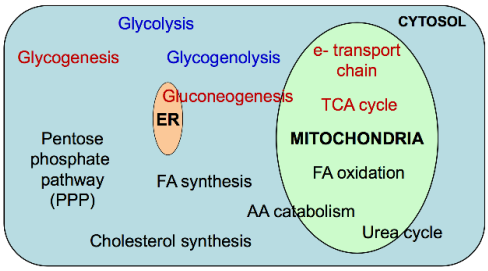
Lecture 32: Regulation of Metabolism: Allosteric Regulation & Tissue Specificity.

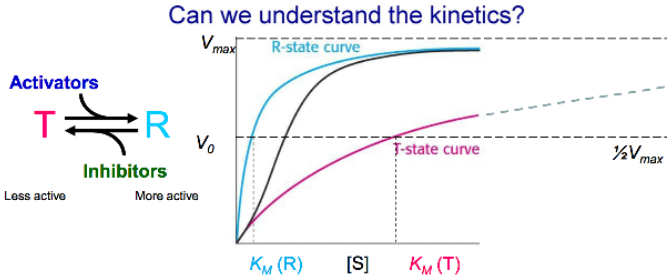
Recurring Motifs in Regulation.

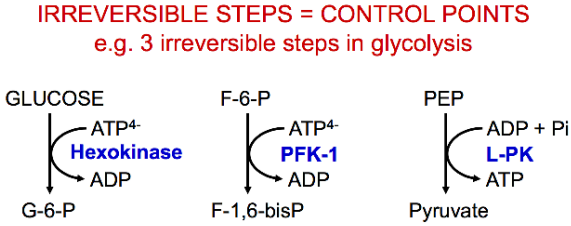
- Compartmentalization – where do the reactions occur? (cytosol, mitochondria, etc.)



- Allosteric regulation – enzymes catalysing committed and usually irreversible steps.

- Definition of allosteric: Of or involving a change in the shape and activity of an enzyme that results from the binding of a regulatory molecule at a site other than the active site.





- Allosteric sigmoidal curve arises from combination of two Michaelis-Menten curves.
- Same V_{max} for R and T-states; but with different K_M values.
  - R-state (more active) lower K_M (uninhibited state)
  - T-state (less active) higher K_M (inhibited state)
- As [substrate] increases, there is a shift from T-state to R-state curve.
- Equilibrium shifts T → R with increased [substrate] as substrate binding stabilizes R-state.

Example 1: Allosteric Regulation in Glycolysis: Phosphofructokinase-1.

- Phosphofructokinase-1 (PFK-1) is the most important control element of glycolysis.
- PFK-1 is deficient in Type VII glycogen storage disease.

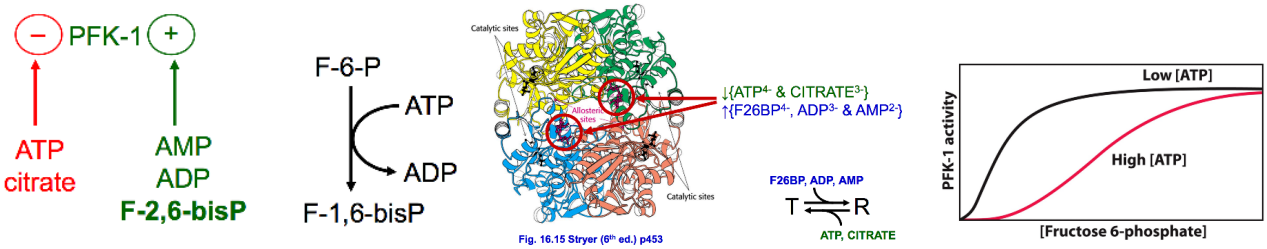
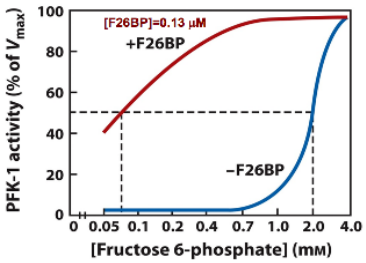
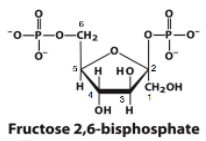


Fig. 16.15 Stryer (6th ed.) p453

- ATP, citrate inhibits while AMP, ADP and F-2,6-bisP activates PFK-1.
  - Purpose of glycolysis is to make ATP, if we've got a lot of ATP this will have a negative feedback on the enzyme. Citrate is the first product of TCA cycle and works in the same way.
  - When inhibitor binds it does not mean no activity; T-state still have some activity but just lower. (which is different to competitive/uncompetitive/mixed inhibitors which 'switches off' the activity)

