فريق طوفان الأقصى

METABOLISM

Modifide N. 13

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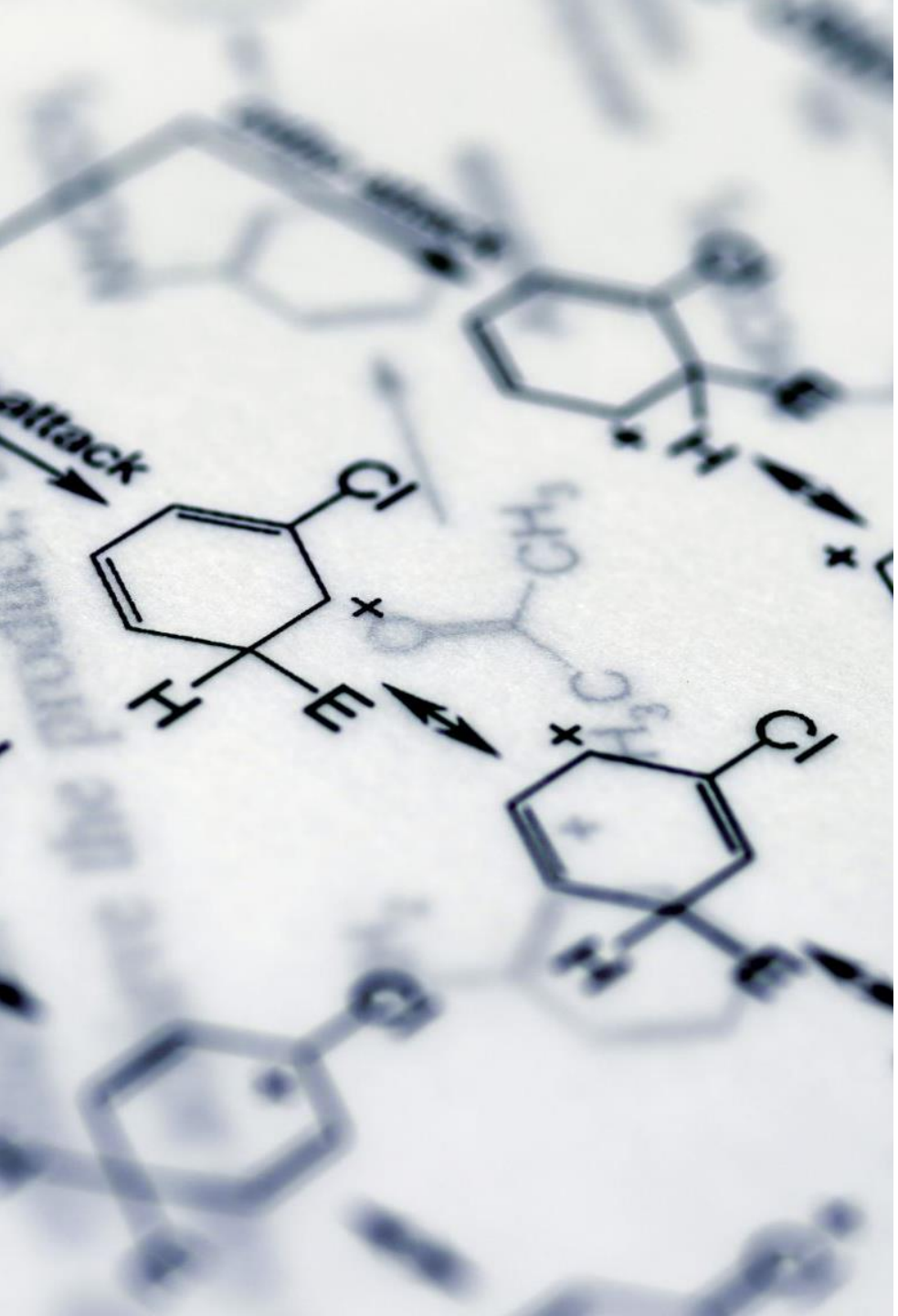
اللهم سخر لأهل غزْة جنود الأرض وملائكة السماء
واحمهم وانصرهم وثبت اقدامهم وأرح قلوبهم
والطف بحالهم إنك بعبادك خبيرٌ بصير.
اللهم سلّط غضبك وعذابك على أعداء الإسلام،
اللهم اقتلهم مددا ولا تبق منهم أحدًا وأرنا فيهم
انتقامك، إنك العزيز الجبّار



Dr. Diala Abu-Hassan

Textbook:

Lippincott's Illustrated reviews: Biochemistry

Gluconeogenesis
(Production of glucose from non- carbohydrate precursors)

NOTE:

Gluco=glucose , Neo=new , Genesis=generation , formation.

Gluconeogenesis means generation of glucose from non-carbohydrate sources

Gluconeogenesis is the reversible pathway of the glycolysis, when we talk about gluconeogenesis we are talking about fasting state for a long time which mean that we don't have a diet , glycogen is consumed , we know that brain , RBC , medulla are dependent on glucose and can't synthesis it so we need to supply them with glucose

NOTE: when you are having a diet , sugar levels will increase then the insulin increases sugar uptake into cells (this happens in 2-3 hours after eating) , sugar levels will decrease and we will have what is called (fasting blood sugar) (the normal range) .

