13

carbohydratesisomersketone starchlipidproteinamine

Bio chemistry

Doctor 2017 | Medicine | JU

Sheet Slides

Done By

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Doctor

Dr.Nayef

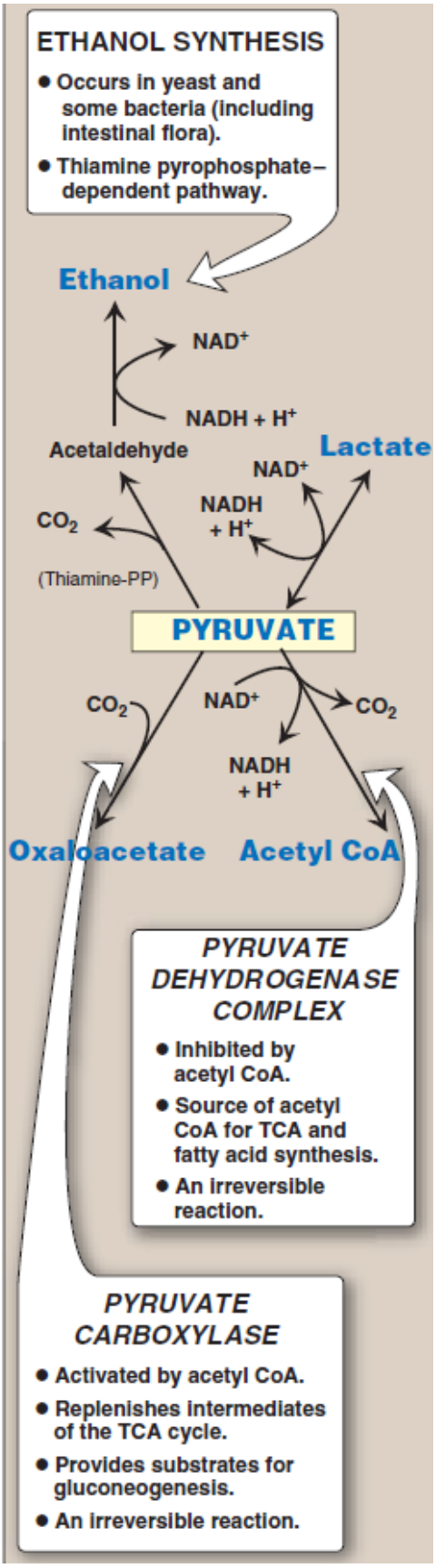
Pyruvate has a central position:

- ⇨ Pyruvate usually comes from carbs, however in low intake conditions (E.g. fasting), it would come from other sources like amino acids.

  We studied in the last lecture that pyruvate has a central position for many pathways:

  1- Fermentation, its importance lies in its ability to oxidation of NADH:
     A- Lactic acid, as in RBCs and some bacteria. Also, our muscles use this method when oxygen supply isn't enough.
     B- Ethanol, as in yeast.
  2- Acetyl CoA, this process is done by pyruvate DH[1]. The importance of Acetyl CoA lies on its ability to:
     A- Start CAC cycle.
     B- Start FA synthesis, which happens after high carbs intake.(stored as triglyceride, mainly in the liver)
  3- Oxaloacetate, which is done by pyruvate carboxylase, it starts Gluconeogenesis pathway (synthesis of glucose), So you would expect it to occur in fasting conditions. But why would we synthesize glucose in fasting conditions? To keep blood glucose lvl constant, hence let more importance organs (E.g. brain) use it.

  Note that in fasting conditions, fat metabolism takes place, which results in high conc of acetyl CoA in the end. This high conc of acetyl CoA inhibits Pyruvate DH while in the same time activates Pyruvate carboxylase. Thus, creating sugar by gluconeogenesis and preventing pyruvate from decreasing.



[1] DH=dehydrogenase…lvl=Level…FA= fatty acid… conc= concentration… PK= pyruvate kinase

Lactate Production:

- ⇨ For cells with low energy demand, for instance RBCs which lack a lot of organelles. Thus, low metabolic activity.
- ⇨ To cope with increased energy demand in rigorously exercising muscle. Here, both aerobic and anaerobic pathways work with maximum potential trying to reach the cell's demand.

Hypoxia, when no sufficient conc of O_2 is available. The cell tries to survive these brief episodes of hypoxia by lactate production (anaerobic respiration) till O_2 is back. However, if the hypoxia is prolonged, the cells would die.