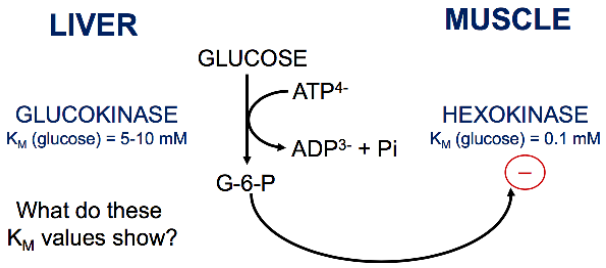




This is different to Fructose 1,6-bisphosphate

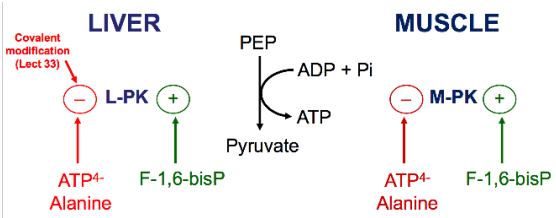
*PFK-1 is allosterically activated by Fructose-2,6-bisphosphate production in regulated differently in Liver and Muscle (lecture 33).
There is always a dynamic balance between T and R state, it's usually not just one or the other.

Example 2: Allosteric Regulation in Glycolysis: Hexokinase.



- In the liver, there is glucokinase and in the muscle, there is hexokinase.
  - HEXOKINASE (and not GLUCOKINASE) is allosterically inhibited by the product of the reaction it catalyses.
  - They have very different K_M. Hexokinase is working near its K_{max} all the time, Glucokinase on the other hand mostly work during fed state.
    *Note blood glucose level is always ~5mM.
  - G-6-P can slow down hexokinase when bounded to it.

Example 3: Allosteric Regulation in Glycolysis: Pyruvate Kinase.



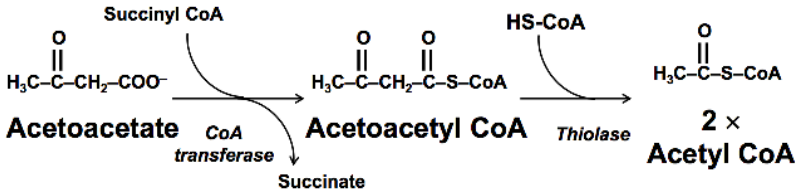
- Fructose-1,6-bisphosphate allosterically activates L-PK and M-PK, and ATP^{4-} & Alanine allosterically inhibit.

- Covalent regulation- phosphorylation.
- Enzyme levels- control of gene expression.

- Specialization of organs – we will compare the metabolism of brain, liver, muscle & adipose.

Brain.

- Fuel sources: Glucose is major fuel for the human brain
  - Brain can adapt to ketone bodies (KBs) during starvation (> 3 days) & danger occurs when [glucose] < 2.2 mM.
- Brain lacks fuel stores.
  - relies on a constant supply of blood glucose (via GLUT3, $K_M \approx 1.0$ mM)



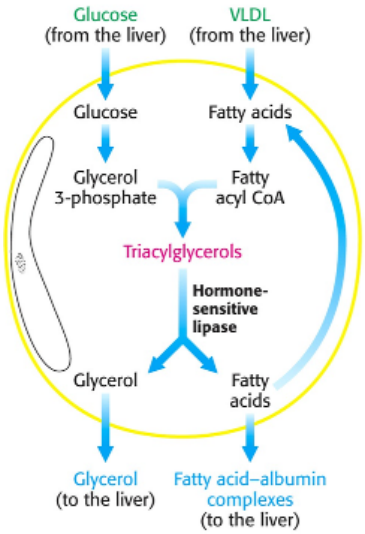
- Resting Conditions.
  - Brain consumes 60% of total GNG glucose = 120 g/day (1.76 MJ) in the resting state

Muscle.

- Fuel sources: Glucose, fatty acids & ketone bodies.
- Muscle stores 75% of total body glycogen (> 5 MJ) and can represent 1% of muscle weight after a meal (other 25% stored in liver).
- Resting Conditions: Muscle utilizes fatty acids (Fas) as the major fuel in the resting state (85% of energy). Heart muscle uses the one of the ketone bodies, acetoacetate, in preference to glucose.
- Muscle & Liver metabolites connected by the Cori Cycle, as we saw in Lect. 30.

Adipose.

- Fuel sources: Requires glucose to perform major task of synthesizing and storing triacylglycerol, which is mobilized during fasting.
- In a 70kg person, adipose stores >80% of total available energy (565 MJ or 15 kg).
- Resting Conditions: Highly active during starvation (↓ insulin activates hormone- sensitive lipase which breaks down TAG - see opposite)



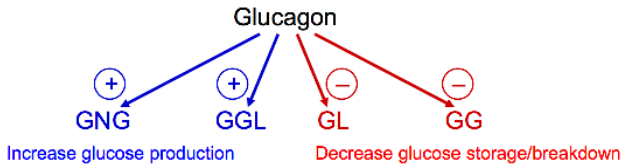
Liver.