If the sugar levels are below the normal range , your body will start getting energy from glycogen

Glycogen levels will start to disappear after (12-18)hrs

A reason that our bodies store a low amount of glycogen is the amount of energy in the fatty acids case will give us large amounts of energy so fatty acids is more suitable as a storage form of energy (1g of fatty acids will give 9kcal , 1g of glucose will give 4kcal)

Glucose Synthesis is Required for Survival

- Brain is dependent on glucose 120g/day
- Body glucose reserve is limited
  ≈ 20 g (extra cellular fluid)
  ≈ 75 g (liver glycogen); enough for 16 hours
  ≈ 400 g (muscle glycogen); for muscle use only
  Main source of energy for resting muscle in post-absorptive state
- 70 Kg man has ≈ 15 Kg fat
  - Fatty acids can not be converted to glucose
  - Utilization of FA is increased 4-5 X in prolonged fasting
  - In prolonged fasting;FA ketone bodies at high rate

NOTE: fatty acids are used to provide energy to cells that don't depend on glucose as a source of energy

why the glycogen source comes before the gluconeogenesis?
Because its a quick source , and the gluconeogenesis is the opposite of glycolysis so I'm going to reverse 10 steps (takes a longer time)

NOTE: glycogen is stored in all cells but mainly in muscles and liver (muscles glycogen > liver's one)

- **NOTE**: muscles can’t release the glucose in the blood stream(بتحتفظ فيه لصالحها) , while liver doesn’t use glucose as a source of energy , just breaking down and releasing it

- **NOTE:** the amount of sugars that we have in our cells is nearly 500g .

A guy with 70kg mass will be having 15kg of fatty acids (15000g) , mass of fatty acids times 9 (kcals that 1g of fatty acid gives) .

Nearly 135000 kcal a 15kg of fatty acids will give , if we divide it by 4 to see how much KGs of carbohydrates we need to provide our bodies with the same amount of energy , we will have nearly 35kg of carbohydrates .

So as we said , its more efficient to store lipids .

Another reason that we store small of amounts of glycogen is for the hydrophilic nature of the glycogen (then the water will go in the direction of the hydrophilic glycogen then the size of our body will be very large with excessive of fluids .

Gluconeogenesis occurs mainly in the liver

Tissues that do not oxidize glc. completely
e.g RBCs
Exercising muscle

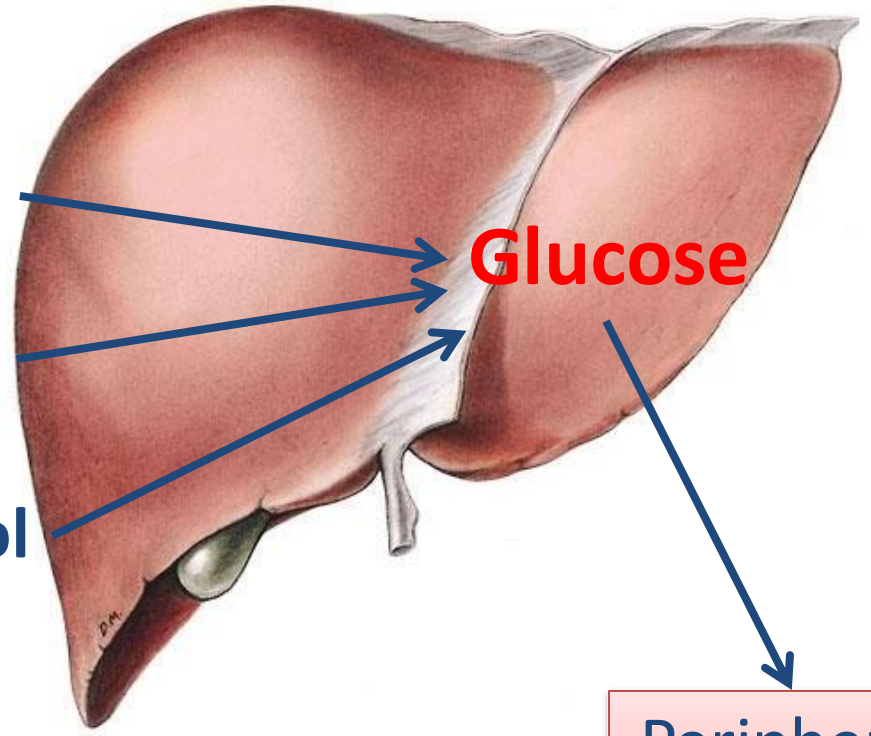
Lactate

Muscle
Glucogenic A.As

Alanine

Glycerol

Adipose tissue



Glucose

Peripheral tissues

Where and when does gluconeogenesis occur?

