فريق طوفان الأقصى

METABOLISM

Modifide N. 12

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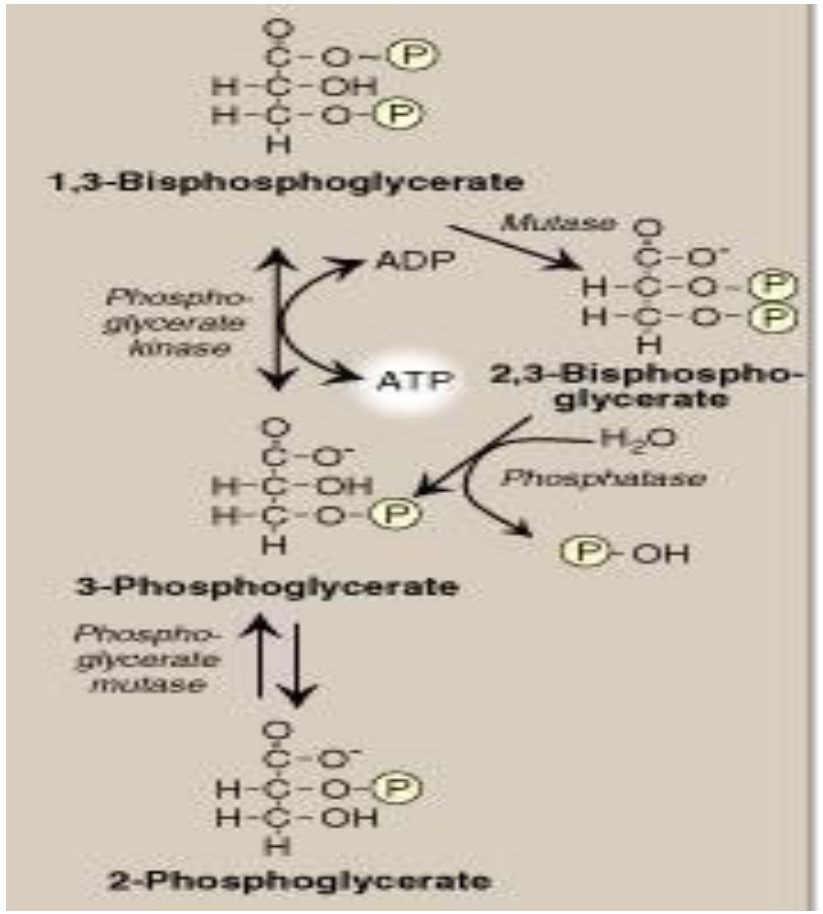
Synthesis of 2,3 bisphosphoglycerate in RBC



↑↑ Oxygen delivery to tissues

By binding to deoxyhemoglobin reducing its affinity to O2 and increasing O2 release to tissues

- **Remember:** glycolysis is a pathway composed of 10 steps, 7 of them are reversible while 3 are irreversile.

- **NOTE:** how much ATP molecules are produced by one glucose molecule that enters glycolysis?

2ATP molecules are produced as a net for the glycolysis reaction

- **The complement in this slide:**there are cells that depend on glycolysis because they exclusively depend on glucose as a source of energy like the adrenal medulla, brain and other tissues

