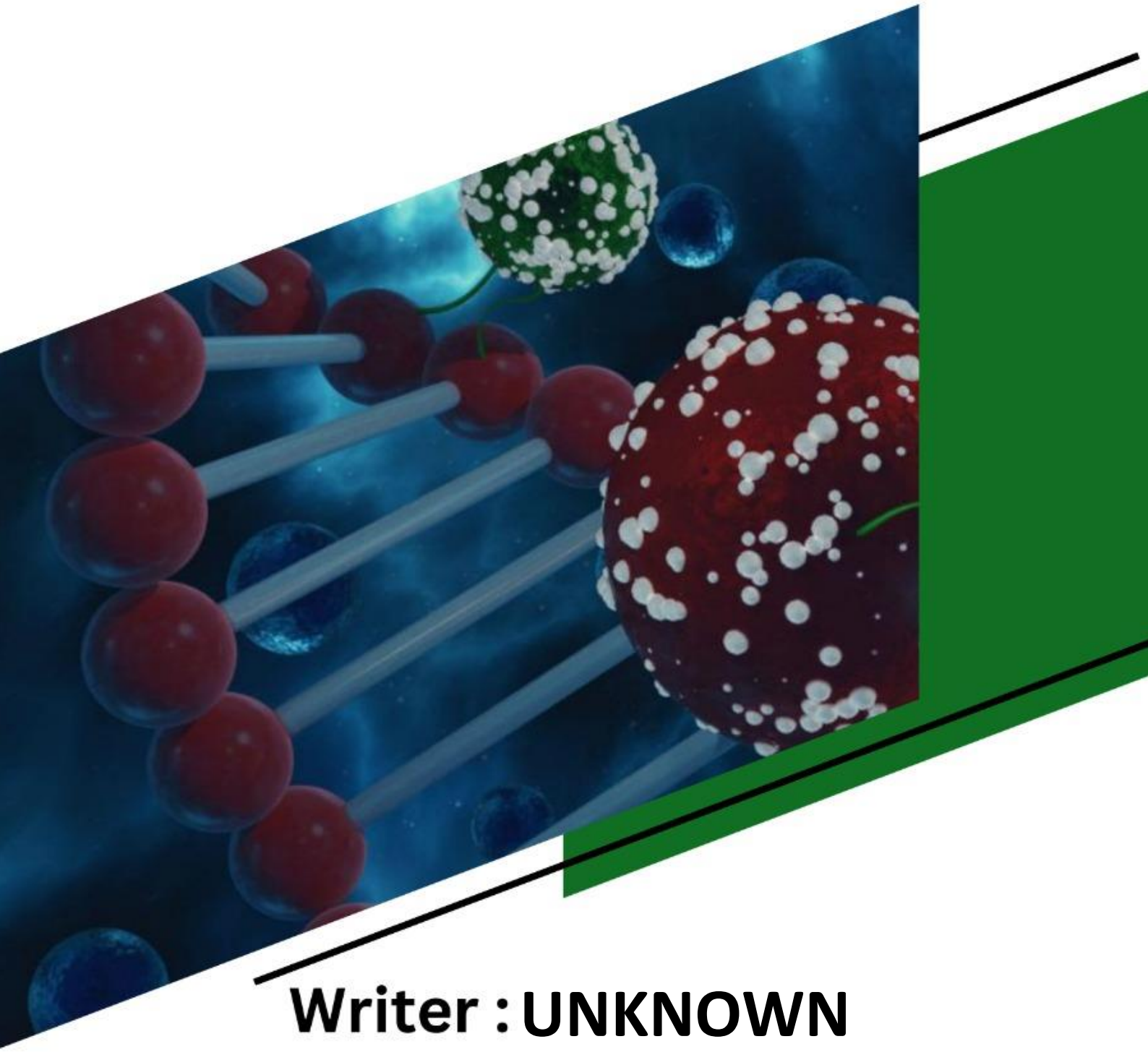
Doctor 021

METABOLISM

Sheet no. 14



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HORMONAL REGULATION OF GLYCOGEN METABOLISM

Today we are going to talk about regulation of glycogen metabolism, Last time we talked about degradation and synthesis of glycogen but today we are going to show how the body coordinate between these opposite pathways and make sure that the two pathways can't occur at the same time

When we say glycogen synthesis we expect to see active hormones such as insulin , in degradation we expect to see glucagon and epinephrine (as a hormone) , we have epinephrine and norepinephrine and both of them are secreted from the adrenal gland , remember that norepinephrine is neurotransmitter

We are talking about hormones specifically epinephrine , epinephrine is secreted in the fight or flight which means that we need energy (we need energy so we need to degrade glycogen rather than synthesis) , and absolutely you won't eat in this position in addition we know that degradation of stored glucose will be faster than synthesis of it

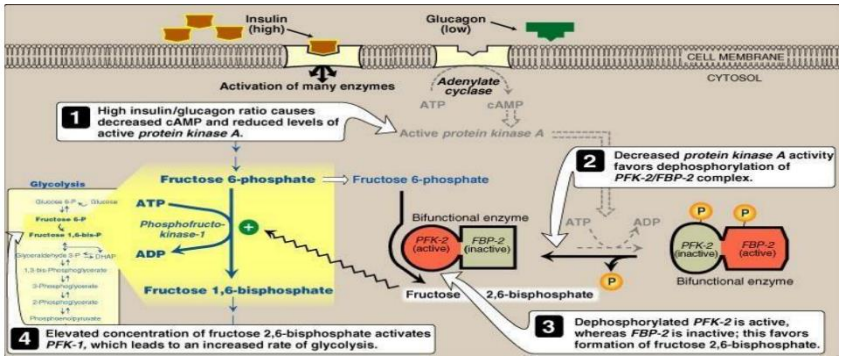
Now epinephrine and glucagon will work at the same time or one of them will , absolutely glucagon will exist in the fast conditions , but epinephrine will exist in the fight or flight conditions sometimes hunger will be an emergent condition of the body and we need to warn the sympathetic system

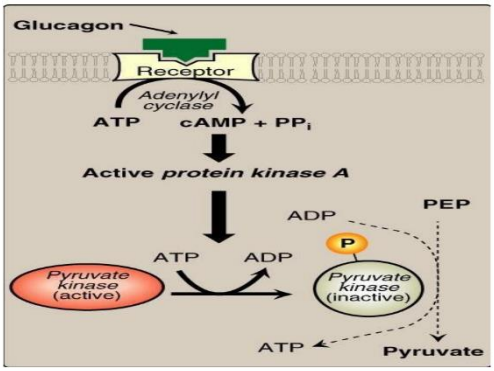
So we have epinephrine and glucagon and both can bind to same surface of the GPCR (g protein coupled receptors), but each one of them has specific receptor to distinguish between them

When they bind of the GPCR then the receptor get activated , g-proteins get activated by exchanging GDP by GTP at the alpha subunit , then the alpha subunit become active and dissociate out of the complex(separate form beta & gamma subunit) , beta & gamma subunit stay attached to membrane and alpha subunit go on a several pathways

