BB 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations — Exam-1 material

Pyruvate — Exam-2 material
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Fat Catabolism — Exam-3 material
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: β-oxidation, 4 steps:

Protein Catabolism
Amino-Acid Degradation
- Dealing with the nitrogen; Urea Cycle
- Dealing with the carbon; Seven Families

Nucleic Acid & Nucleotide Degradation

PHOTOSYNTHESIS:

Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop experiment"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions

ANABOLISM I: Carbohydrates

Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
  - Carboxylase
  - Oxygenase
  - Glycolate cycle

  } Know mechanism
- Stage Two – making sugar
- Stage Three – remaking Ru $1,5P_2$

Overview and regulation
Calvin cycle connections to biosynthesis — Know pathway
C4 versus C3 plants
Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
- precursors
- Cori cycle

Gluconeogenesis — Know pathway
- reversible steps
- irreversible steps – four
  - energetics
  - 2-steps to PEP in mitochondria: Pyr carboxylase-biotin & PEPCK
  - FBPase
  - G6Pase

Glycogen Synthesis
- UDP-Glc
- Glycogen synthase
- branching

Pentose-Phosphate Pathway — Know pathway
- oxidative-NADPH
- non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
- Acetyl-CoA/Pyruvate
- Pyruvate/PEP
- F6P/FBP: Fru $2,6P_2$
- Glc/Glc6P: sequestration
- Glycogen: PKA/PP1
- Insulin signaling

Anaplerotic reactions
Biosynthesis of Lipids

instagram

Regulation of Carbohydrate Metabolism

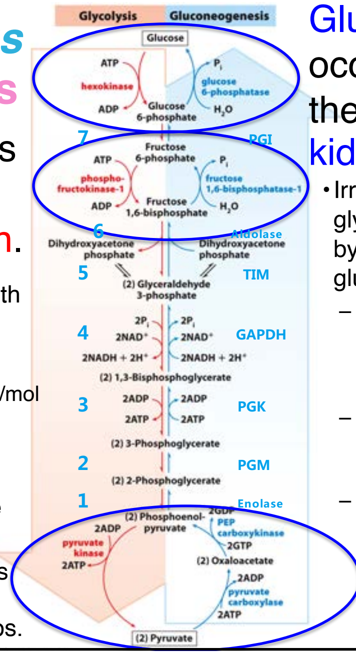
Catabolism *vs.* Anabolism

RECALL: Gluconeogenesis

Glycolysis *versus* Gluconeogenesis

Glycolysis occurs mainly in the muscle and brain.

- Opposing pathways that are both thermodynamically favorable:
- Glycolysis: $\Delta G^{\circ\prime} = -35$ kcal/mol
- Gluconeogenesis: $\Delta G^{\circ\prime} = -9$ kcal/mol
  - operate in opposite direction
    - end product of one is the starting compound of the other
- Seven Reversible reactions are used by both pathways.
- Three "glycolysis-specific" steps are reversed with Four "gluconeogenesis-specific" steps.



Gluconeogenesis occurs mainly in the liver and kidney cortex.

- Irreversible reactions of glycolysis must be bypassed in gluconeogenesis.
  - no ATP generated during gluconeogenesis; instead 6 ATPs and 2 NADH needed per Glc.
  - Some different enzymes results in the different pathways
  - differentially regulated to prevent a futile cycle

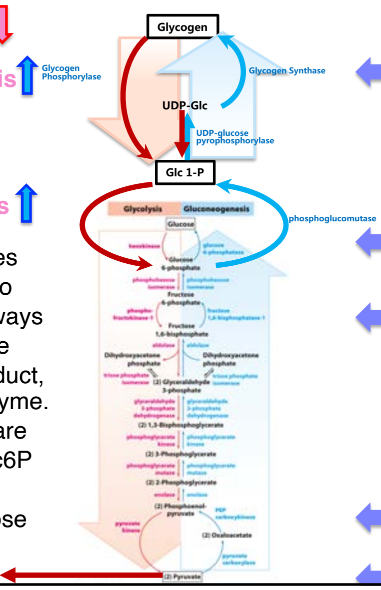
Regulation of Carbohydrate Metabolism

Gene controlled

Glycogenolysis ↓
versus
Glycogen Synthesis ↑

Glycolysis ↓
versus
Gluconeogenesis ↑

- Regulated enzymes often correspond to points in the pathways that have the same substrate and product, but a different enzyme.
- Also where there are junctions; e.g., Glc6P
- Can you name those enzymes?