- Fuel sources: Can utilize glucose, fatty acids, ketone bodies and amino acids.
  - But prefers α-keto acids derived from the degradation of amino acids in preference to glucose.
- Liver stores 1/4 of total body glycogen.
  - Uses lactate & alanine from muscle, glycerol from adipose & glucogenic amino acids from diet to make ~ 200 g of glucose per day via gluconeogenesis.
- Resting Conditions: Highly active during starvation making glucose via GNG to maintain blood [glucose] primarily for the brain & RBCs.
  - Also oxidizes FAs for energy and formation of KBs for the brain, heart muscle & other tissues.
- Other Functions: Synthesizes TAGs, PLs & cholesterol & secretes as VLDL for lipoprotein transport & synthesizes heme.
  *It is an altruistic organ (unselfish)

Lecture 33.

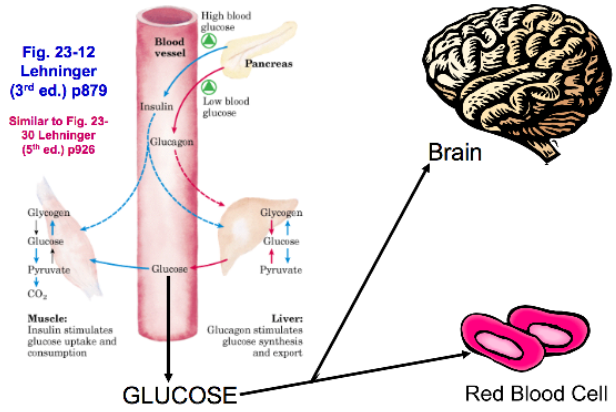
The Fasting State.

• Drop in blood glucose below 4.5mM triggers glucagon release from the pancreas:
  - resting [glucose]blood = 5.0 mM
• Glucagon causes an increase in blood glucose levels.
• Glucagon activates gluconeogenesis (GNG) & glycogenolysis (GGL) in the liver.
• Glucagon inhibits glycolysis (GL) and glycogenesis (GG).



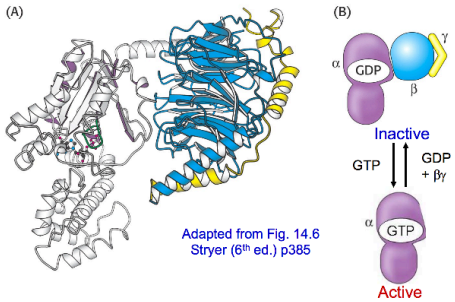
Fasting: Priority to Supply Glucose to Brain and Erythrocytes.

- Brain and rbc needs glucose to function.
- rbc don't have mitochondria.

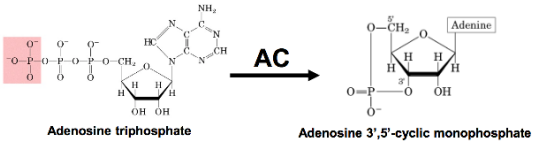


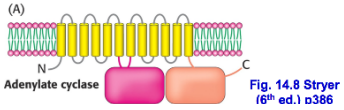
The Glucagon Signal Transduction Pathway in Liver.

- Glucagon (1° messenger).
  - Peptide hormone (29 AAs, Mr = 3,483)
  - • Synthesized in the α cells of the pancreas.
  - • Secreted into the blood when [glucose]blood drops < 4.5mM.
- Glucagon Receptor.
  - The glucagon receptor (& β-adrenergic receptor) is a 7TM receptor since it contains 7 transmembrane α-helices.
- Guanyl Nucleotide (G) Protein.
  - Information is transmitted to G-protein coupled receptor.

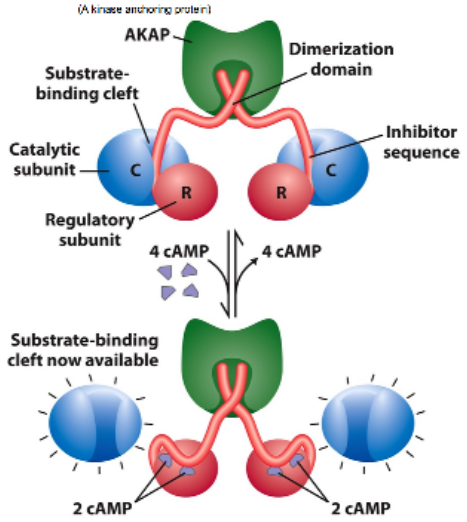


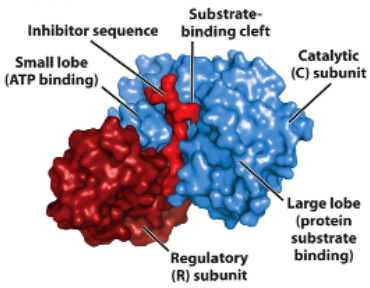
- cAMP (2° messenger).
  - cAMP is made from ATP by the enzyme Adenylate Cyclase (AC).