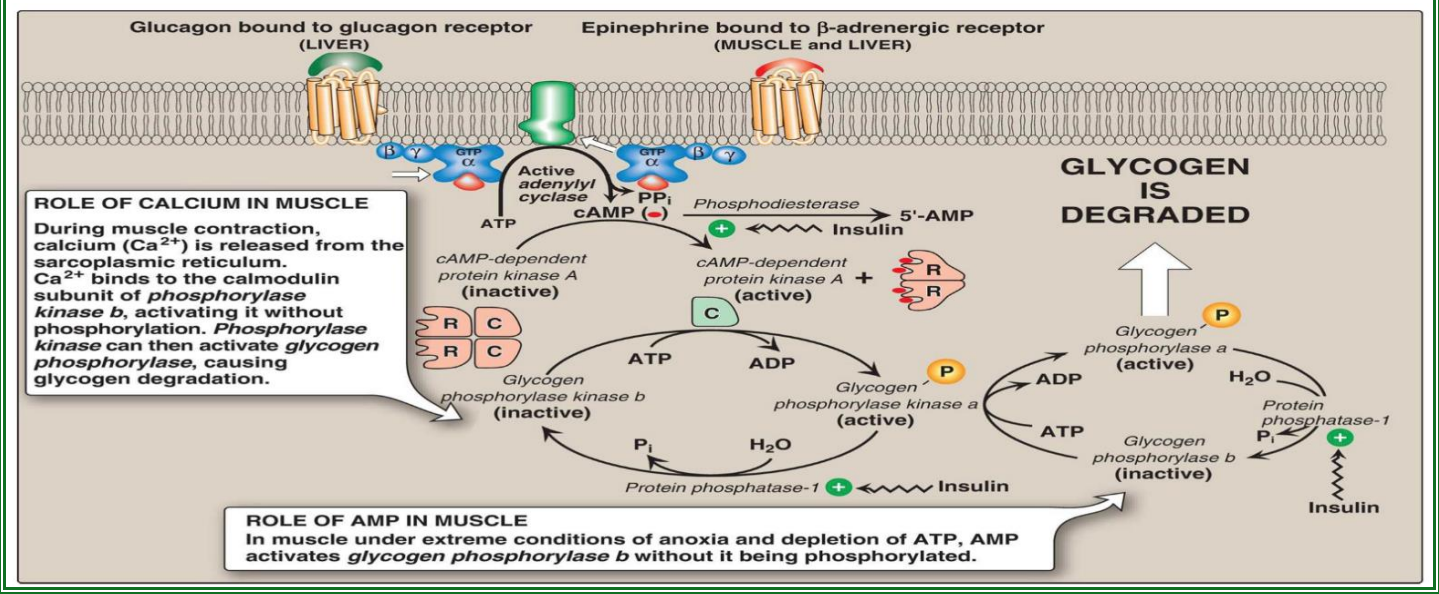
One of these pathways is the activation of adenylyl cyclase to produce cAMP , cAMP is a second messenger because (it's the first molecule that separate from the membrane) , then cAMP attach to the regulatory subunits of the protein kinase A (his name protein kinase **A** :- because its related to cAMP)and releasing the catalytic subunits and making them active , catalytic subunits are kinases so when the get activated they will do phosphorylation and they have so many targets like :- the bifunctional enzyme and pyruvate kinase .

In the fasting condition, pyruvate kinase is inactive so it will stop glycolysis in the tissues which can alternate their source of energy (for example : brain , RBC , medulla can't change their source of energy) , and the kinase domain of the bifunctional enzyme is inactive so it will stop the production of fructose 2,6 bisphosphate to inhibit the glycolysis. (the pics below from lec.11)





The active catalytic sites of the protein kinase A target the (**glycogen phosphorylase kinase**) and phosphorylate it , (**glycogen phosphorylase kinase**) (regulatory enzyme for glycogen phosphorylase) the enzyme which phosphorylate the (**glycogen phosphorylase**) and when the(**glycogen phosphorylase**) get phosphorylated then (**glycogen phosphorylase**) becomes active and start breaking down the glycogen (in this process the phosphorylation process is an activation process , pyruvate kinase phosphorylation process is inhibitory)



REGULATION OF GLYCOGEN SYNTHESIS

When we don't have glucagon & epinephrine all of the previous processes will stop , but remember that we have insulin which bind on the receptor type C kinase , and receptor kinase C phosphorylate a group of proteins they end up with the activation of the enzyme phosphodiesterase which degrades cAMP to AMP , so the second messenger which activates the protein kinase A will be inhibited so there is no activation of glycogen degradation

Insulin affect these proteins or enzymes **indirectly**:

1-phosphodiesterase

2-protein phosphatase-1 (inhibitory protein)

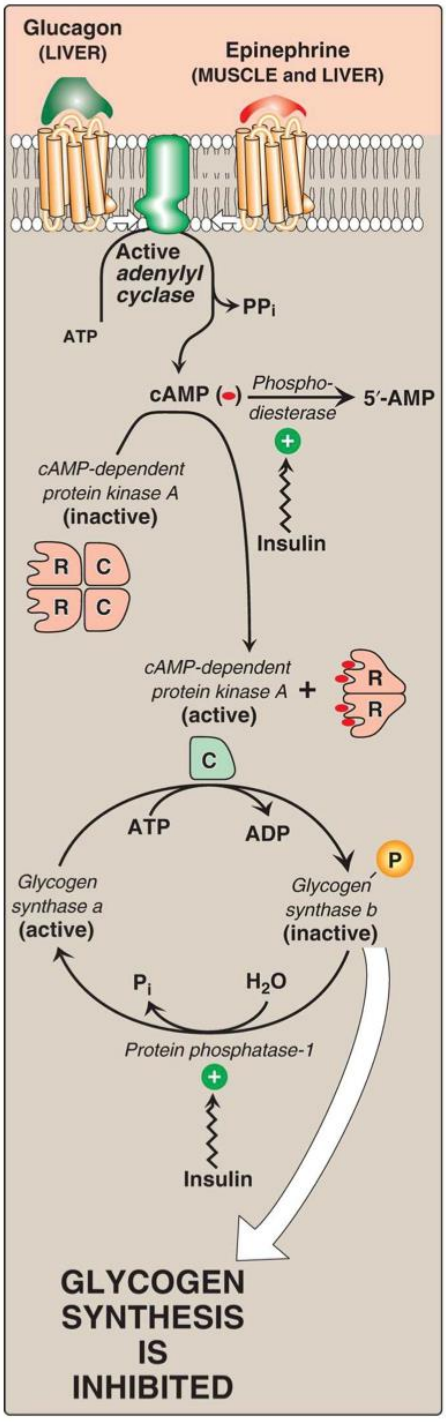
(which Dephosphorylate the glycogen synthase b to become active and called glycogen synthase a) and (it Dephosphorylate **glycogen phosphorylase kinase** and **glycogen phosphorylase** to inhibit them). Look at picture above very important

So usually, we can say that insulin activate the phosphatases.

Then in Fasting state means high levels of glucagon and epinephrine then activation of adenylyl cyclase so producing cAMP and activating protein kinase A , bifunctional enzyme , glycogen phosphorylase Kinase.