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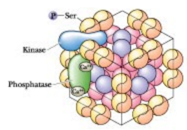
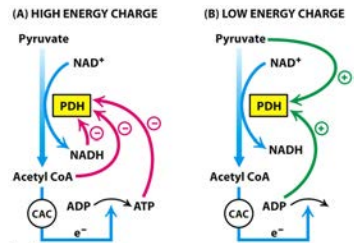
Hexokinase	Glucose 6-phosphatase
Phosphofructo-kinase-1	Fructose 1,6-bisphosphatase-1
Pyruvate Kinase	– PEPCK – Pyruvate carboxylase
Pyruvate Dehydrogenase Complex	

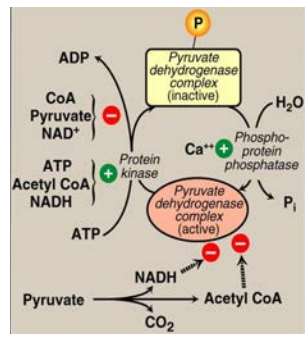
Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Dehydrogenase Complex





- Allosteric regulation by energy charge and substrate/product
  - ADP & pyruvate activates
  - ATP/NADH & acetyl-CoA inhibit
- Regulated by reversible phosphorylation of E1
  - phosphorylation: inactive
  - dephosphorylation: active
- PDH kinase and PDH phosphatase are part of mammalian PDH complex.
  - Kinase is activated by ATP.
    - high ATP → phosphorylated PDH → less acetyl-CoA made
    - low ATP → kinase is less active and phosphoprotein phosphatase removes phosphate from PDH → more acetyl-CoA made



Regulation of Carbohydrate Metabolism

Gene controlled

Glycogenolysis ⇩
versus
Glycogen Synthesis ⇧

Glycolysis ⇩
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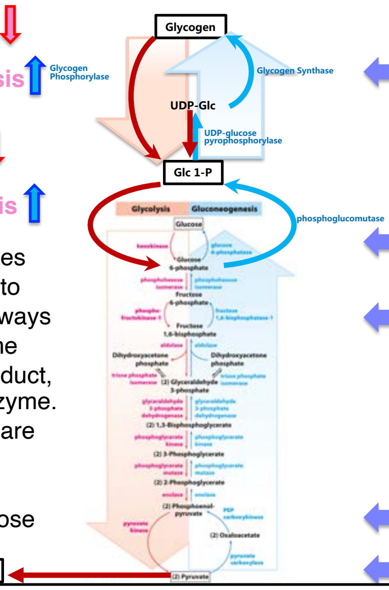

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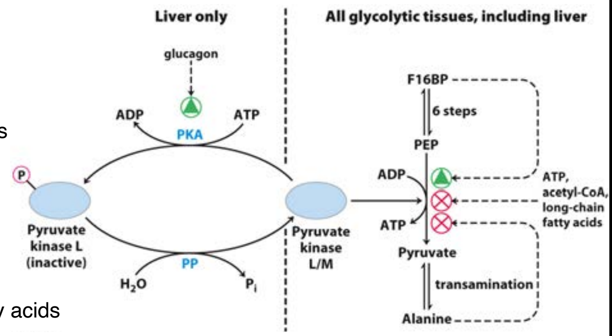
Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Kinase

All tissues

- Allosterically activated by fructose-1,6-bisphosphate
  - increase flow through glycolysis
  - Feed-forward activation
- Allosterically inhibited by signs of abundant energy supply.
  - ATP
  - acetyl-CoA and long-chain fatty acids
  - alanine (enough amino acids)



Liver only (under hormonal control)

Inactivated by phosphorylation in response to signs of glucose depletion (low blood-glucose→glucagon) (liver only)

Glucose from liver is exported to the brain and other vital organs.