- ⇨ lactate level is increased 5 to 10 folds during the anaerobic respiration.

Lactate Acidosis:

- ⇨ it's the most common cause for metabolic acidosis.
- ⇨ ↓ pH of the plasma due to acid accumulation;
  1- ↑ Production of lactic acid (more common)
  2- ↓ utilization of lactic acid (less common)

Pyruvate + NADH $\rightleftharpoons$ Lactate + NAD^+

Remember from Lew Chatlee principle that increasing substrates => increase products

Note that pyruvate can be metabolized to Acetyl CoA, while lactic acid is an end product, meaning that the only way to get rid of it is by regenerating pyruvate (which occurs in liver).

Most common cause of lactic acidosis: Impairment of oxidative metabolism due to collapse of circulatory system;

- Impaired O2 transport as in hemolytic anemia.
- Respiratory failure (common)
- Uncontrolled hemorrhage => a lot of hemolysis in tissues.

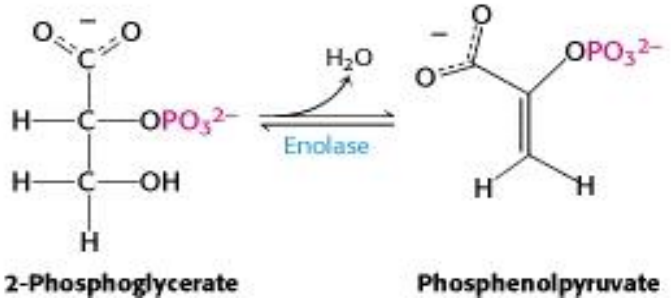
Other less common causes:

- Direct inhibition of oxidative phosphorylation => ↑NADH => ↑ Reduction of pyruvate
- Hypoxia in any tissue
- Alcohol intoxication (high NADH/ NAD^+). Metabolism of alcohol, which is a high energy compound, reduces NAD^+ to NADH, thus increasing reduction of pyruvate.
- ↓ Gluconeogenesis => ↑ [Pyruvate]**=>** ↑ Lactic acid
- ↓ Pyruvate Dehydrogenase=> ↑ [Pyruvate]**=>** ↑ Lactic acid
- ↓ TCA cycle activity => ↑ [Pyruvate]**=>** ↑ Lactic acid
- ↓ Pyruvate carboxylase => ↑ [Pyruvate]**=>** ↑ Lactic acid

Inorganic Inhibitors of Glycolysis:

1- Florida, by inhibiting enolase, it prevents bacteria from metabolizing the remnants of sugar in your mouth.

Fluoridated water ➔ bacterial enolase ➔ Prevention of Dental Carries



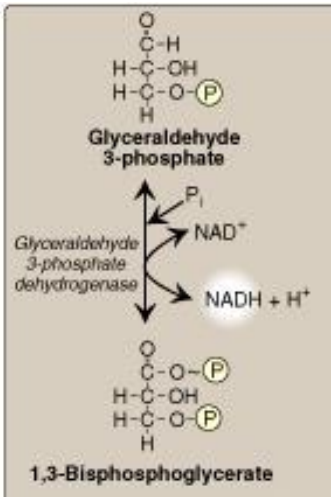
2- Arsenic Poisoning:

a- Pentavalent Arsenic (Arsenate), competes with phosphate as a substrate for GA3PDH => ↓ATP synthesis

b- Trivalent Arsenic (Arsenite), Forms stable complex with -SH of lipoic acid in:

- Pyruvate Dehydrogenase => ↓ energy
- α ketoglutarate Dehydrogenase => ↓ CAC=> ↓ energy

this in turn leads to Neurological disturbances……. DEATH (if severe).



Pyruvate Kinase deficiency:

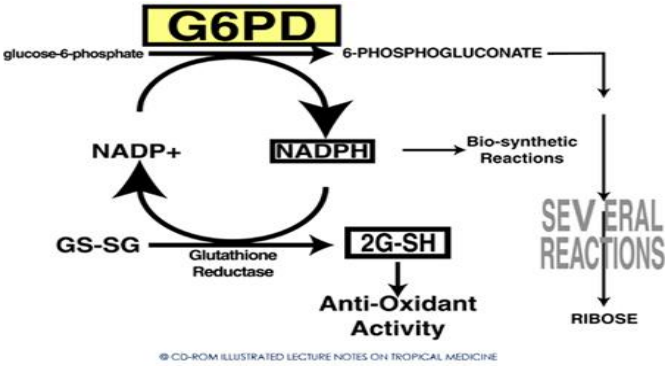
Glycolytic enzyme deficiencies are generally rare. The most common deficiencies among these rare cases => 95% of cases Pyruvate Kinase; 4% PhosphoGlucoisomerase.