In well-fed state (**glycogen synthase**) will be activated by the pathways which is started by insulin .

"look at picture here to more clarification" .



ALLOSTERIC REGULATION OF GLYCOGEN METABOLISM

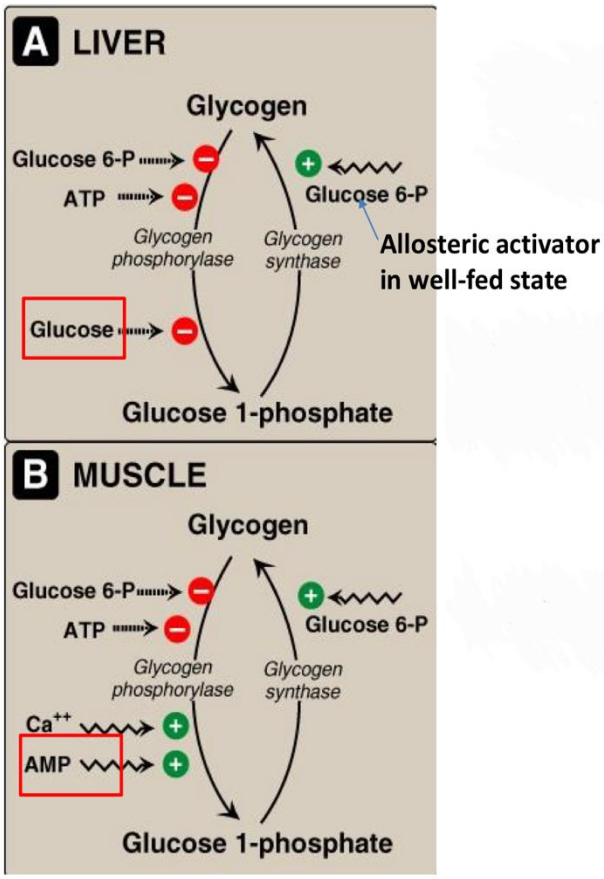
RAPID RESPON TO CELL'S NEEDS

AVAILABLE SUBSTRATE AND ATP

→ SYNTHESIS

LOW GLUCOSE LEVEL MEANS LOW ATP LEVEL

→ GLYCOGENOLYSIS



Hormones as a regulators don't bind to the enzymes by itself It's just activating signalling pathways which will end by activating of kinase & phosphatase which will make a covalent modifications of the enzymes By adding or removing phosphate group.

Allosteric regulators bind to the enzymes Directly and change their activity, liver and muscles (there are differences between them) are the main sites of glycogen metabolism.

When we talk about Liver in the degradation of Glycogen to glucose 1-phosphate this process can be inhibited (in liver and muscles) by glucose 6-p because we use glucose 6-p to produce energy then we have large amount of glucose 6-p we don't need to degrade glycogen.

Glucose work as inhibitor (of glycogen degradation) in the liver but why it doesn't work in the muscles? Because in the muscles we don't have the enzyme glucose 6-phosphatase which convert glucose 6-p to glucose.

So in the muscle we found large amount of glucose 6-p and small amounts of glucose.

In the liver, glucose can form then the large amounts of glucose will inhibit glycogen degradation.

In muscles we have another regulator:

1-AMP: when we have large amounts of AMP then we have small amount of energy, so we need to degrade more amounts of glycogen (another reason because AMP activate glycogen phosphorylase (a form))

why AMP found in muscle and not in liver?? Because the glucose that produced in liver its not for degradation in liver it will go to blood stream that's why we have a glucose 6-phosphotase in liver and no in muscle then we have a lot of AMP in muscle that activate muscle.

2-Ca+2: during muscles contraction Ca+2 is released from sarcoplasmic reticulum then Ca+2 increased in the cytosol then this means the cell is highly active and need energy, so we degrade glycogen.

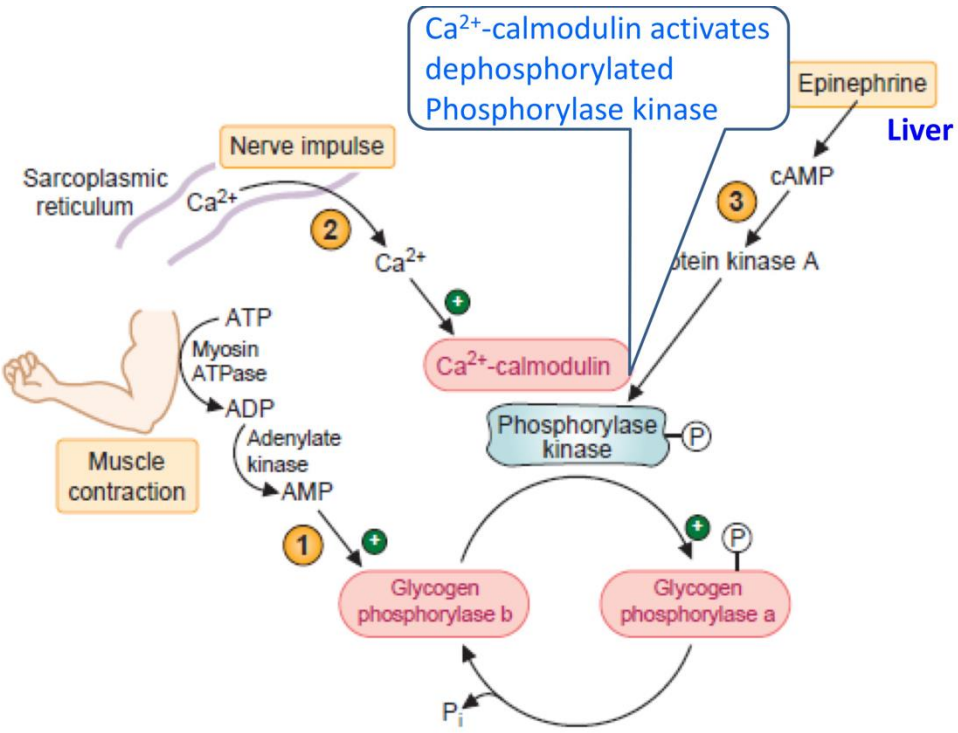
In both sites (liver and muscles) we see that glycogen synthesis can be activated by glucose 6-p , In glycogen synthesis we convert glucose 1-p to glycogen , now we see that glucose 6-p can activate the synthesis of glycogen with presence of insulin in the well fed state now we know that glucose enter the cells then convert to glucose 6-p then we need to convert glucose 6-p to glucose 1-p then to UDP glucose and finally we will start glycogen synthesis (see the figure in the previous page)