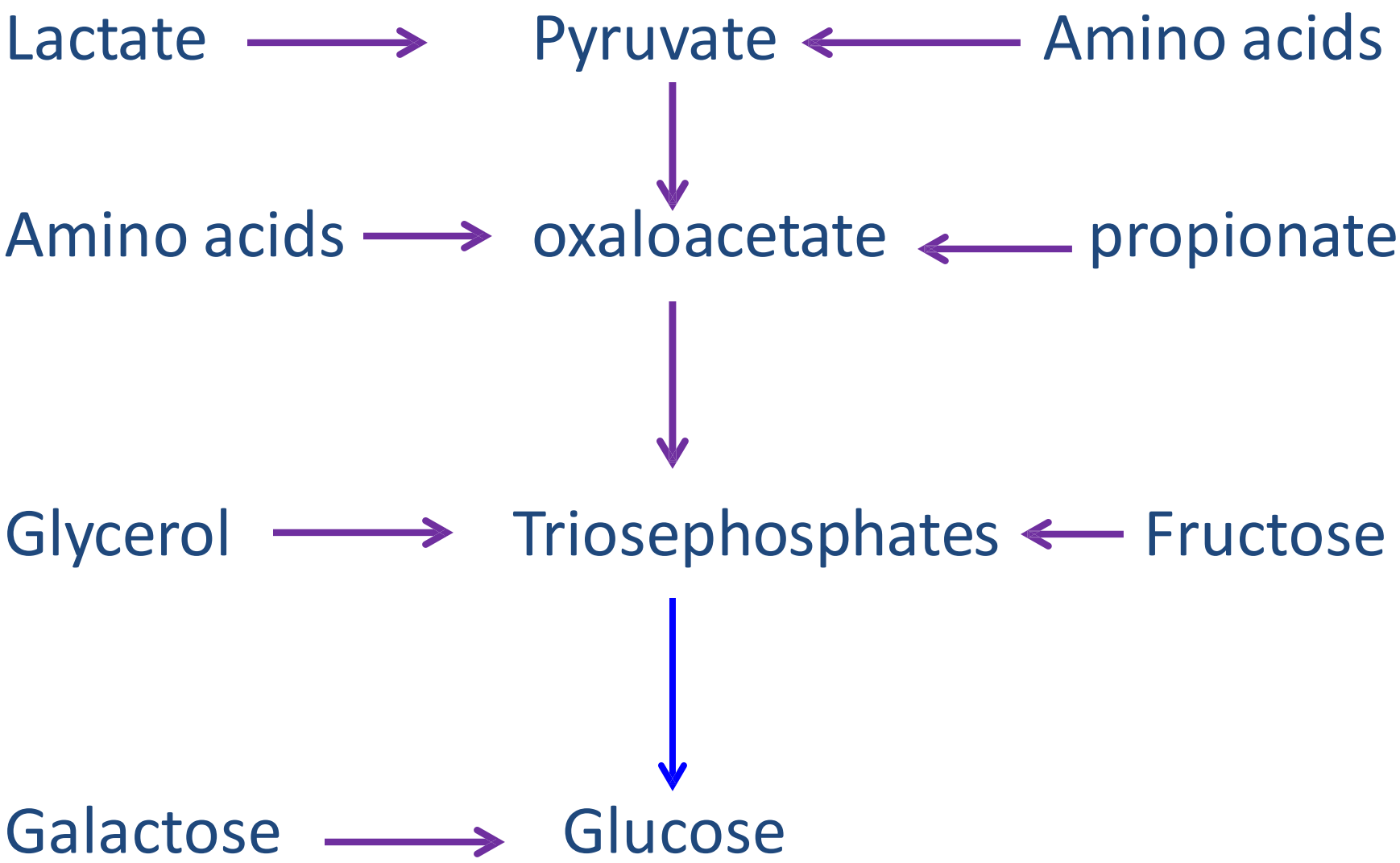
During an overnight fast, ~ 90% of gluconeogenesis occurs in the liver and 10% by the kidneys

During prolonged fasting kidneys become major glucose-producing organs (40% of total glucose production)

- **The complement in this slide:** Glycolysis occurs in all cells (in the cytosol) , while gluconeogenesis occurs in specific sites , when the fasting state starts (the time we start depending on gluconeogenesis) , majority of the glucose is made in liver (90%) , and 10% is made in kidneys . **Note that it doesn't occur in the muscle.**

If we have a prolonged fasting state , kidneys synthesis if nearly 40% instead of 10% and the liver will synthesize 60% instead of 90% .

Entrance of substrates into gluconeogenesis



The complement in this slide: We have more than one precursor to start this process . The storage form of the fats in the adipose tissue must be triacylglycerol(TAG) inside adipocyte fat droplets , triacylglycerol consist of glycerol and fatty acids . TAG will release fatty acids for oxidation (energy production) , glycerol is used as a precursor for gluconeogenesis .

I can also break down muscle's protein to have an amino acids (not all of them) , they can be used to produce glucose and we call them (glucogenic amino acids) e.g. alanine (isn't the only AA but never forget that it is a source of pyruvate) by a transamination reaction .

- **The complement in this slide:** The 3rd precursor which is lactate (anaerobic respiration) , and can be converted into pyruvate by LACTATE DEHYDROGENASE even in RBCs or muscles .

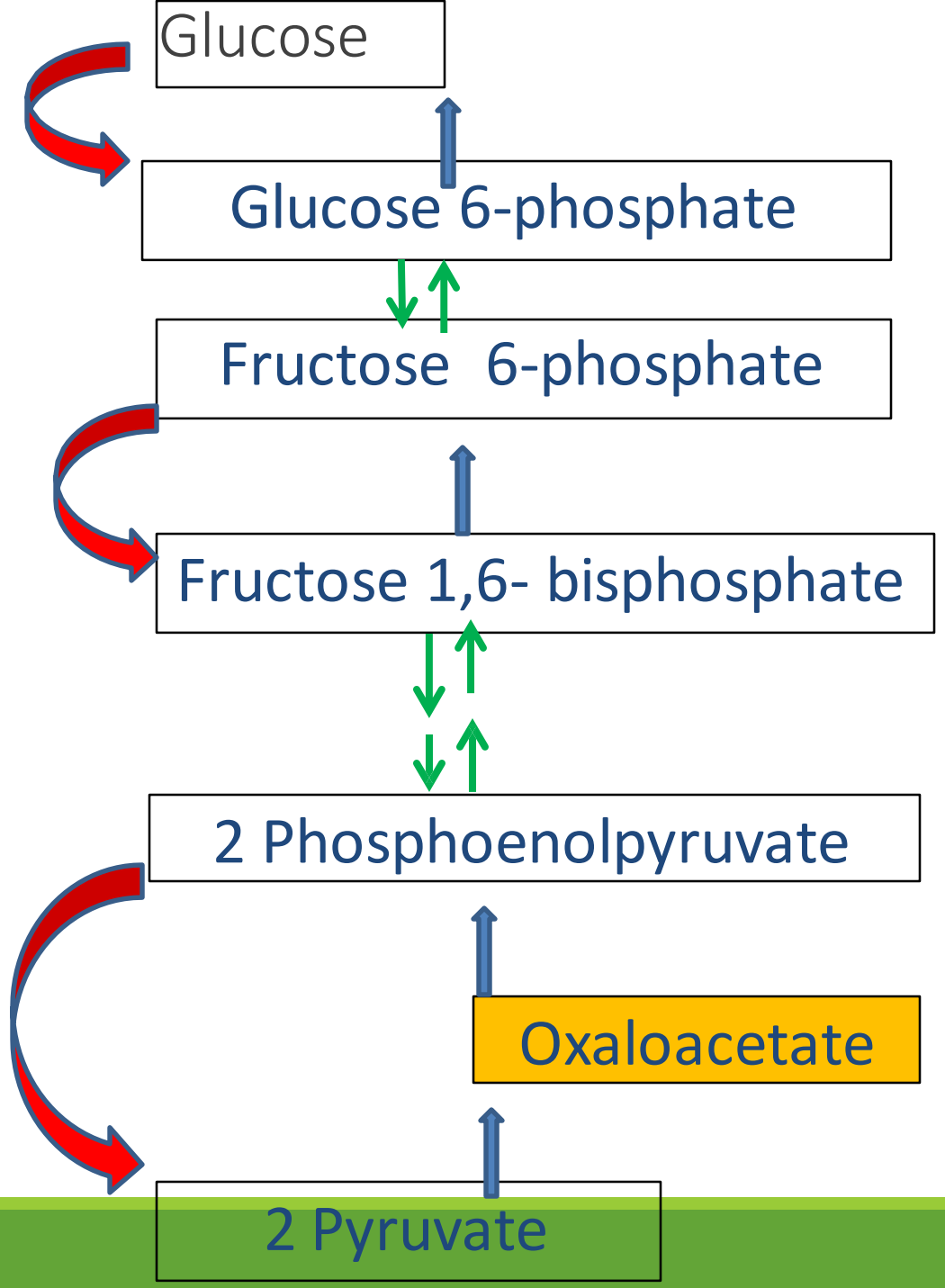
Different precursors can enter the glucogenesis pathway in a different entry points (depends on precursor) , alanine into pyruvate , other AAs can be metabolized into oxaloacetate which is a precursor of gluconeogenesis (this step occurs in gluconeogenesis but not in glycolysis) , in glycolysis it's a 2 steps reaction .

