SHEET NO.

8 part 2

METABOLISM

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

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✓ In the previous sheet, glycolysis was discussed. We now know that it starts with glucose and ends with pyruvate, and if there is not enough oxygen (due to 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.

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

Synthesis of 2,3 bisphosphoglycerate

➢ 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.

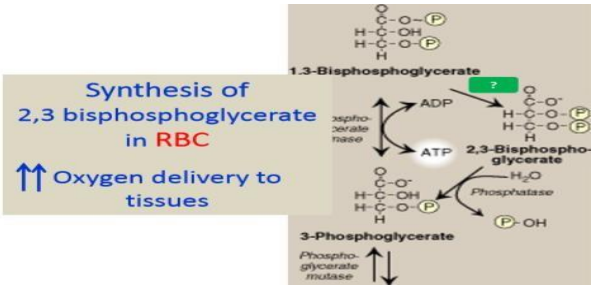
Let's look further into how this conversion happens

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

✓ If 2,3-bisphosphoglycerate is no longer needed, it is degraded by the enzyme **<u>phosphatase.</u>** Phosphatase removes the phosphate group from carbon #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.



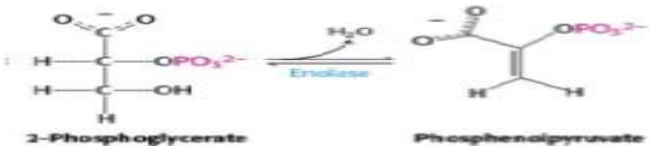
Note:- Mutase is the enzyme that transfers the phosphate group from one carbon atom to another carbon within the same molecule.

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.

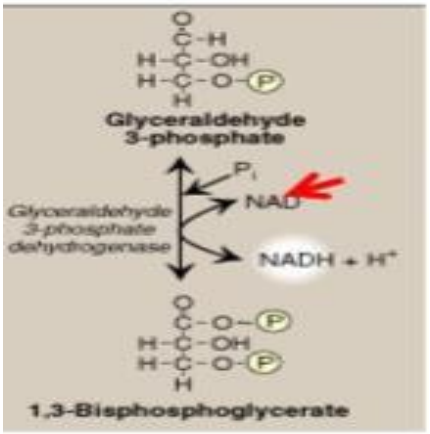
    - Fluoride can be added to toothpaste in order to prevent dental carries. Also, fluoride can be added to water making “Fluoridated water”.

    - 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 the conversion of glyceraldehyde-3-phosphate to 1,3- bisphosphoglycerate.



Glyceraldehyde-3-phosphate is converted into 1,3-bisphosphoglycerate by the 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 a 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. This causes the 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 an 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.

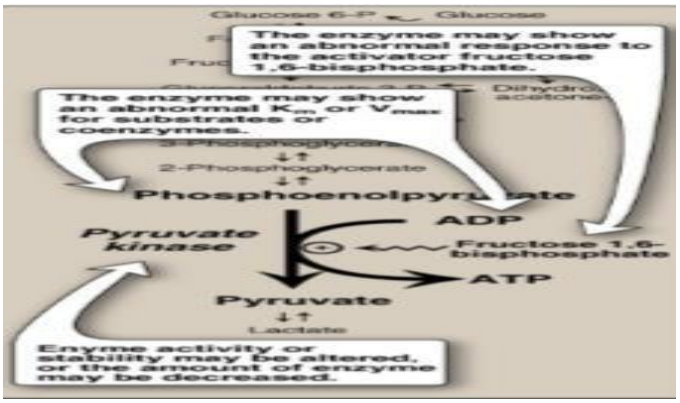
- Energy is needed by the RBCs for active transport (NA+/K+ pump) to maintain the flexible shape of the cell. RBCs need to squeeze through very narrow capillaries, and if they became rigid they would be destroyed, their shape would be disrupted and they would be removed by endothelial cells from the 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 increase in the value of Km or decrease in the value of *V*max for the substrate (phosphoenolpyruvate) or coenzyme (ADP).
  - The enzyme stability or activity may be altered, or the amount of enzyme maybe decreased; for example the enzyme wouldn't be stable in extreme pH levels.