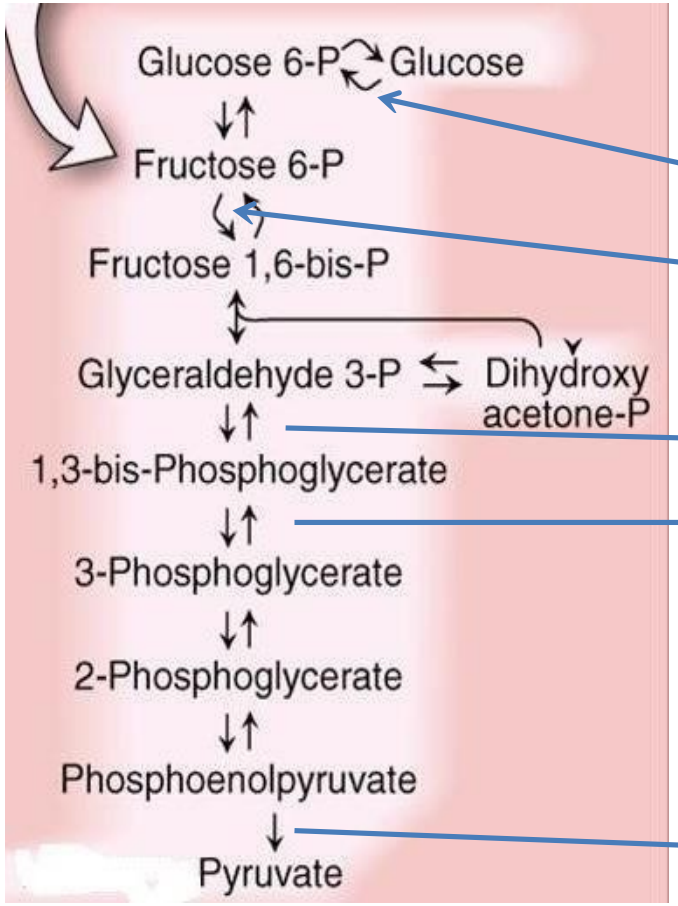
- **The complement in this slide:**The RBCs are totally dependent on glycolysis pathway in it's energy requirements because it doesn't have a mitochondria or a nucleus.
- It could take the pyruvate to anaerobic respiration to produce ATP, but its's energy requirements are generally low, so it doesn’t need that much energy.

- **The complement in this slide:** RBCs can perform their normal metabolic pathway and may have a modified version of it in certain conditions
- This modification happens at the step of going from 1,3-bisphosphoglycerate to 3-phosphoglycerate which is utilized by phosphoglycerate kinase , this step includes the first ATP production , now this is the regular pathway
- we may take an alternative path or shunt, which means that we take a different pathway but produce the same product as the regular pathway (which is 3-phosphoglycerate)

- **The complement in this slide: what are the steps of the alternative pathway?**
- **A mutase transports a phosphate group from carbon number 1 to carbon number 2 producing 2,3-Bisphosphoeglycerate ,then a phosphatase removes the phosphate from carbon number 2 producing 3-phosphoglycerate with no production of ATP like the regular pathway**
- **In this way, we lose ATP, so if we look at glycolysis using this alternative pathway, how much ATP is produced?**
- **The net energy production is zero because we have lost two glyceraldhydes (there are 2 ATP molecules consumed is the first phase)**

- **The complement in this slide:although this alternative pathway doesn't produce energy, the RBCs go through it anyway, why?**
- **When the RBC is present at the site of gas exchange where its going to give oxygen to cells and take co2 from them ,now hemoglobin becomes deoxygenated (deoxyhemoglobin)**
- **Oxygen is a gas, so it's going to spread to the outside, but it could return and bind back to hemoglobin (it still has some affinity for hemoglobin) so In order to prevent it's rebinding, 2,3-Bisphosphoglycerate binds to deoxyhemoglobin and reduces it's affinity to oxygen, so all of oxygen is released to the cell and in this way we have more efficient oxygen transport to the cells.**
- **This pathway doesn't occur for example in the lungs or in the bloodstream because there is no need of it ,so it only occur at the site of gas exchange in the cell.**

Energy Need and Production



- **NOTE:** NADH is only considered as a product of this pathway , **not an energy molecule.**

