◉Sheet ○Slides

Subject :	**Glycolysis 2**
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Number :	**12**

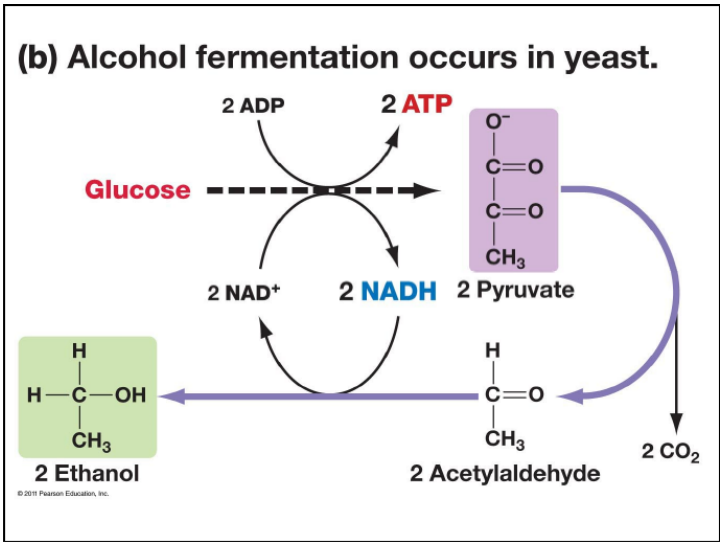
Last time we talked about glycolysis which during its first phase 'preparative phase' consumes 2 ATP molecules and in its second phase 'energy-generation' produces 2 NADH and 4 ATP molecules with a net production of 2 ATP molecules.

In order for glycolysis to continue, we need to regenerate NAD+ from NADH by the oxidation of NADH. If oxygen is available, the regeneration occurs in the mitochondria, but if oxygen is not available, one of these processes takes place:

1- Lactate fermentation: NADH is re-oxidized to NAD+, transferring its electrons to pyruvate to produce lactate. Pyruvic acid and lactic acid are both carboxylic acids made of three carbons each. Therefore, if the final product of glycolysis is lactate instead of pyruvate, NADH will not appear in the net products.

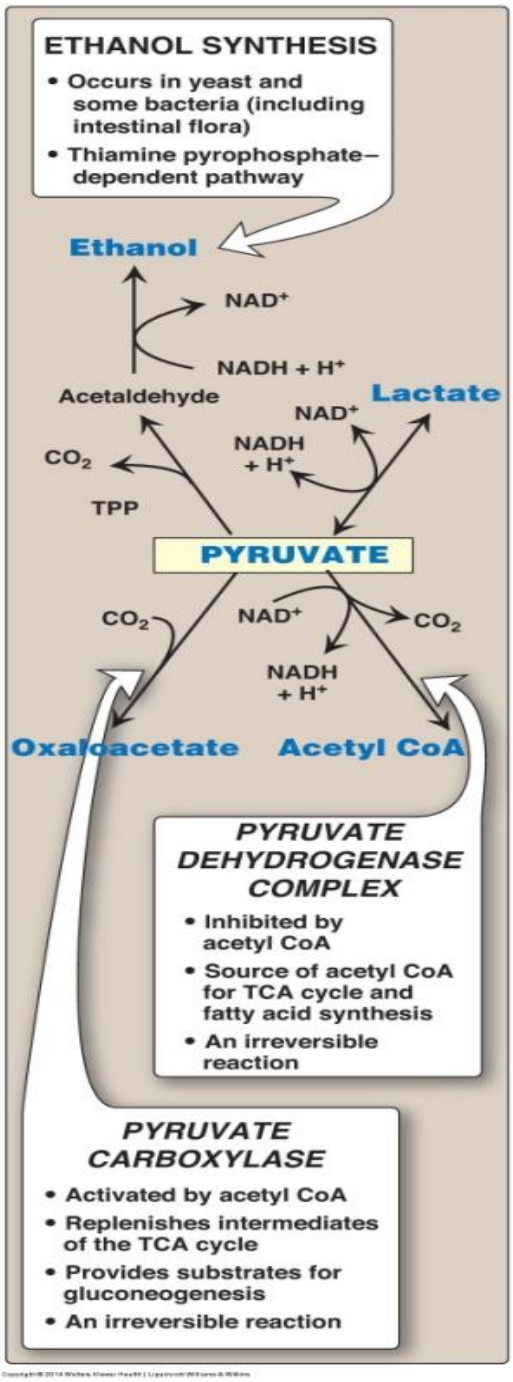
Note that this is a reversible reaction that is catalyzed by lactate dehydrogenase. Once oxygen is available again, lactate is oxidized to pyruvate. So the ratio of NADH/NAD+ determines the direction of the reaction. If NADH level is high, the reaction goes in the forward direction producing lactate.

