DOCTOR 2020 | JU

METABOLISM

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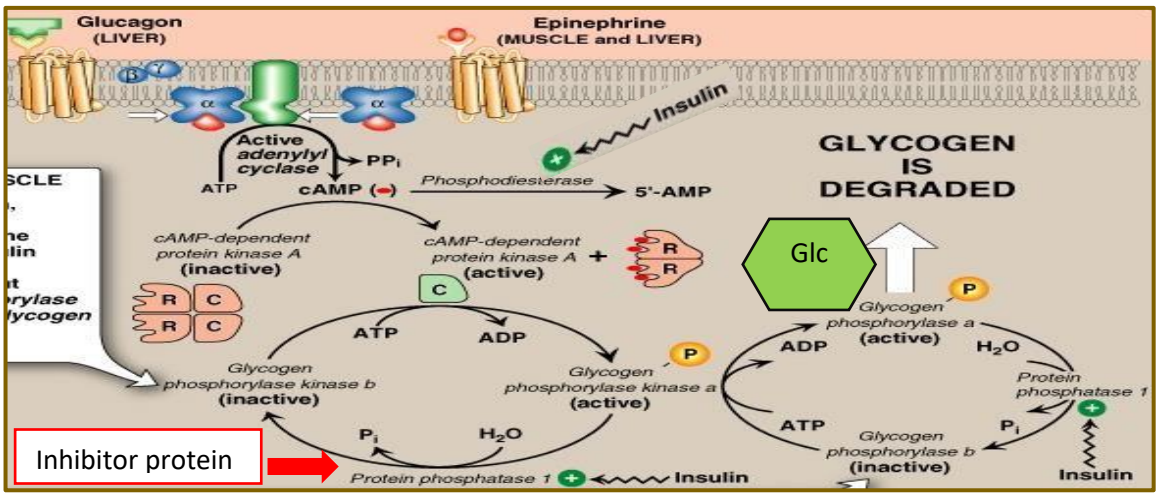
Regulation of Glycogen Metabolism

We've definitely agreed that glycogen synthesis is active at a well-fed state,with a certain fraction of excess sugar being stored as glycogen.Whereas glycogen degradation gets activated under fasting conditions(2h after a meal,followed by no additional external source of energy).



Glycogen degradation gets activated mainly by GLUCAGON (which predominates under fasting conditions, as you know). Another hormone that gets secreted under SAME conditions is EPINEPHRINE. Both of them bind on GPCR, preserving specificity for either ligand.

Once bound to its ligand, GPCR is activated and alpha-GDP gets exchanged to alpha-GTP, therefore alpha dissociates leaving beta-gamma dimeric complex, it continues the pathway by activating adenylyl cyclase that will increasingly synthesize cAMP, which in turn binds to regulatory subunits of protein kinase A (PKA) stimulating catalytic subunits and facilitating their dissociation from regulatory ones. Consequently, they initiate phosphorylation of different protein targets.

****Remember the two targets we previously mentioned, 1.the bifunctional enzyme complex (PFK-2&FBP-2), and 2.Pyruvate Kinase, which both became inactivated after phosphorylated, fitting the situation of low-energy state and low glucose conc.(high presence of glucagon).HERE, WE AIM TOWARDS THE OPPOSITE OUTCOME, utilizing phosphorylation as an activator ,enhancing the progress of glycogen degradation(perfectly consistent with the current situation).**

Our new target protein that gets activated by phosphorylation by PKA as a part of glycogen degradation regulation is a regulatory enzyme called Glycogen Phosphorylase Kinase(a 'kinase' for itself ,modulates the action of glycogen phosphorylase).Once it's phosphorylated, it's active .Then it starts phosphorylating its substrate(glycogen phosphorylase),which becomes activated, catalyzing glycogen degradative pathway.

#Under low conc. Of glucose = glucagon is the ligand → we want to degrade the glycogen:

Glycogen phosphorylase kinase-P(active) → glycogen phosphorylase-P(active) → glycogen degradation

Now let's shed light on Insulin role related to glycogen metabolism…

You and I agree that Insulin must be low under the previous conditions. However, in the well-fed state, how can Insulin turn this pathway off?

1.Insulin will definitely bind to its receptors, RTKs (tyrosine receptor kinases), activating a series of downstream proteins that end up turning on phosphodiesterase enzyme, which is responsible for degrading cAMP, blocking the whole progress (glycogen degradation)

#Under high conc. Of glucose (the ligand is insulin) , so we don't want to degrade glycogen:

cAMP $\xrightarrow{\text{phosphodiesterase}}$ 5-AMP, so there is no cAMP downstream signaling pathways of glycogen degradation (inhibited) , when insulin is an (activator) of phosphodiesterase.