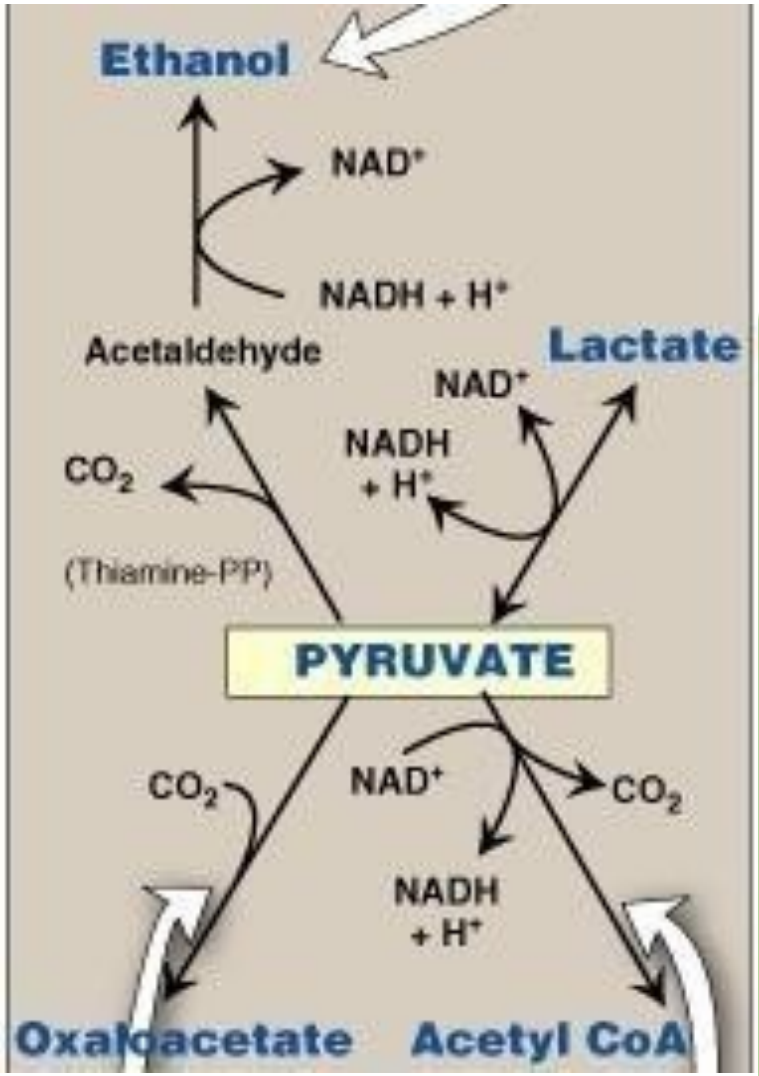
Is Oxygen needed?

- **NOTE:** glycolysis doesn't need oxygen or release oxygen.
- It occurs under aerobic and anaerobic conditions.

- **NOTE:** there is a production of 2 ATP molecules because we have 4 ATPs generated by the second phase of glycolysis (ATP generating phase) ,and 2 ATP molecules are going to be consumed, so the total is going to be 2

- **NOTE:** what happens to the pyruvate?