Glucogenic amino acids : amino acids which can be metabolised into Krebs cycle intermediates or pyruvate and used these can be used in gluconeogenesis (enter as OAA).

glycerol (3C alcohol) then usually we will go in trioses pathway (enters as dihydroxy acetone phosphate which is an isomer), doesn't continue through glycolysis but it acts as entry point for glycerol into gluconeogenesis .

Fructose and galactose(cant be converted into glucose) , fructose enters as triose phosphate while galactose as a hexose but this isn't considered as gluconeogenesis because these are carbohydrate sources .Fructose can enter the pathway as triose and we will discuss this in the next lectures.

Gluconeogenesis is the opposite of glycolysis BUT



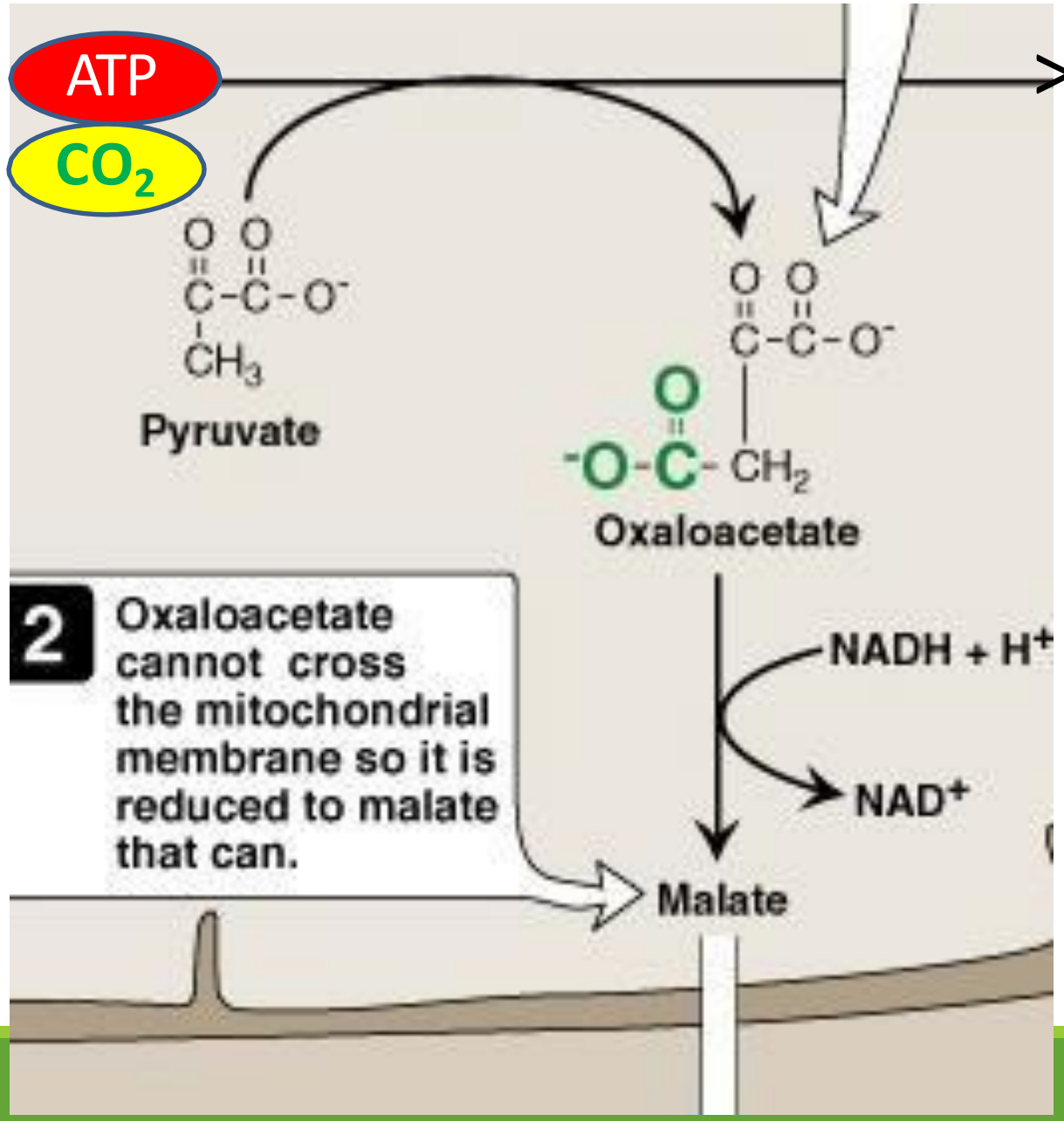
But we have 3 irreversible reactions , irreversible reactions in biochemistry means that reaction can't occur in the opposite way by the same enzyme then we need to use different enzymes , we won't discuss the 7 reversible steps , because every revesible step is reversed by the same enzyme .