2.Insulin acts on another targets, such as phosphatases, which are going to dephosphorylate glycogen phosphorylase kinase as well as glycogen phosphorylase, inhibiting both of them, thus stopping degradation.

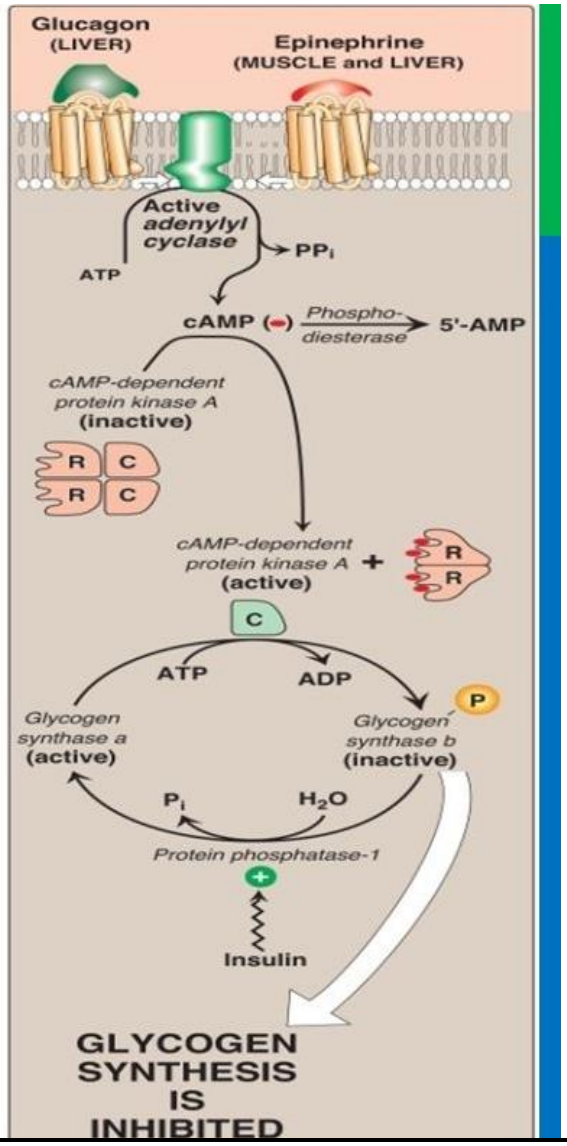
Regulation of glycogen synthesis: A new target

We talked about the bifunctional enzyme, pyruvate kinase and glycogen phosphorylase kinase, as regulatory enzymes modulating glycogen metabolism, activated by PKA under the effect of glucagon. Now we're adding a fourth one^-^, Glycogen Synthase. As the name implies, it turns on glycogen synthesis. So, what do think? Will phosphorylation activate or inhibit the enzyme?

ASURELY IT WILL BE INHIBITORY, because this is a synthetic pathway, it's impossible for both synthesis and degradation to occur simultaneously. (they have opposite functions.)

With respect to Insulin, it's going to dephosphorylate the enzyme (glycogen synthase) by phosphatases, activating it again, and this is certainly logical, because we're in the context of high glucose conc. (well-fed state) that will further activate synthesis.

- **Note that Insulin contributed in dephosphorylation and inactivation of the previously mentioned degradative enzymes (glycogen phosphorylase kinase & glycogen phophorylase) . On the contrary, it aided in glycogen synthesis by activating glycogen synthase.**
- **BUT, PLZ WATCH OUT!! INSULIN AND GLUCAGON SECRETION DON'T HAPPEN TOGETHER. We are talking about two completely opposite situations, but we mentioned Insulin in both cases just to examine what would happen if Insulin was present.**



- Note also that the degree of inhibition is proportional to the degree of phosphorylation, provided that phosphorylation may occur at several sites, because more than 2nd messenger can be activated underneath GPCR serving different effects regarding the same enzymes. We will see this reflected in an example shortly.

LEFT SIDE: GLYCOGEN DEGRADATION

RIGHT SIDE: GLYCOGEN SYNTHESIS

LEFT: You see Glucose 6-P as an inhibitor in both sites (liver& muscles).Why? Because if present at high conc., I won't need it anymore(e.g: I don't need further glycogen degradation).Note that Glucose 6-P keeps degrading into glucose, in contrast to muscle cells, but both are inhibited in this case.