Gluconeogenesis

Glu: glucose, neo: new, gensis: production

Gluconeogenesis is the production of glucose from *non-carbohydrate* sources meaning that glycogen to glucose is **not** gluconeogenesis . Gluconeogenesis is rather **the opposite** of glycolysis

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 takes 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 <u>non-carbohydrate</u> sources such as <u>pyruvate, lactate and amino acids</u>.

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

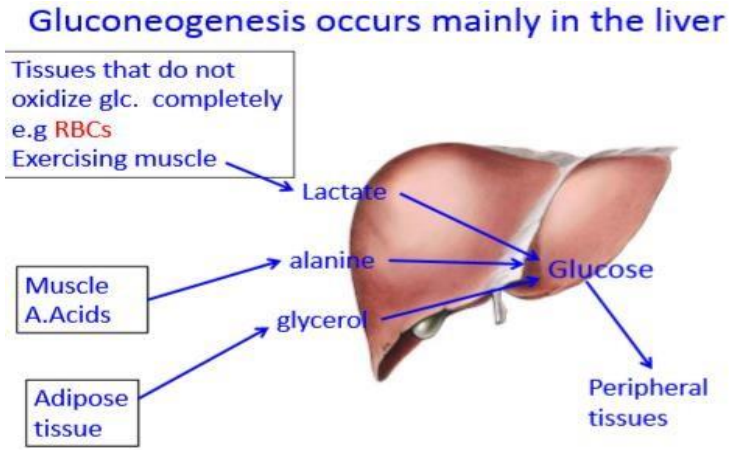
- The liver stores glycogen in lesser amounts, to be released to the blood and used by other tissues.

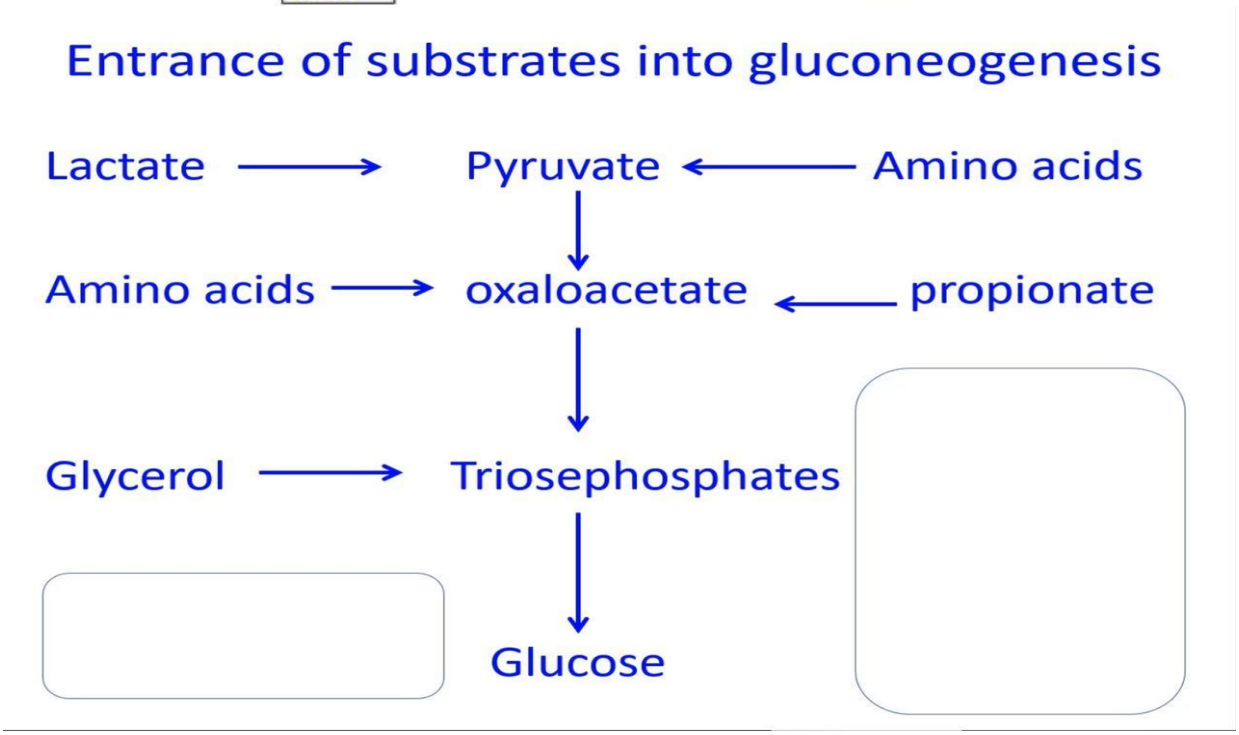
Fatty acids can't be converted to glucose, because fatty acid oxidation will produce acetyl COA which can't be converted to pyruvate (Pyruvate to acetyl COA is an irreversible step). Fatty acids can be produced from glucose.