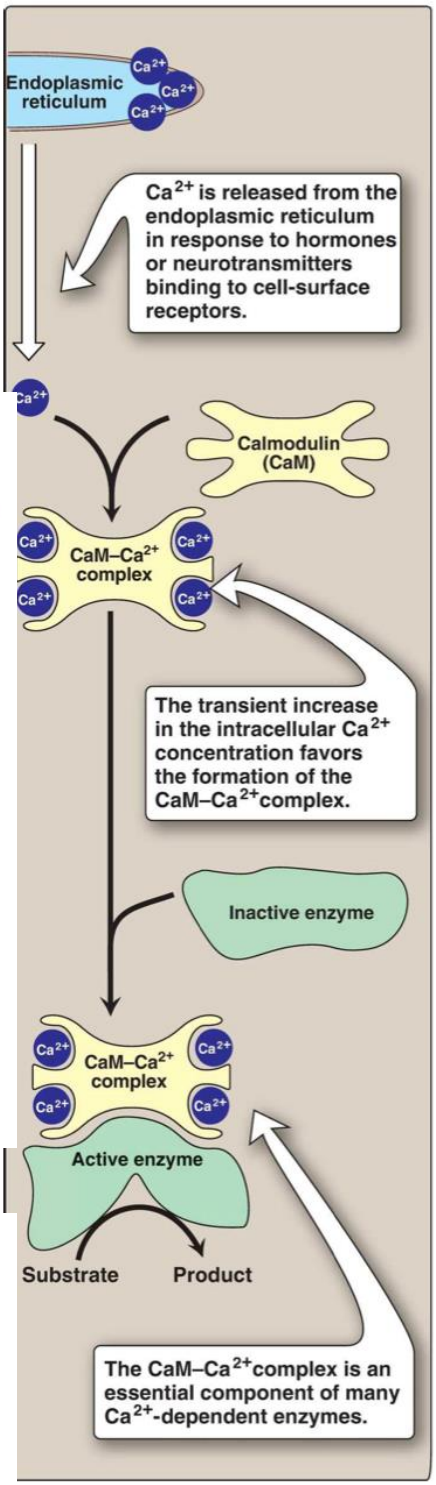
Let's talk about Ca+2 and other proteins which related to muscles function, in muscles contraction we need energy which is produced by hydrolysis of the ATP by using ATP-ase then ADP is produced and ADP can become AMP (which is a regulator of glycogen metabolism , activator of glycogen phosphorylase by binding to the glycogen phosphorylase as allosteric regulator and active at (a state)

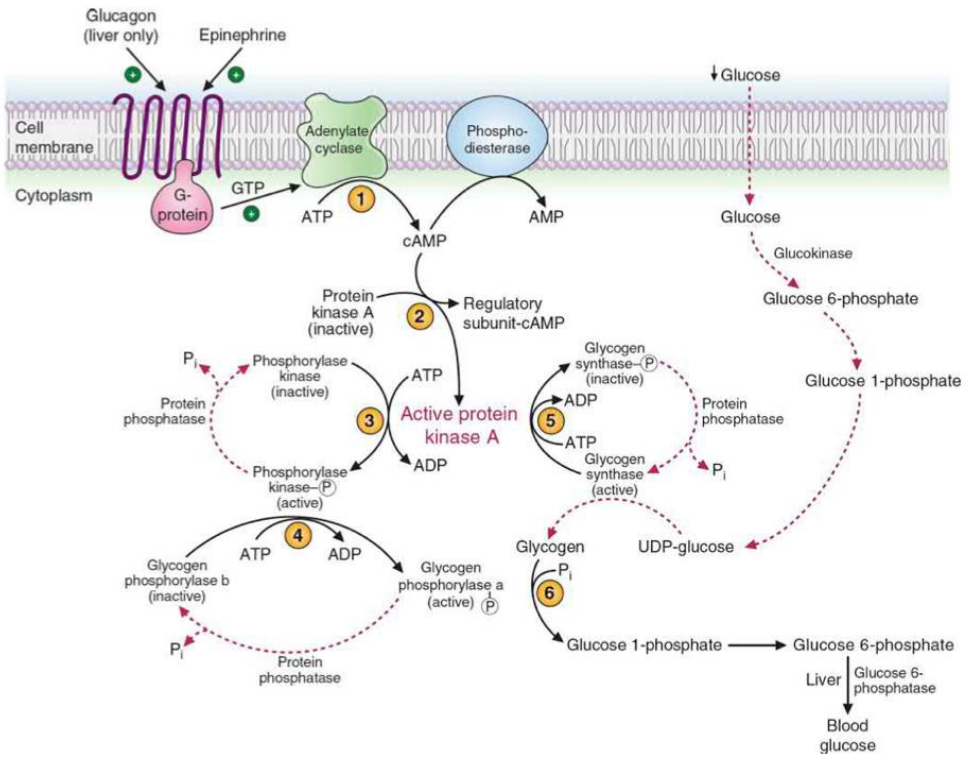
When the nerve impulses reach the muscles , then the muscle contract and calcium is going to released out the sarcoplasmic reticulum (as we said before) , calcium is an allosteric regulator which bind to the glycogen phosphorylase and activate it , another pathway that calcium bind to another protein called calmodulin (cal means bind to calcium) , now Ca+2-calmodulin complex bind to phosphorylase kinase and make it active (partially) , and phosphorylase kinase activates glycogen phosphorylase , this is another way to regulate the reaction (without phosphate) (then phosphorylase kinase can be activated by binding to the Ca+2-calmodulin complex or by phosphorylation by protein kinase A)

Then in regulation we can use 3 ways

in Allosteric regulators we use, Phosphorylation under neath Hormones (like insulin, EP, Glucagon), Ca+2-calmodulin complex (in the muscles only)

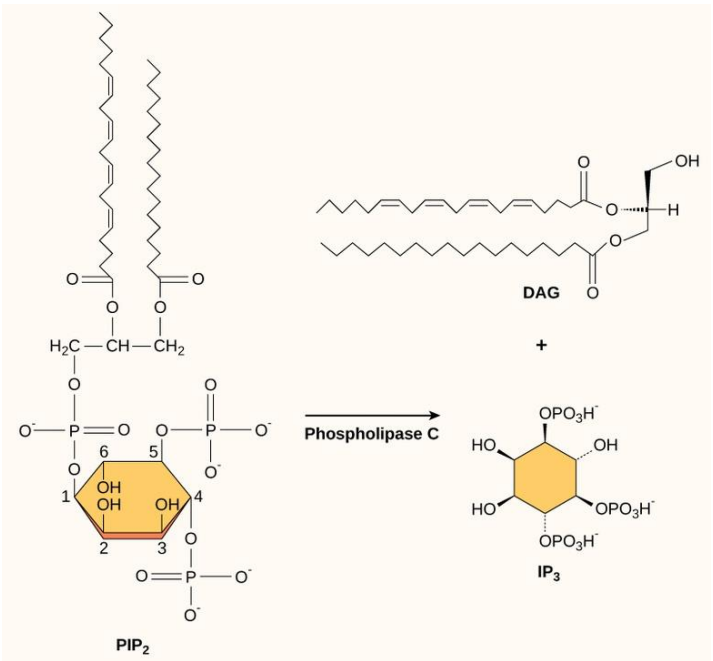






CALCIUM ACTIVATION OF LIVER PHOSPHORYLASE KINASE

Under neath GPCR when glucagon or epinephrine bind to the receptor we mention one of the pathways which is activation of adenylyl cyclase … , but we have another pathway which start when alpha subunit activates enzyme called phospholipase C , this enzyme involved in the metabolism of phospholipids and hydrolyses the phosphatidylinositol bisphosphate (PIP2) to diacylglycerol (DAG)(have hydrophobic part so it stays in the membrane) and IP3 , see the figure below , now DAG activates protein kinase C , IP3 it's a second messenger and can leave into intracellular part and activate the calcium channels (and return to Ca+2-calmodulin complex)