2- Alcohol fermentation: This pathway doesn't occur in the bodies of animals nor human beings. Pyruvate undergoes decarboxylation by the enzyme pyruvate decarboxylase producing acetaldehyde and the carboxyl group is released as CO2. This is facilitated by having the ketone group at the α-carbon. This reaction is a spontaneous exergonic reaction. As a rule of thumb, decarboxylation reactions



are exergonic and favorable. Now acetaldehyde can be reduced by alcohol dehydrogenase to ethanol by gaining two electrons from NADH producing NAD+. This reaction occurs in certain types of bacteria and in yeast. It is termed ‘fermentation' because oxygen is not needed. We use this reaction when we bake to expand the dough due to the release of CO2.

The net products of alcohol fermentation are 2 ATP, 2 ethanol and 2 CO2. NAD+ is not part of the net products because it’s consumed in glycolysis.



The figure here demonstrates the different pathways that pyruvate can undergo. Pyruvate can be converted to lactate or ethanol. In the presence of oxygen, pyruvate can be converted to Acetyl CoA in case energy is needed, this occurs when pyruvate undergoes decarboxylation to give acetaldehyde which is **directly** oxidized to give acetic acid. Acetic acid is then joined with CoA to produce acetyl CoA. This reaction produces NADH, CO2 and Acetyl CoA. Acetyl CoA will then enter the citric acid cycle. This **irreversible** reaction is catalyzed by pyruvate dehydrogenase complex, an enzyme complex that is very similar to α-ketoglutarate dehydrogenase complex which converts α-ketoglutarate to succinyl-CoA. Pyruvate and α-ketoglutarate are both α-keto acids so they undergo similar reactions.

Pyruvate can also go through carboxylation to give oxaloacetate by the consumption of 1 CO2. This reaction is catalyzed by the enzyme

pyruvate carboxylase. This **irreversible** reaction is performed to produce intermediates of the citric acid cycle. The intermediates of the citric acid cycle are not usually consumed during the cycle but if for any reason they are, they should be replenished. These reactions are called “fill-up reactions”.

Lactate production:

The body undergoes this pathway in several cases:

1- **Cells with low energy demands** such as RBCs undergo this pathway. They also *lack mitochondria* so they can’t undergo oxidative phosphorylation.
2- **To cope with increased energy demand in rigorously exercised muscles:** When oxygen that is being supplied to the muscle is not enough to perform oxidative phosphorylation, the muscle undergoes anaerobic glycolysis to generate more energy. So both aerobic and anaerobic pathways run together in order to cope with the high energy demand and produce the maximum possible amount of ATP.
3- **Hypoxia:** Lack of oxygen supply to the tissues forces the cells to undergo anaerobic glycolysis as a temporary solution in order to survive.

Lactate acidosis:

The “sis” suffix implies that this is a disease. Acidosis can happen due to a problem with the lung (respiratory acidosis) or due to the production of acids (metabolic acidosis).

Lactate acidosis is the most common cause of metabolic acidosis. It happens either due to increased production of lactic acid which decreases the pH or decreased utilization of lactic acid.

Pyruvate + NADH <--> Lactate + NAD+

From the above equation, we can see that the increased production of NADH pushed the reaction in the forward direction producing lactate.