- Mild to severe chronic hemolytic anemia
- ATP is needed for Na+/K+ pump➔ maintain the flexible shape of the cell
- Low ATP ➔ premature death of RBC
- Abnormal enzyme; mostly altered kinetic properties

"Pyruvate kinase deficiency: The normal, mature erythrocyte lacks mitochondria and is, therefore, completely dependent on glycolysis for production of ATP. This high-energy compound is required to meet the metabolic needs of the red blood cell, and also to fuel the pumps necessary for the maintenance of the biconcave, flexible shape of the cell, which allows it to squeeze through narrow capillaries."[2]

[2] Lippincott Biochemistry, 7th edition, P103

Glucose 6 -Phosphate Dehydrogenase:



The Dr mentioned G6PD, which is an enzyme used to convert G6P into 6-phosphogluconate while reducing NADPH.

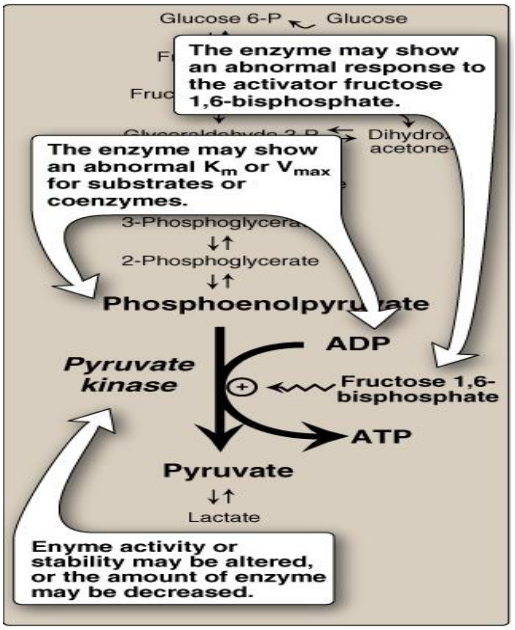
NADPH can then be used to reduce GSH (glutathione) so they can eliminate more ROS.
We will study more about it later…

Alterations observed with various mutant forms of pyruvate kinase:

When we talked about PK deficiency, we didn't only mean decreasing the enzyme amount.
Deficiency may include:

a- decreasing the catalytic property of the enzyme.
b- The enzyme becomes non-sensitive for an activator.
c- Alteration in the Km and Vmax

Remember that in our body the conc of a substrate is around the Km value. (enzymes aren't in lovely conditions).



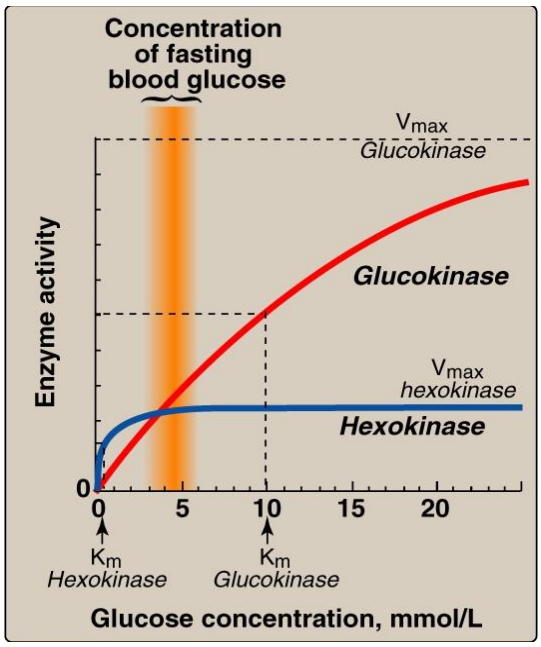
Note: detection of pyruvate kinase deficiency is hard to detect (need a specialized lab)

Regulation of Glycolysis:

⇨ It takes place in 3 irreversible reactions:
1- Hexokinase/glucokinase
2- Phosphofructokinase (MOST Important)
3- Pyruvate kinase

Types of regulation:

1- Allosteric regulation, which is fast, it occurs when one of the metabolites interfere with the enzyme activity.
2- Hormonal regulation, which is slow, mostly occurs when hormones circulate in blood and enter the tissues.



