13

Bio chemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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✓ In the previous lecture, glycolysis was discussed. We now know that it starts with glucose and ends with pyruvate, and if there is not enough oxygen (because of direct inhibition of oxidative phosphorylation or hypoxia) pyruvate will be reduced and converted into lactate by lactate dehydrogenase.

✓ Overproduction of lactate causes a drop in the pH resulting in **lactic acidosis**, which is the most common type of metabolic acidosis.

✓ <u>Alcohol intoxication</u>

Alcohol is used as a source of energy. If somebody drinks alcohol, ethanol will be oxidized to acetaldehyde by alcohol dehydrogenase, reducing NAD^+ into NADH. High levels of NADH or a high NADH/NAD^+ ratio (in case of excessive intake of alcohol) **inhibits** many enzymes and processes that depend on NADH/NAD+ ratio such as:

- ↓Gluconeogenesis
- ↓Pyruvate dehydrogenase
- ↓TCA cycle activity
- ↓Pyruvate carboxylase

In this sheet, we'll mainly cover the regulation of glycolysis and start talking briefly about gluconeogenesis.

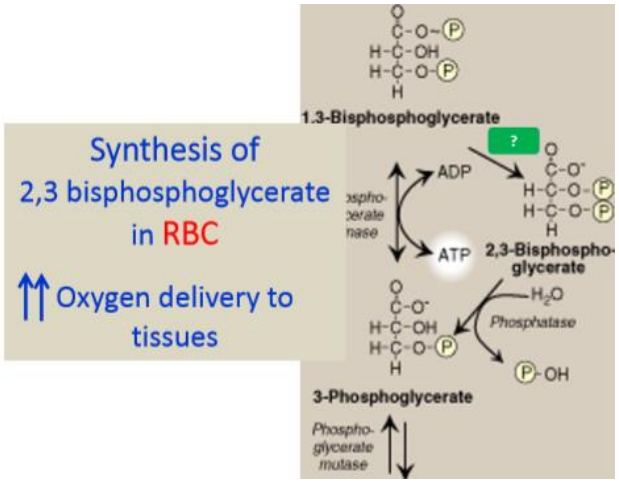
<u>Synthesis of 2,3 bisphosphoglycerate</u>

➢ 1,3-bisphosphoglycerate is one of the glycolysis intermediates which normally transfers the phosphate group from carbon 1 to ADP, producing ATP.

➢ The function of 2,3-bisphosphoglycerate is to increase oxygen delivery to tissues by making it easier for hemoglobin to unload its oxygen.

Let's look further into how this conversion happens:

It is simply done by the transfer of the phosphate group from carbon number 1 of 1,3-BPG to carbon number 2, producing 2,3-BPG. This is carried out by the enzyme bisphosphoglycerate mutase.



- ✓ If 2,3-bisphosphoglycerate is no longer needed, it is degraded by the enzyme **phosphatase.** Phosphatase removes the phosphate group from carbon number 2 by hydrolysis, producing 3-phosphoglycerate.
- ✓ Regulatory molecules (inhibitors or activators) are not required to signal continuously, so they must be easily degradable in order to retain the initial response.
- ✓ The amount of 2,3-bisphosphoglycerate in the cells is usually low.

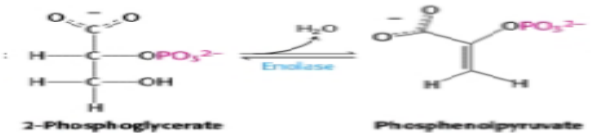
Note:- Mutase is the enzyme that transfers the phosphate group from one carbon atom to another carbon within the same molecule.

Inorganic Inhibitors of glycolysis

1. Fluoride: Fluoride inhibits enolase, which is one of the glycolysis enzymes. Enolase is responsible for the conversion of 2-phosphoglycerate to phosphoenolpyruvate by dehydration.

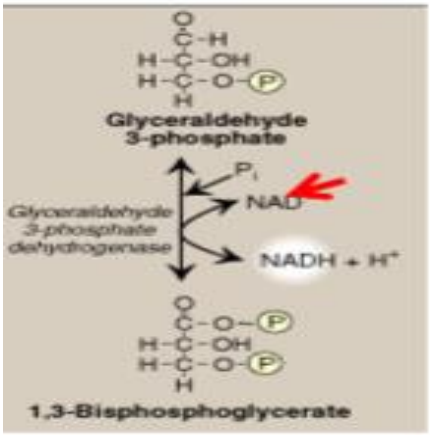
    - Fluoride can be added to toothpaste in order to prevent dental carries. Also, fluoride can be added to water making "Fluoridated water".