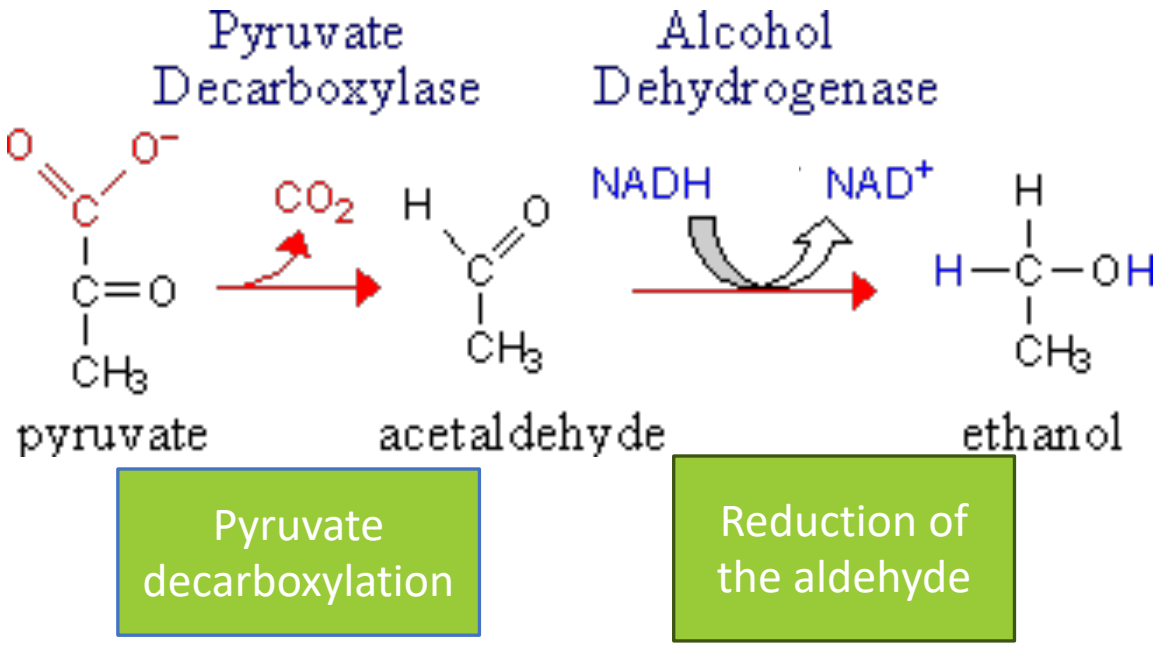
Pyruvate Fates



- **NOTE:** it can either go into the production of acetyl CoA by pyruvate dehydrogenase complex and then continue into krebs cycle, or in anaerobic conditions when there is less oxygen available, the pyruvate is going to be converted to lactate in muscle cells during exercise.
- Another option at different types of conditions: pyruvate can be carboxylated to become oxalacetate, mainly this reaction doesn't occur during the break down of glucose molecules ,it occurs when the reverse reaction(gluconeogenesis) is active because it doesn't make sense when we break glucose to pyruvate and then use it to make glucose(oxalacetate is an intermediate in gluconeogenesis), so the pyruvate go to it in the reverse reaction(pyruvate comes from many sources other than glycolysis like degradation of some amino acids, and this is what is happens in gluconeoenesis, so it can be used as a non-carbohydrate source to produce glucose via glycogenesis