Glycolysis : gluconeogenesis → 10:11 steps

Reversing the irreversible steps

1. From pyruvate to phosphoenolpyruvate (PEP)

Carboxylation of Pyruvate Produces Oxaloacetate (OAA)



- By pyruvate carboxylase
- In mitochondria
- Allosterically activated by Acetyl Co A

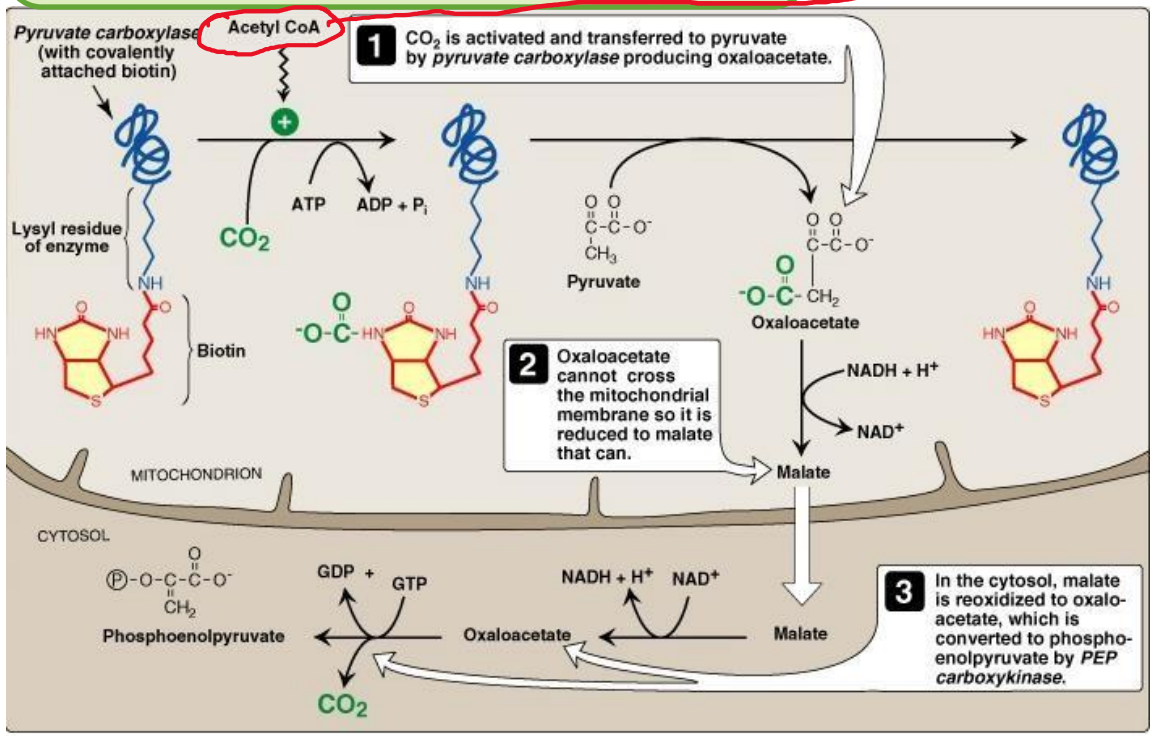
- **The complement in this slide:** Pyruvate not only exist in the cytosol where glycolysis occur, it also exist in the mitochondria (because it is not the same pyruvate that come from the glycolysis, it can be produced from lactate for example!), so it'll proceed some steps in the mitochondria & then exit to continue in the cytosol (because the enzymes of the reversible steps are there.)

- **The complement in this slide:** the first step is converting pyruvate to oxaloacetate but pyruvate contain 3C and oxaloacetate contains 4C so it's a carboxylation reaction by pyruvate carboxylase using CO_2 for sure (**carboxylases need ATP & Biotin Coenzyme**), so we'll have oxaloacetate then we need to convert oxaloacetate to PEP and this reaction occur in the cytosol, so we need to transport oxaloacetate outside but there isn't transporter for it so we reduce OAA to malate using NADH, because malate can cross the membrane.

- **NOTE:** we use Krebs cycle enzymes to reverse this reaction, in Krebs cycle we oxidized malate to OAA but now we reverse this step (reduction using NADH)

NOTE: Note that carboxylase is bound to biotin & the biotin holds the CO2 group ..