    - Fluoride in water or in toothpaste leads to inhibition of the bacterial enolase, preventing dental carries.



2. Arsenic poisoning;

Arsenate - the pentavalent form of arsenic, has five bonds. It competes with phosphate as a substrate for glyceraldehyde-3-phosphate dehydrogenase – one of the glycolysis enzymes responsible for conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate.



Glyceraldehyde-3-phosphate is converted into 1,3-bisphosphoglycerate by oxidation and addition of a phosphate group. However, arsenate competes with phosphate producing **1-arseno-3-phosphoglycerate** which is considered a highly unstable molecule that is rapidly hydrolyzed. As a result, there will be loss of 1,3- bisphosphoglycerate that should have been used to synthesize ATP later on, causing a reduction in ATP levels.

Arsenite - the trivalent form of arsenic- forms a stable complex with the sulfhydryl group (-SH) of lipoic acid that was discussed in previous lectures. This causes inhibition of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase.

Consequently, it leads to Neurological disturbances which can lead to death.

Why does it lead to neurological disturbances? Because of the great dependency of the CNS on glucose.

↑glucose means that we need increased activity of PDH to promote acetyl CoA and ATP production. Since PDH is inhibited in this case by Arsenite, less Acetyl CoA and ATP are produced. For a highly active organ like the brain, decreased ATP levels result in severe disturbances.

Pyruvate Kinase Deficiency

Pyruvate kinase is the last enzyme in glycolysis, it converts phosphoenolpyruvate to pyruvate by dephosphorylation coupled to ATP synthesis. Pyruvate kinase deficiency is the most common among glycolytic enzyme deficiencies.

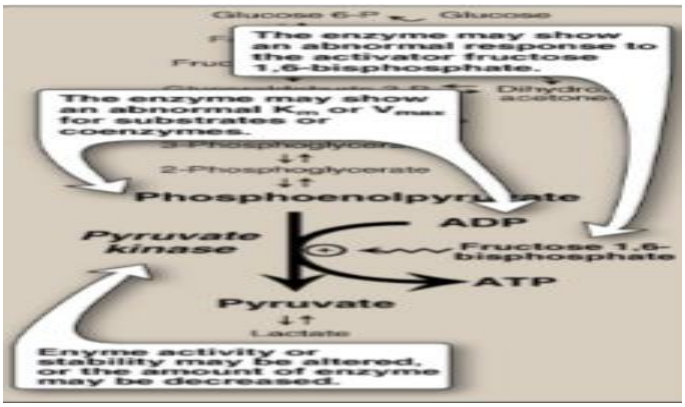
- Mainly, RBCs are affected leading to mild or severe chronic hemolytic anemia. This is because other tissues can compensate by synthesizing more of the defective enzyme, whereas RBCs can't because they lack a nucleus. Note that defective enzyme implies reduced activity but not necessarily zero activity.

- Energy is needed by the RBCs for active transport (NA+/K+ pump) to maintain the flexible shape of the cell. RBCs need to squeeze through very narrow capillaries, and if they become rigid they would be destroyed and removed from the circulation.

- Low ATP levels lead to premature death of RBCs (due to becoming rigid), hence the lifespan of RBCs will greatly decrease (instead of 120 days it will be much less than that) , leading to hemolytic anemia.

- The enzyme may not be deficient but abnormal, causing altered kinetic properties and such alterations are observed with various mutant forms of Pyruvate kinase, such as:

  - The enzyme may show an abnormal response to the activator fructose-1,6-bisphosphate.
  - The enzyme may show an abnormal Km or *V*max for the substrate (phosphoenolpyruvate) or coenzyme (ADP).
  - The enzyme stability or activity may be altered, or the amount of enzyme may be decreased; for example the enzyme wouldn't be stable in extreme pH levels.