Extra function of Ca+2 -calmodulin

That it can work in the **(calmodulin**

Dependent protein kinase)

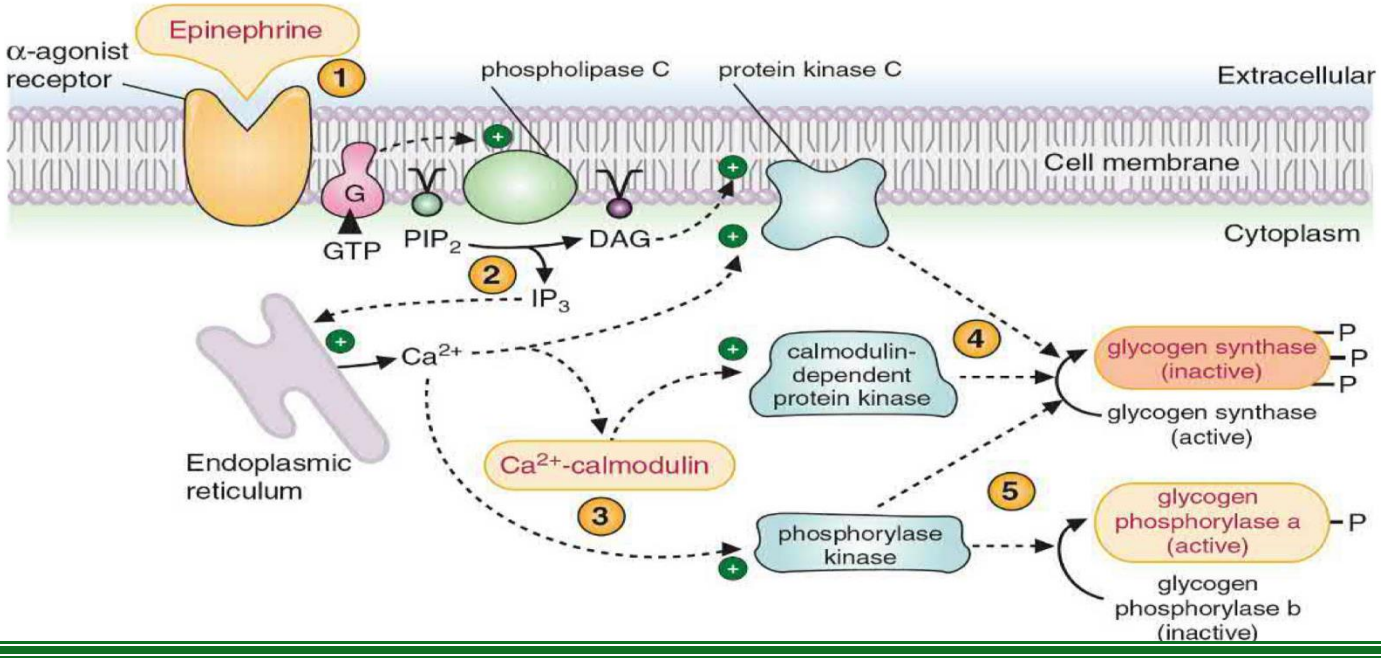
as we see protein kinase c can

Phosphorylate glycogen synthase

So inactive it, remember that

Each regulation way work in both

Pathways (degradation and synthesis) at the same time because we don't want to turn on the two pathways in same time. **(The pic below is very important)**



GLUCONEOGENESIS

GLUCONEOGENESIS (PRODUCTION OF GLUCOSE FROM NON-CARBOHYDRATES PRECURSORS)

Gluconeogenesis is the reversible pathway of the glycolysis, when we talk about gluconeogenesis we are talking about fast condition 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

We say that our body can store around 500 g of glycogen , one of the reasons that we store small of amount 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 ,remember that glucose used in measuring the osmolarity then storage of glucose will change the osmolarity then changing the movement of water and the osmotic pressure) , other reason is the amount of energy in this case fatty acids 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)

- **BRAIN IS DEPENDENT ON GLUCOSE 120G/DAY**
- **BODY GLUCOSE RESERVE IS LIMITED**

≈ 20 G (EXTRA CELLULAR FLUID) (MODIFIED GLUCOSE IN THE GAGS)

≈ 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

The storage form of the fats in the adipose tissue must be triacylglycerol inside adipocyte fat droplets , triacylglycerol consist of

1-glycerol

2-fatty acids

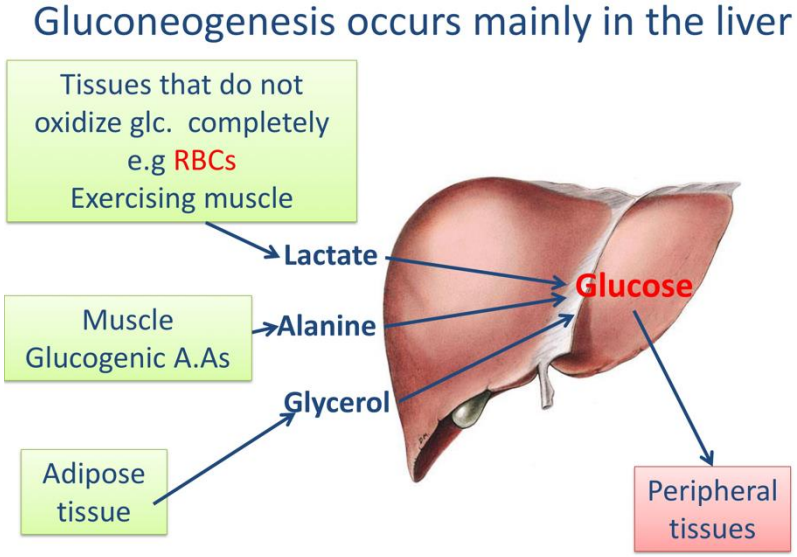
Hormones have receptors of the adipose tissue , now in the fast conditions the changes in the hormones will change the enzymes activity inside the adipose tissue , we start to degrade fatty acids from the adipose tissue and release them to blood stream , size of the fatty acids may determine the way of transporting to the blood , small and medium fatty acids chains with solubility can be released without carriers but for large chain we carry the chains by albumins until reaching the cells.