- 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.

- Another reason for the occurrence of gluconeogenesis mostly in the liver is due to the absence of specific enzymes needed for the process outside the liver like the phosphatase enzyme , which is no where to be found in muscles .

- adipose 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.

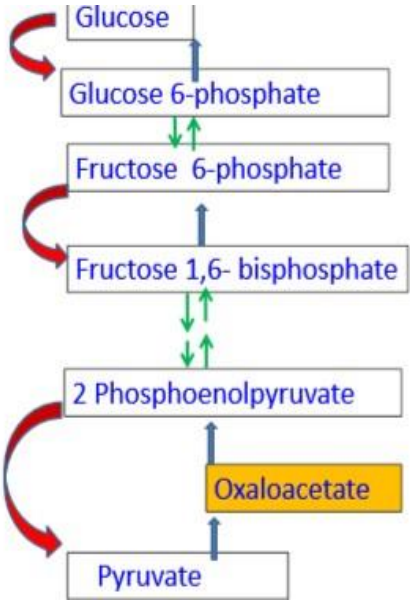




Note: converting of galactose to glucose isn't gluconeogenesis, because galactose is a carbohydrate source. (as Fructose).

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

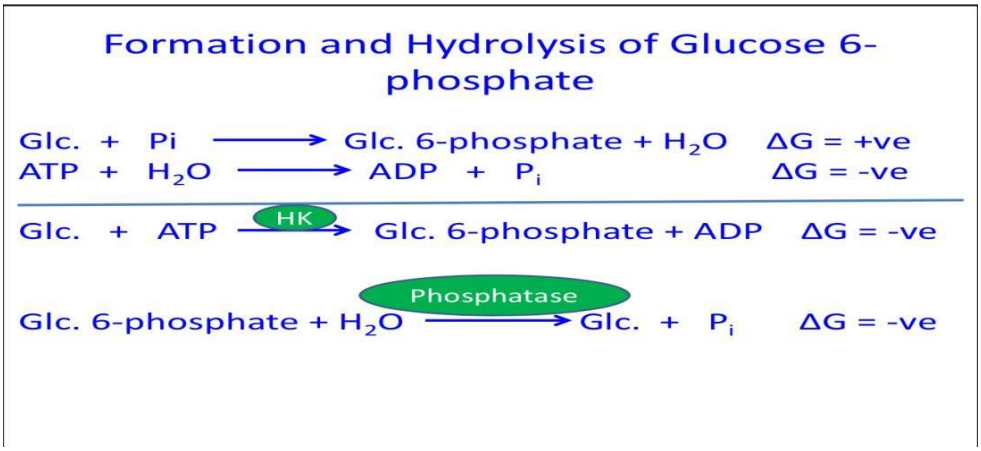

Glucose
Glucose 6-phosphate
Fructose 6-phosphate
Fructose 1,6- bisphosphate
2 Phosphoenolpyruvate
Oxaloacetate
Pyruvate

As we discussed earlier that gluconeogenesis is the process in which we convert Lactate or Pyruvate to Glucose. This is the opposite of Glycolysis. (Gluco: glucose, neo: new, genesis: production of. Altogether, it's the production of a new glucose molecule), and we said that the reactions in glycolysis can be used in gluconeogenesis (the reversible ones can be easily reversed, and the irreversible must be regulated by different mechanisms and different enzymes when we go from pyruvate to glucose).

- Remember that hexokinase produces glucose-6-phosphate from glucose by consuming ATP (it phosphorylates glucose).
- In the figure below we have the formation of glucose from glucose-6-phosphate. This reaction which appears to be the "reverse" of the reaction catalyzed by hexokinase is catalyzed by an enzyme called phosphatase. Phosphatase hydrolyzes G6P to produce glucose and phosphate.
- Keep in mind that the reaction catalyzed by this enzyme **is not** the reverse reaction of hexokinase since hexokinase consumes ATP whereas here in phosphatase it's just simple hydrolysis without any ATP being produced.