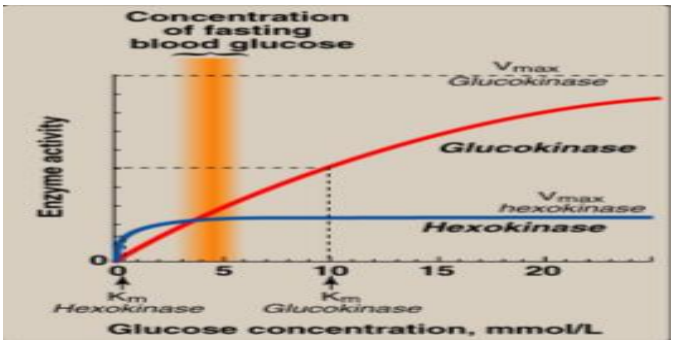
And now , let's start talking about **<u>The regulation of glycolysis</u>**

- The regulation of glycolysis is done at these three irreversible steps:
    1. Adding phosphate to glucose **by** hexokinase and sometimes glucokinase.
    2. Adding phosphate to fructose 6-phosphate **by** phosphofructokinase
    3. Producing pyruvate from phosphoenolpyruvate **by** pyruvate kinase.

<u>Let's start discussing the ***regulation*** of the first step:-</u>

✓ As mentioned in previous lectures, hexokinase has **lower** Km than glucokinase (more affinity to glucose). We can find hexokinase in tissues that require glucose even when blood glucose levels are very low. Glucokinase is found in the liver and is activated when blood glucose levels are high.

- **Low blood glucose level → hexokinase**
- **High blood glucose level → glucokinase**

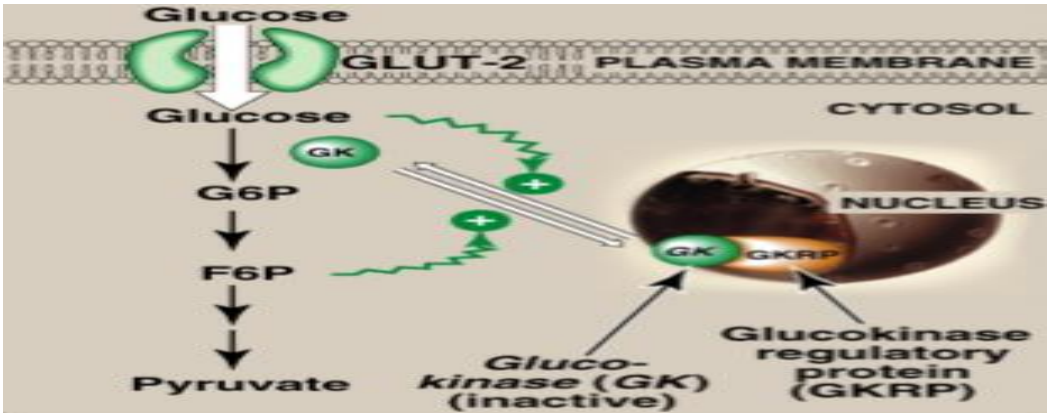


- Usually, the blood glucose level is 5.5 mmol/L (90mg/dl). Glucokinase can only be activated when blood glucose is **double the normal** amount and this is where glucose should be used by the liver; for storage as glycogen or as a source of energy or to be converted into fatty acids.

- Glucokinase isn't directly inhibited by glucose-6-phosphate like other hexokinases. Instead, it is *indirectly inhibited* by fructose-6-phosphate, which is in equilibrium with Glucokinase product; G-6-P. It is *indirectly stimulated* by glucose, a substrate of Glucokinase. Regulation is achieved by reversible binding of Fru-6-P and glucose to glucokinase regulatory protein (GKRP), a hepatic protein.
- In the presence of Fru-6-P, glucokinase binds to GKRP, migrates to the nucleus and becomes inactive.
- In the presence of glucose, glucokinase is released from GKRP and re-enters the cytosol where it phosphorylates glucose to G-6-P.

Note: Regulation of GK is needed because the cell should NOT overproduce phosphorylated sugars.

