrow \text{FA metabolism} \)
  - Ketosis: NEFA converted to ketone bodies
  - Insulin resistance: leads to \( \uparrow \text{insulin production/release} \) and hyperinsulinemia

**Glucagon**
- Structure: 29 aa residue peptidic hormone
- Synthesis: proglucagon in \( \alpha \) cells \( \rightarrow \) protease processing \( \rightarrow \) mature glucagon
- Actions: ↑glucose concentration in blood; stimulates gluconeogenesis, lipolysis, ketone formation, aa uptake; glycogenesis inhibition in liver
- Receptor: G-protein linked receptor → activation → ↑cAMP and (+)PKA

**Somatostatin**
- Structure: 14 aa peptidic hormone
- Synthesis: in δ pancreatic cells as well as certain gut and neuronal cells
  - Starts as a preprohormone → alternate ways of cleavage depending on tissue source and cell type
- Actions: depends on which of 5 subtypes
  - (–) Insulin & glucagon secretion
  - (–) Self-secretion
  - (–) Pituitary hormone secretion: TSH, ACTH, & GH
  - (–) GI secretion: gastrin, secretin, cholecystokinin, etc.
  - (–) Salivary secretion, acid and pepsin secretion, and ↓GI tract motility

**Other relevant hormones**
- **GLP-1**: glucagon-like peptide
  - Release stimulated by food intake
  - Actions: ↑insulin release, ↓glucagon levels
- **IGF-1**: insulin-like growth factor
  - Produced by liver
  - Actions: similar to insulin, but to a much smaller degree
- **Amylin**
  - Co-secreted with insulin
  - Actions: promotes postprandial glucose control

**PHARMACOLOGY: DIABETES TREATMENT**

**TREATMENT TYPES!**
- **Type I**: exogenous insulin that mimics both basal and bolus insulin secretion in response to glucose
- **Type II**: maintenance of glucose concentrations within normal limits via ↓weight, ↑exercise, diet changes, oral hypoglycemic agents, and sometimes exogenous insulin therapy

**EXOGENOUS INJECTABLE INSULINS!**
- For both Type I & Type II DM

- Insulin’s aa sequence similar among humans, pigs, and cows → therefore, we can use their insulin extracts
- However, impurities caused humans to produce antibodies to foreign insulin, so now semi-synthetic human insulin is most commonly used
- Semi-synthetic preparation: porcine insulin → enzymatic conversion (replace Ala with Thr) → “human” insulin
- Methods currently used: recombinant DNA methods (Humulin), yeast (Novolin)

**Insulin kinetics**
- Low basal rate
- High rate in response to meals (prandial + postprandial)
- Half life: 3-5 minutes (degraded by insulinase, removed from bloodstream by liver & kidneys)
- P’kinetics variable: very difficult to mimic (50% variance)
  - Variability due to rate of subcutaneous absorption, which is dependent on:
    - Formulation (concentration, additives, dosage form)
    - Injection conditions (site, injection depth, delivery device)
    - Other factors (smoker, exercise, temp, stresses)

**Insulin properties & preparations**
- At low concentrations: monomer
- At high concentrations: dimers
  - Readily diffuses into blood
- In presence of Zn²⁺: hexamers → Poorly diffuses, storage form in β cells
- Addition of a protamine (basic protein): prolonged effects, slow release
Different insulin preparations take advantage of different combinations of additives
  - Addition of Zn\(^{2+}\) and protamines → varying onset and durations of action
  - Insulin analogues
    - Manipulation of onset and duration of action by varying aa residues of C-terminus of the β chain
      - Does not affect biological activity
      - Does affect rate of dimer formation/separation
    - Produced by recombinant DNA methods
  - Four types: rapid acting, short acting, intermediate acting, long acting (and combinations)
    - **Short acting (regular):** Regular Humulin, Novolin R, Velosulin BR
      - Soluble crystalline zinc insulin
      - PK: onset @ 30mins, peaks @ 2 and 3hrs, duration of 5-8hrs
    - **Rapid acting:** Lispro, Aspart, Glulisine
      - ↓Self-association: # of monomers > # of dimers and hexamers
      - ↑Rate of absorption
    - **Intermediate acting:** NPH Humulin, Novolin N
      - Suspension of crystalline zinc insulin combined with protamine
      - Smaller doses: lower, earlier peaks, short duration
      - Larger doses: bigger, later peaks, longer duration
      - Unpredictable, high variability of absorption
    - **Long acting:** Detemir, Glargine
      - ↑Self-aggregation
      - ↑Albumin binding (reversible)
      - ↑Prolonged availability
      - Fatty acid part helps it stick to albumin and keeps it away from insulinase
    - **Premixed combinations (%NPH/%Reg):** Humulin 70/30 or 50/50, Novolin 70/30
      - Readily miscible: lispro, aspart, glulisine + NPH
      - Must be given separately: glargine, detemir