This is not the only time we'll see hormonal control of these pathways.

Regulation of Carbohydrate Metabolism

Gene controlled

Glycogenolysis ⇩ *versus* **Glycogen Synthesis** ⇧

Glycolysis ⇩ *versus* **Gluconeogenesis** ⇧

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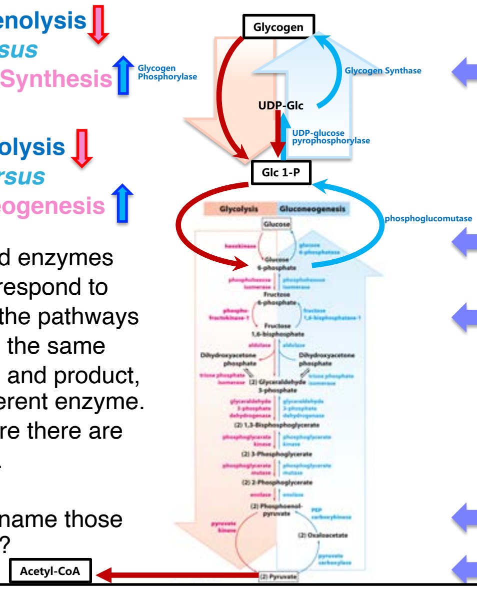

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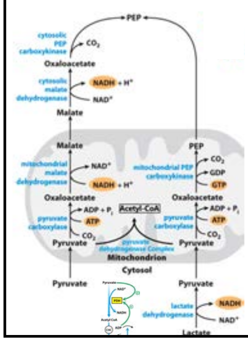
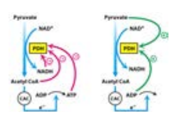
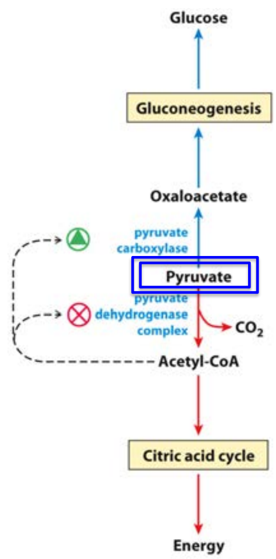
Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Carboxylase

- Allosteric activation of pyruvate carboxylase by **acetyl-CoA**
  - stimulates glucose synthesis via gluconeogenesis because plenty of acetyl-CoA signals no need to make more and save pyruvate.
- Notice the reciprocal control of PC & PDHC by **acetyl-CoA**







Regulation of Carbohydrate Metabolism

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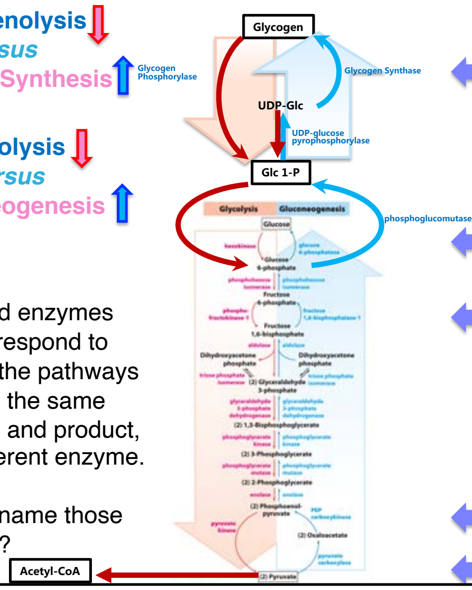