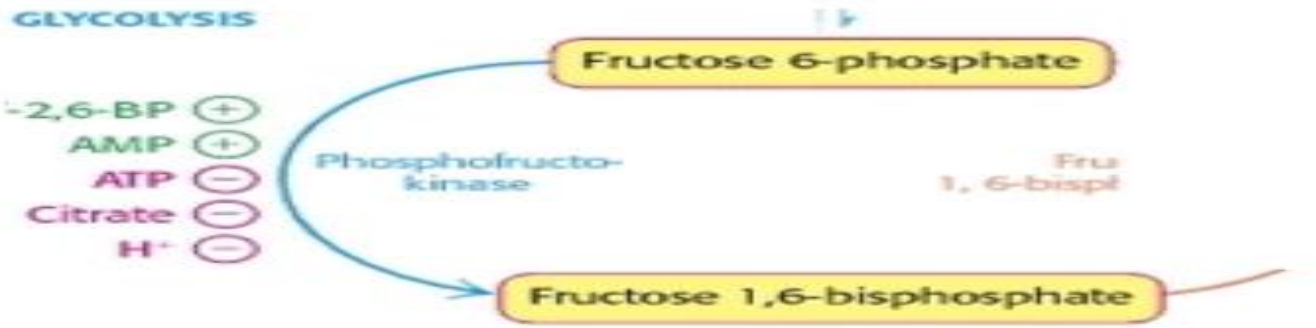
Why? Phosphorylating sugars consumes the phosphate group supply of the cell (they trap the phosphate with them). Therefore, overproduction of phosphorylated sugars may cause depletion of our phosphate group stores. This will lead to a new problem, which is the inability to produce ATP, because ATP synthesis requires phosphate groups.



✓ The second enzyme is Phosphofructokinase-1:

- PFK-1 is **inhibited by**;
  1. **ATP**; since the goal of glycolysis is to produce ATP, if the level of ATP is high the glycolysis should be switched off
  2. **Citrate**; high citrate levels mean high amount of building blocks.
  3. **H+** which indicates low pH since the end product of glycolysis is either pyruvate or lactate (both of them are weak acids that decrease the pH) so if the PH is decreased , glycolysis should stop

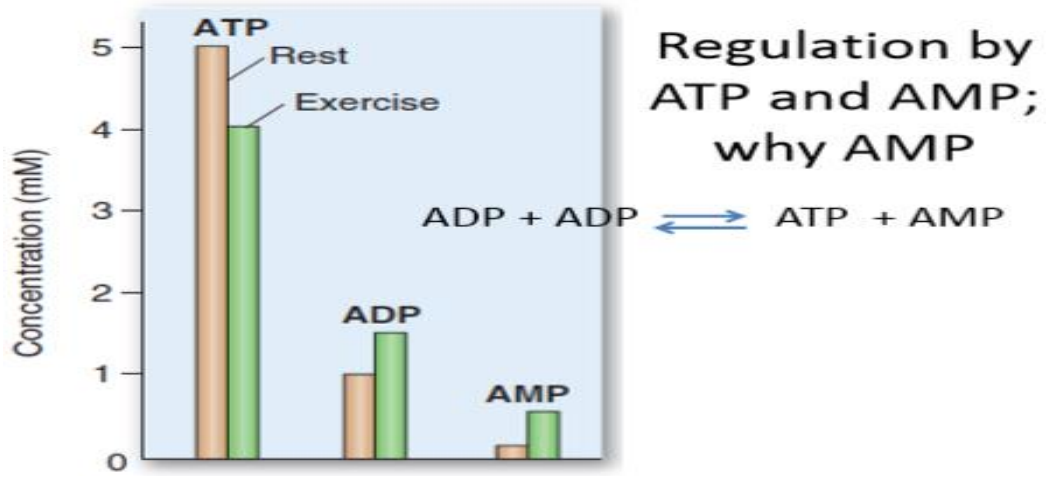
- PFK-1 is **activated by**;
  1. **AMP**; it indicates a low energy state in the cell.
  2. **2,6-**bisphosphate; it is a regulator (activator) of glycolysis not an intermediate.



❖ Regulation is done by ATP and AMP.
❖ Why not ADP, although it also indicates a high energy state in the cell?

If ATP is used up at a very high rate, ADP levels will increase but ADP cannot be used to replace ATP even though it has a lot of energy.
The enzymes that depend on ATP can't use ADP to carry out the reaction.