Check the figure and try to spot the differences between the 2 isoenzymes:

1- Note that the Vmax of Glucokinase (in liver) is too high so it can cope with increasing sugar levels after a meal. Why doesn't it have low Km as in hexokinase? If it had, it would take glucose from blood even in low conc and turn it into glycogen.

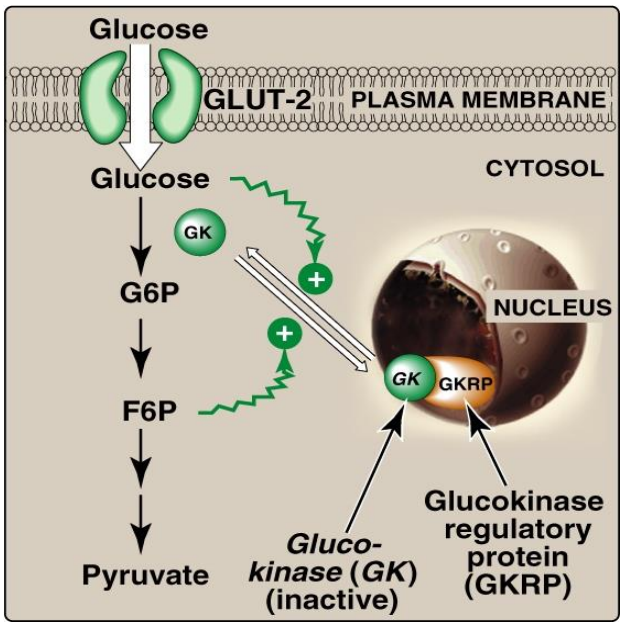
2- What would happen if Hexokinase got similar Vmax? Since the Km of the enzyme is too low (↑ affinity) it can work even in low blood glucose conditions, if it got high Vmax as in Glucokinase it would metabolize all glucose in blood.

Note that Hexokinase is inhibited by G6P (feedback-inhibition) and by ATP. Why inhibited by ATP? If we got a lot of ATP in a cell, it means that we have enough energy. Thus, we don't need to start glycolysis.

Glucokinase regulation:

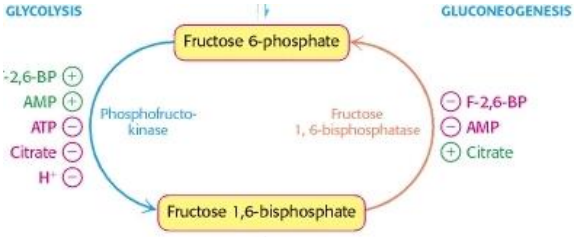
When high amounts of G6P are present in the cell, its isomer F6P (fructose 6 phosphate) would also accumulate. Accumulation of F6P relocates GK from cytoplasm to the nucleus, there it would bind to a protein called glucokinase binding protein (GKRP). thus, preventing its function.

On the other hand, when a lot of glucose is present, glucokinase is relocated from the nucleus to the cytosol to perform its function.



Glucokinase can also be regulated by insulin

Phosphofructokinase regulation:

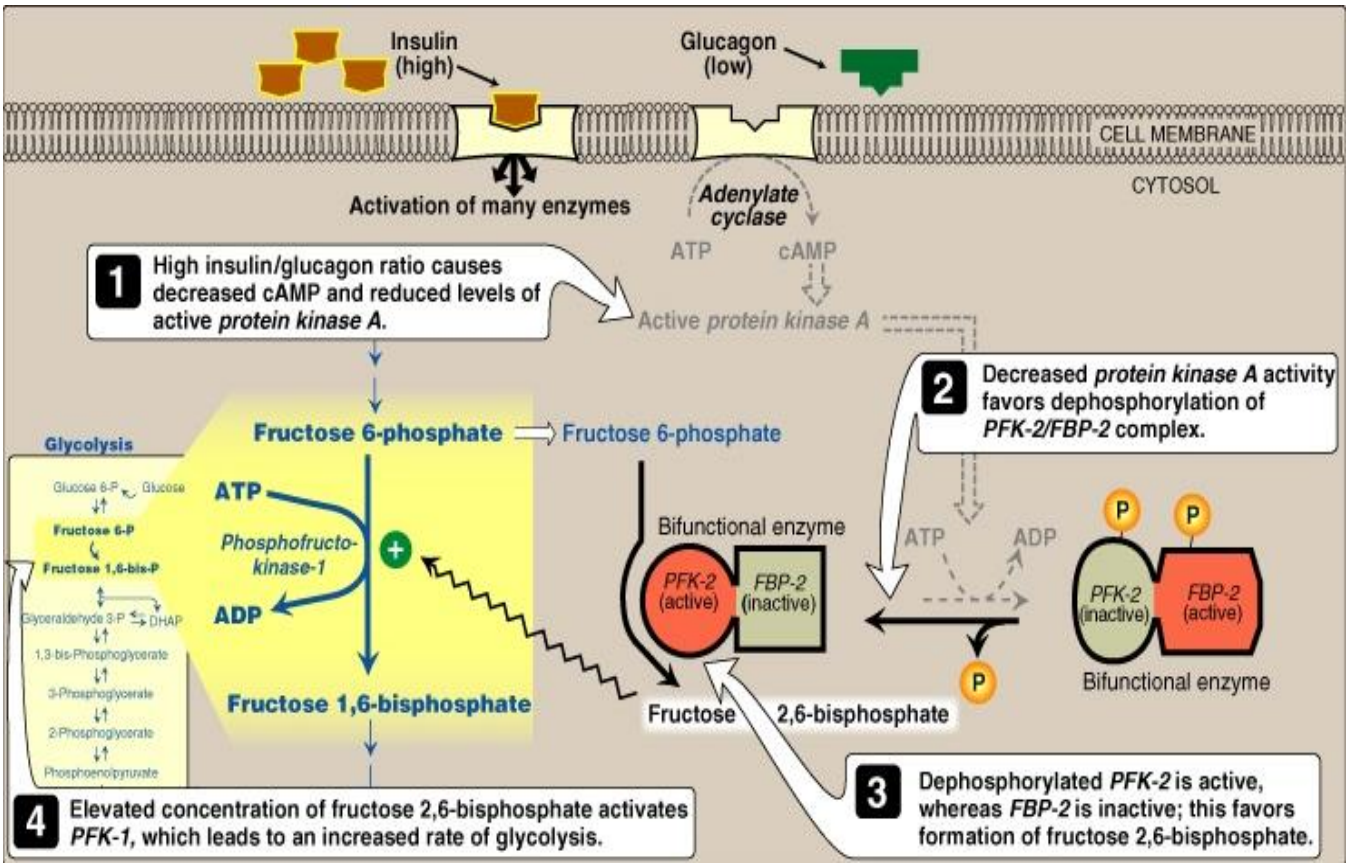


- ⇨ Phosphofructokinase catalyzes an irreversible reaction, and it is sensitive to many compounds.
- ⇨ You should understand why each molecule inhibit/activate the enzyme:

1- ATP: when we have high ATP in the cell this means that we have enough energy, so there is no need for synthesizing more=> the enzyme should be slowed down, that's why ATP is an inhibitor.
2- AMP: high AMP levels mean we have low ATP, so the ATP won't inhibit the reaction. In the same time AMP means that we don't have sufficient amount of energy for the cell. That's why we should activate glycolysis, and that's why AMP is an activator.
3- Citrate: high citrate level indicates high metabolism of fats, which happens when we don't have enough carbs -this is the basis for low carbs diet-, so the liver shouldn't consume any glucose, so more vital organs (brain) can use it.
4- H+: inhibitor, the doctor didn't talk about it.

Note: activators of phosphofructokinase inhibit fructose 1,6-bisphosphatase and vice versa, this happens so we don't reach a futile cycle.