We need to know that fatty acids is used as source of energy and we don't use them to synthesis glucose, the remain part of the triacylglycerol is the glycerol and this part is used for gluconeogenesis (glycerol is non carbohydrate residue)

Fatty acids produce huge amounts of acetyl coA because most common fatty acids contains 16-18 carbons, acetyl coA consist of 2 carbons then we can use each fatty acids to produce 8-9 acetyl coA which is larger than the amount that glucose can produce (2)

Acetyl coA needs oxaloacetate to run the Krebs cycle , but in these conditions we don't have enough amounts of oxaloacetate (OAA is consumed in these conditions) then acetyl coA accumulate and result in ketogenesis or ketone bodies formation

- **WHERE AND WHEN DOES GLUOCNEOGENSIS OCCUR?**
- **DURING AN OVERNIGHT FAST, ~ 90% OF GLUCONEOGENESIS OCCURS IN THE LIVER** (HYPATOCYTES) **AND 10% BY THE KIDNEYS**
- **DURING PROLONGED FASTING KIDNEYS BECOME MAJOR GLUCOSE-PRODUCING ORGANS (40% OF TOTAL GLUCOSE PRODUCTION)**



We will use all available source such as lactate (mostly in exercising muscles which will go through anaerobic glycolysis , and RBC), remember that gluconeogenesis take place in kidney and liver only

We can also use glucogenic amino acids (like alanine which came from muscles) , amino acids metabolism leads to alpha keto acids formation (such as pyruvate)

Amino acids can be

1-Glucogenic amino acids : amino acids which can be metabolised into Krebs cycle intermediates or pyruvate and used these can be used in gluconeogenesis

2-Ketogenic amino acids : amino acids which can be metabolised into acetyl coA or Acetoacetyl-CoA which mean that they can used in synthesis of ketobodies

3- glucogenic + ketogenic (the major amino acid used is alanine)

We said that we can degrade fats by breaking ester bonds and using glycerol for gluconeogenesis precursor , producing glucose go from the liver to the blood stream and distribute to the tissues according to the priority , small fraction of glucose remain in the bloodstream

Generally gluconeogenesis is the opposite of glycolysis

According to the substrates we use in the gluconeogenesis we have many entry points , if the substrate is glycerol (3C alcohol) then usually we will go in trioses pathway

For amino acid , amino acids can enters as oxaloacetate , pyruvate , lactate and another Krebs cycle intermediates .

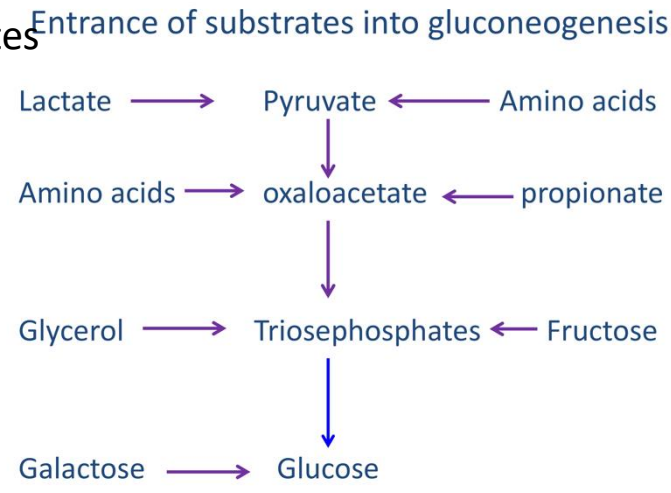
Fructose and galactose are carbohydrates

So we can't use them as precursors in

Gluconeogenesis , but we can synthesis

them by non carbohydrates precursors

Then convert them to glucose



Galactose (aldose) is more similar to glucose (aldose) than fructose(ketose).

Fructose can enter the pathway as triose and we will discuss this in the next lectures.

GLUCONEOGENESIS IS OPPOSITE TO GLYCOLYSIS BUT ?

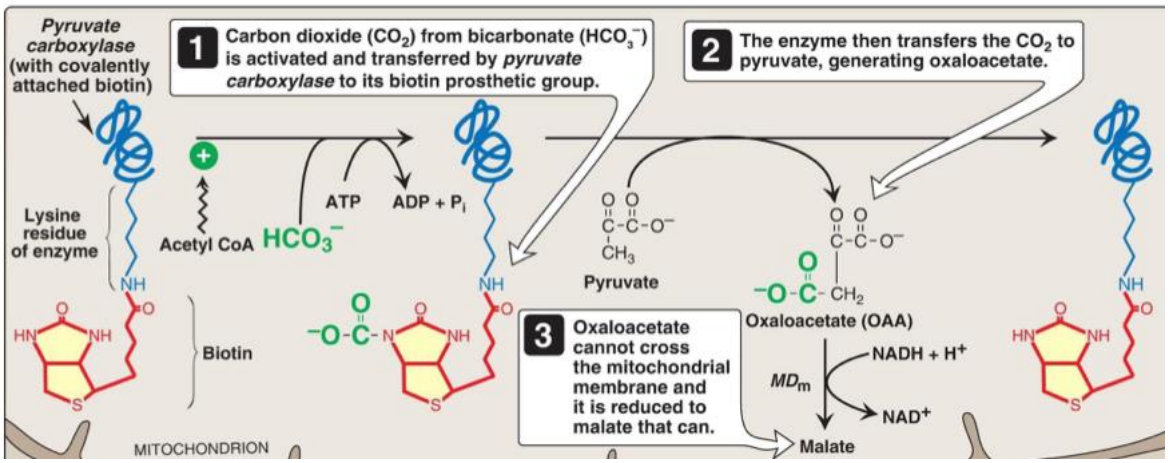
But we have 3 irreversible reactions , irreversible reactions in biochemistry means that reaction can't occur in the opposite way by the same enzyme then we need to use different enzymes , we won't discuss the 7 reversible steps