**Chemical degradation of insulin**
- Acidic conditions: Asn (of C-terminus) → cyclization to anhydride → reacts with H\(_2\)O → deamidation → inactive
- Preparations generally kept at pH 7.2-7.4 (except glargine at pH 4)
- Neutral pH: may undergo deamidation at Asn

### ORAL HYPOGLYCEMIC AGENTS!
**Classification**
- **Insulin secretagogues:** sulfonylureas, meglitinides
- **Insulin sensitizers:** thiazolidinediones (TZDs), biguanides (metformin, drug of choice)
- **α-glucosidase inhibitors:** acarbose, miglitol
- **Incretin based:** GLP-1 analogues, DPP-IV inhibitors
- **Amylin analogues**

### INSULIN SECRETAGOGUES

#### SULFONYLUREAS
- **Drugs:** glipizide & glyburide (intermediate acting), glimepiride (long acting)
- **SAR:** para substituted aromatics with bulky substituent
- **MOA:** bind to functioning β cell receptors → block ATP sensitive K\(^+\) channels → depolarization → (+)endogenous insulin secretion from β cells; enhances peripheral insulin receptor sensitivity; ↓glycogenolysis
- **P’kinetics:** hepatically metabolized, renally excreted, highly protein bound
- **Drug interactions:** some drugs may inhibit their metabolism/excretion or displace it from bound protein

#### MEGLITINIDES
- **Drugs:** repaglinide (Prandin), nateglinide (Starlix)
- **Compared to sulfonylureas:** 2 common binding sites + 1 unique binding site; less hypoglycemia
• **MOA:** similar to sulfonylureas: (+)endogenous insulin secretion from β cells
• **P'kinetics:** rapid onset, short acting, t½ <1hr, taken immediately before meals
• **Drug interactions:** drugs that affect CYP3A4 (inhibition ↑effects, induction ↓effects)

### INSULIN SENSITIZERS

#### BIGUANIDES (metformin)
- **Drug:** metformin (Glucophage)
- **MOA:** activates enzyme AMPK → ↓hepatic glucose production; ↓hyperlipidemia
- **SE:** lactic acidosis (rare but serious/fatal)
- **P'kinetics:** not metabolized, renally excreted, t½ 1.5-3hrs
- **Drug interactions:** cimetidine competes for renal excretion and can ↑metformin plasma levels
- Requires presence of insulin, does not promote its secretion
- Low risk of hypoglycemia
- The only oral agent shown to ↓CV mortality

#### THIAZOLIDINEDIONES
- **Drugs:** pioglitazone (Actos), rosiglitazone (Avandia)
- **MOA:** activation of PPAR-γ → (+) insulin responsive genes → ↑insulin sensitivity in adipocytes, hepatocyte, and skeletal muscles
- **SE:** hepatotoxicity, CV events (serious)
- Requires presence of insulin, does not promote its secretion
- Low risk of hypoglycemia

#### α-GLUCOSIDASE INHIBITORS  “Starch inhibitors”
- **Drugs:** acarbose (Precose), miglitol (Glyset)
  - Acarbose: poorly absorbed, remains in intestines
  - Miglitol: absorbed, but not metabolized/excreted by kidney
- **MOA:** delays digestion of carbohydrates → ↓postprandial blood glucose concentrations
- **SE:** flatulence, diarrhea, abdominal pain

### INCRETIN BASED THERAPIES

#### GLP-1 ANALOGUES
- **Drugs:** exenatide (Byetta), liraglutide (Victoza)
- **MOA:** (+)Insulin release when there are high glucose concentrations; ↓glucagon secretion, slows gastric emptying time, ↓appetite

#### DPP-IV INHIBITORS
- **Drugs:** sitagliptin (Januvia), saxagliptin (Onglyza)
- **MOA:** inhibits the enzyme responsible for degrading GLP-1 by cleaving after proline residues next to active site

#### AMYLIN AGONISTS
- **Drug:** pramlintide
- **MOA:** slows gastric emptying, ↓glucagon release