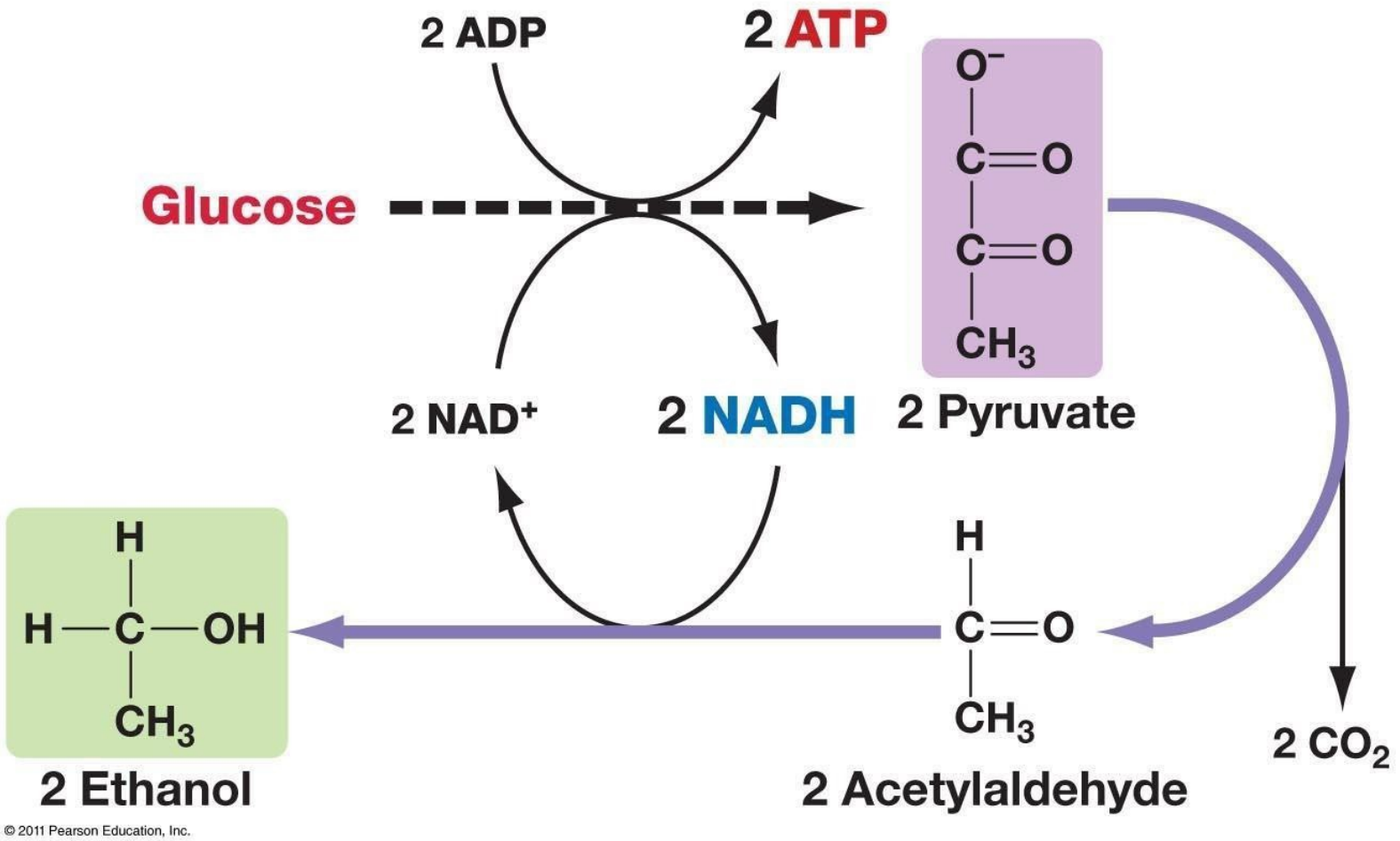
From Pyruvate to Ethanol

- **NOTE:** Another fate that doesn't occur in our cells but occurs in yeast cells is the decarboxylation of pyruvate to acetaldehyde and then reduce it to ethanol by alcohol dehydrogenase and convert NADH to NAD.+