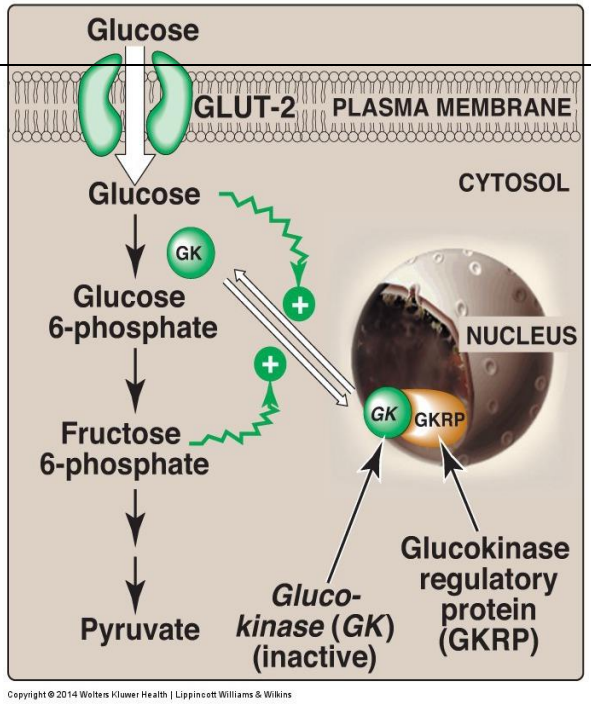
Lactate acidosis can happen due to several reasons:

1- Impairment of oxidative phosphorylation: The most common cause of lactate acidosis. It might occur due to the following reasons:
   a- Impaired O2 transport: oxygen is not reaching the cells adequately.
   b- Respiratory failure.
   c- Uncontrolled hemorrhage.
   d- Direct inhibition of oxidative phosphorylation: as in CO/CN^- poisoning or increasing the concentration of NADH.
2- Alcohol intoxication: characterized by high NADH/NAD+ ratio.
3- Decrease in gluconeogenesis which causes accumulation of lactate.
4- Decrease in pyruvate dehydrogenase activity.
5- Decrease in the citric acid cycle activity.
6- Decrease in pyruvate carboxylase activity.

Regulation of glycolysis:

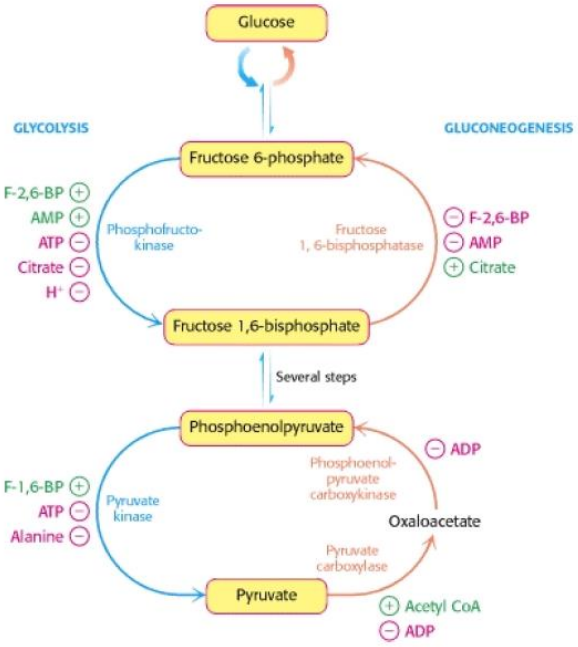
We have mentioned before that there are 3 irreversible steps in glycolysis. The first step is the phosphorylation of glucose. The second step is adding a phosphate group to fructose 6-P (this is the most important step to be regulated). The last step is conversion of phosphoenolpyruvate to pyruvate. Regulation usually happens in irreversible steps because reversible ones can go back and forth depending on the concentration.

1- **Regulation of the hexokinase/glucokinase:** Phosphorylation is either done by hexokinase or glucokinase. Glucokinase is an enzyme found in the liver, it is specific for glucose and involved in the storage of it (note that Lippincott book states that both enzymes have the same substrate specificity but it's being under research). On the other hand, hexokinase is found in all tissues and is involved in the use of glucose for energy. Glucokinase has high Km which means that this enzyme works when blood glucose is high whereas hexokinase has low Km meaning that it works even when blood glucose is low. On the contrary, Vmax for glucokinase is high which means that it's very efficient when it works.



