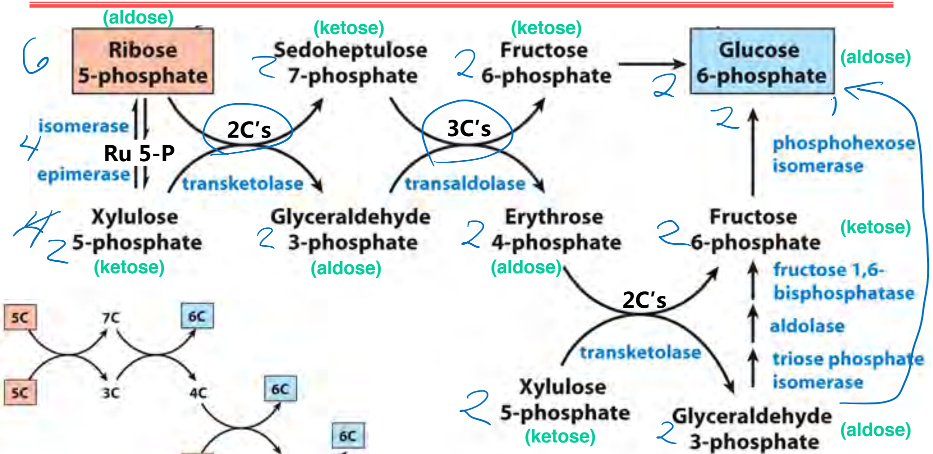
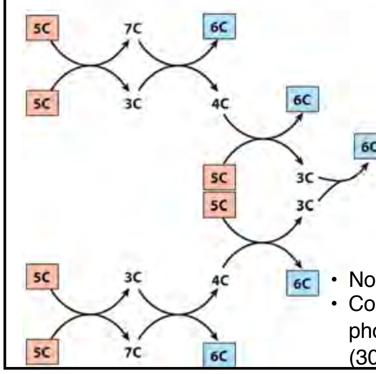
Pentose Phosphate Pathway



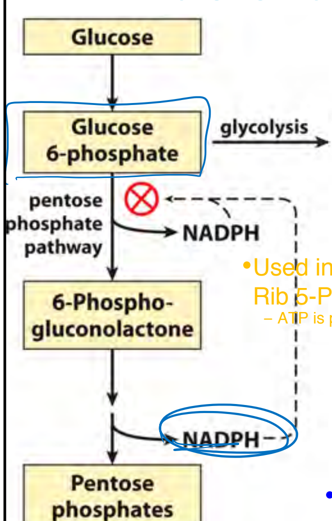


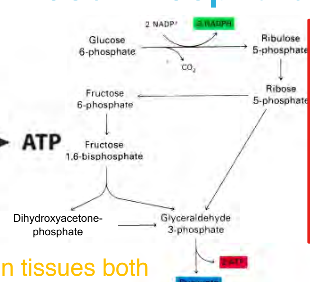
Non-oxidative Phase
Interconverts Rib 5-P to form Glc 6-P

- No ATP is used in the non-oxidative phase.
- Complete conversion of ribose-5-phosphate to glucose-6-phosphate requires 6 Rib 5-P (30 carbons) to make 5 Glc 6-P (30 carbons).

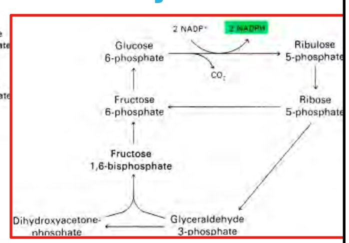
Pentose Phosphate Pathway

NADPH Regulates Partitioning into Glycolysis versus Pentose Phosphate Pathway

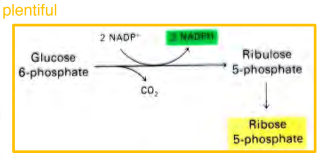




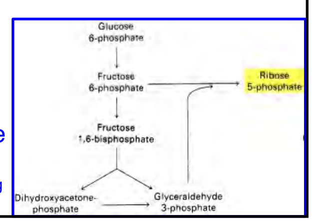
•Used in tissues both Rib 5-P & NADPH
– ATP is plentiful



•Used in tissues requiring more NADPH than Rib 5-P
– such as the liver and adipose tissue

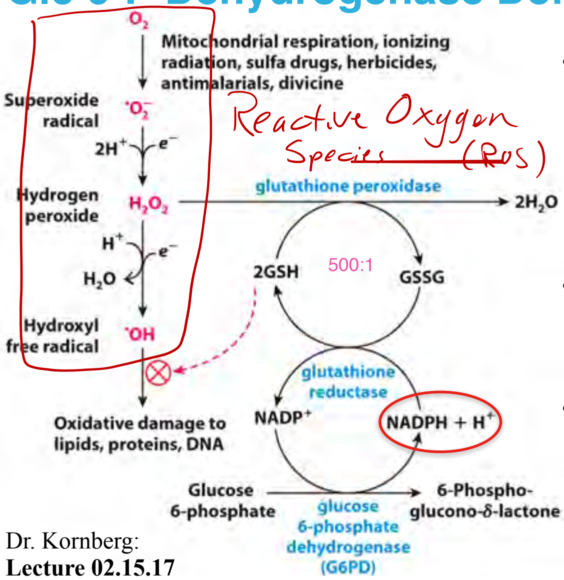


•Used in tissues requiring more Rib 5-P than NADPH
– such as the lymphocytes and fast growing tissues/cancer cells



Pentose Phosphate Pathway

Glc 6-P Dehydrogenase Deficiency*



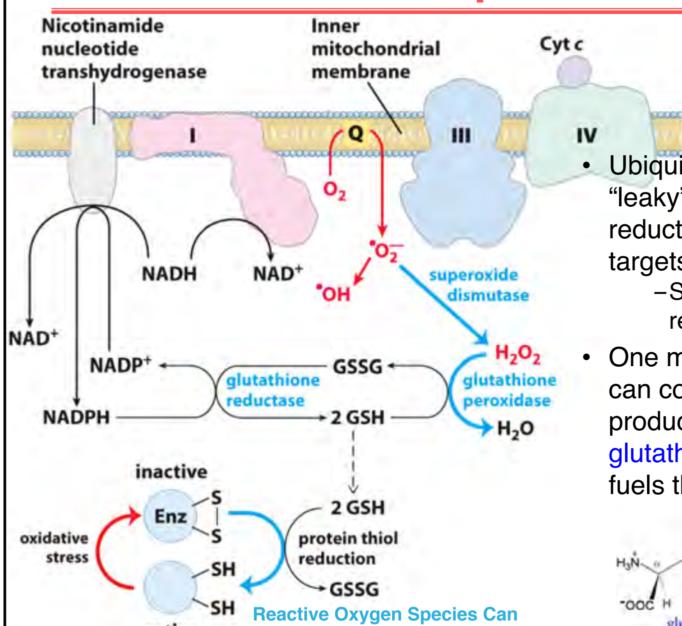
- Can be fatal in cases of high oxidative stress. Hemolytic anemia
  - certain drugs, herbicides, and some foods (fava beans)
- Resistance to malaria due to high oxidative stress in red blood cells
- X-linked heterozygous advantage

Essential for red-blood cells

Dr. Kornberg:
Lecture 02.15.17
(35:00-41:36) 6.5 min

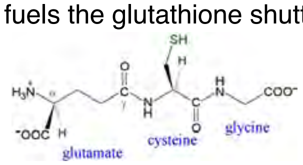
*Total lack of G6PDH is lethal

Pentose Phosphate Pathway



Reactive Oxygen Species Can Damage Biological Macromolecules

- Ubiquinone is naturally "leaky" and facilitates partial reduction of non-Complex III targets.
  - Single electron transfers result in free radicals.
- One method by which the cell can correct free-radical production of reduced glutathione (GSH), which fuels the glutathione shuttle

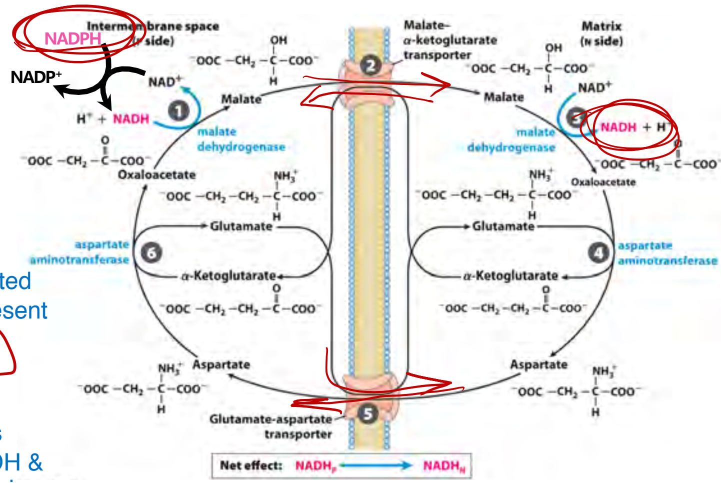


Glutathione

Pentose Phosphate Pathway

Malate-Aspartate Shuttle

Converting Cytosolic Electron Carriers (NADH & NADPH) to the Mitochondria



- This more complicated shuttle is mostly present in liver, heart, and kidney.
- It moves NADH & NADPH equivalents from cytosol to NADH & NADPH equivalents in the mitochondria.

Regulation of Carbohydrate Metabolism

Catabolism *vs.* Anabolism

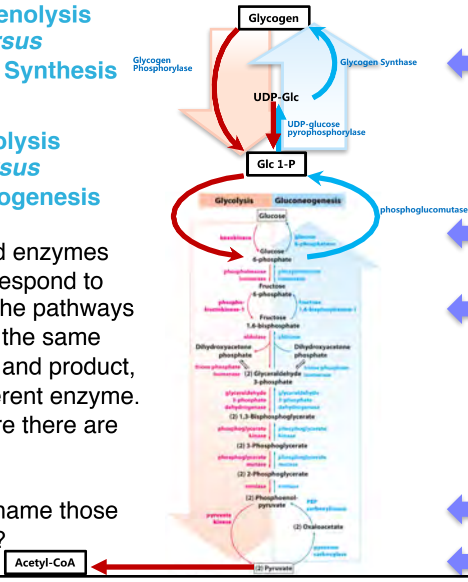
Regulation of Carbohydrate Metabolism

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.

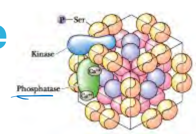
- Can you name those enzymes?

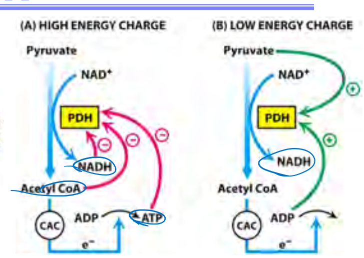


Catabolism	Anabolism
Phosphorylase	Glycogen Synthase
Hexokinase	Glucose 6-phosphatase
Phosphofructo-kinase-1	Fructose 1,6-bisphosphatase
Pyruvate Kinase	– PEPCK
Pyruvate Dehydrogenase Complex	– Pyruvate carboxylase

Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Dehydrogenase Complex

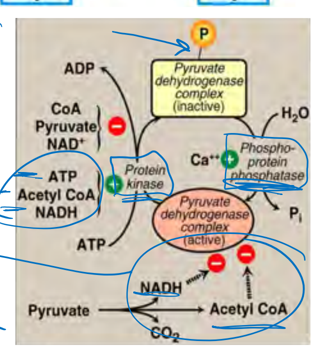




- Allosteric regulation by energy charge and substrate/product
  - –ADP & pyruvate activates
  - –ATP/NADH & acetyl-CoA inhibit
- Regulated by reversible phosphorylation of E1
  - –phosphorylation: inactive
  - –dephosphorylation: active
- PDH kinase and PDH phosphatase are part of mammalian PDH complex.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

Covalent

allosteric

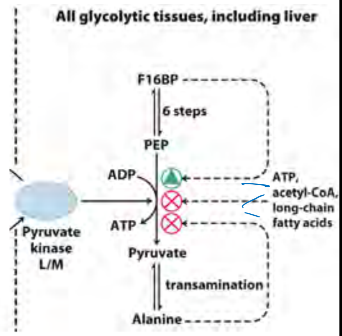


Regulation of Carbohydrate Metabolism

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

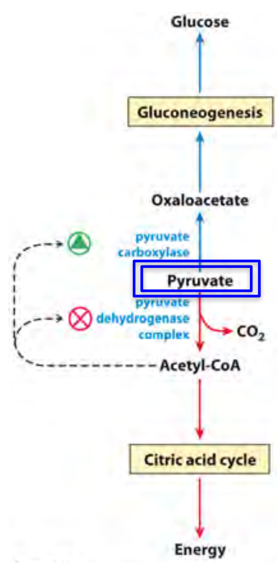
Inactivated by phosphorylation in response to signs of glucose depletion (low blood-glucose→glucagon) (liver only)

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

Regulation of Carbohydrate Metabolism

Regulation of Pyruvate Carboxylase

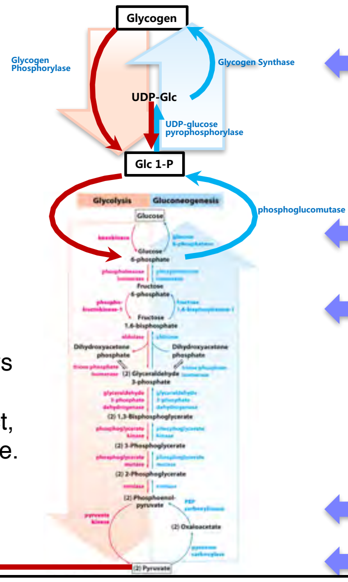
- Acetyl-CoA activates pyruvate carboxylase
  - stimulates glucose synthesis via gluconeogenesis by
- Notice the reciprocal control of PDH Complex by acetyl-CoA



Regulation of Carbohydrate Metabolism

Catabolism | Anabolism

Glycogenolysis
versus
Glycogen Synthesis



Glycolysis
versus
Gluconeogenesis

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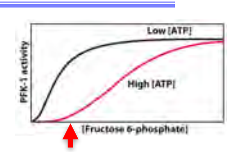
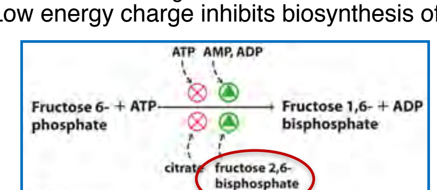
Regulation of Carbohydrate Metabolism

Regulation of Phosphofructokinase-1



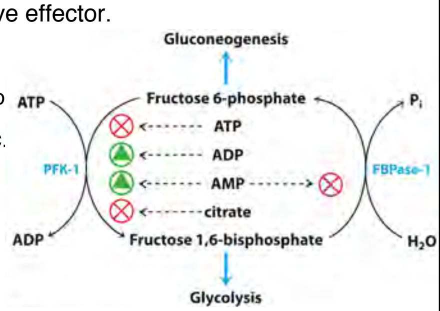
Homeostatic level of Fru 6-P

- 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?

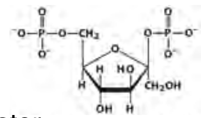
Bumble bees are missing an FBPase that responds to AMP

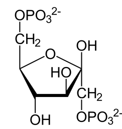


- Go glycolysis if AMP is high and ATP is low.
- Go gluconeogenesis if AMP is low.

Regulation of Carbohydrate Metabolism

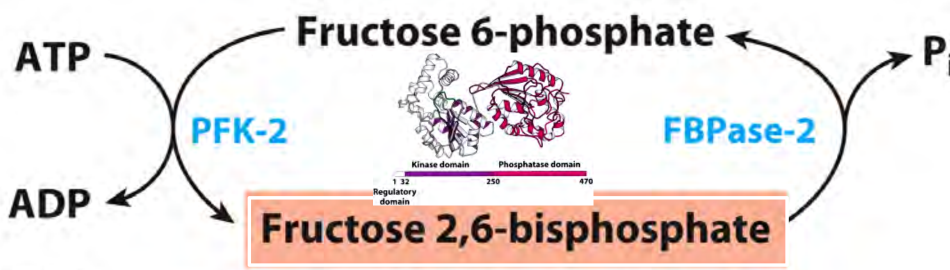
Fructose 2,6-*(bis)*phosphate (Fru-β2,6P_2)





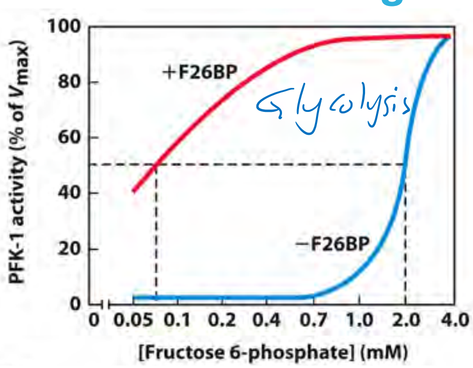
- NOT a glycolytic intermediate, only a regulator
- Produced specifically to regulate glycolysis and gluconeogenesis
  - activates phosphofructokinase-1(PFK-1) (glycolysis)
  - inhibits fructose 1,6-bisphosphatase (FBPase) (gluconeogenesis)

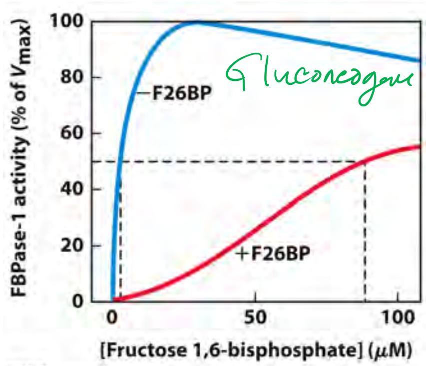
Enzyme for synthesis and degradation of Fru 2,6P_2 done with a dual-function enzyme



Regulation of Carbohydrate Metabolism

Regulation of Glycolysis and Gluconeogenesis by Fru-2,6P_2



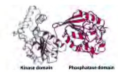


- With Fru 2,6P_2 (130 nM), Go glycolysis.
- With Fru 2,6P_2 (1300 nM), Stop gluconeogenesis.

- Without Fru 2,6P_2, STOP glycolysis GO gluconeogenesis.

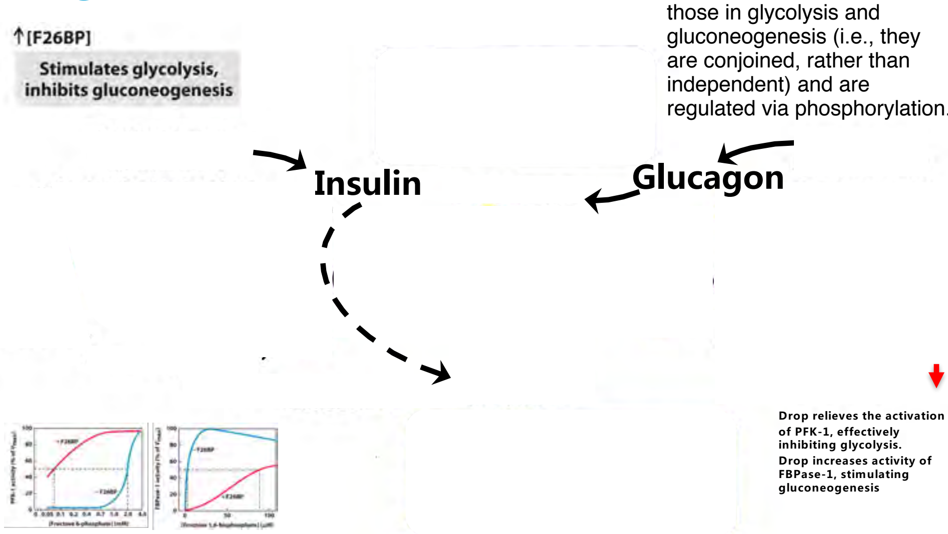
What controls PFK-2/FBPase-2?

Regulation of Carbohydrate Metabolism

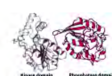


Regulation of Fru-2,6-P_2 Levels

Structurally, these two enzymes are different than those in glycolysis and gluconeogenesis (i.e., they are conjoined, rather than independent) and are regulated via phosphorylation.



Regulation of Carbohydrate Metabolism



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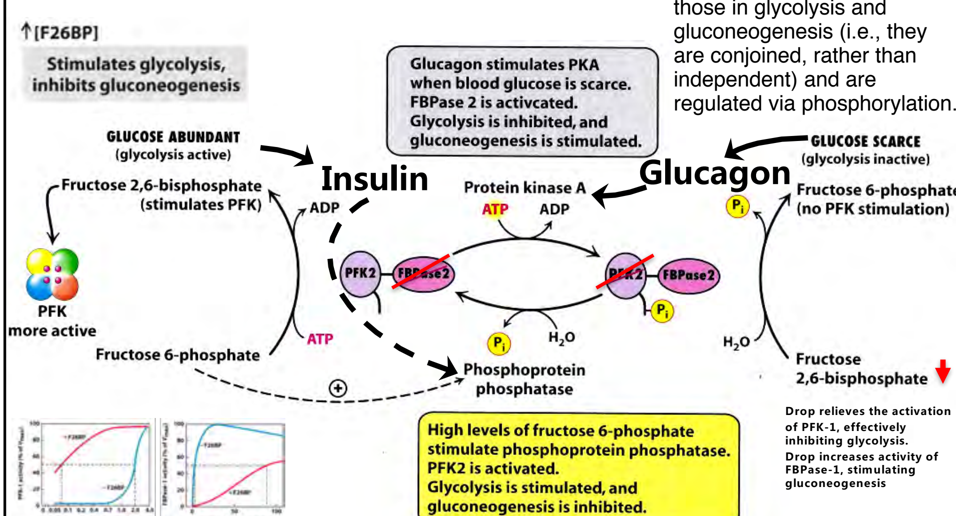

Regulation of Carbohydrate Metabolism

Glycogenolysis *versus* Glycogen Synthesis

Glycolysis *versus* Gluconeogenesis

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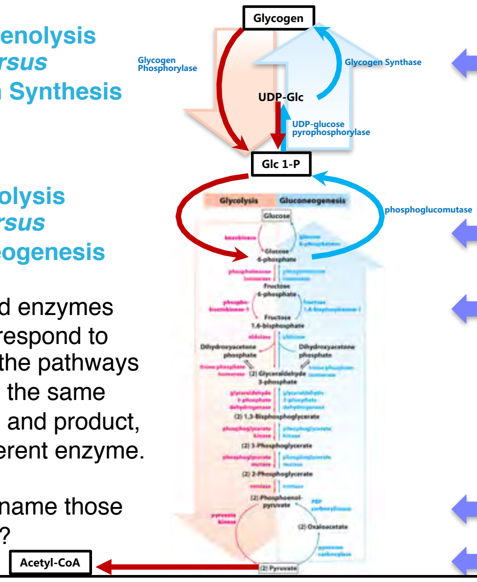

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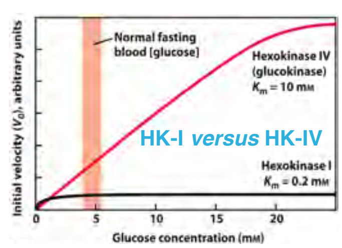
Pyruvate Dehydrogenase Complex	

Regulation of Carbohydrate Metabolism

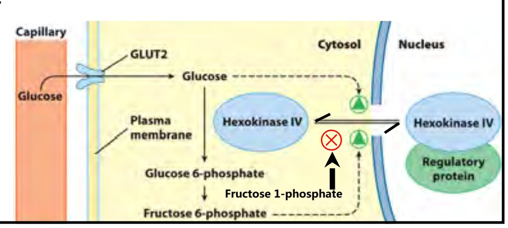
Regulation of Hexokinase

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



Glucokinase Is Regulated by Sequestration



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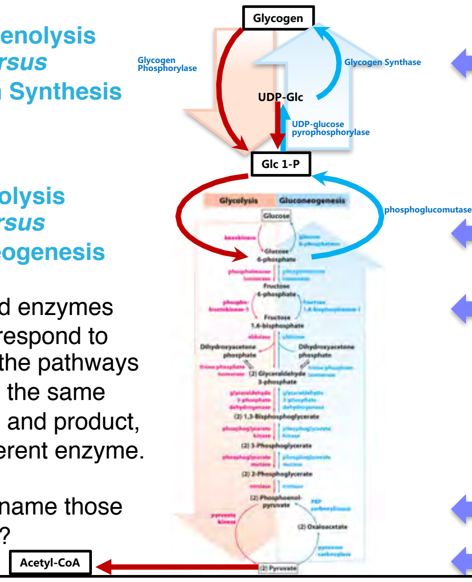

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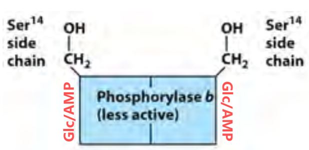
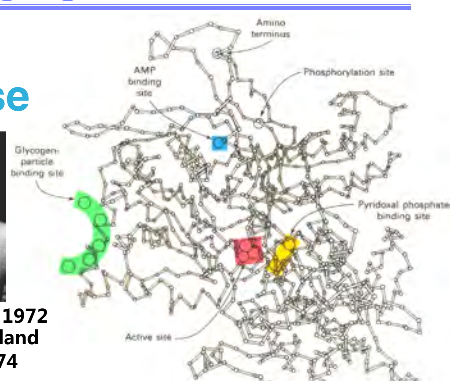
Regulation of Carbohydrate Metabolism

Regulation of Glycogen Phosphorylase



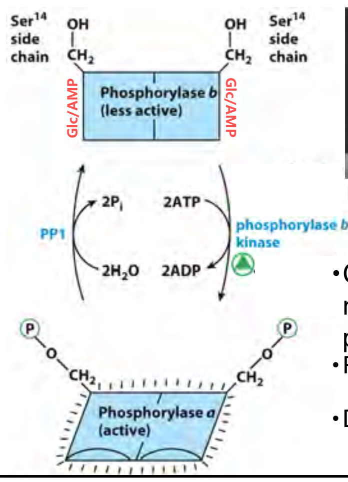
Nobel Prize 1972
Earl Sutherland
1915-1974



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

Regulation of Carbohydrate Metabolism

Regulation of Glycogen Phosphorylase



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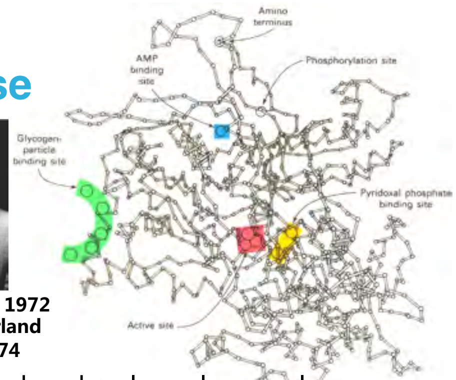

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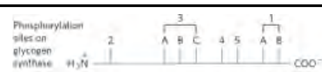
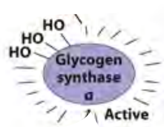
Regulation of Carbohydrate Metabolism



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	+

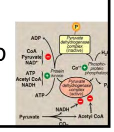
Regulation of Glycogen Synthase



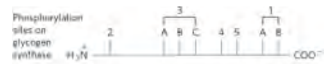
- Glycogen synthase adds glucose residues to glycogen using UDP-Glc.
- Phosphorylation inhibits glycogen synthase-*a*
  - Its complicated, responding to multiple signals
  - Example: First Casein Kinase-2 (CKII), then Glycogen Synthase Kinase-3 (GSK3)

- 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



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Casein kinase I	At least nine	+ + + +
Casein kinase II	5	0
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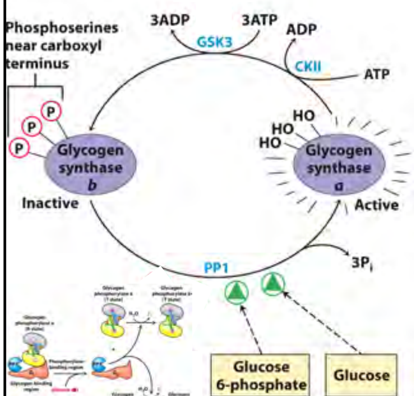
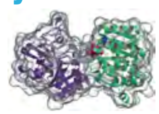
Glycogen synthase kinase 4	2	+

Regulation of Glycogen Synthase





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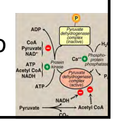
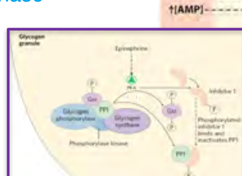


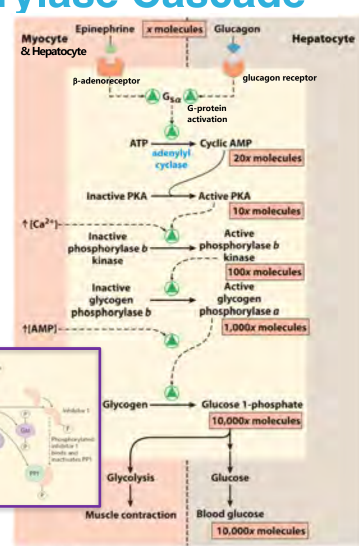
Regulation of Carbohydrate Metabolism

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→Glc
- 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 inactive





Regulation of Carbohydrate Metabolism

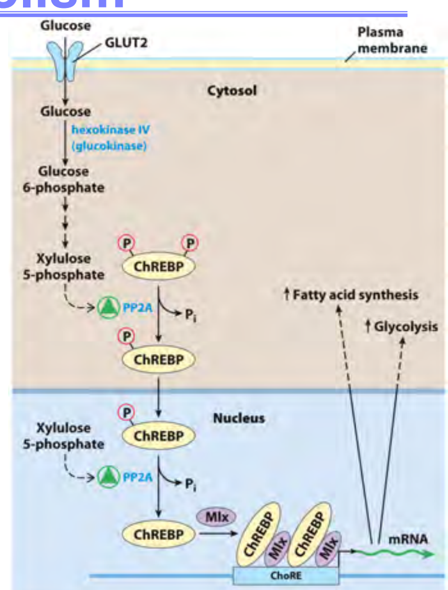
The Amount of Many Metabolic Enzymes Is Controlled by Transcription

TABLE 15-5 Some of the Many Genes Regulated by Insulin

Change in gene expression	Role in glucose metabolism
Increased expression Hexokinase II Hexokinase IV Phosphofructokinase-1 (PFK-1) PFK-2/FBPase-2 Pyruvate kinase	Essential for glycolysis, which consumes glucose for energy
Glucose 6-phosphate dehydrogenase 6-Phosphogluconate dehydrogenase Malic enzyme	Produce NADPH, which is essential for conversion of glucose to lipids
ATP-citrate lyase Pyruvate dehydrogenase	Produce acetyl-CoA, which is essential for conversion of glucose to lipids
Acetyl-CoA carboxylase Fatty acid synthase complex Stearoyl-CoA dehydrogenase Acyl-CoA–glycerol transferases	Essential for conversion of glucose to lipids
Decreased expression PEP carboxykinase Glucose 6-phosphatase (catalytic subunit)	Essential for glucose production by gluconeogenesis

Regulation of Carbohydrate Metabolism

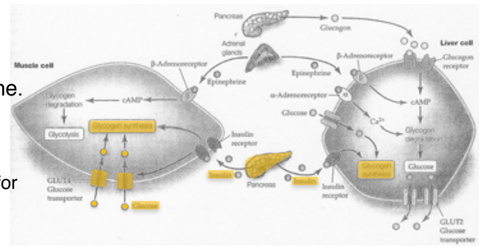
The Carbohydrate Response Element Binding Protein (ChREBP) Activates Transcription in Response to Glucose

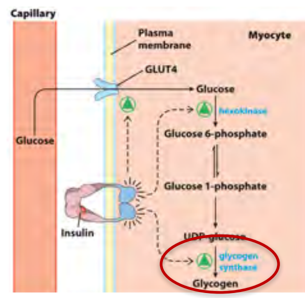


Mice with KO of the ChREBP have interesting phenotype. They are intolerant to fructose

Regulation of Carbohydrate Metabolism

- Insulin signaling pathway activated when there is a need for energy STORAGE
  - Causes the translocation of GLUT4 from internal membranes to the plasma membrane.
    - Glucose enters muscle cells
  - Increase intracellular [Glc]
    - In liver, releases glucokinase (liver HK) from binding protein, thus it migrates to the cytosol for activity
    - In muscle, higher [S] is higher activity





Regulation of Carbohydrate Metabolism

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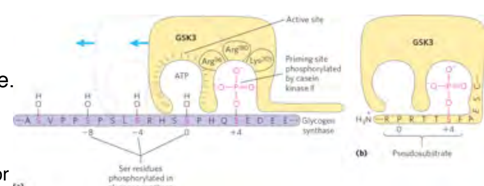
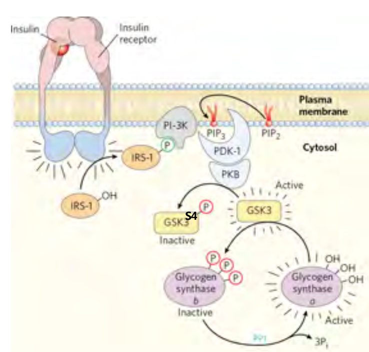
  - Stimulates Protein Kinase B (PK-B) to phosphorylates GSK-3 at the Ser-4 in the pseudo-substrate site.
    - This inactivates GSK-3
    - Prevents the re-phosphorylation of GS so that it remains ACTIVE





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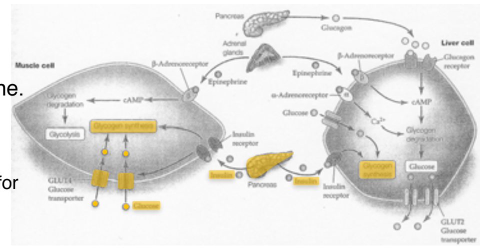
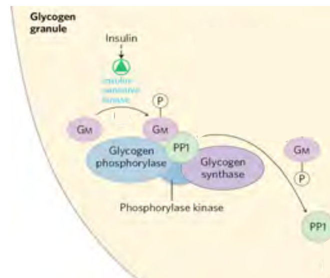
  - Stimulates phosphoprotein phosphatases
    - Example: ISK phosphorylates the glycogen-targeting proteins in muscle (G_M)
    - This dissociates it from PP-1: PP1 is more active





Regulation of Carbohydrate Metabolism

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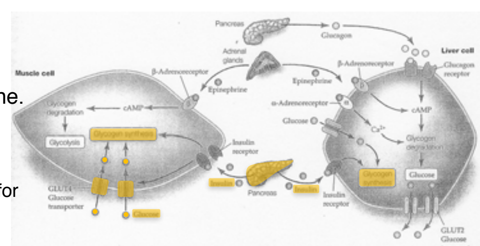
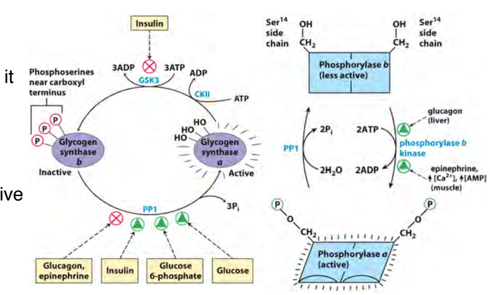
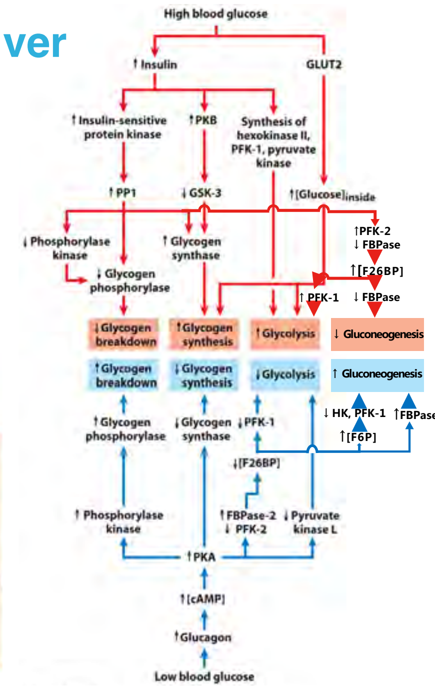
    - Example: ISK phosphorylates the glycogen-targeting proteins in muscle (G_M)
    - This dissociates it from PP-1: PP1 is more active
    - PP-1 dephosphorylates several proteins
      - Phosphorylase kinase: inhibits
      - glycogen phosphorylase: inhibits
      - Glycogen synthase (GS): activates
- GS makes glycogen for energy storage.





Regulation of Carbohydrate Metabolism

In the Liver



Liver versus the Muscle