In the figure below there is a comparison between ATP, ADP and AMP levels at rest and during exercise:



- ✓ Let's start with ATP: ATP levels during exercise decrease from 5→4 mM because exercise needs energy which can be obtained from ATP hydrolysis.
- ✓ ATP (as a regulator of glycolysis) indicates a high energy state, inhibiting glycolysis.

- ✓ ADP levels increase slightly during exercise because ATP hydrolysis leads to formation of ADP and inorganic phosphate. ADP can't be used for regulation purposes.

- ✓ AMP activates glycolysis because it indicates a low energy state in the cell. It's used as a regulator of glycolysis due to its sensitivity to the energy state in cells.
  AMP can be produced during ATP synthesis by the enzyme adenylate kinase, by combining 2 ADP molecules:

ADP + ADP → ATP + AMP

It can also be produced by hydrolysis of one high energy phosphate bond of ADP.

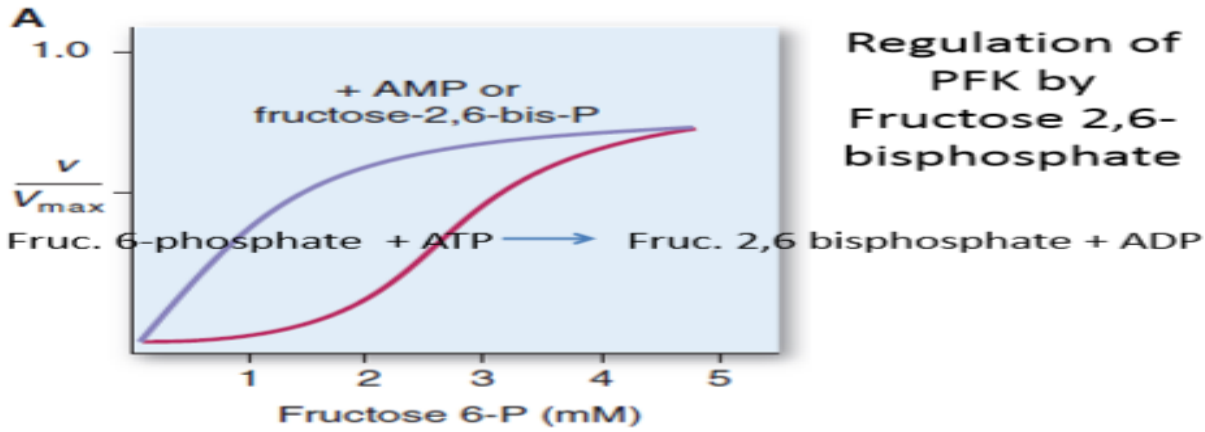
- **<u>2,6-bisphosphate</u>** is an activator of glycolysis, produced by adding a phosphate group to fructose-6-phosphate by the enzyme phosphofructokinase-2.
- How can we distinguish between PFK-1 and PFK-2?

- Phosphofructokinase **1** → Adds a phosphate group to carbon number **1** of Fru-6-P.
- Phosphofructokinase **2** → Adds a phosphate group to carbon number **2** of Fru-6-P.

Looking at the **red** curve in the figure below, we can see the relationship between the velocity and the PFK substrate concentration which is Fru-6-P.

If we increase the substrate concentration, the velocity increases until it reaches *V*max. But as we can see in the **blue** curve (with the addition of AMP and/or fructose-2,6-bisphosphate), the **red** **sigmoidal** curve becomes **hyperbolic** due to increased activity of enzymes indicated by increased velocity at the same substrate concentration.

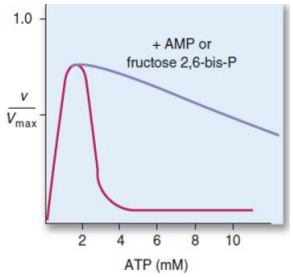
Fructose-2,6-bisphosphate and AMP bind to the enzyme on the regulatory site activating the binding of the substrate, resulting in increased enzymatic activity.



Fru-6-P + ATP → Fru-2,6-BP + ADP

SUMMARY:- AMP and fructose-2,6-bisphosphate act as regulatory molecules by decreasing the Km (higher affinity) and shifting the curve to the left (increasing activity).