5- Fructose **2,6**-bisphosphate (powerful activator) (important mechanism)

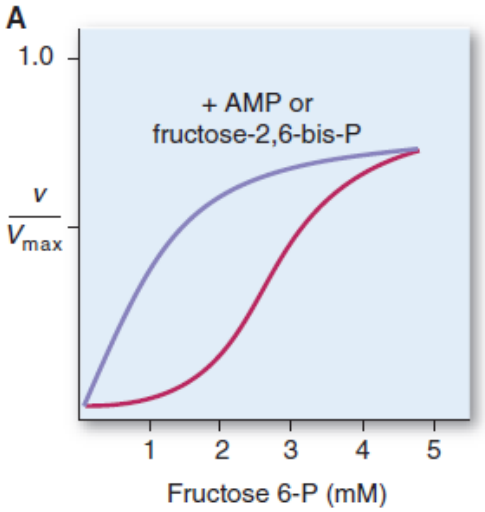


1- When insulin conc is high (E.g. after meal), it binds to insulin receptors. (Glucagon conc is low)
2- The binding of insulin causes a decrease in cAMP conc by decreasing protein kinase A activity.
3- This promotes the dephosphorylation of a bifunctional enzyme (enzyme with 2 functions), which in the case of liver, activates the phosphofructokinase-2 and deactivates fructose 2,6-bisphosphatase. So, we would end up with high conc of fructose 2,6-bisphosphate.

4- The resulting fructose 2,6-bisphosphates activates phosphofructokinase-1 to synthesize fructose 1,6-bisphosphate.

Note: the reverse of this process happens when we have a fasting condition (high glucagon)

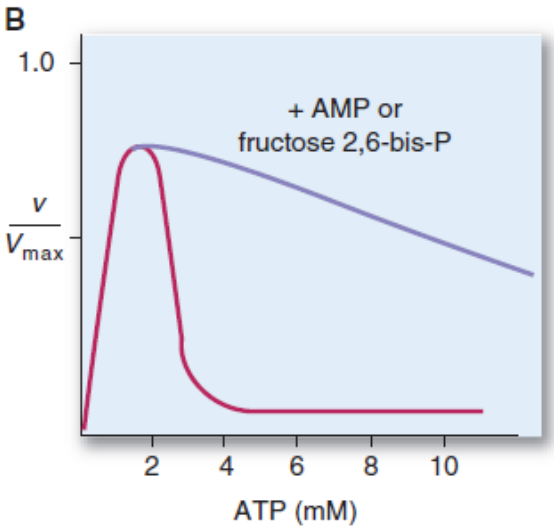
Note 2: be sure you understand the difference between phosphofructokinase 1 and 2

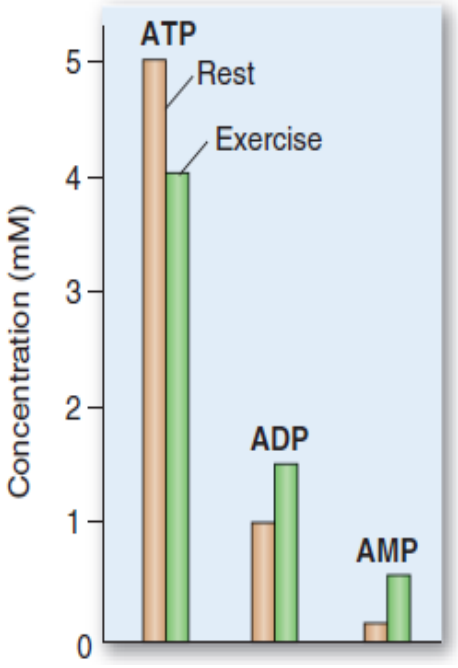


The Km of the enzyme decreases when AMP/ Fructose-2,6-bisphoshpate are present

Fruc. 6-phosphate + ATP $\xrightarrow{\text{PFK-2}}$ Fruc. 2,6 bisphosphate + ADP

- ⇨ Red curve: phosphofructokinase-1 activity with increasing ATP (X axis) conc. The normal ATP conc in a cell= 5mmol/L.
- ⇨ Blue curve: phosphofructokinase-1 activity with increasing ATP and with the presence of an activator (AMP or fructose 2,6-bisphosphate)
- ⇨ Isn't it fascinating that ATP, which is a substrate inhibits its reaction??





ATP: ADP + ADP → ATP + AMP

Normal ATP lvl= 5 mmol

Exercise ATP conc= 4 mmol, why didn't it decrease that much? Because even when we are utilizing ATP for muscle contraction, we synthesize new ATP at the same time.

