PHYSIOLOGY OF DIABETES: PANCREAS

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

Islets of Langerhans

- **α** → glucagon, GLP-1, GLP-2
- **β** → insulin, C peptide, amylin
- **δ** → somatostatin
- **PP** → pancreatic polypeptide

Functions of pancreatic peptide hormones:

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

Type 1: Viral infection or chemical triggers → immune attack → loss of β cells → ↓insulin *IDDM/juvenile onset*
Evidence: antigens on β cells; chemical triggers include zinc chelators, nitrates, rodenticides

Type 2: Genes/exogenous stimuli → defect in signaling/metabolic pathways → 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 ↑metabolic needs and ↑glucose output, she may develop gestational DM

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

Endocrinopathies

- **↓Insulin secretion:** somatostatinoma, aldosteronoma, pheochromocytoma (**tumors** that ↓insulin secreted)
- **↑Insulin resistance:** acromegaly, Cushing's syndrome, hyperthyroidism (**hormone problems** ↑resistance)

↑[Glucose] = ↑osmotic pressure = ↑bp → polyuria/polydipsia to excrete glucose → ↑protein filtration rate → ↑pore size

AGE: Advanced Glycosylation End-products

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

Trauma → stress → catecholamines + cortisol

- ↑Catabolism
- ↓Insulin release (↓signal)
- ↑Glucose production from liver
- ↑Lipolysis → ↑plasma fatty acids
- ↑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 → vasopressin insensitivity → ↓reabsoprtion of H_2O → ↑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 (>200mg/dL)

Prediabetes

- Not quite there yet, but ↑ 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 $\xrightarrow{glucose\ oxidase}$ hydrogen peroxide $\xrightarrow{Pt\ (oxidation)}$ O_2
  - Electrical current: produced, amplified, measured
  - Amount of current $\xrightarrow{proportional\ to}$ amount of glucose oxidized
- **Photometric glucose monitors**
  - Measures color change
  - Test strip embedded with glucose oxidase + peroxidase + dye
  - Glucose $\xrightarrow{glucose\ oxidase}$ hydrogen peroxide $\xrightarrow{Peroxidase}$ Dye oxidized → color change
  - When test strip instertedi nto meter, color change measured and converted to equivalent glucose level
- **Hb A_{1c}**
  - Glucose + hemoglobin $\xrightarrow{nonenzymatic\ rxn}$ Hb A_{1C}
  - Hb A_{1C} measures average blood glucose level in past 4-6 weeks
  - Amoutn 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:**

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

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-synthesization & secretion:** TG + lipoproteins (from lymph to blood, passing by liver and peripheral tissues)

- **Hydrolyzation:** TG $\xrightarrow{lipases}$ non-esterified fatty acids (NEFA) + glycerol
- **Storage:** FA stored as TG in fat droplets
- **Liver action:** ***NEFA*** $\xrightarrow{\beta-oxidation}$ acetyl CoA $\xrightarrow{TCA\ cofactors,\ \ oxidative\ phosphorylation}$ ATP generation
  Glycerol → 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 tirglycerides/fat

Randle Hypothesis

- ↑FA metabolism → ↑acetyl CoA + ↑citrate → (–) PFK & pyruvate dehydrogenase → ↓glycolysis rate → ↑intracellular glucose → (–) hexokinase → ↓glucose usage
- Overall: ↓glucose uptake, ↑resistance
- Contrary evidence: incorrect for muscle tissue
  - ↑FA → ↑acyl CoA + ↑diacylglycerol → (–) 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 → causes accumulation of acyl CoA → leas to interference with insulin signals

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 → proinsulin
- Proinsulin folds, forming 3 S-S bonds
- C peptide cleaved in golgi apparatus and 4 more aa removed → insulin

Insulin release

- GLUT2 mediates glucose uptake in β cells
- Glucose metabolism in β cells → ATP production → causes K+ channels to close → membrane depolarization → Ca^{2+} entry into βell → IP_3 + DAG → exocytosis of insulin stimulated
- Ca^{2+} activates CREB protein → insulin gene expression (CREB = Ca^{2+} responsive Element Binding)
- Kinetics: 1st phase=immediate bolus; 2nd phase=lower levels but elongated plateau

Insulin receptor

- Transmembrane receptor: tyrosine kinase class
- Heterodimer: 2α + 2β subunits
  - α subunits: extracellular, binds hormone
  - β 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 ↑cellular respiration
- Aspects of the cascade: autophosphorylation of tyrosine kinase, ↑glucose transporters, GLUT4 in peripheral tissue
- Interruption of insulin signals
  - Apparent starvation: X insulin → ↓glucose entry into peripheral tissues → energy starved
  - ↑FA metabolism
  - Ketosis: NEFA converted to ketone bodies
  - Insulin resistance: leads to ↑insulin production/release and hyperinsulinemia

Glucagon

- Structure: 29 aa residue peptidic hormone
- Synthesis: proglucagon in α cells → protease processing → 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 foregin 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 Zn^{2+}: 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_2O → 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! — Only for Type II DM

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 substitutent
- **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