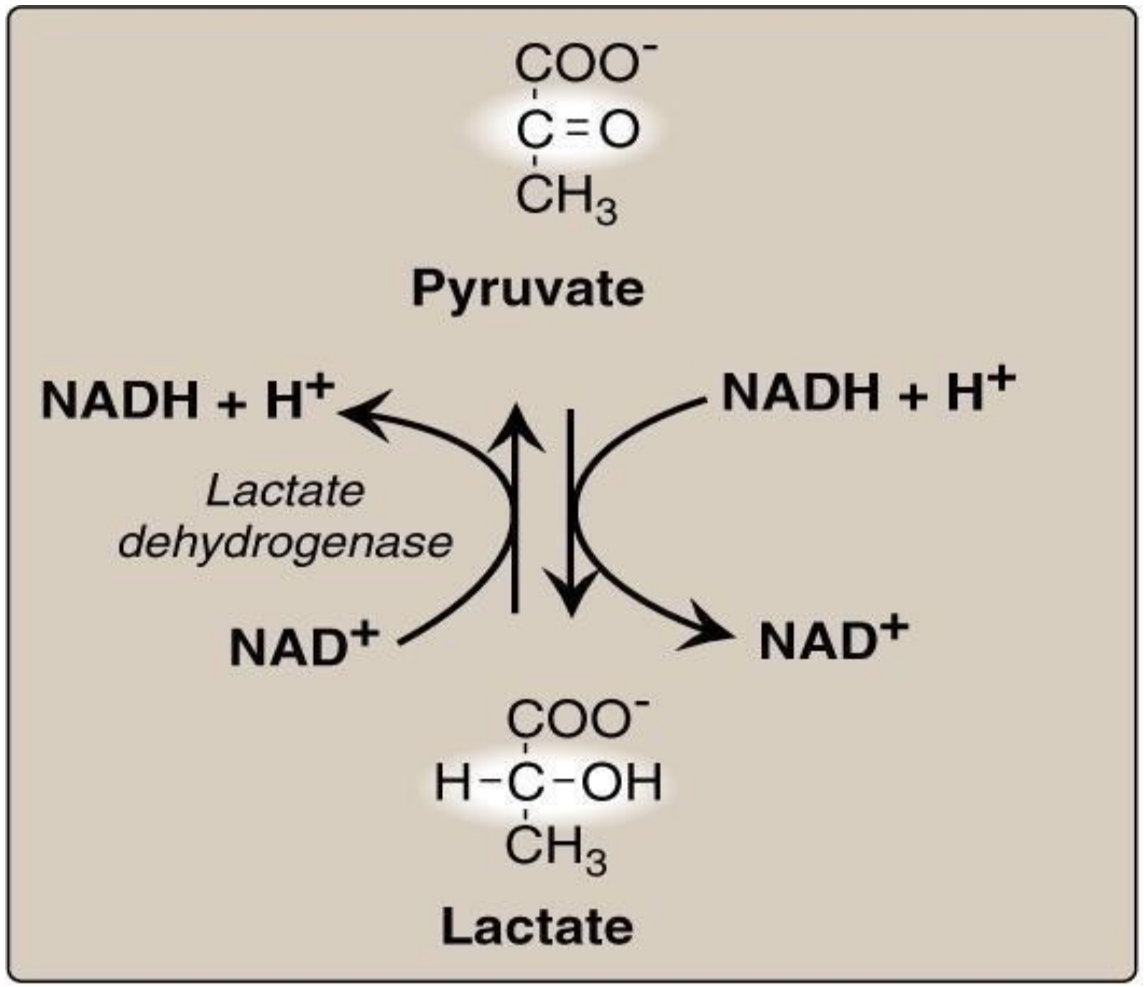


- **Additional information:** بصير بعملية تخمير العجين من خلال وضع السكر على الخميرة وبندفيها بماء ساخن او بجو ساخن وبنتركها فترة بمكان مناسب ,بعد فترة بتنفخ العجينة لانه الجلوكوز رح يتكسر ل **Pyruvate** الي بصير اله ازالة لمجموعة كربوكسيل وهذا التفاعل بنتج ثاني اكسيد الكربون الذي يعتبر غاز فبنتشر بس بلاقي مقاومة من العجينة فبتنفخ

(b) Alcohol fermentation occurs in yeast.



From Pyruvate to Lactate



- **NOTE: lactate dehydrogenase can reverse the reactions using co-enzymes, so if we want to convert lactate to pyruvate we need NAD+ while if we want to convert the pyruvate to lactate the enzyme will use NADH.**

Pyruvate + NADH ⇌ Lactate + NAD^+

When is Lactate Produced?

NOTE: it occurs mainly in RBCs (cels with low energy demand (there is no fatigue)The fatigue occurs mainly in the muscles as a result of lactate accumulation)

- Cells with low energy demand
- To cope with increased energy demand in rigorously exercising muscle, lactate level is increased 5 to 10 folds
- Hypoxia (Lack of oxygen)

to survive brief episodes of hypoxia

NOTE: lactate production doesn't happen in a resting muscle.

When the muscle starts to work frequently ,it will need high amounts of energy.

NOTE: in cases of hypoxia we cant carry out aerobic respiration because we have a limited amount of oxygen , therefore we carry out anaerobic respiration with the production of lactate

Clinical Hint: Lactic Acidosis

- ↓ pH of the plasma
- The most common cause of metabolic acidosis
  - ↑ Production of lactic acid
  - ↓ utilization of lactic acid

- Most common cause: Impairment of oxidative metabolism due to collapse of circulatory system.
  - Impaired O_2 transport
  - Respiratory failure
  - Uncontrolled hemorrhage

- **The complement in this slide:** whenever the concentration of lactate increases so much due to over production of lactate or reduced utilization of it, we can convert it to pyruvate and take advantage of it, but if it remained accumulated without making use of it , this will result in lactic acidosis.
- Lactate has a carboxyl grouo and an OH group , both are acidic groups, so it's an acid.

- **The complement in this slide:** reasons of lactic acidosis : Impaired O2 transport: any problem in the function of hemoglobin or the function of RBCs reduces the transport of oxygen, so there will be hypoxia in the tissue and thus there will be a higher dependance on anaerobic respiration and production of lactate resulting in increased ph.
- Respiratory failure: leads to ineffeciency in oxygen transport which result in relying on anaerobic respiration and production of lactate.
- Uncontrolled hemorrahage: results in loss of blood , hemoglobin and RBCs resulting in inefficient transport of oxygen and hypovolumic shock.