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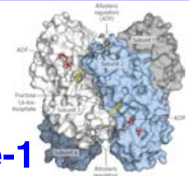
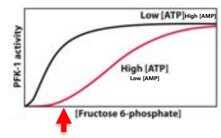
Regulation of Carbohydrate Metabolism

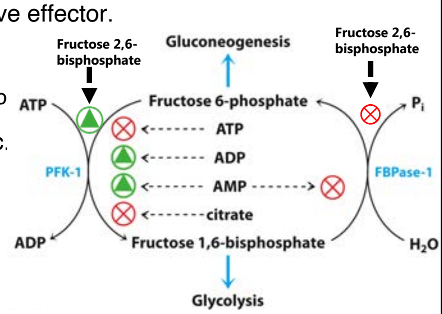
Regulation of Phosphofructokinase-1 *versus* Fructose 1,6-bisphosphatase-1

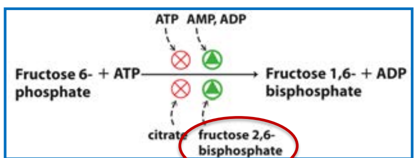




Homeostatic level of Fru 6-P

- Fructose-6-phosphate → fructose 1,6-bisphosphate is the commitment step in glycolysis
- While ATP is a substrate, ATP is also a negative effector.
  - Do not spend glucose in glycolysis if there is plenty of ATP.
  - Same for citrate, if there is plenty of citrate, do not waste glucose
- Low energy charge inhibits biosynthesis of Glc.





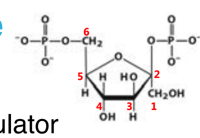
Is this a typo?

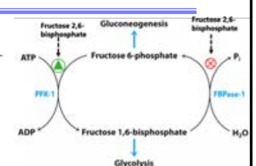
~~Bumble bees~~ are missing an FBPase that responds to AMP

- Go glycolysis if AMP is high and ATP is low.
- Go gluconeogenesis if AMP is low.

Regulation of Carbohydrate Metabolism

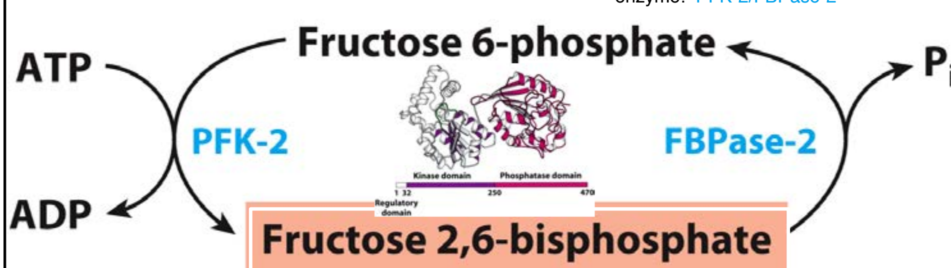
Fructose 2,6-*(bis)*phosphate (βD-Fru-2,6P_2)





- NOT a glycolytic intermediate, only a regulator
- Produced specifically to regulate glycolysis and gluconeogenesis
  - activates phosphofructokinase-1(PFK-1) (glycolysis)
  - inhibits fructose 1,6-bisphosphatase (FBPase-1) (gluconeogenesis)