Hexokinase is inhibited by its product, glucose 6-phosphate. When glucose 6-phosphate accumulates, hexokinase becomes less efficient whereas glucokinase is inhibited by fructose 6-phosphate rather than glucose 6-phosphate which is in equilibrium with it. When fructose 6-phosphate accumulates, it promotes glucokinase to be translocated to the nucleus where it binds to glucokinase regulatory protein (GKRP). On the other hand, as a result of the increase in glucose and insulin levels, glucokinase reenters the cytosol and is active.

2- Regulation of phosphofructokinase: Fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate. This allosteric enzyme is inhibited by ATP (when its concentration is high), citrate and protons (acidity). It is stimulated by fructose 2,6-bisphosphate and AMP. 5 different signals regulate this enzyme, meaning this is a very important enzyme.

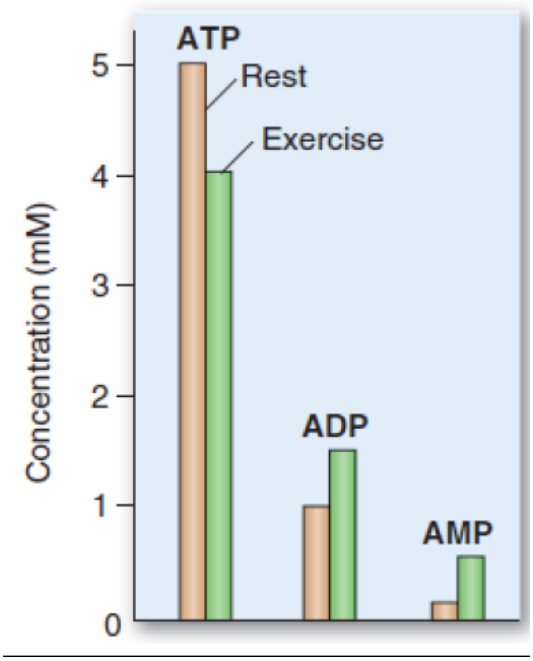


This enzyme is inhibited by ATP because high ATP means that there is no need for glycolysis to occur. On the other hand, high ADP means that the cell needs energy and should activate the enzyme, but in reality, AMP activates it instead of ADP.

ADP + ADP -> ATP + AMP

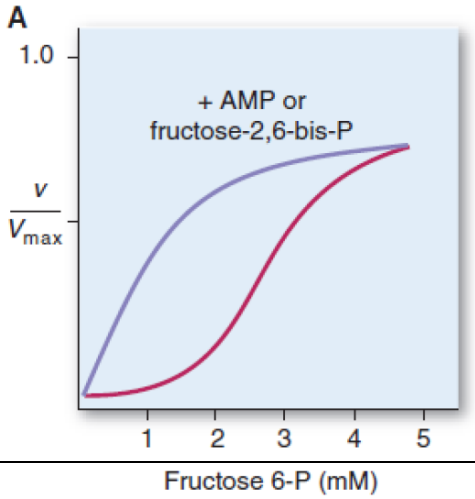
When ATP is consumed, ADP is produced. ADP has energy but still it cannot be used as a source of energy for reactions that use ATP. Therefore, one ADP molecule transfers one phosphate group to another ADP to produce ATP and AMP. This reaction is catalyzed by Adenylate kinase. So when ADP accumulates, it is rapidly converted to AMP and ATP. AMP then can stimulate PFK-1.

This figure shows the concentration of ATP, ADP and AMP during rest and exercise. During exercise, ATP is only reduced by about 20%. On the other hand, concentration of AMP is about 300% higher during exercise than its concentration while resting. Because the change in AMP is much higher, it is used as a signal to activate PFK instead of ADP (powerful activator). This difference in concentrations is enough to overcome the ATP inhibition and allow glycolysis to continue because even if ATP =4mM, it's enough to inhibit PFK-1).