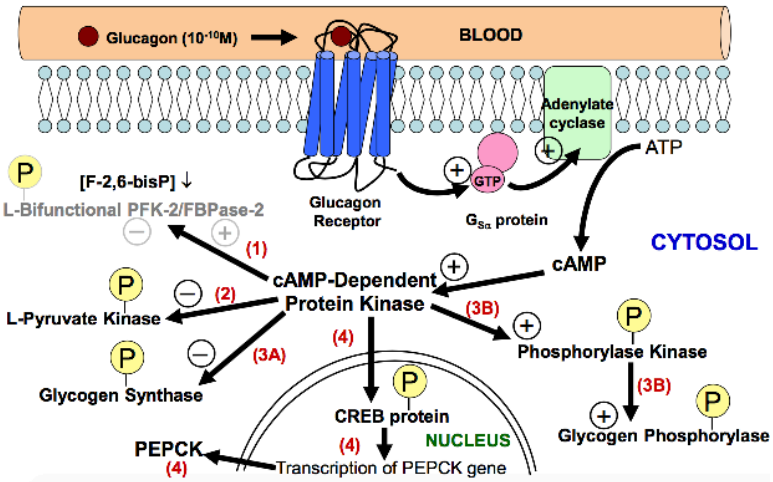


- cAMP-dependent protein kinase or Protein Kinase A (PKA)
    - cAMP activates PKA allosterically & when activated PKA phosphorylates several protein targets on Ser/Thr residues



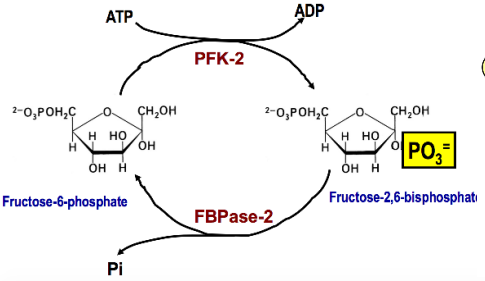


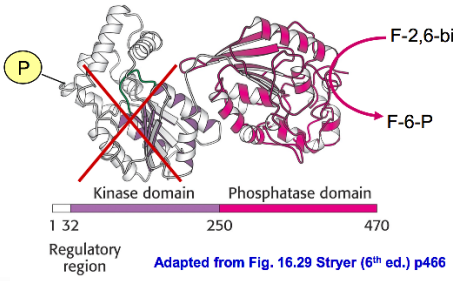
key targets.
Glucagon Signalling in the LIVER.

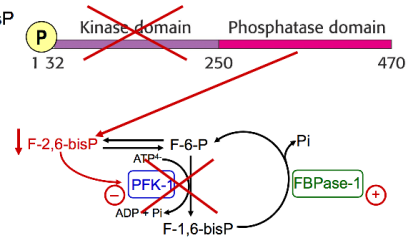


Once PKA is activated by cAMP, it will now bind to multiple targets:

1. Bifunctional PFK-2/FBPase-2 enzyme.

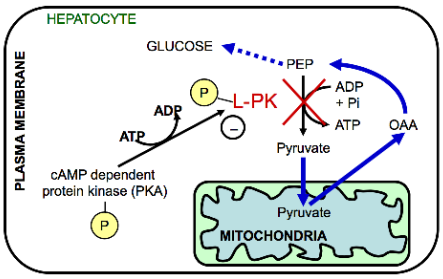




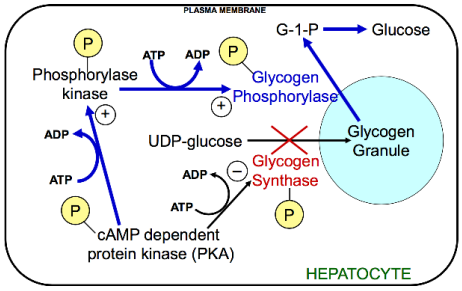


- Liver PFK-2 is inactivated and FBPase-2 activated when a Ser residue in the regulatory domain is phosphorylated by PKA.
- Kinase domain is phosphorylated thus inactivated.
- effectively lowering the concentration of F-2,6-bisP which affects glycolysis and slows it down.

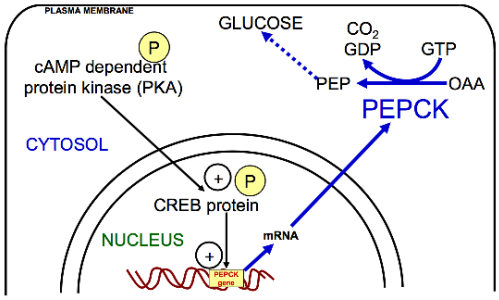
2. L-Pyruvate Kinase is phosphorylated.
   - make more PEP for glucose and GNG.