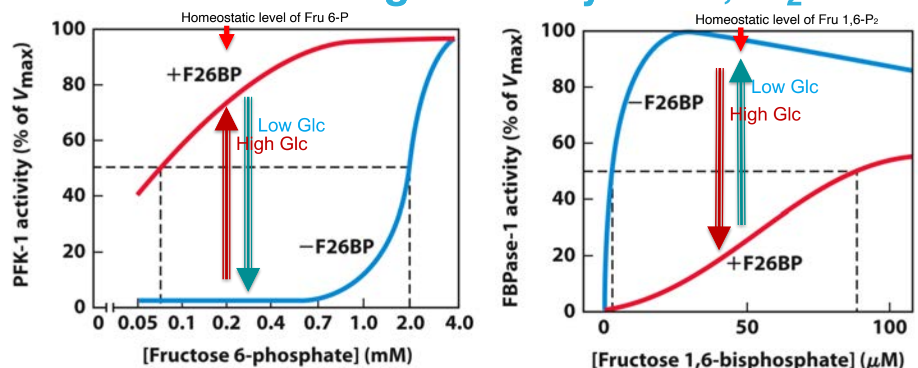
Enzyme for synthesis and degradation of Fru 2,6P_2 done with a dual-function enzyme: PFK-2/FBPase-2*



*6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase

Regulation of Carbohydrate Metabolism

Regulation of Glycolysis and Gluconeogenesis by Fru-2,6P$_2$



- Without Fru 2,6P$_2$, STOP glycolysis
  GO gluconeogenesis.

This would be the state of low Glc

- With Fru 2,6P$_2$ (130 nM), Go glycolysis.
- With Fru 2,6P$_2$ (1300 nM), Stop gluconeogenesis.

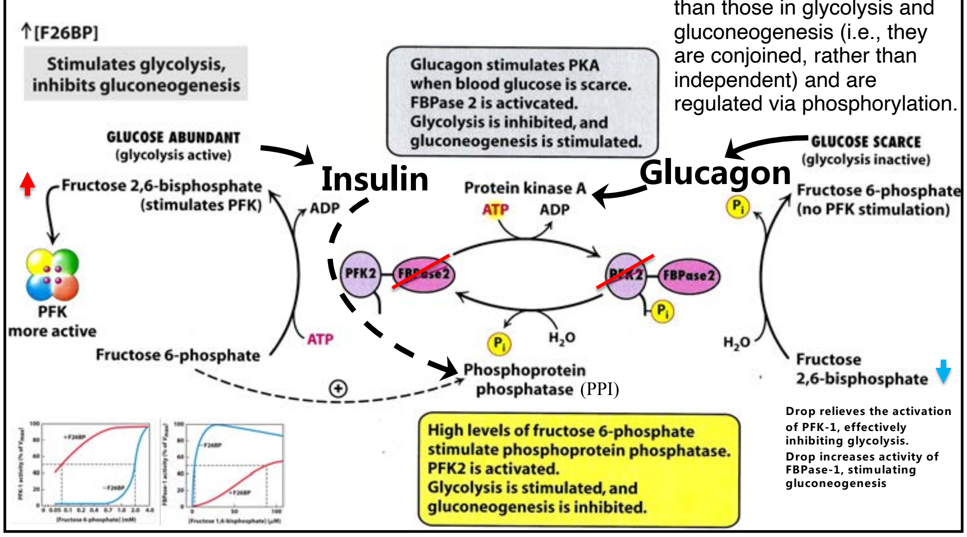
This would be the state of high Glc

What controls PFK-2/FBPase-2?

Regulation of Carbohydrate Metabolism

Regulation of Fru-2,6-P$_2$ Levels

Structurally, this enzyme, with its two activities is different than those in glycolysis and gluconeogenesis (i.e., they are conjoined, rather than independent) and are regulated via phosphorylation.



Regulation of Carbohydrate Metabolism

Gene controlled

Glycogenolysis ⇩ *versus* **Glycogen Synthesis** ⇧

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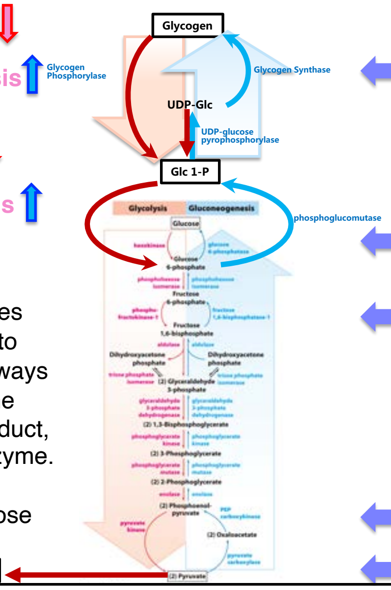