Fructose 2,6-bisphosphate is a regulatory molecule and is not an intermediate in glycolysis.

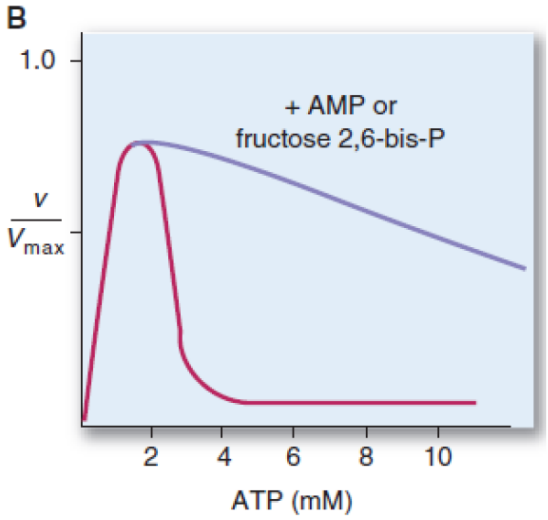
This curve relates the concentration of the first substrate for PFK (fructose 6-P) and the velocity of the enzyme as a fraction of Vmax. Usually the proportion between the velocity and the substrate concentration is a direct proportion. The curve is hyperbolic if it follows Michaelis-



Menten kinetics, but if it's sigmoidal, then the enzyme is an allosteric enzyme.

When we add AMP or fructose 2,6-bisphosphate (positive effectors), the curve becomes hyperbolic. The enzyme is much more active regardless of the substrate's concentration as this addition causes the reduction of Km.

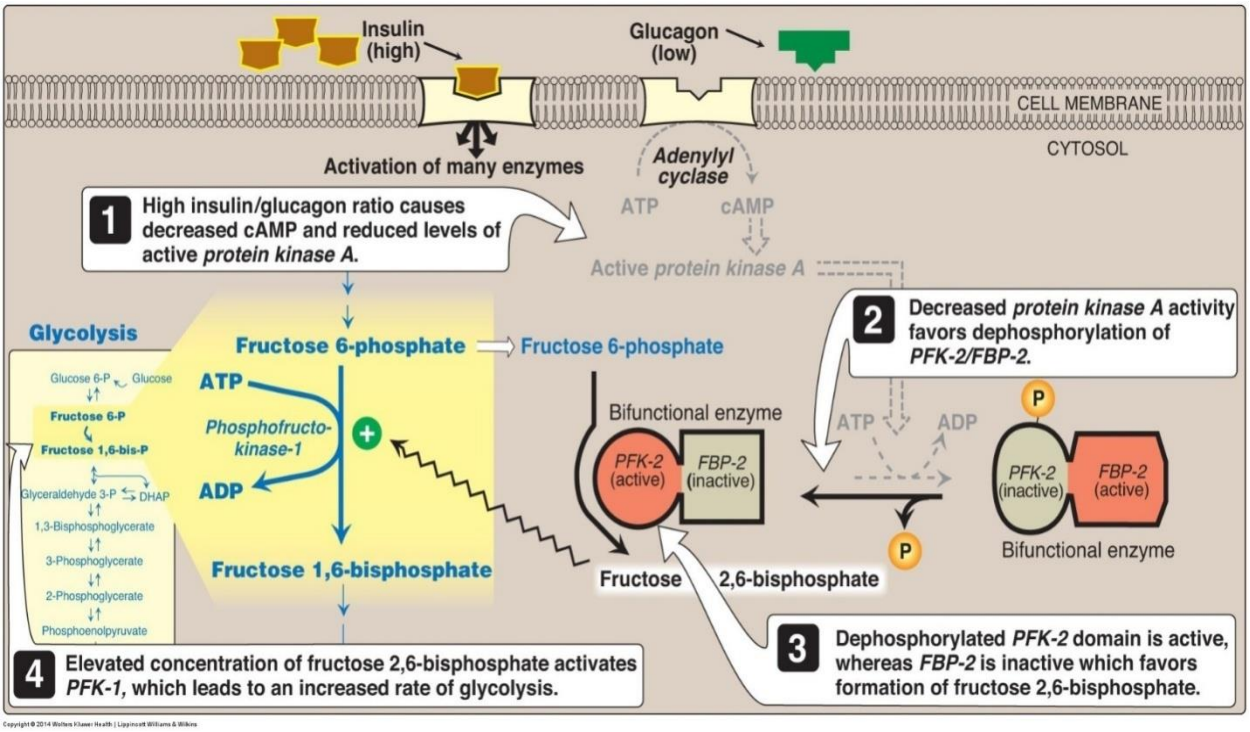
This curve relates the concentration of the second substrate for PFK (ATP) and the velocity of the enzyme as a fraction of Vmax. As we increase the substrate concentration, the velocity of the enzyme increases then suddenly drops because ATP is an inhibitor of PFK. AMP and fructose 2,6-bisphosphate prevent the inhibition of PFK.