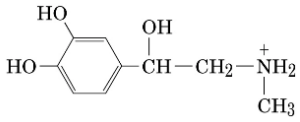
3. (A) Glycogen Synthase.
   (B) Glycogen Phosphorylase.
   - chop up more and store less glycogen.



4. Phosphoenolpyruvate carboxykinase (PEPCK).
   - part of gluconeogenesis, target of PKS.
   - more PEPCK, more PEP, make more glucose.

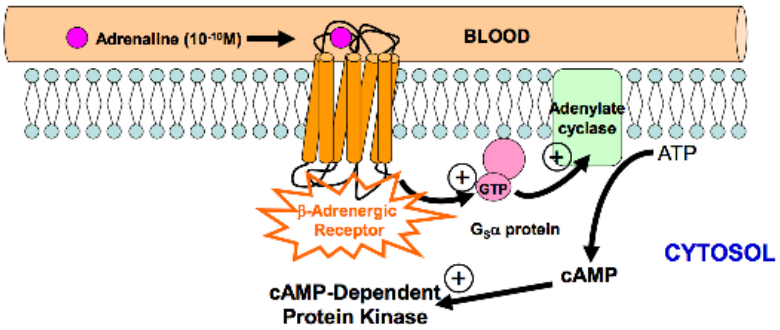


The Adrenaline Signal Transduction Pathway in Liver:
Adrenaline (Epinephrine).
• Synthesized from tyrosine in 4 steps via dopamine intermediate (see structure below)
• Catecholamine released from the adrenal gland.



• Involved in the fright-flight-fight response largely targeting muscle, but also hits liver cells.
- "Fright" → Release of adrenaline from adrenal gland
- "Flight" & "Fight" → Adrenaline stimulates glycogenolysis and glycolysis in muscle so that ATP can be produced quickly for energy to "fly" away or "fight" with predator

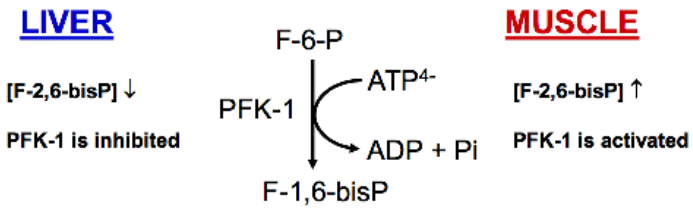
Adrenaline Signalling.
cAMP dependent kinase & everything downstream are also switched on by adrenaline binding to the β-adrenergic receptor, but hepatocytes are more responsive to glucagon because they have more glucagon receptors relative to adrenaline receptors.



Adrenaline Signalling in Liver versus Muscle.
• In Liver: Adrenaline switches on gluconeogenesis & glycogenolysis.
• In Muscle: Adrenaline switches on glycolysis and glycogenolysis.

How does Adrenaline Signalling in Muscle differ from Liver to create different responses?
- to understand this, we need to know what's happening to the molecules:

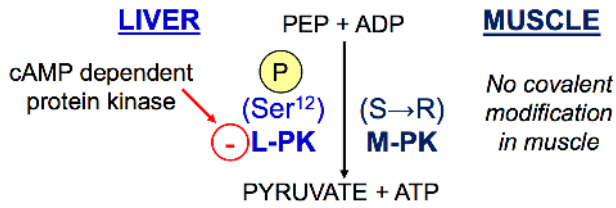
(1) [F-2,6-bisP] down in liver; up in muscle.



• Liver and muscle express different isoforms of the enzyme that controls F-2,6-bisP levels (bifunctional PFK-2/FBPase-2).
• PKA phosphorylation has opposite effect on these enzymes.
• Adrenaline signalling causes ↓[F-2,6-bisP] in liver, but ↑ [F-2,6-bisP] in muscle
• Glycolysis is suppressed in liver, but increased in muscle.

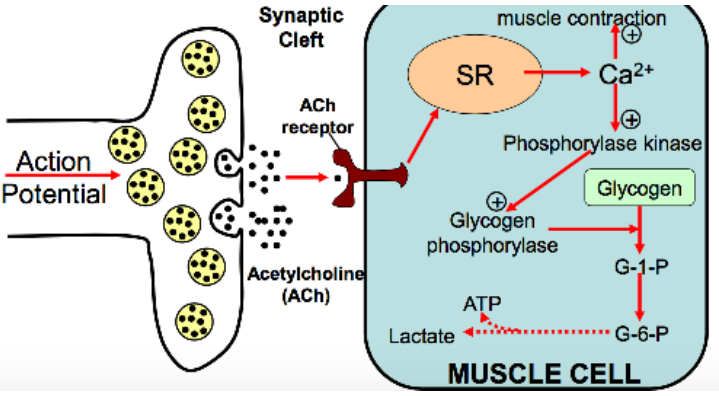
(2) M-PK is not Phosphorylated.