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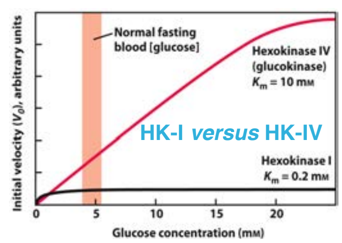
Pyruvate Dehydrogenase Complex ✓	

Regulation of Carbohydrate Metabolism

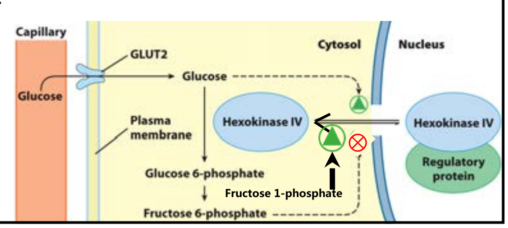
Regulation of Hexokinase

There Are Four Isozymes of Hexokinase (I-IV)

- Isozymes are different enzymes that catalyze the same reaction.
  - typically share similar sequences
  - may have different kinetic properties
  - can be regulated differently
- HK I is expressed in all tissues, to different levels.
- HK IV (glucokinase) is only expressed in the liver and pancreas.
  - has higher K_m, so responsive to higher [glucose]
  - not inhibited by glucose-6-phosphate, so can function at higher [glucose]
  - functions to clear blood glucose at higher [glucose] for storage as glycogen (also Fru1P)
  - Glc activates release/Fru6P inhibits



Glucokinase Is Regulated by Sequestration



Regulation of Carbohydrate Metabolism

Gene controlled

Glycogenolysis ⇩
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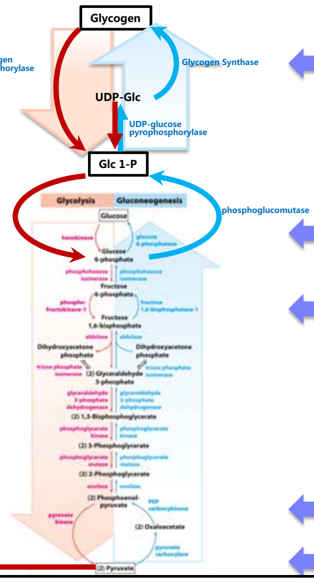

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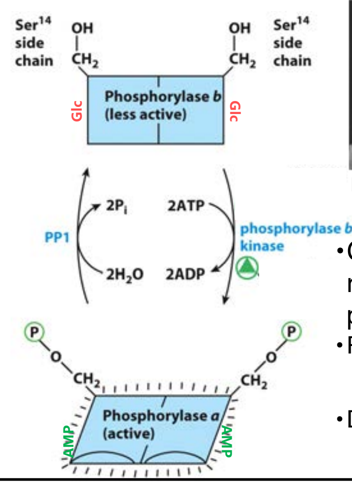
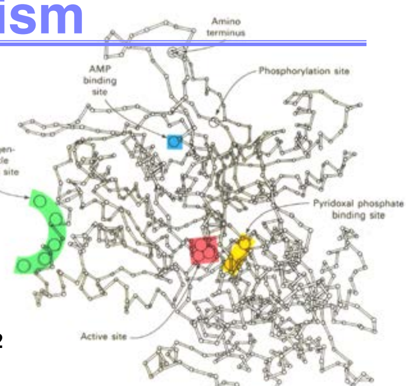
Regulation of Carbohydrate Metabolism