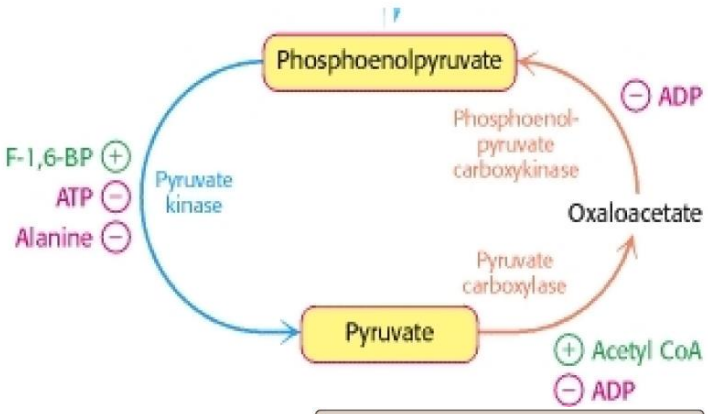
Fructose 2,6-bisphosphate is formed by the enzyme phosphofructokinase-2. This enzyme has 2 domains (bifunctional enzyme): a kinase and a phosphatase. The *kinase converts fructose-6-phosphate into fructose-2,6-bisphosphate*, while the phosphatase converts *fructose-2,6-bisphosphate to fructose-6-phosphate* (removal of phosphate).

PFK-2 requires regulating, this is achieved by the phosphorylation or dephosphorylation of the enzyme. When a phosphate group is added to PFK-2 by protein kinase A, the kinase is inactivated and the phosphatase is activated and vice versa. When insulin levels are high, protein kinase A is inactivated which favors dephosphorylation of PFK-2. Dephosphorylation of PFK-2, thus, activates the kinase and inactivates phosphatase activity which is for the purpose of accelerating glycolysis (by increased production of Fructose-2,6-bisphosphate). Glucagon does the opposite (it favors phosphatase activity) by activating PKA and adding phosphate to the enzyme. Note that high insulin means the abundance of glucose so more need for glycolysis ,while high glucagon (like in fasting) indicates the scarcity of glucose so breaking down of glucose must be reduced and

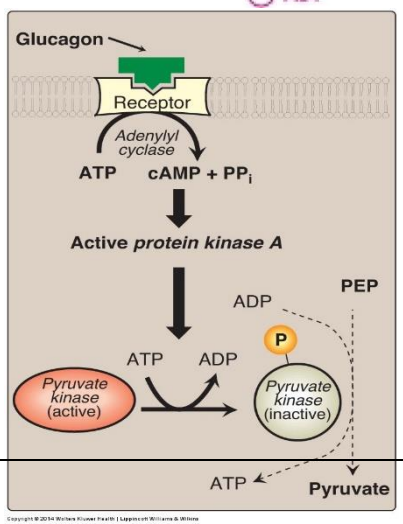
gluconeogenesis must get activated (glucose should be preserved). The heart and muscles actually start burning fat in this case.



3- Regulation of Pyruvate kinase: This enzyme is activated by fructose 1,6-bisphosphate, an early intermediate, by feed-forward activation. It is inhibited by ATP and alanine. Alanine indicates the abundance of amino acids, and because the role of glycolysis is to produce building blocks, it inhibits glycolysis. Note that pyruvate and alanine are very similar (the first is a keto-acid and the second is an amino-acid).