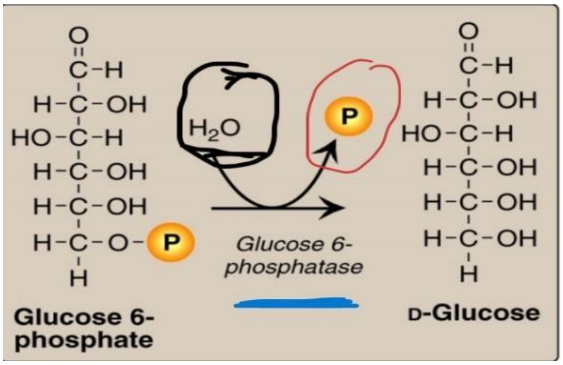
❖ The reverse of a reaction involves reversing all reactants and products (it occurs in liver only).

- In conclusion, the hydrolysis of glucose-6-phosphate is carried out by G6P - phosphatase, and since it's a hydrolysis reaction, this means its exergonic, feasible and spontaneous with negative delta G.



➲ This reaction occurs in the endoplasmic reticulum. G6P enters the lumen of the ER, it gets hydrolyzed to glucose there and then glucose gets transported to the cytosol.

Addition of water>> breaking the bond >> release of P_i + glucose. Hydrolysis is exergonic reaction.



- The next reaction is **Carboxylation of Pyruvate to produce Oxaloacetate**.

As we know, the reaction that converts phosphoenolpyruvate (PEP) to pyruvate is an irreversible reaction that's highly exergonic, which means that it can't be reversed directly.

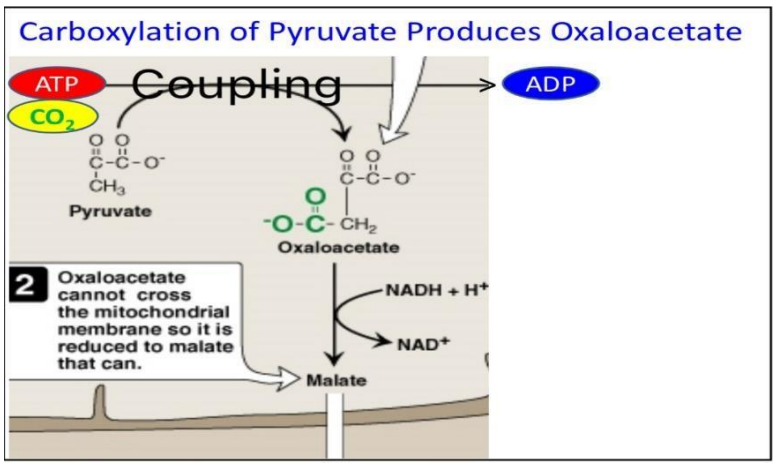
The formation of phosphoenolpyruvate from pyruvate can occur in an alternative pathway that involves two steps:

1. Carboxylation of pyruvate to produce Oxaloacetate.

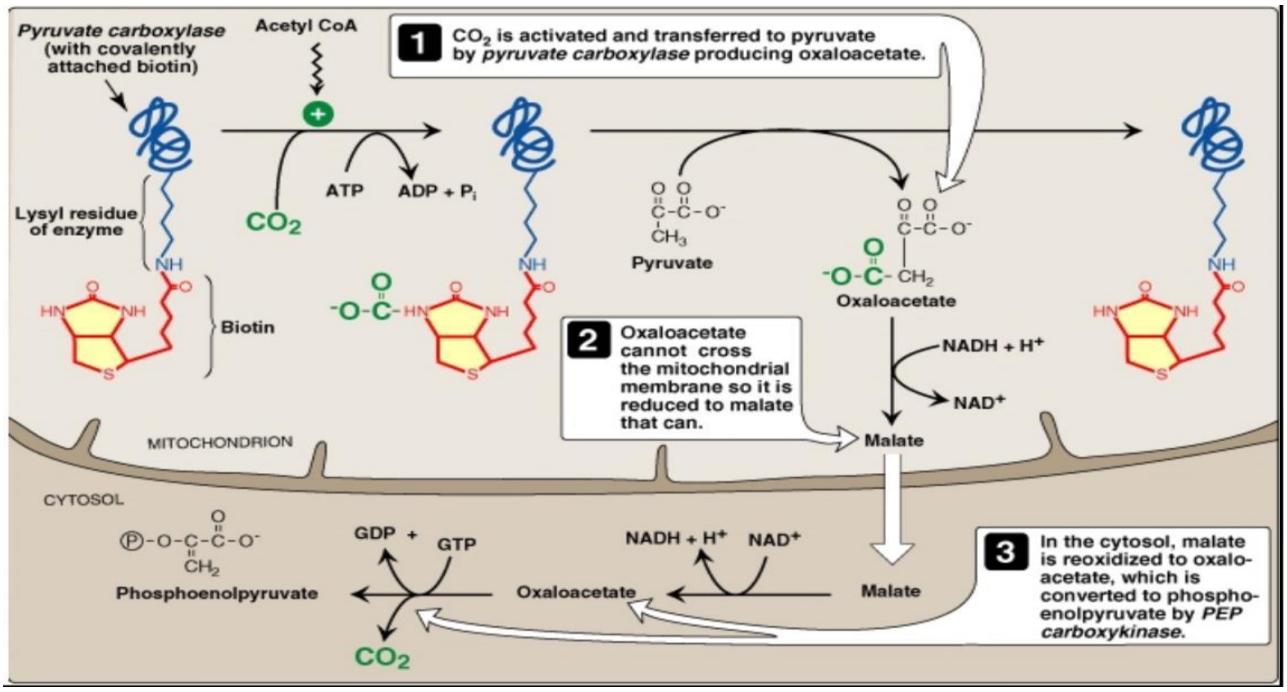
2. Conversion of oxaloacetate into PEP.

HOW?

- Pyruvate is a keto acid* composed of **3** carbons, and Oxaloacetate is a di-carboxylic acid composed of **4** carbon atoms with a ketone group attached to the alpha carbon.
- For this conversion to occur, we have to add CO_2 in a process known as carboxylation.
- We said in the recap that decarboxylation reactions are exergonic, here we have carboxylation which is the reverse so it's an endergonic rxn with positive delta G, so for the endergonic rxn to proceed it should be coupled with an exergonic rxn.
- In this case, carboxylation (endergonic) is coupled to the hydrolysis of ATP → ADP (exergonic) .



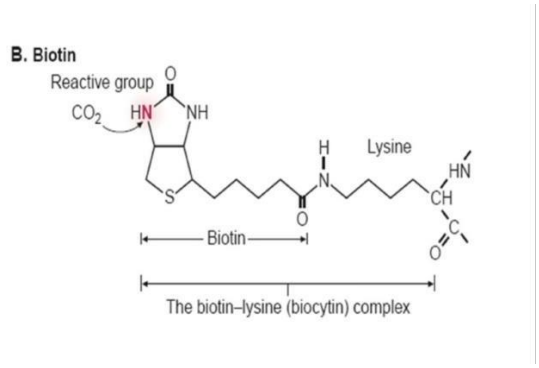
- For glucogenesis to continue, Oxaloacetate must be converted to PEP, but the reaction that produces Oxaloacetate takes place in mitochondria whereas **gluconeogenesis** occurs in cytoplasm, so oxaloacetate has to be transported to the cytosol.
- There's no transporter for oxaloacetate in the mitochondrial membrane, but there's one for malate. In other words, Malate can be transported from the mitochondria into the cytosol, while oxaloacetate cannot. That's why **oxaloacetate** must be converted to **malate.**
- Remember that the conversion of malate to oxaloacetate is the last reaction in TCA cycle in which **malate** is oxidized into **oxaloacetate,** and NAD+ is reduced into NADH. Here we want to reverse that step (we want to reduce oxaloacetate into malate and oxidize NADH into NAD+).
- Once Malate is transported to the cytosol, it gets re-oxidized to OAA, Oxaloacetate is then phosphorylated (a phosphate group is transferred from GTP to oxaloacetate) and decarboxylated to produce PEP. The conversion of oxaloacetate to PEP is catalyzed by PEP carboxykinase.
- This action is pushed in the forward direction by decarboxylation.



- Addition of CO2 to pyruvate is catalyzed by an enzyme known as pyruvate carboxylase (PC). This enzyme catalyzes carboxylation by the help of the co-enzyme BIOTIN.