Regulation of Glycogen Phosphorylase

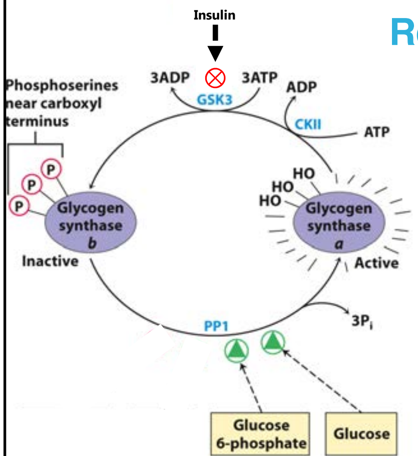


Nobel Prize 1972
Earl Sutherland
1915-1974

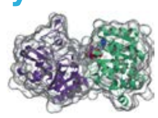


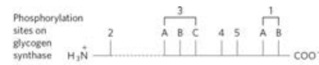
- Glycogen phosphorylase cleaves glucose residues off glycogen, generating glucose-1-phosphate (Glc 1P).
- Phosphorylation activates glycogen phosphorylase-*b*
  - Phosphorylase-*b* **Kinase**
  - Accentuated by allosteric binding of AMP (muscle only)
- Dephosphorylation inhibits glycogen phosphorylase-*a*
  - Phosphoprotein phosphatase-1 (PP1)
  - Accentuated by allosteric binding of Glc (in liver only)

Regulation of Carbohydrate Metabolism



Regulation of Glycogen Synthase



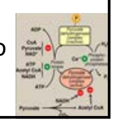


Kinase	Phosphorylation sites	Degree of synthase inactivation
Protein kinase A	1A, 1B, 2, 4	+
Protein kinase G	1A, 1B, 2	+
Protein kinase C	1A	+
Ca^{2+}/calmodulin kinase	1B, 2	+
Phosphorylase *b* kinase	2	+
Casein kinase I	At least nine	+ + + +
Casein kinase II	5	0
Glycogen synthase kinase 3	3A, 3B, 3C	+ + +
Glycogen synthase kinase 4	2	+

Need energy: stop storing & release

- Glycogen synthase adds glucose residues to glycogen using UDP-Glc.
- Phosphorylation inhibits glycogen synthase-*a*
  - Its complicated, responding to multiple signals
  - Example: First Casein Kinase-2 (CKII), then Glycogen Synthase Kinase-3 (GSK3) [inactivated by insulin]
- Dephosphorylation activates glycogen synthase-*b*
  - Phosphoprotein phosphatase-1 (PP1) (in liver it's a different PP)
  - PP1 is bound to GS-b
- Also, feedforward control by glucose and Glc-6P
  - Binding causes a conformation favorable for PP-1 binding
  - Binding does not allow GSK-3 access to phosphorylation sites

Regulation of glycogen synthase/glycogen phosphorylase is somewhat similar to regulation of PDH complex