Pyruvate kinase (in the liver only) is converted from the **active form to the inactive** form by phosphorylation catalyzed by protein kinase A which is activated by cAMP which in turn is



activated by glucagon. High glucagon level means that blood glucose is low (hypoglycemia) and glycolysis should be inhibited.
Note: Alanine also inhibits this enzyme because it is produced during gluconeogenesis which indicates low glucose level (that's why your body is making it!). Glycolysis must be reduced in tissues like the liver or even muscle tissue → blood sugar is low so we have to reserve glucose for the completely glucose-dependent **brain and RBCs** (critical tissues) and decrease the breaking down of glucose by tissues that can utilize fat for energy production.

Pyruvate kinase deficiency: Severe deficiencies in any of the glycolytic enzymes cause babies to die early in the fetal life. Pyruvate kinase deficiency is the most common among the glycolytic enzymes deficiencies (95%). RBCs only require energy to keep ion pumps working to maintain the flexible shape of the cell (RBCs don't contain protein synthesis machinery nor do they have nuclei so only a small amount of energy is required but energy is crucial for them) and because they are *absolutely dependent* on glycolysis (due to the lack of mitochondria), they are highly affected by Pyruvate kinase deficiency causing mild to severe hemolytic anemia (due to *premature death* –decreased life span-of RBCs because they don't make enough ATP to keep the ion pumps working) and the patient needs periodic blood transfusion.
Enzymes that are deficient aren't always absent, sometimes they're present with abnormalities such as altered/abnormal Km or Vmax for the substrate or coenzyme. The enzyme might show an abnormal response/sensitivity to the activator fructose 1,6-bisphosphate. The enzyme activity & stability may also be altered (decreased stability decreases the life span of the cell). The amount of the enzyme also may decrease. This is because of the presence of several mutations in the enzyme gene that may affect the enzyme. **Any mutation will change something regarding the enzyme (its kinetics-Km or Vmax-/its amount/ its regulation/its stability).**

***Final and important notes:*
1-Catabolism is a convergent process; a wide variety of molecules are transformed into a few common end products. On the contrary, anabolism

is a divergent process in which a few biosynthetic precursors form a wide variety of complex biomolecules.

2- Intracellular regulators like feedback inhibitors (excess end products) and allosteric modulators are very fast in action, while intercellular communication -for the purpose of regulation- is slower.

3-Vmax of glucokinase is much higher than hexokinase to minimize hyperglycemia in blood after a carbohydrate-rich meal (phosphorylation is efficient).

4- Citrate inhibits PFK-1 because high citrate leads to fatty acid influx and usage for energy. Fat as an energy source means there isn't enough glucose in the cell (to be reliable for energy production) so the glycolytic pathway must be slowed down to preserve cellular glucose.
5- High insulin/glucagon ratio **induces** more synthesis of glucokinase, PFK-1 and Pyruvate kinase.
6- There are some inorganic inhibitors of glycolysis like fluoride ion (added to tooth paste) which inhibits enolase, thus bacterial growth on teeth is stopped by blocking glycolysis to prevent dental caries. Also, arsenic poisoning can be fatal as trivalent arsenic, **arsenite**, binds to the SH group in lipoic acid and inactivates many enzymes including Pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex leading to neurological disorders and potential death. On the other hand, pentavalent arsenic, **arsenate**, prevents production of ATP and NADH in glycolysis without stopping the pathway. It does so by competing with Pi as a substrate for G3P dehydrogenase forming a complex that is spontaneously hydrolyzed to 3-phosphoglycerate.
7- Pyruvate kinase deficiency will cause accumulation of metabolites before this specific step happens (if PEP can't turn to Pyruvate, then we'll have an accumulation of upstream metabolites). One of them is 2,3-bisphosphoglycerate (2,3-BPG) which enhances the release of oxygen from RBCs to tissues. So although we are having hemolytic anemia → decreased oxygen carrying capacity, the release of oxygen towards tissues increases (as a source of condolence).

The End