Positive regulator



From OAA to PE

NOTE: pyruvate carboxylase

- Enzyme is found in both cytosol and mito
- The generated PEP in the mitochondria is transported to the cytosol by a specific transporter
- The PEP that is generated in the cytosol requires the transport of
  OAA from the mitochondria to the cytosol

NOTE: Acetyl CoA concentration under this fasting conditions is high (come from oxidation of fatty acids)

- **The complement in this slide:** After reducing oxaloacetate to malate , malate cross the mitochondrial membrane, now we don't ever need malate so we use malate dehydrogenase and end up with oxaloacetate (by reduce NAD+), after that we want to reach the form of PEP (3C and 1 phosphate) we need to decarboxylase +phosphorylase the oxalacetate , we use GTP to add the phosphate group (the enzyme catalyzes this reaction called PEP-carboxykinase), and finally we have PEP, and the reversible steps will occur until we reach the 2nd irreversible step

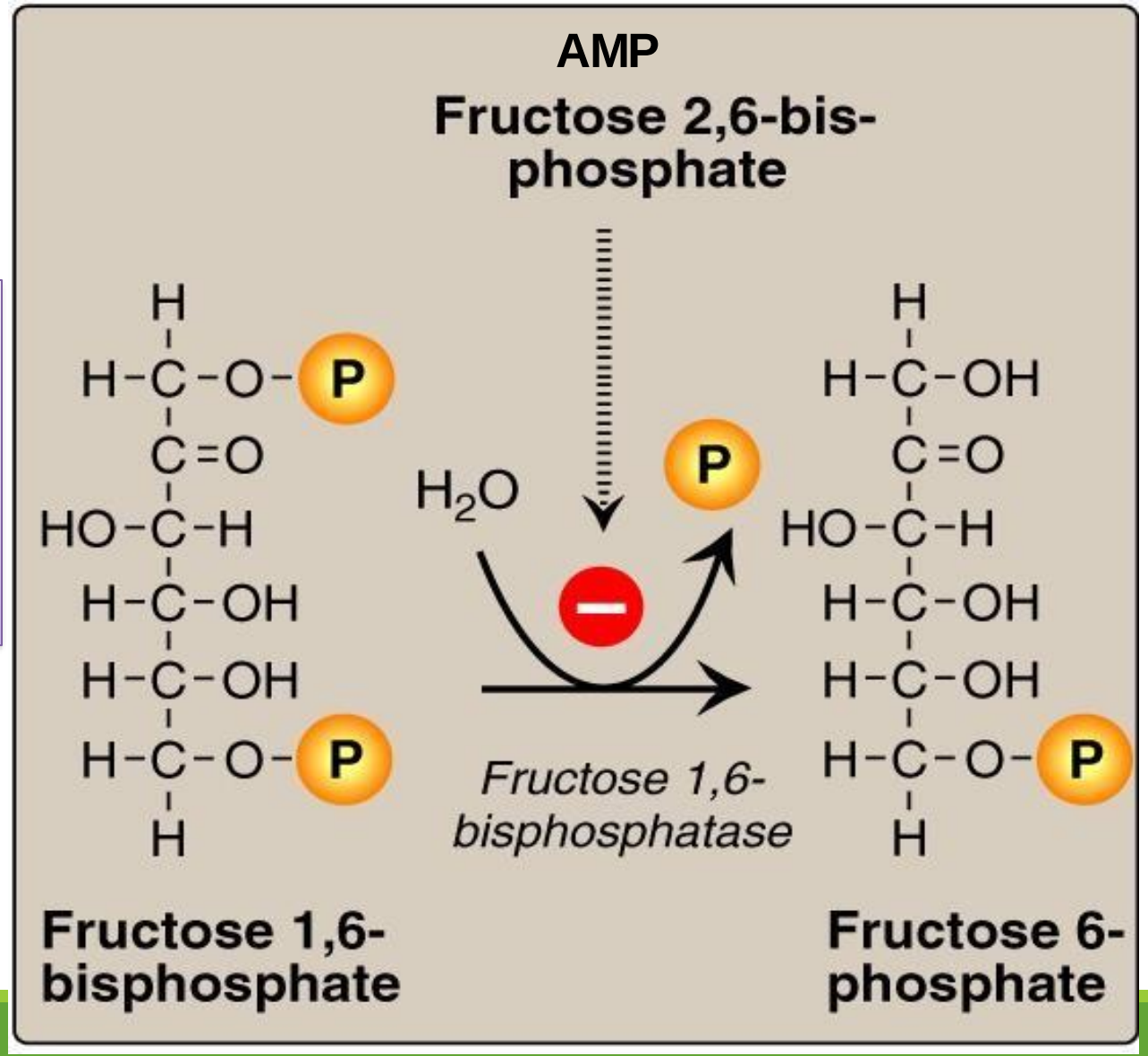
- **NOTE: If you are asking why we need to enter the mitochondria to convert pyruvate into oxaloacetate, it's related to the enzymes we need to use in each step**

Reversing the irreversible steps

2. From fructose-1,6-bisphosphate to fructose-6-phosphate

Dephosphorylation of fructose 1,6-bisphosphate

This reaction bypasses the irreversible phosphofructokinase -1 reaction



- The complement in this slide: THIS REACTION catalyzed by fructose 1,6-bisphosphatase, this enzyme is the opposite of PFK-1 enzyme that catalyzes the glycolytic step
- Also this enzyme is negatively regulated by AMP & Fructose 2,6-bisphosphate (Notice that they are the positive regulators of PFK-1 as mentioned in the previous lecture) so they turn on the glycolysis and turn off the gluconeogenesis

- NOTE: Under gluconeogenic (fasting) condition, the concentration of AMP & Fructose 2,6-bisphosphate is low, so gluconeogenesis occurs more often