*In muscle, it is arginine instead of serine.

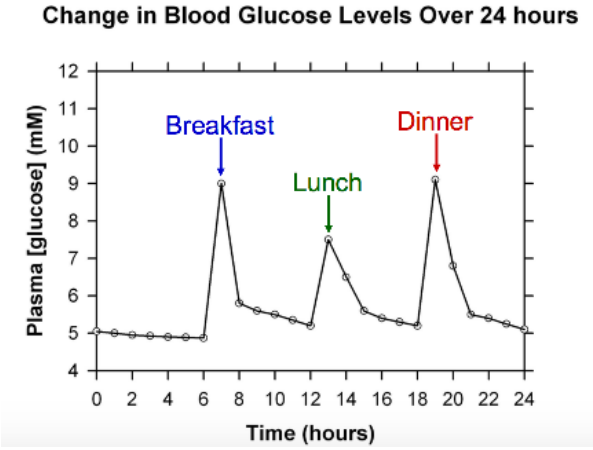


• M-PK lacks the key serine residue that is phosphorylated by PKA in L-PK.
• Muscle PK is not inhibited by adrenaline signaling (PEP>pyruvate into TCA>oxPhos will continue)
• Glycolysis is suppressed in liver, but not in muscle.

(2) Neuronal Signalling in Muscle via Ca2+ as a 2° Messenger.
- Brain tells muscles to get moving (NOW!) Ca2+ release stimulates glycogenolysis.



The Post-Prandial State & Insulin.



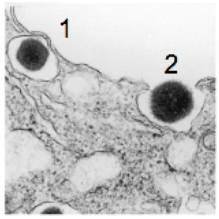
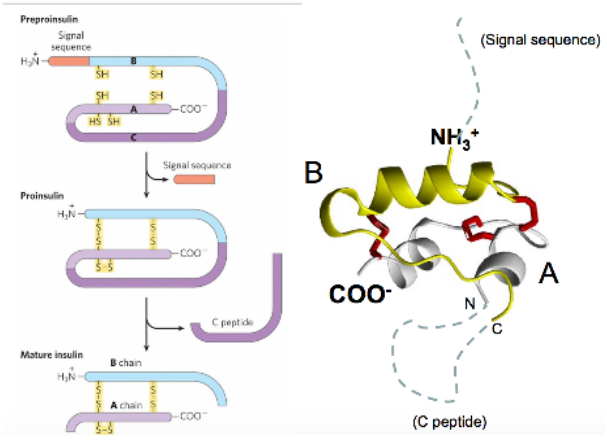
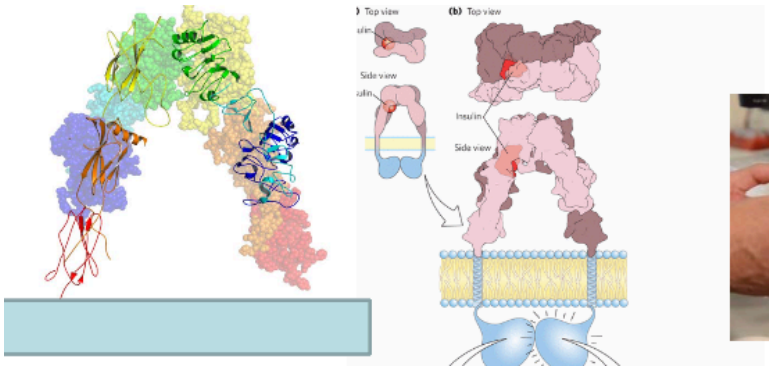


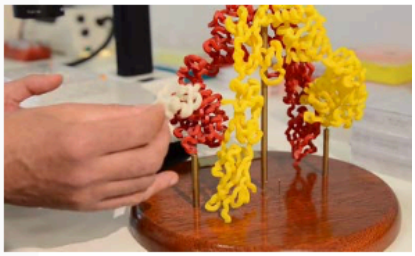
Fig. 27.15 Stryer (6th ed.) p770
EM of insulin being released from a pancreatic β cell. One secretory granule is on the verge of fusing with the plasma membrane (1) and releasing the insulin cargo & the other (2) has already released the hormone.