First step in glycolysis will be the last step in gluconeogenesis

These irreversible steps

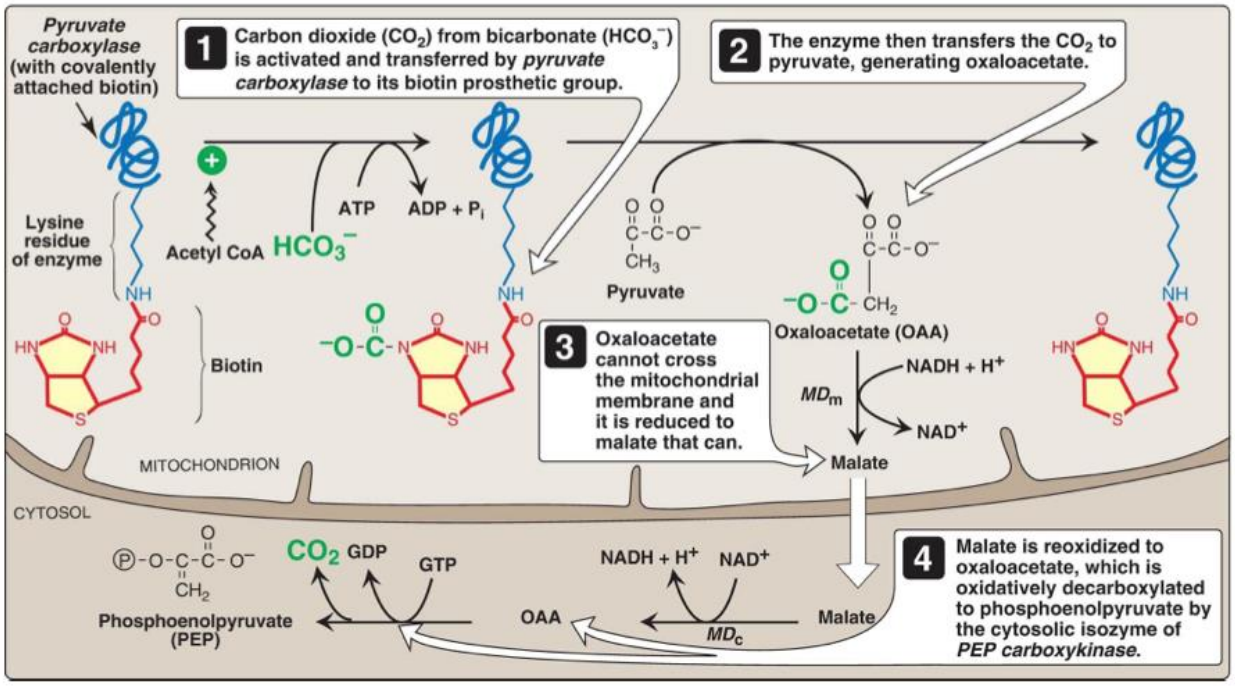
1-FROM PYRUVATE TO PEP

Pyruvate exist in the cytosol where glycolysis occur , now we convert pyruvate to acetyl coA to run the Krebs cycle (in the well fed state) , so pyruvate entered the mitochondria then the first step is converting pyruvate to oxaloacetate but pyruvate contain 3C and oxaloacetate contains 4C so it's a carboxylation reaction by pyruvate carboxylate (carboxylases need ATP) , so we convert oxaloacetate then we need to convert oxaloacetate to PEP and this reaction occur outside the mitochondria then we need to transport oxaloacetate outside it , but we know that can't cross the mitochondrial membrane and don't have any transporter so we need to convert oxaloacetate to another molecule which has a transporter like malate , so we use Krebs cycle enzymes to reverse this reaction , in Krebs cycle we oxidized malate to oxaloacetate but now we reduce oxaloacetate into malate (by oxidation of NADH)



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 + phosphorylation the oxalacetate , we use GTP but we are using 2 molecules of GTP because we want to end up by 2PEP so we are in synthetic pathway and using energy during fast conditions.

Acetyl coA is an activator of Pyruvate carboxylase, in this case large acetyl coA which won't be used then this pathway occurs to produce oxaloacetate



- **BY PYRUVATE CARBOXYLASE**
- **IN MITOCHONDRIA**
- **ALLOSTERICALLY ACTIVATED BY COA**
- **ENZYME IS FOUND IN BOTH CYTOSOL AND MITOCHONDRIA**
- **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**

Remember that we have additional step relative to conversion of pyruvate to oxaloacetate , two steps to reverse the last step of glycolysis (first step in gluconeogenesis)

(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)

2-FROM FRUCTOSE 1,6 BISOPHOSPHATE TO FRUCTOSE 6-PHOSPHATE

• DEPHOSPHORYLATION OF FRUCTOSE 1,6-BISPHOSPHATE

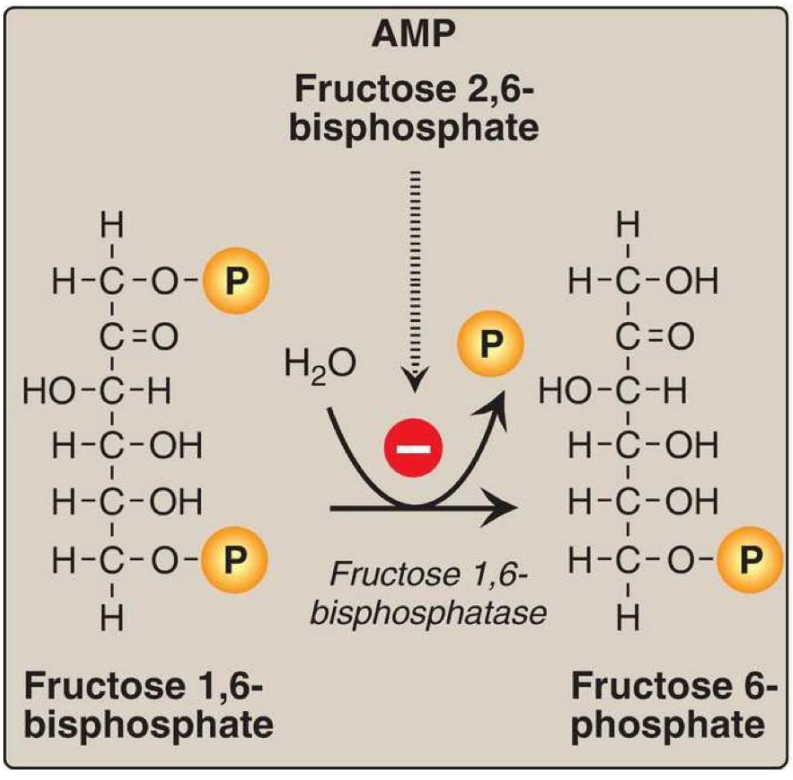
• THIS REACTION BYPASSES THE IRREVISIBLE PHOSPHOFRUCTOKINSE -1

In glycolysis we add phosphate by phosphofructokinase-1 so now we need to use phosphatase (one step), phosphatase can be inhibited by

1 -AMP

2-fructose 2,6-bisphosphate

(remember that both of them activate phosphofructokinase-1) , so in the same molecule we have opposite pathways



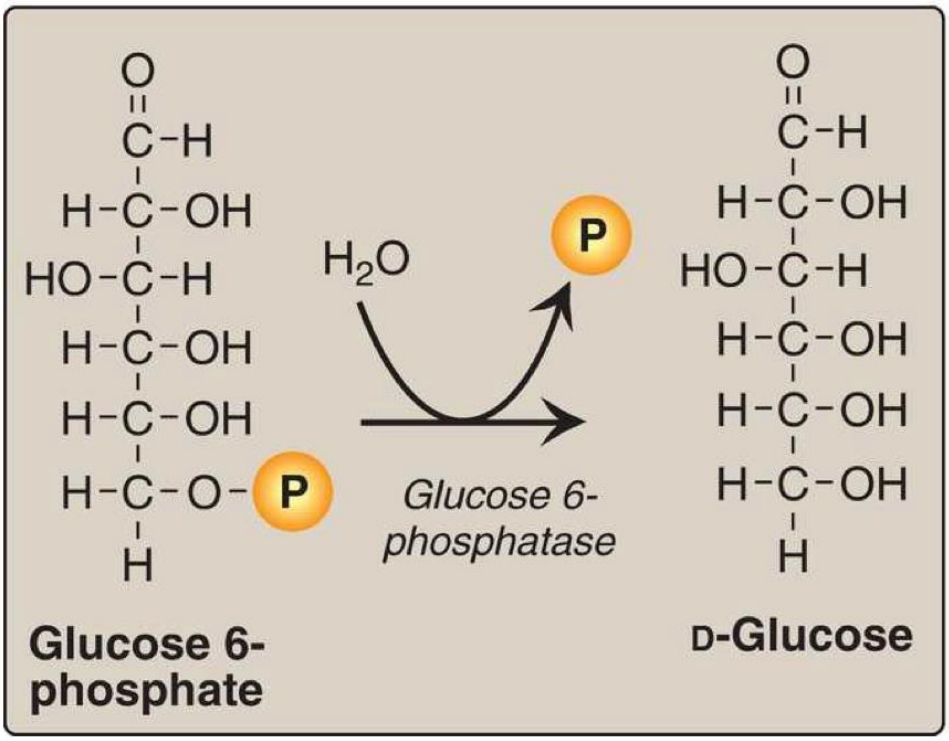
3-FROM GLUCOSE 6-PHOSPHATE TO GLUCOSE

- **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.