ATP is a substrate of the enzyme PFK, so we expect that if we increase the substrate (ATP) concentration, the rate of the reaction will increase.
But as we can see in the figure below, after the reaction gets to *V*max, it sharply decreases which indicates that ATP is an *activator* of PFK at low concentrations but has an *inhibitory* effect at some high substrate (ATP) concentrations.



however, the presence of AMP and/or Fru-2,6-bp removes the inhibitory effect of ATP and the reaction of the enzyme (PFK) proceeds.

Unlike PFK-1, PFK-2 is a bifunctional protein that has both the kinase activity (PFK-2) that produces Fru-2,6-bp and the phosphatase activity (fructose-2,6-bisphosphatase FBP-2) that dephosphorylates Fru-2,6-bp to Fru-6-p.

Phosphorylated form of PFK-2 is inactive, while the dephosphorylated form is active.
Phosphorylated form of FBP-2 is active, while the dephosphorylated form is inactive.

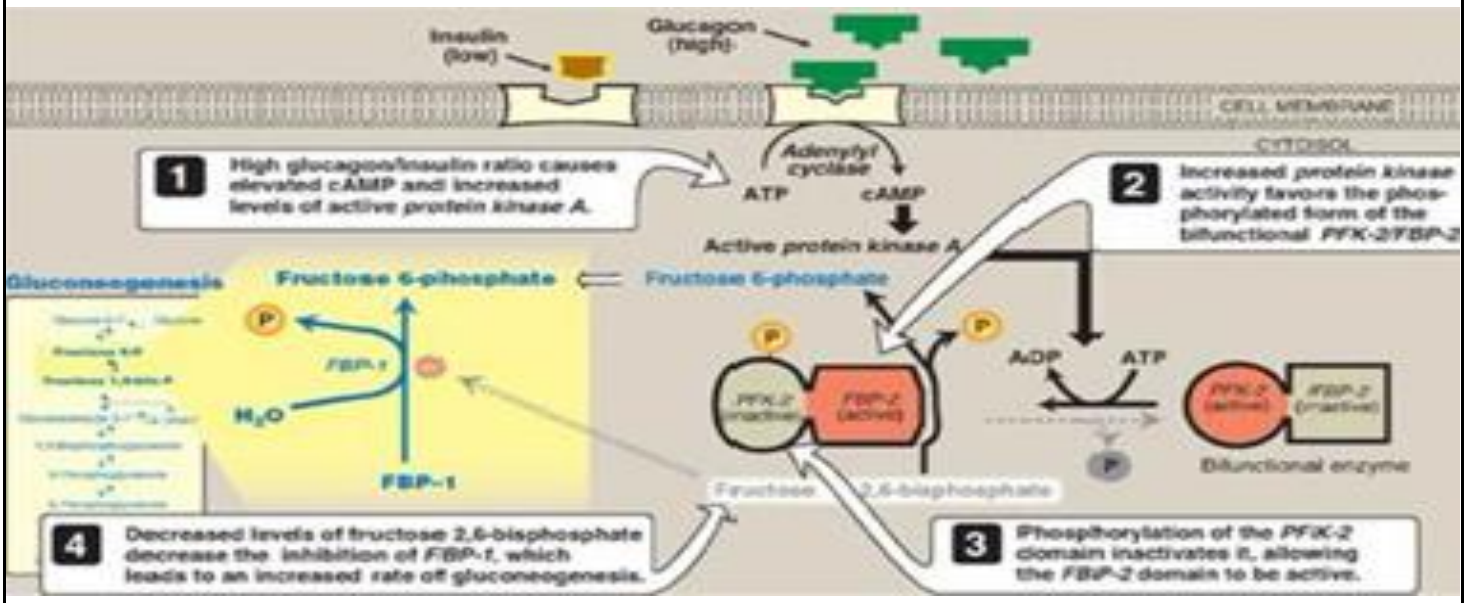
During the well-fed state: decreased levels of glucagon and elevated levels of insulin cause an increase in hepatic Fru-2,6-bp indicating high activity of PFK-2 (dephosphorylated active form) and thus Fru-2,6-bp activates PFK-1 leading to the activation of glycolysis.
*Glucagon indicates low blood sugar, Insulin causes an increase in glucose uptake.

So we can say that Fru-2,6-bp is an intracellular signal of glucose abundance leading to activation of glycolysis.