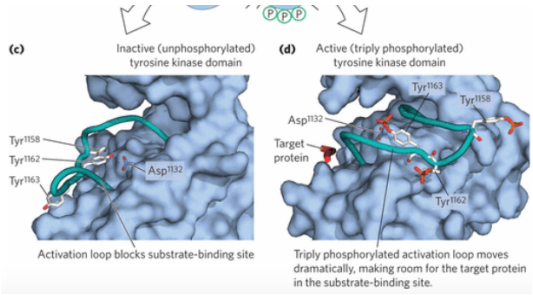
Insulin Signal Transduction Pathway.
(1) Insulin.
• Peptide hormone (51 amino acids, Mr = 5,808).
• Two chains (A and B) linked by two –S–S– bonds.
• Synthesized as preproinsulin in pancreas.
• Pre-peptide (signal) and C-peptide cleaved to yield active insulin hormone.
• Mature form is secreted by β-cells of pancreas.



(2) Insulin Receptor.

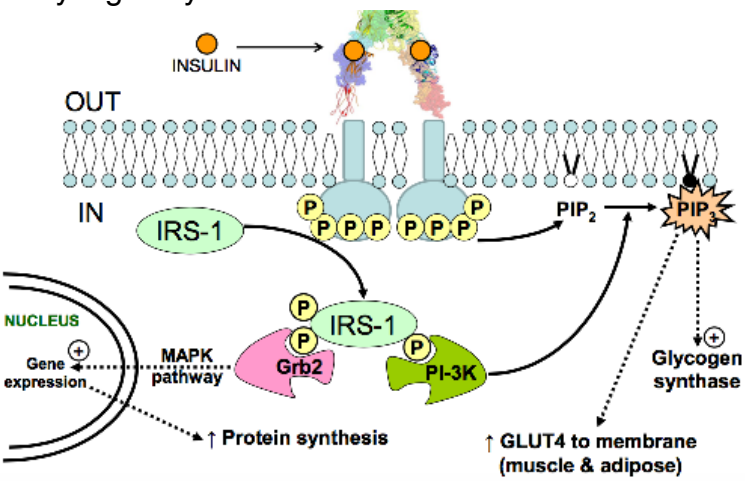






(3) Insulin Receptor Substrate-1 (IRS-1) &
(4) Phosphatidylinositol 3,4,5 triphosphate (PIP3).

- insulin binds, we get a conformational change, causes 2 kinase domains to come closer and able to phosphorylate each other.
- Phosphorylates a bunch of other sites as well, IRS-1 is phosphorylated and is bound to Grb2 and PI-3K.
- PI-3K converts PIP2 to PIP3.
- Glycogen synthase activated and GLUT 4 activated.



Pathways Regulated by Insulin.

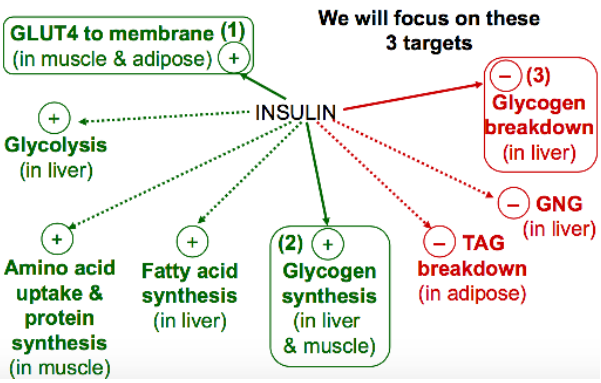
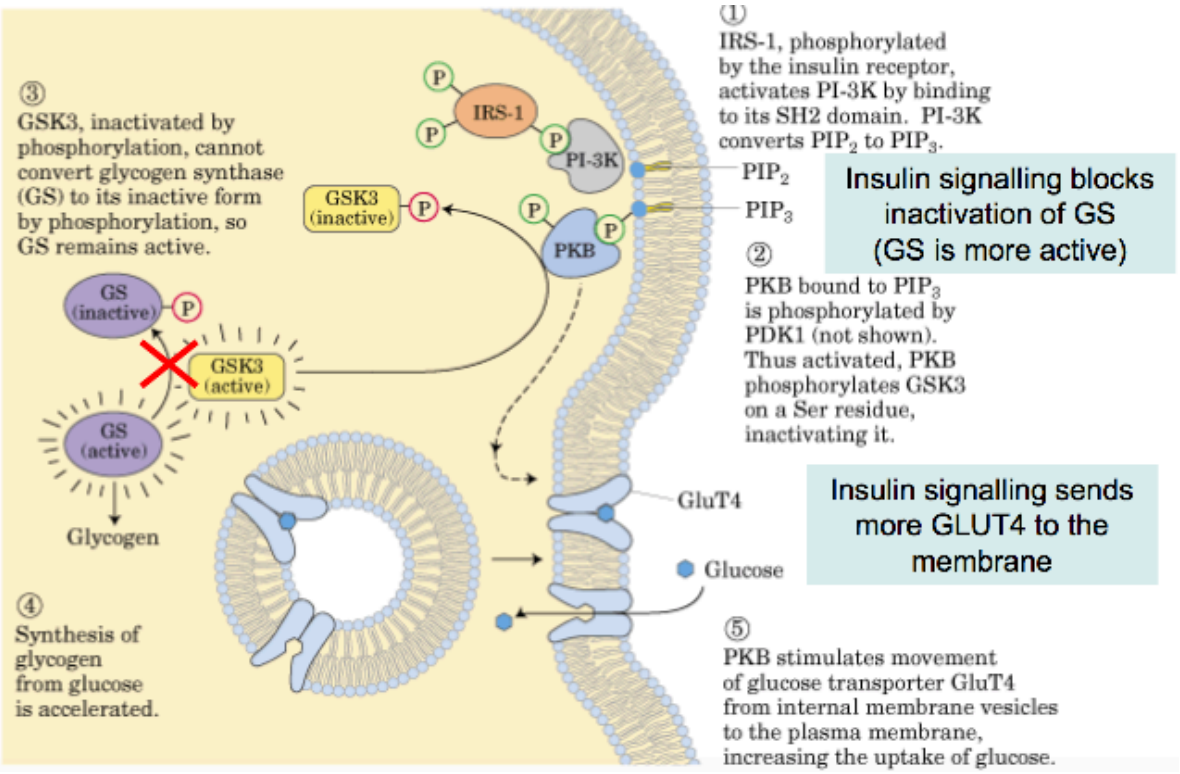