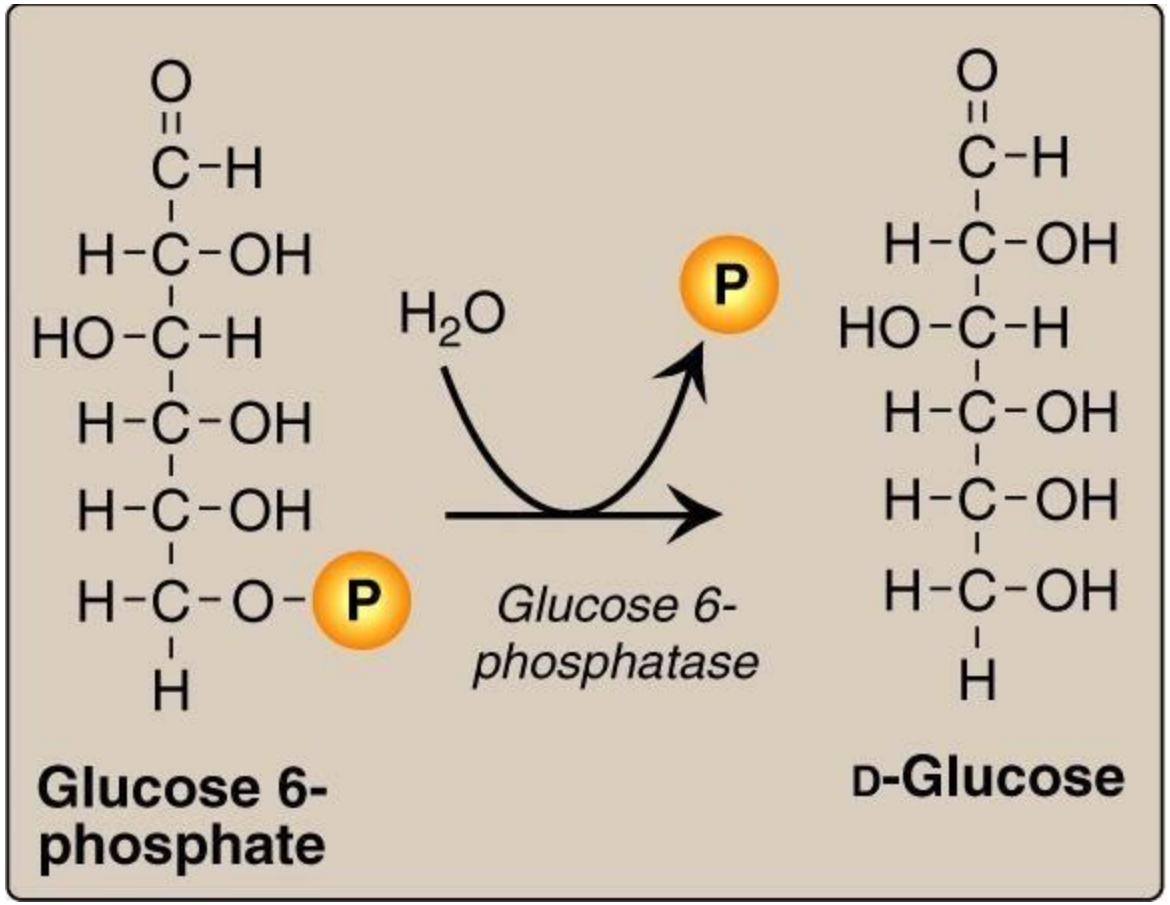
- NOTE: What makes the Fructose 2,6-bisphosphate low is the glucagon effect on the bifunctional enzyme, will be phosphorylated and that activates the phosphatase that degrades Fructose 2,6-bisphosphate to Fructose 6-phosphate

- NOTE: Why AMP is low in fasting?
- هسا الفكرة انه المشكلة مش مشكلة طاقة، لانه في كثير مصادر بأمن فيهم طاقة، المشكلة انه بدنا نأمن الدم والخلايا الي بتعتمد على الغلوكوز كمصدر وحيد فقط.

Additional information: Fructose 6-phosphate is isomerized to glucose-6-phosphate and then it will be dephosphorylated :-

Reversing the irreversible steps

3. From glucose-6-phosphate to glucose



Dephosphorylation of glucose 6-phosphate

- Bypasses the irreversible hexokinase reaction
- Only in liver and kidney
- Glucose 6-phosphate translocase is needed to transport G-6-P across the ER membrane

Glucose 6-phosphatase in Endoplasmic Reticulum (ER)

Hint: Muscle lacks glucose 6-phosphatase, and therefore muscle glycogen can not be used to maintain blood glucose levels.

- **The complement in this slide:** This Step will be catalyzed by glucose-6-phosphatase THAT IS PRESENT IN THE ER, so Glu-6-P should enter the ER to be dephosphorylated, it can't enter using GLUTs because it is phosphorylated, it is transported by Glucose-6-phosphate translocase.
- There, in the ER, Glu-6-P will be dephosphorylated by the ER enzyme to Glucose, it'll leave the ER by GLUT 7 (notice that it is dephosphorylated), so GLUT7 serves in gluconeogenesis.

- **The complement in this slide: Glucose is now in the cytosol, it can be transported to liver and kidneys by GLUT2 and then to the blood stream.**