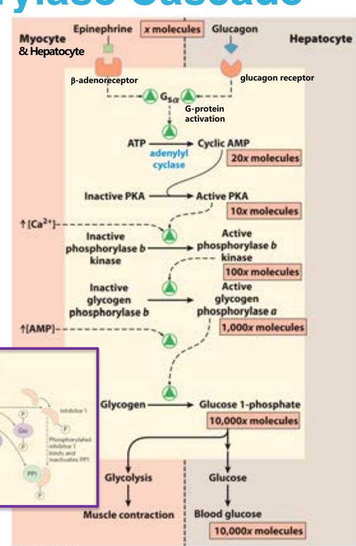


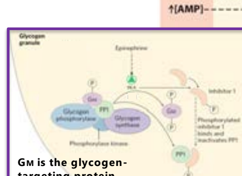
Regulation of Carbohydrate Metabolism

Glycogen Phosphorylase Cascade

Nobel Prize 1992
Edwin Krebs
1918-2009

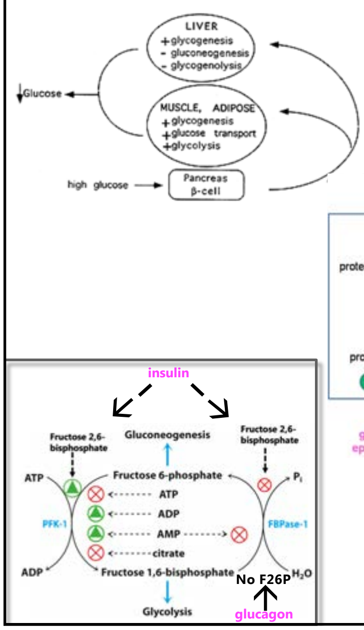
- Glucagon/epinephrine signaling pathway activated when there is a NEED for energy
  - starts phosphorylation cascade via cAMP
  - cAMP activates PKA
  - PKA activates phosphorylase-b kinase
  - this kinase activates glycogen phosphorylase
  - Massive degradation of glycogen
    - In muscle: Glc1P→Glc6P→glycolysis
    - In liver: Glc1P→Glc6P→Blood glucose
- There is reciprocal inhibition of GS
  - PKA phosphorylates G_M, which is bound to PP1 on GS, thus dissociating it.
  - PKA also phosphorylates PP1-inhibitor protein, which binds and inactivates the free PP1, thus leaving GS-Ⓟ and inactive

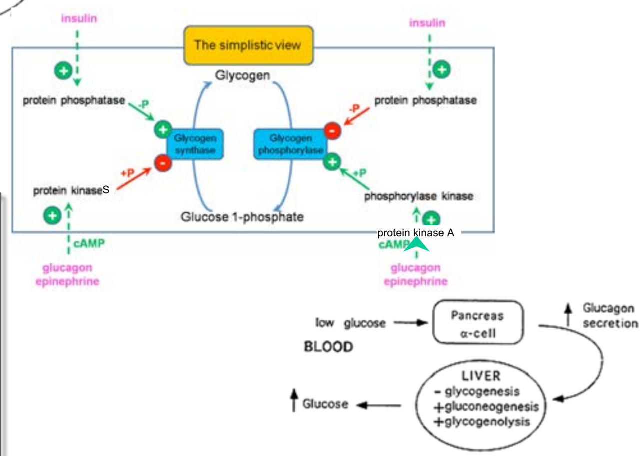




Regulation of Carbohydrate Metabolism

Reciprocal regulation of glycogen synthesis and glycolysis





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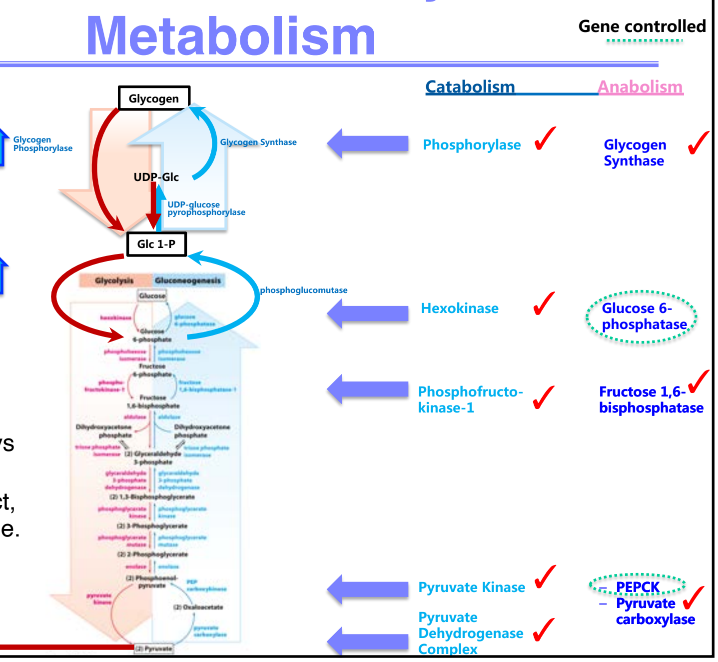

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