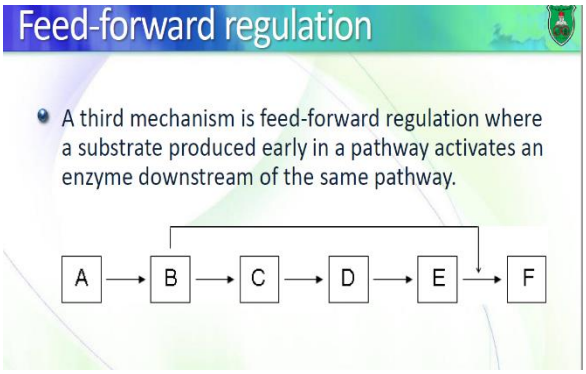
ADP: its increase activates the reaction (a little).

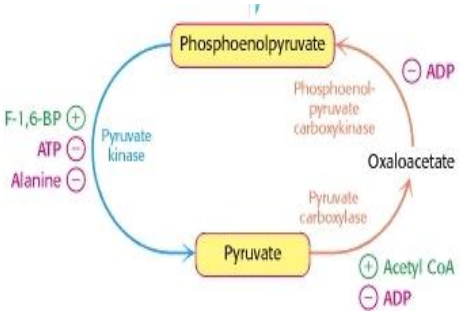
AMP: it increased 3 folds! AMP activates glycolysis for both aerobic and anaerobic pathways.

Note that the increase in PFK-1 is due (mostly) to increase in AMP not decrease of ATP.

Regulation of Pyruvate kinase:

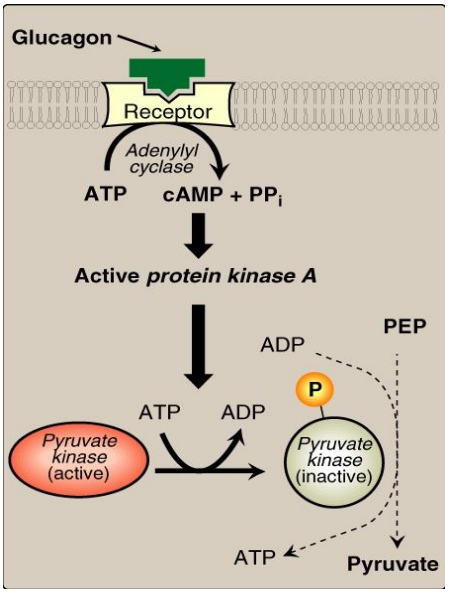
1- Inhibited by ATP, same reasons that were mentioned previously.
2- Activated by Fructose-1,6-BP NOT Fructose-2,6-BP. Forward activation is a process that we learned in summer semester.
3- Inhibited by Alanine, which is an amino acid that is synthesized in fasting conditions to make glucose from it. Its presence means low blood sugar.

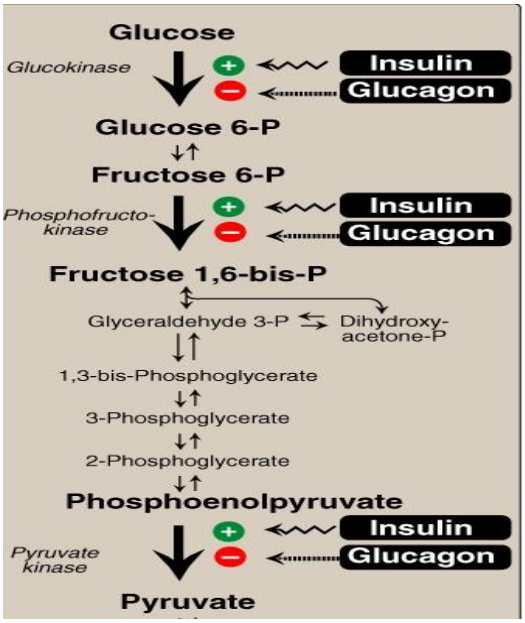




Pyruvate kinase can also be regulated by hormones:

1- Hypoglycemia conditions
2- High glucagon conc
3- Protein kinase A is active
4- Protein kinase phosphorylates many enzymes:
   a- Bifunctional enzyme (Page 6)
   b- Pyruvate kinase (ONLY IN LIVER), when phosphorylated it becomes inactive.





Hormonal Regulation:

1- Hyperglycemia ->
2- High insulin
3- All 3 regulatory steps are activated (NOT HEXOKINASE, only GLUCOKINASE)

On the other hand:

a- Hypoglycemia
b- High glucagon
c- All 3 process are inhibited