Also ATP is a shared as an inhibitor in both, since it marks the high-energy state in the cell, which doesn't necessitate more glycogen degradation.

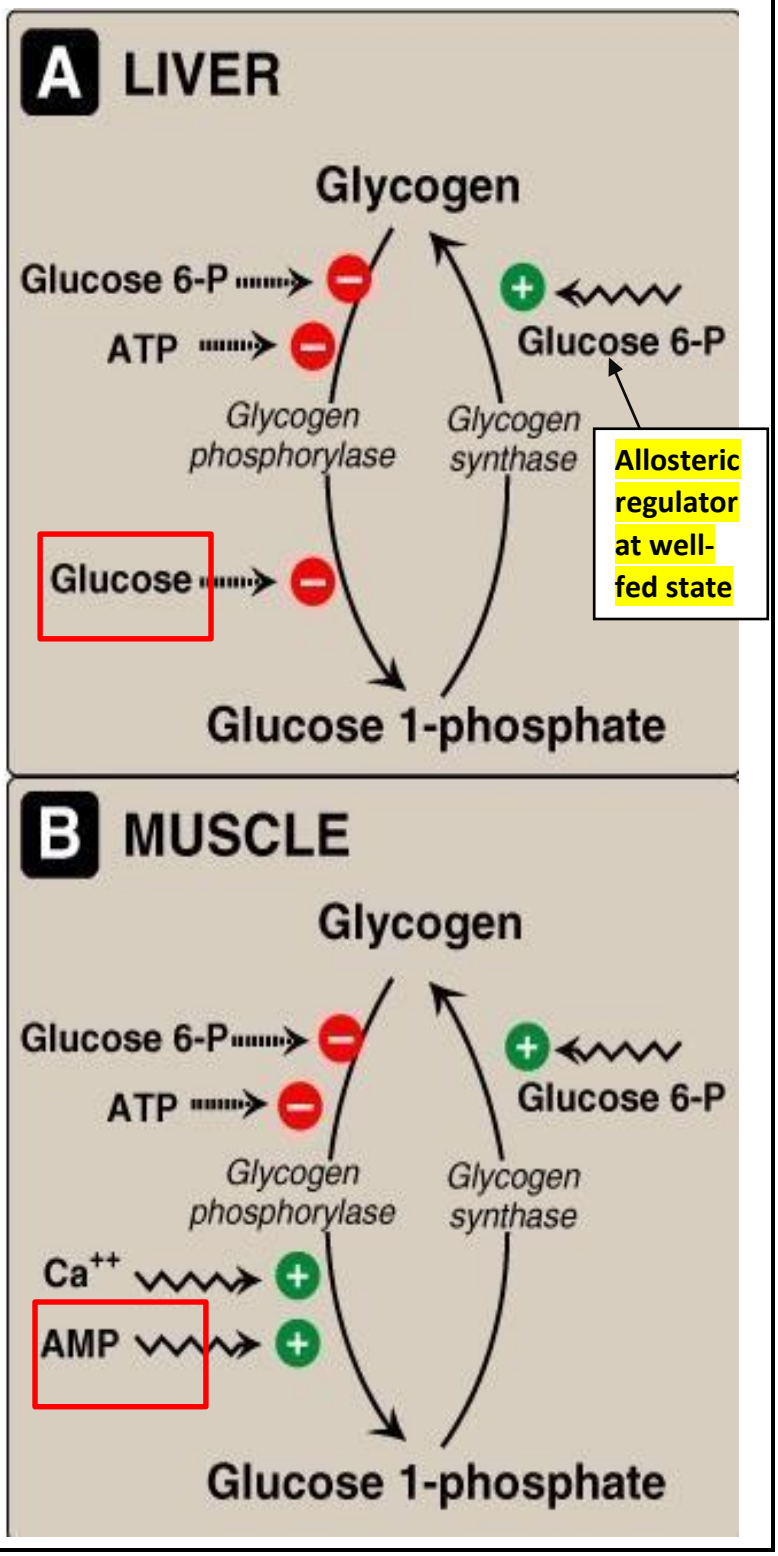
Differing from muscles, glycogen degradative pathway in the liver gets inhibited by GLUCOSE, for the same reason as G6P.Remember that muscles don't reach the point of glucose formation.(so it is regulatory for liver only)

KEEP YOUR FOCUS ON THE LEFT: See muscle side, positive allosteric regulators for degradation are AMP (a marker of low-energy state),also you've noticed Ca^{+2}.

AMP::

AN IMPORTANT QUESTION: Why AMP isn't present as an activator of glycogen phosphorylase in the liver?

Because liver won't use the glucose it produces as a source of energy serving its own needs, rather it'll distribute to tissues (that are dependent on it ,maintaining fasting blood sugar at the same time.)



Ca++:

We said that Calcium is an activator for glycogen degradation in muscles, contributing to muscle contraction.Ca^{+2} are released from sarcoplasmic reticulum (ER of the muscles) to the cytosol,where its conc. is elevated,then it activates contraction.As a consequence,the significant presence of Calcium in the cytosol indicates that the muscle is active (contracting) and it needs energy,that's why glycogen intervenes by its degradative energy-yielding pathway (ends up with G6P).

Summary of glycogen degradation:
1.Shared between muscles and liver:
a. inhibitor: glu-6-p and ATP
2. only liver:
a.inhibitor:glucose
3.only muscles:
a.ativator:Ca++ and AMP.

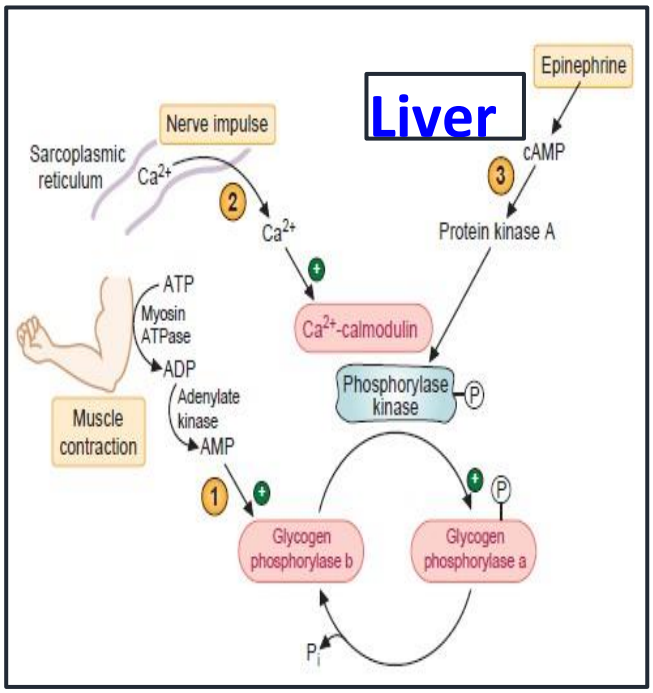
Now let's get into details related to the right side: synthetic pathway

- **Liver: G6P acts as an allosteric activator. Note that G6P manipulates the two processes (degradation and synthesis) possessing two opposite roles:an inhibitor and activator,respectively.G6P is the product of degradation(feedback inhibition) while it respresents the substrate of synthesis (thus enchances activation).**
- **The previous point applies for muscle cells:G6P is an activator.However,keep in your mind that we're talking about the well-fed state.**
- **Generally, available substate and ATP direct us towards synthesis(glycogenesis), whereas low glucose and ATP lead to glycogenolysis(glycogen degradation).**

Regulation of muscle contractility utilizing Ca^{+2}

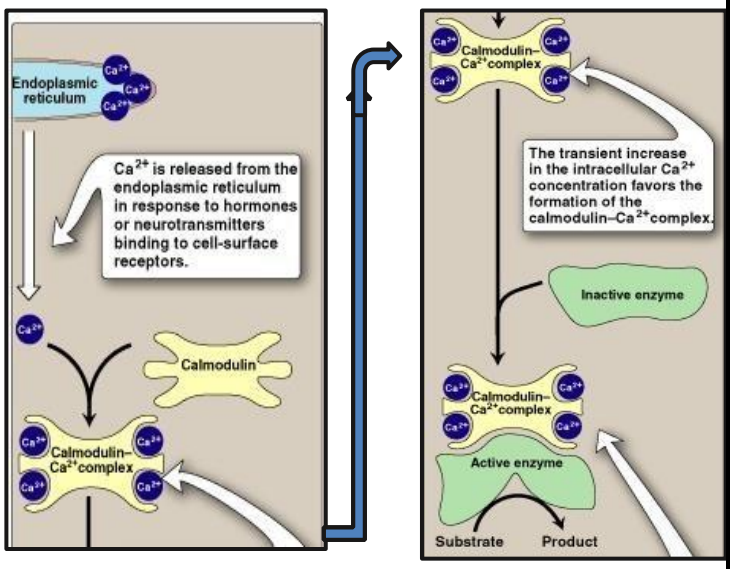
- **(1^{st} pathway) ,The figure below respresents a contracting muscle that needs ATP for the sliding and movement of myosin against actin filaments- which plays a role in contractility- Futhermore,ATP gets hydrolyzed forming ADP,ADP further produces AMP(by adenylate kinase),this will eventually activate glycogen phosphorylase.**

→ (2nd pathway)Another pathway could start by Calcium release from ER during contraction, pushing up its cytosolic conc. in order to facilitate binding to calmodulin forming (Ca+2 – calmodulin) complex which will activate phosphorylase kinase (without phosphorylation) enabling it to phosphorylate glycogen phosphorylase, so it will be activated (boosting the degradative process).



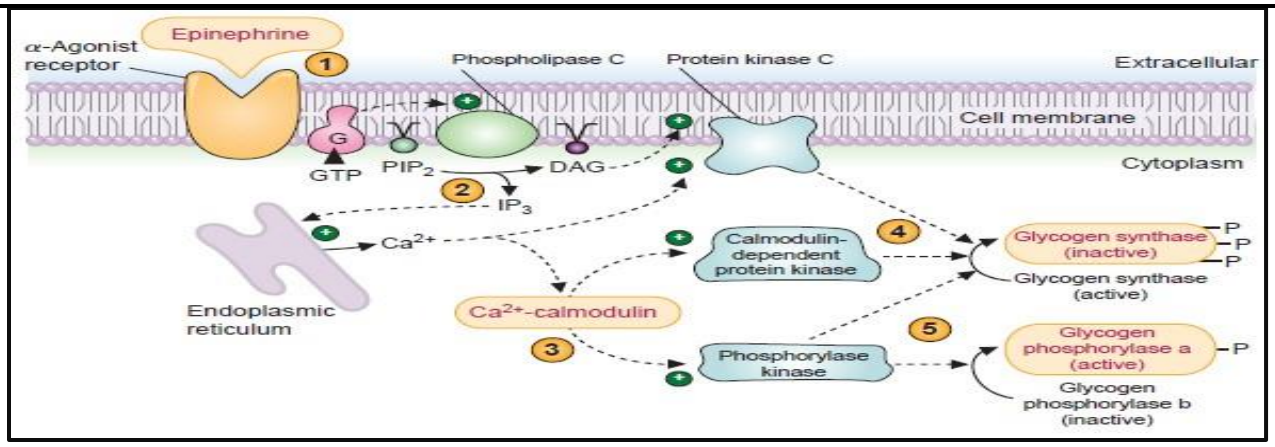
- The role of epinephrine(liver)(3rd pathway)
  When muscle activity is high, epinephrine may be increasingly produced, especially under fight-or-flight conditions-where you are fasting or run out of food-,catalyzing glycogen degradation (cAMP-PKA-phosphorylase kinase(gets activated to phosphorylate glycogen phosphorylase)),in order to generate a sufficient amount of energy in this situation.

- A little bit info about this figure. Once Calcium leaves ER it binds calmodulin, Ca^{+2}-Calmodulin complex is going to induce conformational changes in the target protein, which allows a subsequent cascade of activation.



Calcium activation of liver phosphorylase kinase

We discussed the role of Epinephrine activating GPCR-cAMP pathway,but it's not the only 2nd messenger produced under the effect of GPCR,note that the same GPCR can activate more than one pathway (each controlled by a 2nd messenger). cAMP induces phosphorylation either by activating degradative enzymes(producing energy) or inhibiting synthetic enzymes.



A new pathway Epinephrine will undergo is activation of GPCR followed by Phospholipase C stimulation.This enzyme cleaves PIP2(Phosphatidylinositol 4,5-bisphosphate) into IP3&DAG,the latter stays within the membrane because of its hydrophobic nature(diacylglycerol,2 fatty acids+glycerol).

On the other hand, IP3(Inositol triphosphate) is going to leave as a 2nd messenger, since its almost polar (Inositol is a modified sugar + phosphate groups that are negatively charged). In fact, IP3 is responsible for opening Ca^{+2} channels, because these channels are IP3-gated, their activation is confined to IP3 binding.

Once bound to the Calcium channels, channels will open on the ER surface, releasing Calcium to the cytosol. Again, Ca^{+2} binds to calmodulin forming a complex that will act on glycogen phosphorylase kinase, which will modulate glycogen phosphorylase (by activating it).

Ca^{+2}-Calmodulin can also activate Calmodulin-dependent protein kinase, which phosphorylates glycogen synthase, inhibiting it.

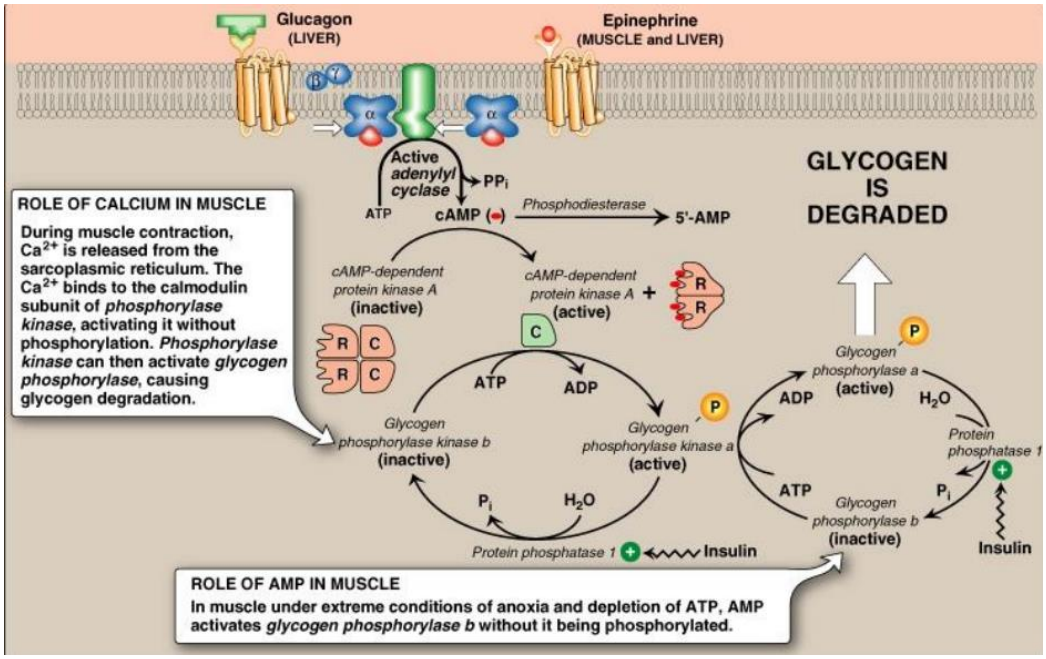
In addition to that, Calcium can activate PKC that consequently phosphorylates glycogen synthase, inhibiting it.

A BRIEF SUMMARY

- **Under fasting conditions, glycogen degradation is active while synthesis is inactive.**
- **Activation of glycogen degradation (and inhibition of synthesis) occurs through glucagon or Epinephrine binding to their receptors (GPCR)**
- **Once active, they can activate different 2nd messengers, either cAMP or IP3.**
- **If cAMP-dependent pathway, it will activate PKA which will phosphorylate glycogen phosphorylase kinase(activated) as well as glycogen phosphorylase. In contrast, PKA phosphorylates glycogen synthase resulting in an inhibition.**
- **If IP3-dependent pathway, IP3 is going to open Calcium channels, which release Calcium into the cytosol, where it binds to calmodulin. The formed complex proceeds by activating glycogen phosphorylase kinase or**

calmodulin-dependent protein kinase that exerts an inhibitory effect by phosphorylating glycogen synthase

- Glycogen synthase as mentioned above can be inactivated by 2 ways: cAMP (by PKA), Calmodulin-dependent protein kinase and the 3rd way by the utilization of PKC that phosphorylates and inhibits glycogen synthase.
- Insulin antagonizes the effect of glucagon and epinephrine , by phosphodiesterase & phosphatases (see page 3 ^-^)
- Phosphorylating degradative enzymes activates them, whereas phosphorylation of synthetic enzymes does inhibit their function.



You can watch this video ☺ : https://youtu.be/pYrbLjgqdTY

It is defined as production of glucose from non-carbohydrate sources

Glucose is a very important molecule in our bodies and all tissues depend on it but by variable degrees (Brain is dependent on glucose 120g/day)

The main source that supplies us glucose is diet. However, if someone has been fasting and hadn't had a meal for a long time, the body will resort to glycogen degradation to cover its need from glucose, but that is a temporary (limited) solution (only for 12-18 hours) then the glycogen will run out. What happens if he fasts for a longer period?

The answer is the body will start synthesizing glucose form other molecules to maintain fasting blood sugar and provide tissues that are exclusively dependent on glucose as a source of energy.

Body glucose reserve is limited. Glycogen is stored in other tissues beside the liver and skeletal muscles (the main ones). It can be stored in heart, smooth muscles, kidney, red and white blood cells but in small amounts

(even the brain stores small amount of glycogen specifically in the astrocytes, but that's small amount doesn't last for a long period because it requires high level of glucose, so it depends mainly on glucose that comes from glycogen degradation in the liver).

How much there is stored glycogen in the body?

≈ 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)

The overall stored glycogen is around 500g. We can't store more glycogen on that because they are hydrophilic molecules and they will attract water causing swelling, so we prefer to store fats instead because fats are hydrophobic and stores higher energy (1 gram of sugar produces 4 cal while 1 gram of fats produce 9 cal)

70 Kg man has ≈ 15 Kg fat – Fatty acids cannot be converted to glucose

Utilization of FA is increased 4-5 X in prolonged fasting

In prolonged fasting; ((FA → ketone bodies at high rate))

Gluconeogenesis occurs mainly in the liver (90% in the liver), 10% occurs in the kidney specially in short-term fasting or (overnight fast) (after 18 hours)

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

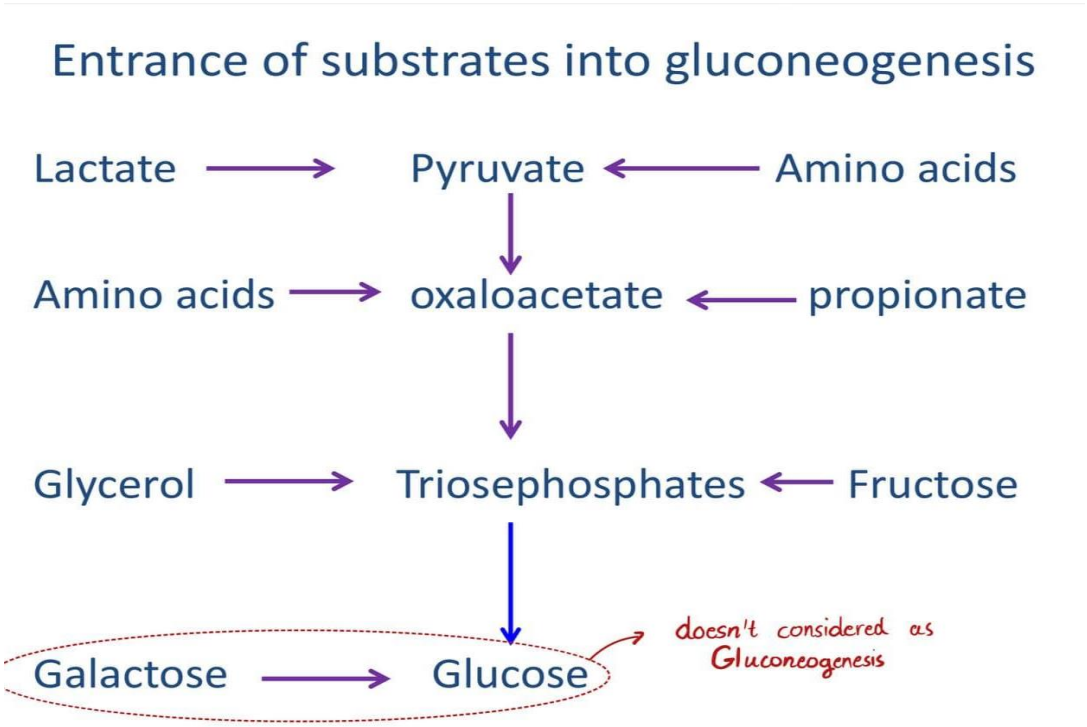
Gluconeogenic pathway can use different precursors to synthesise glucose:

1. **Lactate in RBCs and exercising muscle (they produce high amount of lactate relatively to other cells)**
2. **Alanine in muscles rich in protein (alanine is a source of pyruvate)**
   there are other amino acids that can be converted either to pyruvate or krebs cycle intermediate, we call them *glucogenic amino acids.*
3. **Glycerol in adipose tissues (in this situation, we produce energy from lipids in**

some tissues (doesn't happen in the brain and tissues that are exclusively dependent on glucose)).

→ By hormonal stimulation (glucagon), adipose tissue will start degrading its lipids (the storage form of lipids is triacylglycerol) to fatty acids which are oxidised to produce energy (we can't convert them to glucose) and glycerol which it can be used in gluconeogenesis.

Each precursor has certain site of entrance into gluconeogenesis



Galactose and fructose can be converted to glucose but that is NOT gluconeogenesis because they are carbohydrates

Gluconeogenesis is the opposite of glycolysis BUT there are 3 steps which are irreversible one in glycolysis(step 1 & 3 and the last step) which will differ between these 2 pathways

The last step in glycolysis is reversed by 2 steps, so gluconeogenesis has 11 steps.

The first irreversible reaction (From pyruvate to phosphoenolpyruvate (PEP)) (2 steps):

(Pyruvate → oxaloacetate → phosphoenolpyruvate)

Pyruvate is synthesized in cytosol. In well-fed state, pyruvate will be transported to the mitochondria then be oxidized by pyruvate D.H. to produce acetyl CoA which enters Krebs cycle

Pyruvate (3C) → Oxaloacetate (4C)

- **This step can occur in cytosol or mitochondria by pyruvate carboxylase (exist in both)**
- **Pyruvate carboxylase needs ATP and it is attached covalently to biotin (vitamin B7)**

- **If it happens in mitochondria, oxaloacetate cannot cross the mitochondrial membrane, so it will be reduced to malate by (mitochondrial malate dehydrogenase)**
- **When malate reaches cytosol, it will be reoxidized (by cytosolic malate dehydrogenase) forming oxaloacetate.**
- **Allosterically activated by Acetyl CoA.**

Oxaloacetate (4C) → Phosphoenolpyruvate (3C)

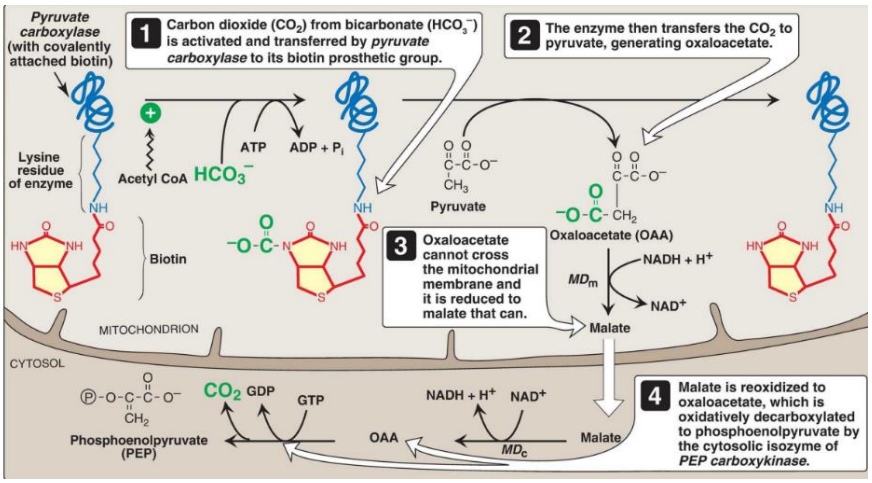
it includes decarboxylation and phosphorylation (using GTP) by enzyme called PEP carboxykinase (exist in both cytosol and mitochondria)

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

The second irreversible reaction (fructose-1,6-bisphosphate to fructose-6-phosphate) by 1,6-bisphosphatase.

- **Dephosphorylation reaction**
- **Catalyzed by fructose-1,6-bisphosphatase**
- **The activators of phosphofructokinase-1 (AMP, fructose-2,6-bisphosphate) are inhibitors to fructose-1,6-bisphosphatase**

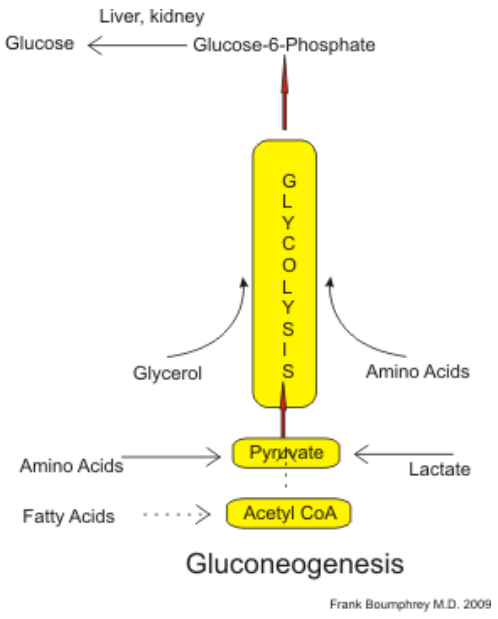


NICE VID(OVERVIEW)

https://youtu.be/ydhr0QAyxYg

NICE VID (DETAILED)

https://youtu.be/YpV96faQOZc



THANK YOU 😊