Quick Recap 2

- ✓ **Biotin>> is the cofactor needed for the PC enzyme in the carboxylation process.**
- ✓ **The functional group in Biotin is: NH in the ring; the nitrogen atom covalently binds to CO2 in an energy-requiring reaction.**
- ✓ **Biotin is covalently bound to a lysine residue in enzymes.**



Now to add a carboxyl group to Pyruvate, it needs to be added in its activated form . (it can't be added directly).

The activated form is first carried by the coenzyme (biotin), the carboxyl

group is added to biotin to form **carboxylated biotin.**

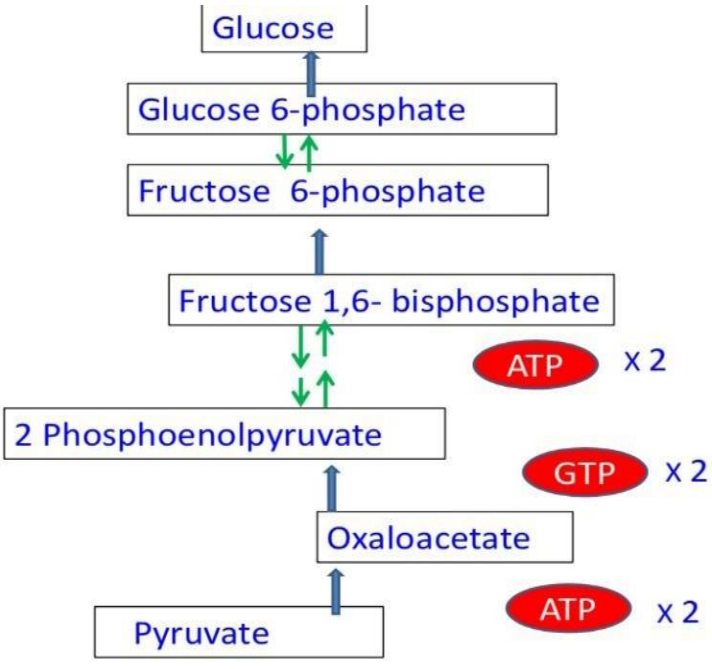
This step requires energy in the form of ATP (hydrolysis of ATP to ADP) and requires acetyl COA.

So, we can say that the PC enzyme and its cofactor have two activities, first biotin is carboxylated and then the carboxyl group carried by the carboxylated biotin is transferred to pyruvate to form oxaloacetate.

To sum it up,
ATP hydrolysis drives the formation of an enzyme – biotin – carbon dioxide intermediate (aka carboxylated biotin), which then carboxylates pyruvate to form OAA.
The PC reaction occurs in the mitochondria of the liver and kidney cells, and has two purposes:
1.To allow production of PEP, an important substrate for gluconeogenesis.
2.To provide OAA that can replenish the TCA cycle intermediates that may become depleted.

Back to the pathway of gluconeogenesis:

- ✓ pyruvate is converted to OAA then to PEP.
- ✓ 2 PEP molecules are converted to Fructose 1,6-bisphosphate in a reversible reaction consuming 2ATP.
- ✓ Fructose 1,6-bisphosphate is then hydrolyzed by phosphatase to Fructose 6-phosphate.
- ✓ Fructose 6-phosphate is converted to Glucose-6-phosphate.
- ✓ Glucose-6-phosphate is hydrolyzed by phosphatase to glucose.



<u>ATP/GTP molecules consumed in these steps:</u>

2 ATP >> in the conversion of pyruvate to OAA.

2 GTP>> (which is equivalent to 2 ATP) in the conversion of OAA into PEP .

And 2 ATP in the reversible reaction that produces **1,3 bisphosphoglycerate.**

So , the net is>> 6 ATP molecules being consumed in gluconeogenesis (pyruvate to glucose) , whereas in glycolysis (glucose to pyruvate) 2 ATP are produced , and this makes sense because the overall rxns from glucose to pyruvate is exergonic , but here to go from pyruvate into glucose ,we have to overcome the irreversible rxns by spending more energy.

Glycolysis and gluconeogenesis shouldn't occur at the same time, to prevent a futile cycle. Meaning that when a cell requires glycolysis, gluconeogenesis stops and vice versa.