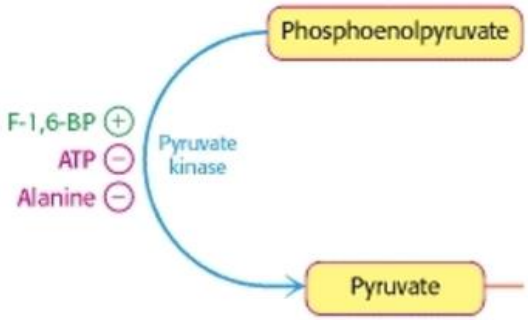
During fasting: elevated levels of glucagon and low levels of insulin lead to a decrease in hepatic Fru-2,6-bp indicating low activity of PFK-2 (phosphorylated inactive form). Low Fru-2,6-bp leads to less activation of PFK-1 causing inhibition of glycolysis but activation of gluconeogenesis (production of glucose from non-carbohydrate sources).

- Steps of PFK-2/FBP-2 regulation;

1. High insulin/glucagon ratio causes decreased cAMP and reduced levels of active protein kinase A.
2. Decreased protein kinase A activity favors dephosphorylation of bifunctional protein PFK-2/FBP-2.
3. Dephosphorylated PFK-2 domain is active, whereas FBP-2 is inactive. The dephosphorylated active PFK-2 favors formation of fructose-2,6-bisphosphate.
4. Elevated concentration of Fru-2,6-bp activates PFK-1 which leads to an increased rate of glycolysis; needed to reduce the large amount of glucose.
   (increased uptake due to high insulin concentrations)
   As you can see in the figure ..



- ✓ Now let's start talking about the third and last irreversible reaction which is the conversion of phosphoenolpyruvate into pyruvate by <u>pyruvate kinase</u>. This reaction is inhibited by ATP and Alanine (which indicates abundance of building blocks) and is activated by fructose-1,6-bisphosphate.
  1,6-bisphosphate is an early intermediate (activator of the enzyme downstream).

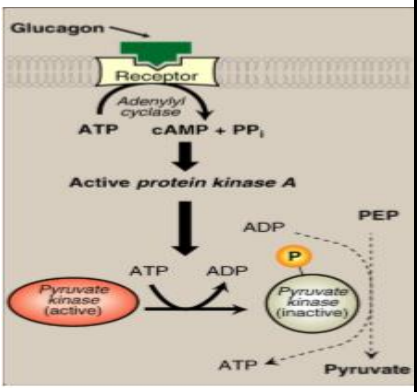


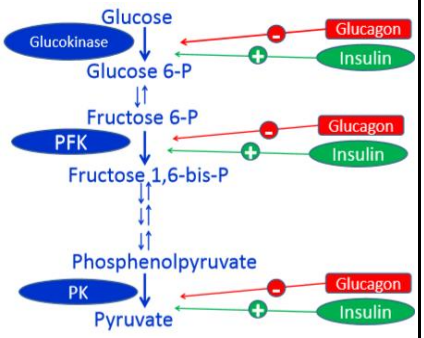
Pyruvate kinase (PK) is also regulated by phosphorylation;

high level of glucagon →stimulates adenylyl cyclase → activates protein kinase → adds a phosphate group to pyruvate kinase (phosphorylation) → inactivates the enzyme PK.

Adding a phosphate group to many enzymes such as pyruvate kinase will spare the glucose (decrease its degradation, utilization, glycolysis AND increase its availability).

- Insulin and glucagon regulate the amount of expression of enzymes (i.e. affect the amount of transcription into mRNA and its translation), thereby regulating metabolism of carbohydrates.
- Glucagon decreases the amount of enzymes (inhibition of enzymes → inhibition of glycolysis).
  Glucagon indicates low blood sugar, meaning we need to keep the glucose.
- Insulin increases the amount of enzymes (activation of enzymes → activation of glycolysis).
  As we have a huge amount of glucose uptake by insulin which needs to go through glycolysis.





Gluconeogenesis

Gluconeogenesis is the production of glucose from *non-carbohydrate* sources (glycogen →glucose is **not** gluconeogenesis).

Gluconeogenesis starts with Pyruvate, so gluconeogenesis is the conversion of **pyruvate and lactate to glucose.**

> In grape trees, pyruvate is found as *hasram* حصرم. When pyruvate turns into glucose it becomes the grape which is full of glucose. What happens in the grape trees happens in our livers.