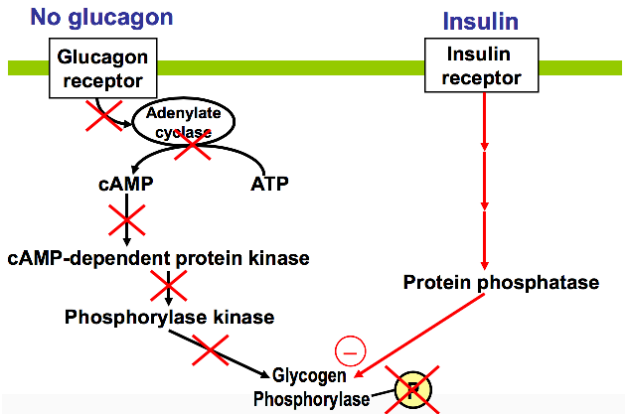
TABLE 11–3 Glucose Transporters in Humans

Transporter	Tissue(s) where expressed	K_t (mM)*	Role†
GLUT1	Ubiquitous	3	Basal glucose uptake
GLUT2	Liver, pancreatic islets, intestine	17	In liver and kidney, removal of excess glucose from blood; in pancreas, regulation of insulin release
GLUT3	Brain (neuronal), testis (sperm)	1.4	Basal glucose uptake
GLUT4	Muscle, fat, heart	5	Activity increased by insulin

Regulation of GLUT4 & Glycogen Synthase.



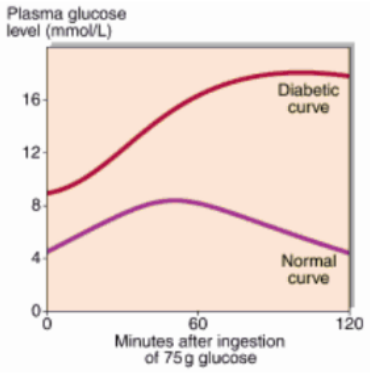
Insulin Inhibits Glycogen Phosphorylase.



*Protein phosphatase activated and deactivates glycogen phosphorylase by snipping off a phosphate group.

Diabetes Mellitus

• Incidence: ~7% of the Australian population (estimated to double by 2031).
• Clinical types:
  -Type 1 (11%).
  - Type 2 (87%).
  - Gestational (2%).
• Causes:
  - Type 1 is caused by autoimmune destruction of insulin secreting pancreatic β cells;
  - Type 2 is prevalent in obese middle-aged people and has a genetic component.
  - Gestational due to insulin deficiency (2-3x insulin required)
• Clinical Symptoms:
  - thirst, excessive urine, dizziness, lethargy, headaches, slowly healing cuts, leg cramps
• Biochemical Symptoms:
  - Elevated blood glucose (i.e. fasting blood [glucose] ≥ 7.0mM – see next slide)
• Treatment:
  - Injection of recombinant insulin post-prandial (Type I); low carbohydrate diet, weight loss & exercise
  - (Type 2); Gestational: combination diet, exercise, sometimes insulin.



Fasting blood glucose		
Non-diabetic	Impaired fasting glucose	Diabetes
<6.0mM	6.0-6.9mM	≥7.0mM
Oral glucose tolerance test		
	Fasting	2 hour
Impaired Glucose tolerance	<7.0mM	7.8-11.0mM
Diabetes	≥7.0mM	≥11.1mM

Summary of metabolism that we've done: