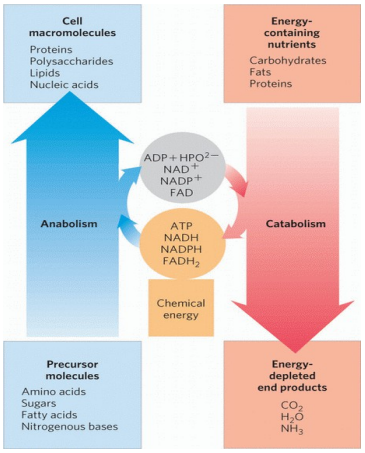
Part C - Carbohydrate Metabolism

PART 1- Glycolysis, Gluconeogenesis and Regulation

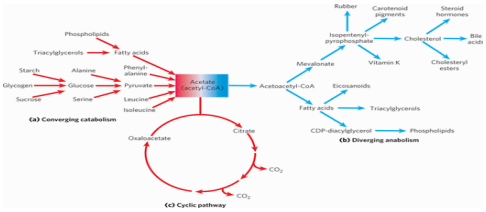


Metabolism – Concepts

- Metabolism is the ***sum*** of chemical transformations
- Metabolic pathways involve ***enzymes***
- Catabolism – degradation of biomolecules
- Anabolism – synthesis of biomolecules

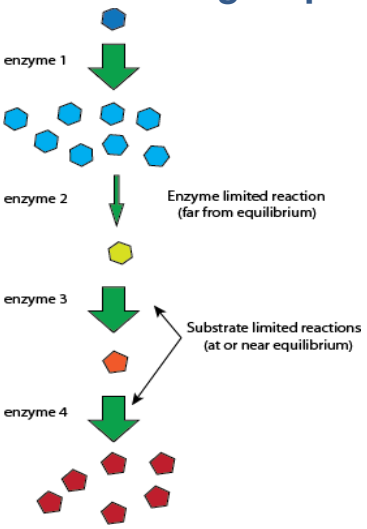
Metabolism= sum of breakdown and build up of molecules= chemical transformations

Types of Pathways



Converging, Cyclical and Diverging Pathways

Rate-Limiting Steps



- **Rates** of biochemical pathways depend on the **activities of enzymes** that catalyze each step
- In any pathway, the rate of most steps is limited by substrate availability (enzyme is in excess)
- However, reactions catalysed one or more enzymes in any pathway will be limiting
- These **rate limiting steps** will set the overall speed of the pathway

Coloured shapes show substrates/ products
The number of shapes corresponds to the relative conc of the substrate/product
Substrate limited= if more sub was available, it could go faster

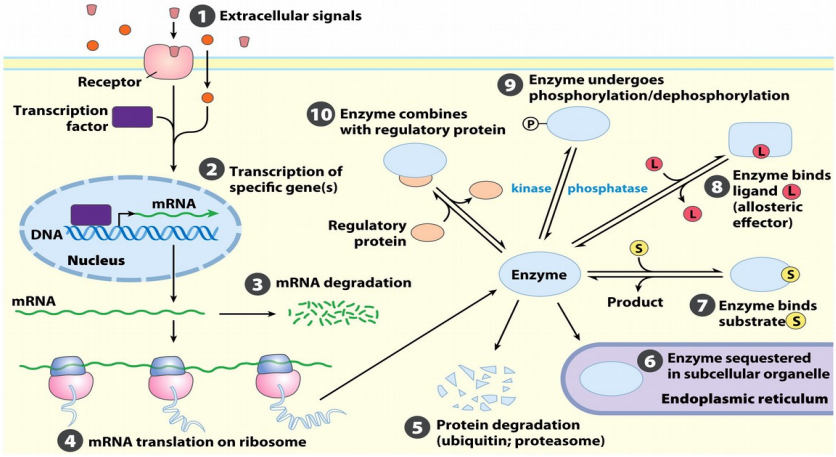
- One step in every pathway is rate limiting
- The thickness of the arrow indicates speed, thicker= faster

Pathways Are Typically Regulated at Rate Limiting Steps

- Rate-limiting steps are often **exergonic** and **irreversible** under cellular conditions

- Enzymes catalyzing exergonic, rate- limiting steps are targets of **Metabolic regulation**
- This allows the cell to regulate the overall rate of metabolic pathway without regulating every single enzyme involved

Exergonic= release energy
RLS= favourable
Irreversible under cellular conditions = under the concentrations of substrate and products present- though the enzyme can work backwards, it is rare



How Are Enzymes Regulated?

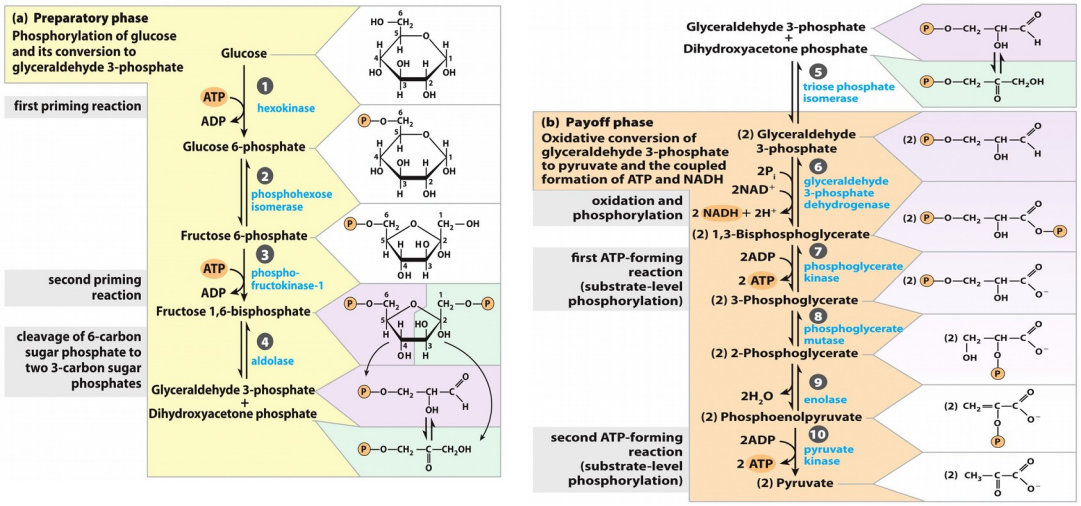
Anything that can be upregulated can be down regulated

Metabolism –Concepts Review

1. Metabolism is the sum of reactions
2. Catabolism is the breakdown of molecules
3. Anabolism is the synthesis (biosynthesis) of molecules
4. Rate-limiting steps determine the overall speed of a pathway
5. Rate-limiting steps represents points of regulation, and are exergonic
6. Being (strongly) exergonic makes reactions essentially irreversible

There can be more than 1 rate limiting step

Review – Glycolysis



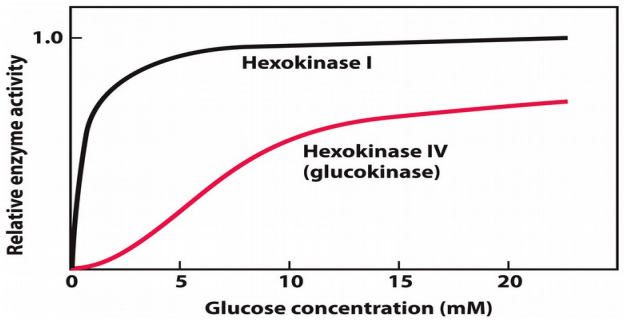
- Box d reactions are highly exergonic (Under cellular conditions)
- These are the steps in glycolysis that are regulated

Hexokinase is Allosterically Inhibited by its Product

- Hexokinase catalyzes the reaction that allows entry of glucose into glycolysis
- **In muscle**, hexokinase I is expressed
  - normally has **maximal activity**
  - if [Glucose 6-P] **increases**, enzyme is inhibited (-ve feedback)
- **In liver**, hexokinase IV (glucokinase) is expressed
  - an *isozyme* (different gene)
  - lower affinity for glucose
  - inhibited by **fructose 6-P** not glucose 6-P
  - inhibition by fructose 6-P effected through **glucokinase regulatory protein**

Enzyme is inhibited by its product- not an all or nothing event – modulation of activity
There are different isoforms of hexokinase- have same activity but are regulated differently

Hexokinase IV has a Relatively Larger K_m

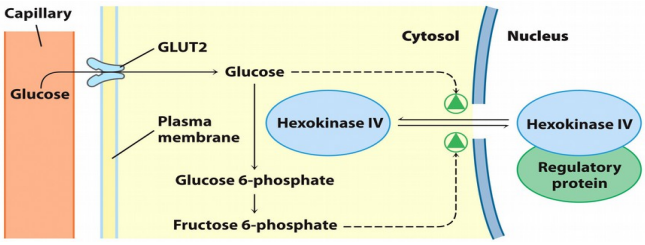


- The muscle enzyme (I) does not increase its rate when blood [glucose] is higher than optimal (~5 mM)
- Hexokinase IV has a much higher K_m (~10 mM)
- With hexokinase IV, the liver responds directly to increasing blood [glucose] with increased turnover

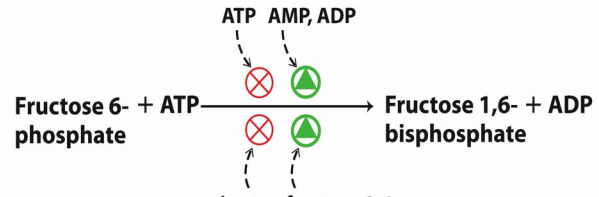
Lower Km= better enzyme
Hexokinase IV actually has a larger Km
In the graph, IV has a sigmoidal curve indicating allostery
Don't use Km for allosteric enzymes, we use K0.5 – doesn't follow Michaelis menten kinetics
Turn over= glucose to glucose 6 P

Regulation of Hexokinase IV

- When [fructose-6-P] is high, glucokinase regulatory protein sequesters hexokinase IV in the nucleus
- High [glucose] weakens the enzyme/regulator interaction, encouraging cytosolic localization
- Glucose weakens the interaction



Phosphofructokinase-1 Allostery



- Glucose-6-P has several possible fates in the cell

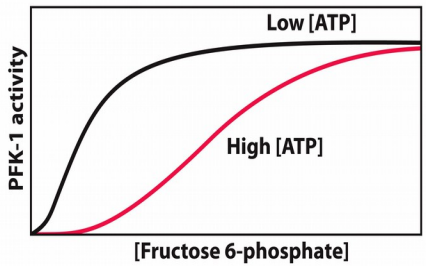
- Phosphorylation by PFK-1 commits fructose 6-phosphate (in equilibrium with G6P) to glycolysis

- Allosteric regulation of PFK-1 is complex:
  - **ATP** binds to an allosteric site on PFK-1 and **lowers affinity** for fructose 6-P
  - ADP and AMP **relieve inhibition** by ATP
  - **Citrate** increases the inhibition by ATP
  - **fructose 2,6-bisphosphate** is a strong activator

- Regulated and highly exergonic
- High ATP= high energy state
- High AMP and ADP= low energy state
- Remember this is glycolysis – energy is being created
- The equilibrium is the isomerase
- This is the committing step to the glycolysis pathway
- Substrate and regulator is the same (ATP)- has a negative effect- makes harder for others to bind

Regulation of PFK-1 by ATP

- High [ATP] greatly reduces the affinity of PFK-1 for fructose 6-phosphate
- When [ATP] is low, higher F6P affinity allows PFK-1 to be more active
- Can see the reduced affinity by the shift to the right- shows increase in K0.5



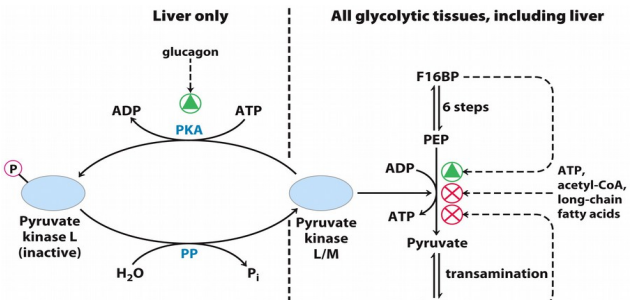
Pyruvate kinase is inhibited By ATP

- Pyruvate kinase (PK) catalyzes the last step in glycolysis
- PK transfers P_i from phosphoenolpyruvate to ADP
- This yields pyruvate and a molecule of ATP
- High **[ATP] allosterically inhibits PK, decreasing its affinity for PEP**

Pyruvate Kinase Inhibition

- **Acetyl-CoA** and **long-chain fatty acids** also **inhibit PK**
  - important fuels for citric acid cycle
  - when plentiful, so is ATP
- Other allosteric modulators of PK:
  - **alanine (-)**
  - **F 1,6-BP accumulation (+)**
- Lots of acetyl CoA= lots of potential energy

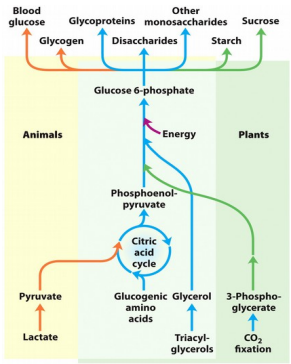
Summary of Pyruvate Kinase Regulation



Pka phosphorylates it in response to glucagon – stops glycolysis to help increase blood glucose levels

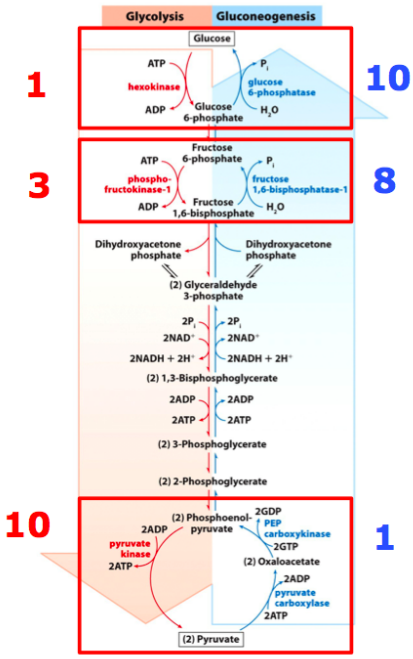
Gluconeogenesis: The Solution

- The body needs a steady supply of glucose to fuel certain key organs (e.g. the brain)
- However, our bodies can only store about a 1 day supply (glycogen!)
- When this is depleted, glucose needs to be made from other molecules
- The body also needs to resynthesize glucose from the lactic acid produced by anaerobic exercise
- Gluconeogenesis is the synthesis of glucose from non-hexose precursors
- Non-6 carbon sugar molecules are used to make glucose



Gluconeogenesis – Precursors

- The main precursors for gluconeogenesis are
  - lactic acid (via pyruvate)
  - glycerol (from lipids)
  - some amino acids (termed glucogenic a.a.)



- In **gluconeogenesis**, 7 of 10 steps are the **glycolytic** reactions but run in **reverse**
- The 3 irreversible steps of glycolysis are bypassed
- Here, different enzymes catalyze one or more different steps to enable the reverse reaction

Lehninger 6th Ed. Fig. 14-17

Don't want both pathways occurring at the same time = futile cycles because youd be making it and breaking it down- would lead to a loss of energy

In the Mitochondria

Pyruvate Carboxylase

Pyruvate + HCO_3^- + ATP ⇌ oxaloacetate + ADP + P_i

Terminal phosphate is cleaved

Mitochondrial Malate Dehydrogenase

oxaloacetate + NADH + H^+ ⇌ L-Malate + NAD^+

In the Cytosol

Cytosolic Malate Dehydrogenase

L-malate + NAD^+ ⇌ oxaloacetate + NADH + H^+

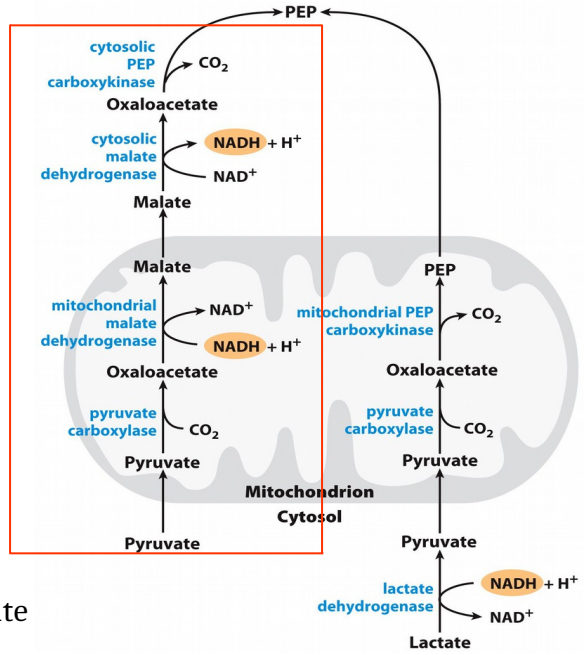
Phosphoenolpyruvate Carboxykinase

Oxaloacetate + GTP ⇌ phosphoenolpyruvate + CO_2 + GDP

Summary of Step 1

Conversion of **pyruvate** to **phosphoenol- pyruvate (PEP)** (bypass for step 10 of glycolysis)

NADH is consumed in the mitochondria and producing it in the cytosol



Why a Malate Intermediate?

- Gluconeogenesis consumes NADH (glyceraldehyde-3-phosphate dehydrogenase)
- The (liver) mitochondria will be degrading fatty acids during gluconeogenesis, producing lots of NADH
- NADH cannot be directly exported to the cytosol
- Instead, mitochondrial malate DH consumes NADH, while cytosolic malate DH produces it
- When lactate is the feedstock for gluconeogenesis, lactate produces cytosolic NADH
- Liver PEP carboxykinase will then produce PEP directly, as extra NADH is not needed in the cytosol

DH= dehydrogenase

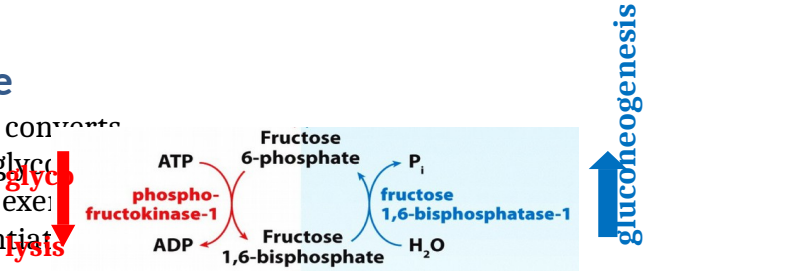
Step 8: Fructose 1,6-bisphosphatase

- Fructose 1,6 bisphosphatase (FBPase-1) con
- F 1,6-BisP to F 6-P (bypass for step 3 of glyc
- This reaction is a phosphatase reaction (exe

Need to know full names for the exam – differentiat

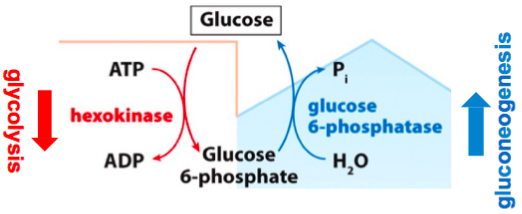
Diposphate= connected to each other

Bisphosphate= different positions



Step 10: Glucose 6-Phosphatase

- **Glucose-6-phosphatase** catalyzes the dephosphorylation of **Glucose 6-phosphate** (bypass for step 1 of glycolysis)
- This enzyme is expressed in few tissues (liver, kidney, small intestine) = gluconeogenic tissues

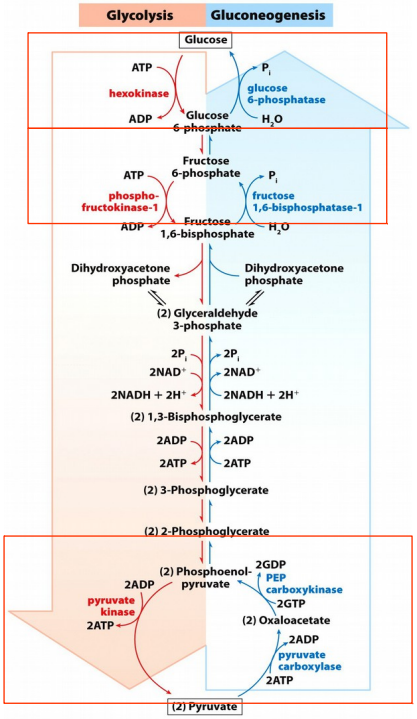


G6P is a substrate for many different pathways
Glucose 6 phospatase is found only in few tissues- ones that are able to make glucose
Thus after glycolysis, G6P is used in other processes

Glycolysis and Gluconeogenesis Regulation is Coordinated

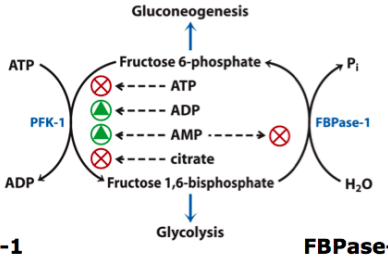
- Glycolysis and gluconeogenesis are opposing cellular processes
- Running both in parallel would simply waste energy
- Regulation of these two processes is therefore coordinated

When one is turned on, the other is turned off



Step 3 (Glycolysis) / Step 8 (Gluconeogenesis)

- The molecules listed represent different levels of energy
- ATP and citrate= sign of high energy- no point in glycolysis going forward
- The AMP and ADP= energy depleted molecules
- The unbolded ones are like modulators- have a bit of an effect
- **F2, 6BP dominates control**
- On the right side we are making glucose- AMP=low energy, so we do not want to store glucose- need to break it down for energy therefore negative regulation of gluconeogenesis

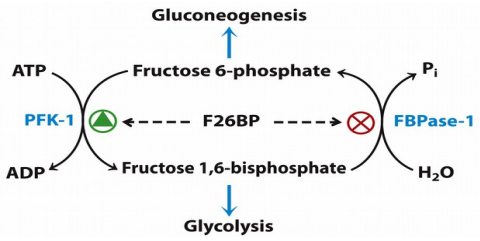


PFK-1
- **F 2,6-BP** (+)
- AMP (+)
- ADP (+)
- ATP (-)
- citrate (-)

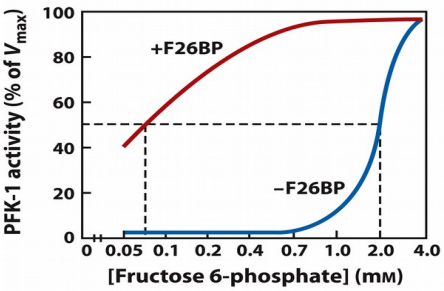
FBPase-1
- **F 2,6-BP** (-)
- AMP (-)

Lehninger 6th Ed. Fig. 15-17

Fructose 2,6-BP allosterically regulates PFK-1 and FBPase-1 in a reciprocal manner

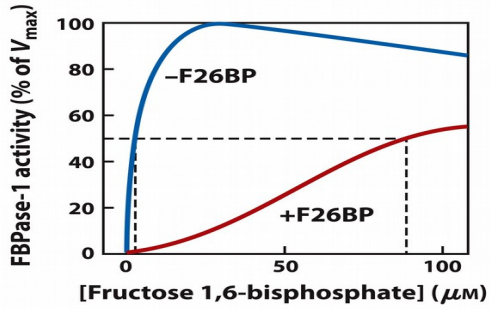


Fructose 2,6-bisphosphate Greatly Increases Phosphofructokinase-1 Activity



has low affinity for fructose-6-P in the absence of F2,6BP BP, affinity increases more than 100x (note log scale)

Fructose 2,6-bisphosphate Drastically Reduces Fructose 1,6-bisphosphatase Activity



- Fructose bisphosphatase-1 has high affinity for fructose- 1,6-BP in the absence of F2,6BP
- In the presence of Fructose 2,6-BP, affinity for Fructose 1,6-BP decreases more than 10x

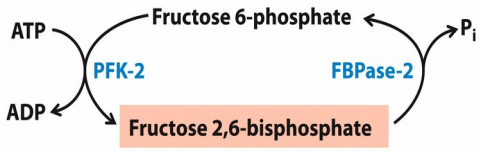
Looks like vmax was affected
High substrate concentrations can sometimes lead to inhibition (blue)

Fructose 2,6-bisphosphate Control

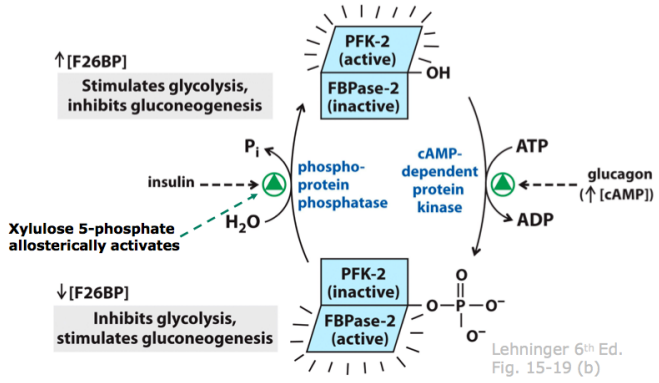
Fructose 2,6-BP concentration is controlled by two opposing enzyme activities:

- **phosphofructokinase-2 (PFK-2)**
- **fructose 2,6-bisphosphatase (FBPase-2)**

Two opposing enzyme activities



PFK-2/FBPase-2 Regulation



Bifunctional enzyme bc it has two different functions

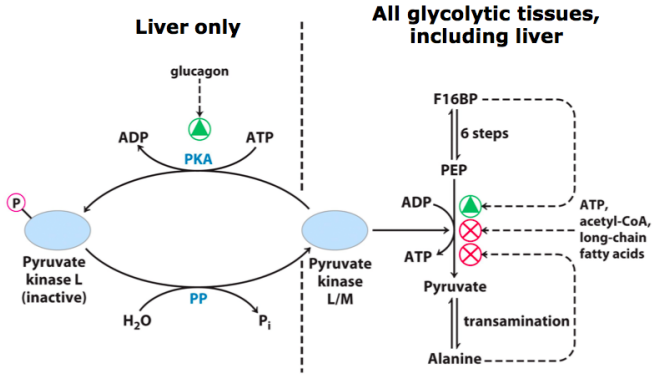
PFK-2/FBPase-2 is a <u>bifunctional</u> enzyme where the two activities are reciprocally regulated:

- Phosphorylation by protein kinase A (in response to glucagon) activates **FBPase-2** and inactivates **PFK-2**
- <u>De</u>phosphorylation by phosphoprotein phosphatase (in response to insulin) activates **PFK-2**, and inactivates **FBPase-2**
- Xylulose 5-phosphate (pentose pathway) also allosterically upregulates phosphoprotein phosphatase

Isozyme-Specific Responses of PFK-2/FBPase-2 to Phosphorylation

- Even while gluconeogenesis occurs in the liver, other tissues (e.g. cardiac muscle) continue glycolysis
- This requires that key enzymes are differentially regulated in these tissues
- These tissues have different PFK-2/FBPase 2 isozymes
  - **Liver isozyme** (Fig. 15-19b): Phosphorylation on Ser 32 activates FBPase-2
  - **Cardiac muscle isozyme**: Phosphorylation on Ser 406 and Thr 475 activates PFK-2
- Liver has different isozyme than muscle

Step 10 of Glycolysis / Step 1 of Gluconeogenesis are Reciprocally Regulated By Acetyl-CoA

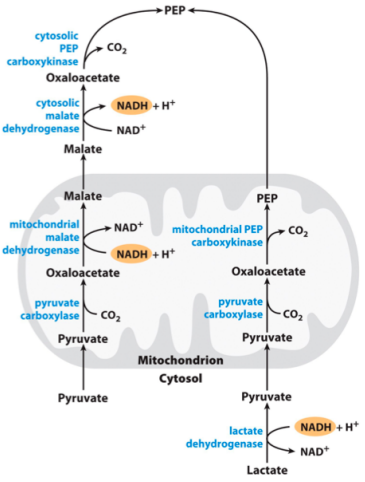


Pyruvate Kinase Regulation, again but different

- Pyruvate kinase is allosterically activated by F1,6-BP – the first molecule committed to glycolysis
- Pyruvate kinase is allosterically inhibited by ATP, acetyl-CoA, long chain fatty acids and alanine (1 step from pyruvate)
- These all signal abundant energy
- The liver has a different pyruvate kinase isoform
- This isoform is phosphorylated by PKA in response to the hormone glucagon (which signals low blood sugar)
- This slows liver PK, reserving scarce sugar for organs that need it

Pyruvate carboxylase
- acetyl-CoA (+)
- ADP (-)

PEP carboxykinase
- ADP (-)



Lehninger 6th Ed.
Fig. 14-20

PART 2: Pentose Phosphate Pathway, Glycogen Metabolism Regulation

Pentose Phosphate Pathway

Two phases:

1. **Oxidative:** oxidation of glucose 6-P
   - produces 2 **NADPH** and **ribulose 5- phosphate (=> ribose-5-phosphate)**
2. **Non-oxidative: isomerization/rearrangements**
   - glycolytic intermediates
   - glucose 6-phosphate
   - **xylulose 5-phosphate** (modulator of phosphatase that stimulates liver PFK-2)

LEO says GER
NADPH= biosynthetic pathways
No electron carriers in the second phase

Products of the Oxidative Phase

- for the synthesis of nucleotides (ribose 5-phosphate)
- reductive biosynthesis (NADPH), for e.g. fatty acids

Non-Oxidative Phase

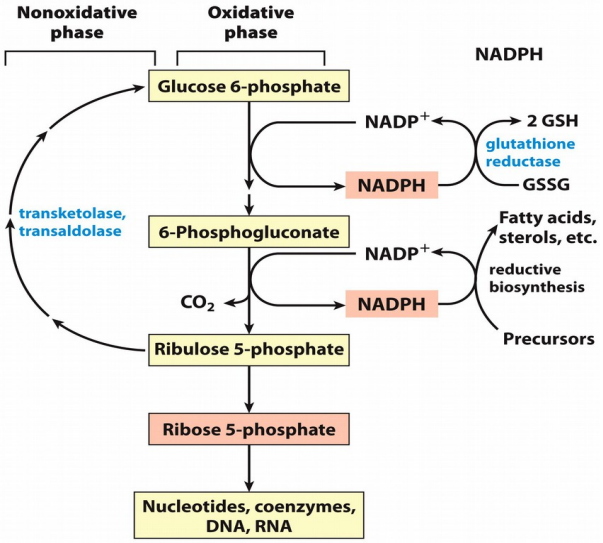
- replenish glucose 6-phosphate and glycolytic intermediates
- source of xylulose 5-phosphate

Nucleotides are good leaving groups- moved in metabolic pathways, not just nuclear information
Reductive= gain e-

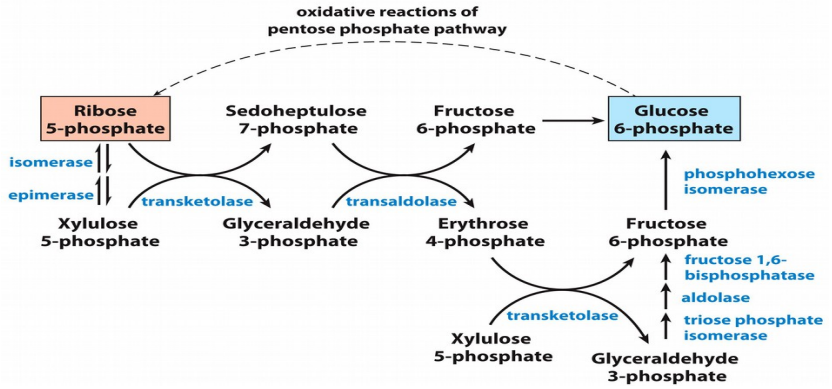
The second G6P made by trransketolase is not the same as the one that starts the oxidative phase

Know the enzymes here

But the new G6P can reenter into the pathway even tho it's a little diff

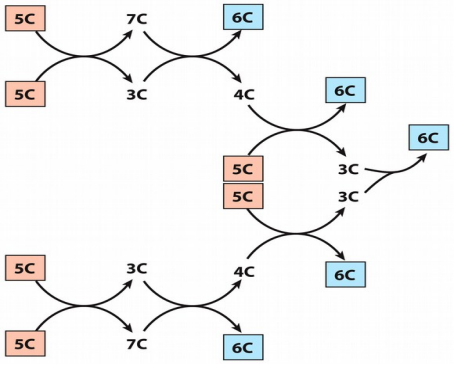


Non-Oxidative Phase



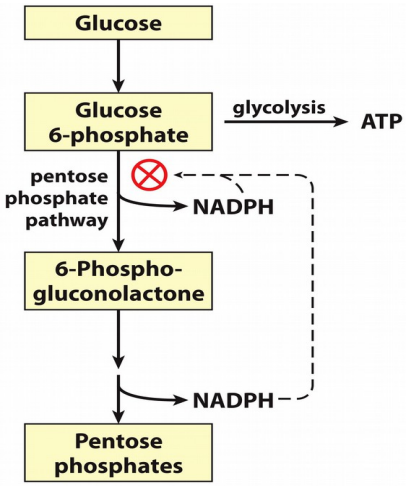
Don't need to know enzymes- just need to know whats going on Shuffling carbons around to reach the product- glycolytic intermediates

- Transaldolase/transketolase can transfer 2/3 carbon atoms between sugar phosphates
- This allows you to rearrange 5C molecules into 6C molecules
- The final 3C sugars are glyceraldehyde-3P, which can turned into glucose-6P by gluconeogensis steps
- Net: 6 x 5C ⟹ 5 x 6C



The 5C starts off as glucose
Main idea is the rearrangement of carbons
The pathways occur simultaneously
Start with six 5Cs and end with five 6Cs

Regulation of the Pentose Phosphate Pathway



Glucose 6-phosphate dehydrogenase is:
- stimulated by $NADP^+$
- inhibited by NADPH

regulated by redox state of the cytosol

If you're low in NADP+ then you're high in NADPH and vice versa

Glycogen Metabolism

Glycogen: **polymer of glucose (storage)**
- quick source of energy
Liver: glycolysis + **control of blood [glucose]**
Muscle: glycolysis
Can't store sugar in the form of glucose in the body because of osmolarity

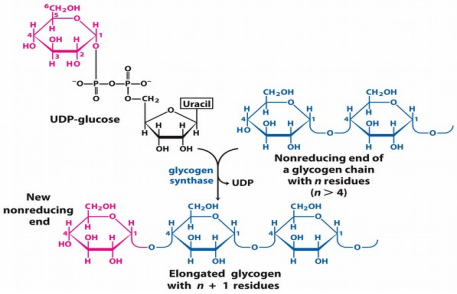
Glycogen Synthesis – making the precursor:

1. glucose 6-P ⟹ glucose 1-P
   Phosphoglucomutase
2. glucose 1-P + UTP ⟹ UDP-glucose + PPi
   UDP-glucose pyrophosphorylase

UDP-glucose then acts as an activated sugar donor

Phosphate moves from the 6 position to the 1 position
Ppi= pyrophosphate, not favourable
But its cleavage is very favourable
UTP= nucleoside?? UMP is actually added to the the 1-P of glucose
Mutase enzyme= rearrangement

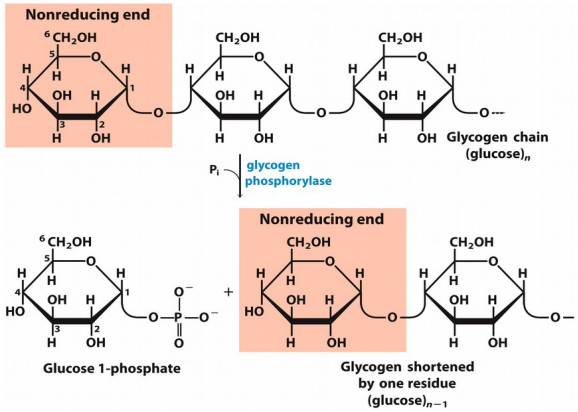
Glycogen Synthase Reaction



UDP is a good leaving group
Glucose gets added into the glycogen chain
1,4 bond between the residues, branching can also occur

Glycogenolysis (Glycogen Breakdown)

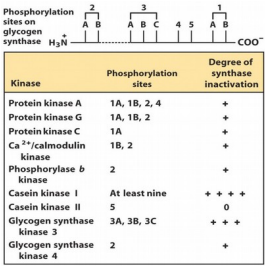
Glycogen Phosphorylase Reaction



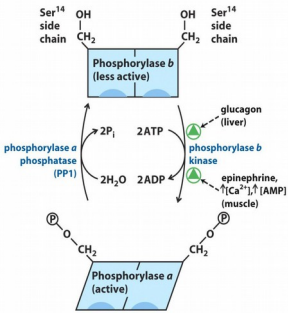
Phosphate is used to cleave a bond by phosphorylase

Regulation of Glycogen Metabolism

Recall from a previous lecture:
Glycogen synthase is inhibited by phosphorylation.



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	+



Recall from a previous lecture:
Glycogen Phosphorylase is activated by phosphorylation

Tissue specificity- kind of tissue dictates what kind of regulation takes place
Muscle- epi, calcium and amp regulate it

Glycogen Metabolism

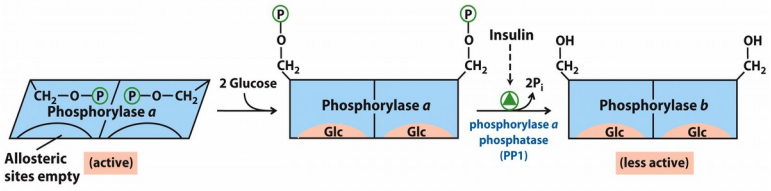
Glycogen phosphorylase *b* kinase
- **phosphorylates** glycogen phosphorylase
- activates glycogen phosphorylase

Glycogen phosphorylase *a* phosphatase
- **dephosphorylates** glycogen phosphorylase
- inhibits glycogen phosphorylase

The activity of **glycogen phosphorylase *b* kinase** is **stimulated by PKA-mediated phosphorylation**

The last part of the enzyme name tells us its action
The first part tells us its substrate

Glycogen Phosphorylase is a Glucose 'Sensor'



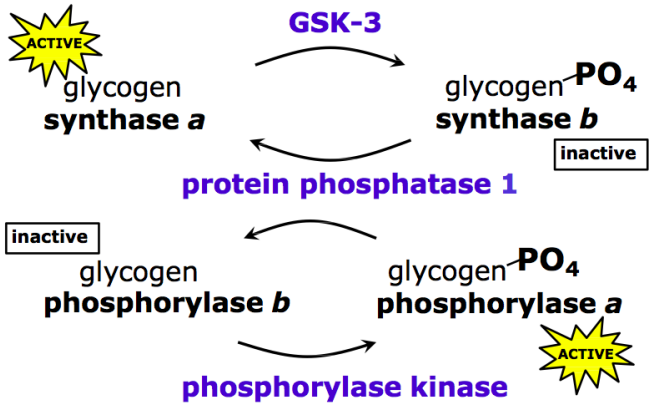
- Glucose binding of phosphorylase a has an allosteric effect
- This favours dephosphorylation (and inactivation) by phosphorylase *a* phosphatase

Has a dimer structure

The serines become more surface exposed when glucose is bound- conformational change

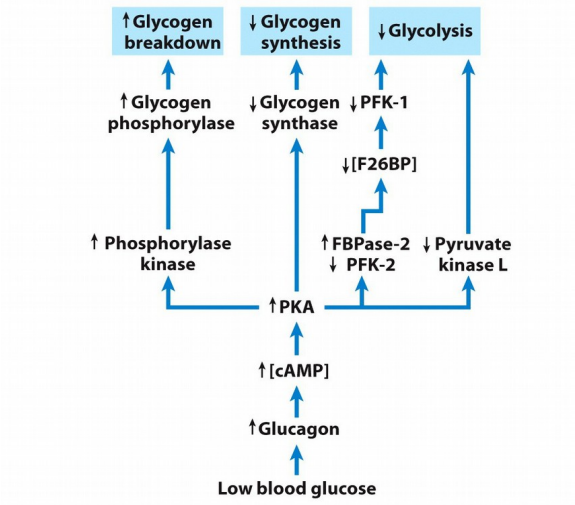
This is an example of regulation enzymes

Glycogen synthase and glycogen phosphorylase are *reciprocally* regulated by phosphorylation



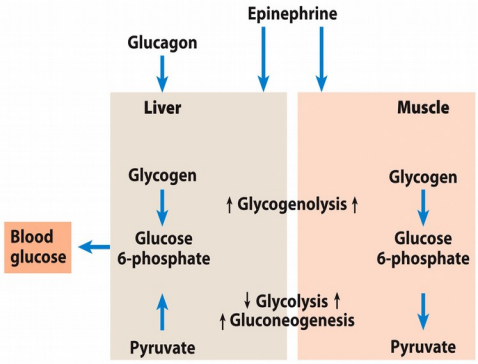
reactions in the middle

PKA-mediated regulation of carbohydrate metabolism in the liver



PKA regulation ensures that low blood sugar leads to increased glycogen breakdown, and decreased glycogen synthesis and glycolysis

Hormone-regulated enzyme activity coordinates tissue-specific metabolism



Tissue-specific Metabolism

Why are muscle and liver metabolism different?

- Myocytes lack glucagon receptors
- Muscle pyruvate kinase is not phosphorylated by PKA
- Muscle lacks gluconeogenic enzymes
- Muscle lacks a key enzyme for glucose export

→ Muscle uses stored glycogen and glucose for itself