Also we need to use phosphatase, this phosphatase called glucose 6-phosphatase this enzyme found only in liver and kidney (not in muscles)

Glucose 6-phosphate exist in the cytosol and glucose 6-phosphatase exist in the ER so we need to move glucose 6-phosphate by using translocase (glut only transport glucose) , so translocase let the glucose 6-p enter the ER and become glucose then glucose leave by glut-7 (transporter not translocase) then glucose reach the cytosol and then on the cell membrane other gluts (glut 2) can transport glucose , then glucose is released to the blood stream



Dephosphorylation of glucose 6-phosphate to glucose, delta G of the reaction in negative so we can run the reaction

Phosphorylation of glucose to glucose 6-phosphate, the delta G of this reaction is positive (without ATP hydrolysis) so we had to couple the reactions, ATP hydrolysis and phosphorylation of glucose then when we add two reactions together then the over all delta G is negative and that's why we can run the reaction

Formation vs. Hydrolysis of Glucose 6-phosphate

- Formation

$$\text{Glc.} + \text{Pi} \longrightarrow \text{Glc. 6-phosphate} + H_2O \quad \Delta G = +ve$$

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \quad \Delta G = -ve$$

Hexokinase

$$\text{Glc.} + \text{ATP} \longrightarrow \text{Glc. 6-phosphate} + \text{ADP} \quad \Delta G = -ve$$

- Hydrolysis

Phosphatase

$$\text{Glc. 6-phosphate} + H_2O \longrightarrow \text{Glc.} + P_i \quad \Delta G = -ve$$

•ENERGY RECUIREMENTS OF GLUCONEOGENESIS

Up to down (glycolysis) (10 steps)

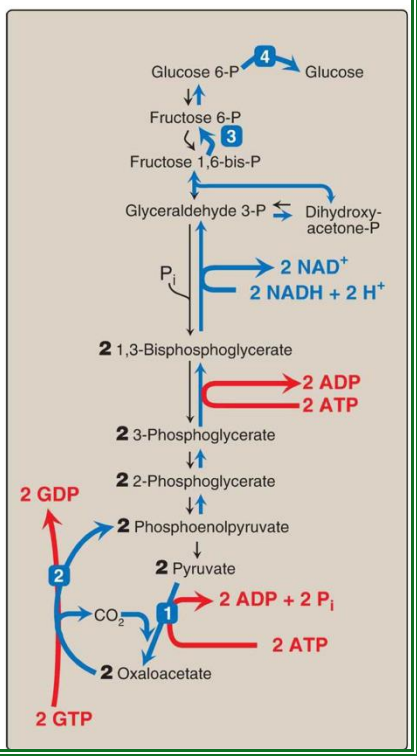
Down to up (gluconeogenesis) (11 steps)

Blue for NADH

Red for ATP

In glycolysis we use 1ATP in (glucose to glucose 6-p)

1ATP in (fructose 6-p to fructose 1,6-biosp), But in Gluconeogenesis we produce inorganic phosphate rather than ATP , and consumed 4 ATP and 2GTP (We can say 6 ATP) and 2NADH , 2ATP (pyruvate to oaa)



2GTP (oaa to pep) , 2ATP (3-phosphoglycerate to 1,3-bisphosphoglycerate)

so the net consumed are 4ATP , 2GTP , 2NADH (remember that we use 2 pyruvate molecules to produce 1 glucose , that's why we need to consume 2ATP&2GTP&2NADH)

we are in fasting state, and we consumed energy (we use fatty acids as a source of energy) , but we produce glucose for the tissues that depend on it as a source of energy (like brain) and to balance the osmotic pressure

(أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ ثَمَرَاتٍ مُخْتَلِفًا أَلْوَانُهَا ۚ وَمِنَ الْجِبَالِ جُدَدٌ بِيضٌ وَحُمْرٌ مُخْتَلِفٌ أَلْوَانُهَا وَغَرَابِيبُ سُودٌ ﴿27﴾ وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ كَذَٰلِكَ ۗ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ۗ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ ﴿28﴾) صدق الله العظيم

CHECK YOUR PROGRESS

1.under conditions of glucagon release, the degradation of liver glycogen normally produces which one of the following?

A. More glucose than glucose 1-p
B. More glucose 1-p than glucose
C. Equal amounts of glucose and glucose 1-p
D. Neither glucose nor glucose 1-p
E. Only glucose 1-p

2.consider a person with type 1 diabetes who has neglected to take insulin for the past 72 hours and also has not eaten much, which one of the following best describes the activity's level of hepatic enzymes involved in glycogen metabolism under these conditions?

	Glycogen Synthase	*Phosphorylase Kinase*	*Glycogen Phosphorylase*
A	Active	Active	Active
B	Active	Active	Inactive
C	Active	Inactive	Inactive
D	Inactive	Inactive	Inactive
E	Inactive	Active	Inactive
F	Inactive	Active	Active

3.Without a steady supply of glucose to blood stream, a patient would become hypoglycaemic and , if blood glucose levels get low enough , experience seizures or even a coma , which one of following is necessary for the maintenance of normal blood glucose ?

A. Muscle glucose 6-p
B. Liver glucose 6-p
C. Glycogen in the heart
D. Glycogen in the brain
E. Glycogen in the muscle

4.which of the following reactions in unique to gluconeogenesis?

A. 1,3-bisphosphoglycerate –> 3-phosphoglycerate
B. Lactate –> pyruvate
C. Oxaloacetate –> phosphoenolpyruvate
D. Phosphoenolpyruvate –> pyruvate

5.which enzyme would be impaired in case of biotin deficiency?

A. Pyruvate kinase
B. PEP carboxykinase
C. Pyruvate carboxylase
D. Fructose 1,6-phosphatase

6.which of the following is true regarding to pyruvate carboxylase?

A. Requires acetyl CoA for activity
B. Occurs in the cytosol
C. Catalyse an irreversible reaction in glycolysis
D. Produce carbon dioxide

7.During fight or flight conditions. which of the following is observed?

A. cAMP synthesis is activated, and downstream phosphorylation takes place
B. Glycogen synthase is activated
C. Inhibitor protein becomes inactive
D. Decreased rate of glycogenolysis

8.the enzyme which is involved in glycogen metabolism and doesn't exist in the muscles?

A. Glycogen synthase
B. Glucose 6 phosphatase
C. Glucose 1 phosphatase
D. Glycogen phosphorylase

1.B	2.F	3.B	4.C
5.C	6.A	7.A	8.B