Formation vs. Hydrolysis of Glucose 6- phosphate

- Formation

Glc. + Pi → Glc. 6-phosphate + H_2O ΔG = +ve

ATP + H_2O → ADP + P_i ΔG = -ve

Hexokinase

Glc. + ATP → Glc. 6-phosphate + ADP ΔG = -ve

- Hydrolysis

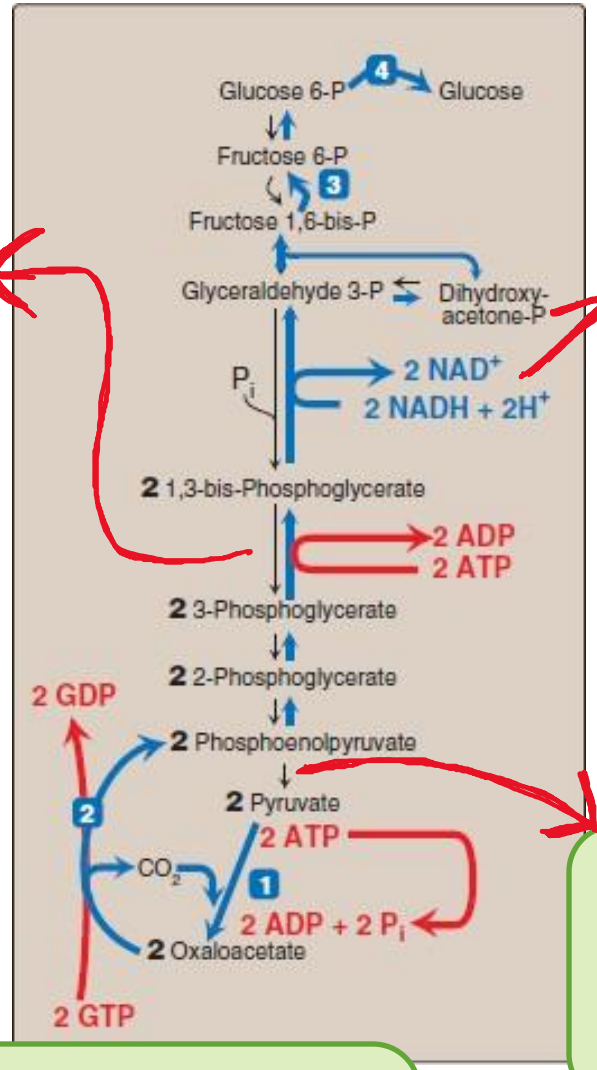
Phosphatase

Glc. 6-phosphate + H_2O → Glc. + P_i ΔG = -ve

- **The complement in this slide:** We will make comparsion between reaction occurs in glycolysis vs gluconeogenesis (1st step, last step, respectively)
- If I phosphorylated glucose using inorganic Pi, delta G will be +ve, so I need to use ATP instead (couple it with ATP hydrolysis that is –ve delta G)
- In gluconeogenesis, when I dephosphorylated Glu-6-P it didn't produce ATP, it released inorganic Pi
- The step that consumed ATP in glycolysis DID NOT produced ATP in gluconeogenesis

Energy requirements of gluconeogenesis

- Note: Here we need 2ATP in addition to inorganic Pi
- Delta G is +ve
- Why 2 ATP? The reaction is repeated (I started with 2 pyruvate and will end in Glucose…)



- Note: NADH produced in glycolysis
- NADH consumed in gluconeogenesis

- Note: Pyruvate→OAA need ATP
- OAA→PEP need GTP
- Don't forget this steps occur twice.

- Note: Here in glycolysis I've earned 2 ATP but in gluconeogenesis it's totally different reverse steps.

- Note: Overall we consumed 4 ATP & 2 GTP

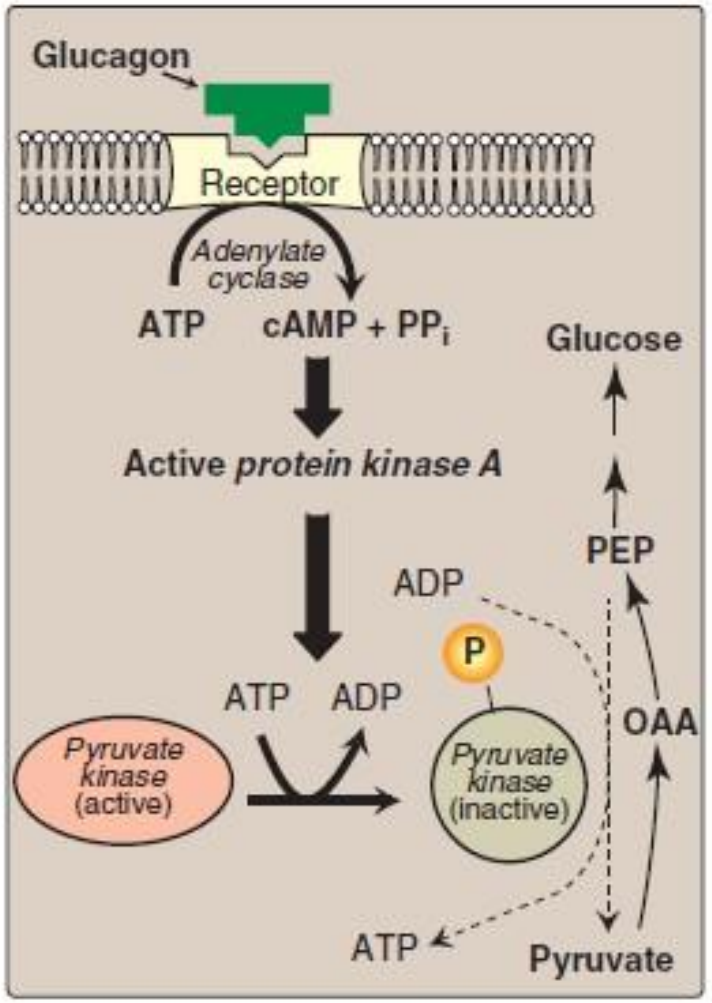
Regulation of gluconeogenesis

- Mainly by:

1. The circulating level of glucagon

- Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bisphosphatase and inhibition of PFK-1
- Inhibition of pyruvate kinase
- Glucagon increases the transcription of the gene for PEP-carboxykinase

2. The availability of gluconeogenic substrates



3.Slow adaptive changes in enzyme activity due to an alteration in the rate of enzyme synthesis or degradation, or both

- **The complement in this slide:** Glucagon is high in fasting condition and turn on gluconeogenesis, activates adenylyl cyclase A & cAMP production and then PK-A that have 2 targets
- 1-Bifunctional enzyme (will be phosphorylated & that will activate phosphatase part of it, lowering Fructose-2,6-bisphosphate, lowering glycolysis and elevating gluconeogenesis).
- 2-Pyruvate kinase: phosphorylation of it —> inactivating it, inhibiting glycolysis and activating gluconeogenesis

- **The complement in this slide: There's regulation by effecting gene expression of PEP-carboxykinase elevating it.**
- **The avilability of gluconeogenesis substrates & their types also play a role in regulation**
- **And the concentration of enzymes for sure.**

V2

Page.9

Page.27

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