Why do we need this conversion?

The brain is dependent on glucose and it requires about 120g/day. We eat about 200g of starch and carbs each day and the brain will take most of it.

So what about in-between meals when there is no more glucose coming from the GI tract, how can we provide glucose to the brain?

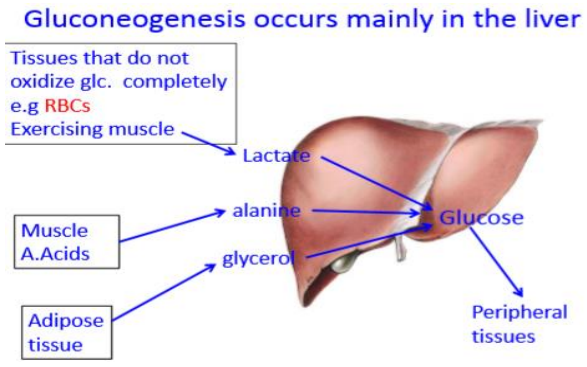
It has to be provided by degradation of glycogen. The body stores 75g of glycogen which is sufficient for less than a day. So, we must produce glucose from non-carbohydrate sources such as pyruvate, lactate and amino acids.

- Muscles contain about 400 g of glycogen for their own use. This is NOT considered a source of glucose for other tissues.

- The liver stores glycogen in lesser amounts, to be released to the blood and used by other tissues.

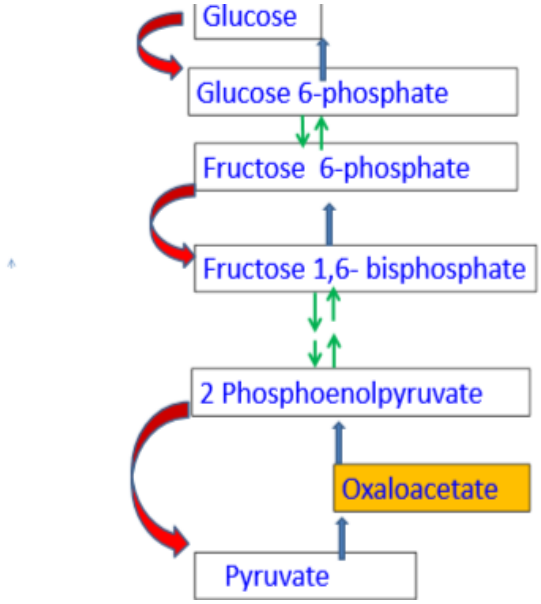
Fatty acids can't be converted to glucose, because fatty acid oxidation will produce acetyl COA which can't be converted to pyruvate (Pyruvate →acetyl COA is an irreversible step). Fatty acids can be produced from glucose.

- Gluconeogenesis occurs mainly in the liver and kidneys, as well as tissues that don't oxidize glucose such as:
  - RBCs - produce lactate.
  - Exercising muscles - produce lactate and alanine.
  - dipose tissue - by the increase in glycerol, fatty acids can't be converted to glucose, but the glycerol part of the fats can be converted to glucose.



Glycolysis occurs in 10 steps, 3 of which are irreversible. Glucose is converted to pyruvate by glycolysis, but we can't directly convert pyruvate to glucose. We need a detour, going first from pyruvate to OAA then to PEP.

This process will be discussed in details in the next sheet.



QUIZ:- (Added by Ameen Alsaras)

Q1:- A child was diagnosed after accidentally ingesting a lot of fluoridated water, which of the following enzymes might be affected in this patient?

A) Pyruvate dehydrogenase
B) Enolase
C) PFK-1
D) PFK-2

Q2) How can ATP molecules be involved in phosphorylation of fructose 6-phosphate?

A) As a substrate
B) As a product
C) As an inhibitor
D) More than one of the above

Q3)Which of the following pairs is mismatched?

A) Higher PH – Lower PFK-1 activity
B) More citrate – Lower PFK-1 activity
C) More glucose – Lower GKRB activity
D) None of the above is a mismatch

Q4) Which of the following processes is regarded as gluconeogenesis?

A) Lactate → Glucose
B) Fructose → Glucose
C) Galactose → Glucose
D) Glucose → Glycogen

Answers

1)B 2)D 3)A 4)A

Good luck