Clinical Hint: Lactic Acidosis

In all the body and fluid(intracellular and extracellular)

- Direct inhibition of oxidative phosphorylation
- Hypoxia in any tissue
- Alcohol intoxication (high NADH/ NAD+)
- ↓ Gluconeogenesis
- ↓ Pyruvate Dehydrogenase
- ↓ TCA cycle activity
- ↓ Pyruvate carboxylase

They are the same molecule, but they alternate between 2 states (oxidized or reduced)

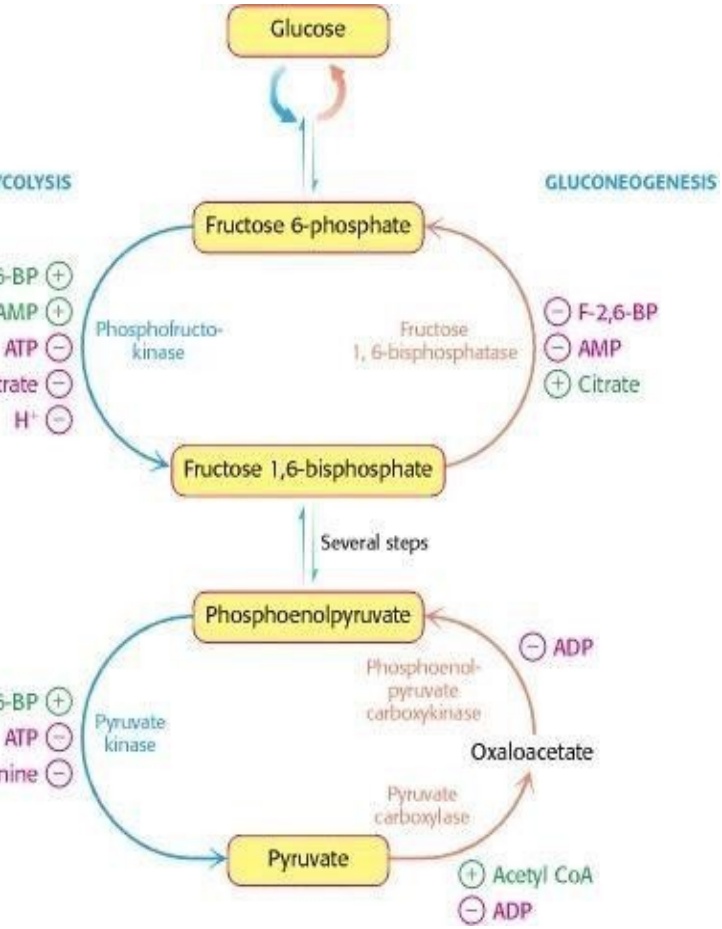
- **The complement in this slide:Direct inhibition of oxidative phosphorylation: a mutation in any complex of the ETC or a problem in it's function results in the inhibition of oxidative phosphorylation, so aerobic respiration doesn't work (kreb's cycle will works, but it produces just one energy molecule which is GTP, so it is a small amount as the majority of energy comes from ETC, so this results in less production of ATP and more rely on anaerobic respiration).**
- **Alcohol intoxication(high NADH/NAD+) :the people who drink alcohol will metabolise in several ways, one of the most important way is oxidation of alcohol to acetaldehyde and this reaction is associated with the reduction of co-enzyme NAD + to NADH, so the ratio NADH/NAD+ increases which resuls in the inhibition of many reactions and one of the most important reactions to be inhibited is kreb's cycle because it needs NAD+, so there is less kreb's running , less energy production from aerobic respiration, so we go to anaerobic respiration.**
- **Decreasing of gluconeogenesis :it is the opposite pathway of glycolysis in which we produce glucose from non-carboydrate sources because we are under fasting conditions as in them we don't have carbohydrates to use them as source sof energy, so we are obligated to use these sources to supply the brain and tissues that are exclusively dependent on glucose as a source of energy.**
- **If it decreased (gluconeogenesis) the pyruvate will accumulate, so this will activate anaerobic respiration resulting in more lactate production.**

- **The complement in this slide:Decreasing of pyruvate dehydrogenase: the enzyme that converts pyruvate to acetyl CoA which enters kreb's cycle, so when we decrease it, we turn off this way and go to anaerobic respiration.**

- **Decreasing of TCA cycle activity: the absence of co-enzymes or a mutation in any enzyme results in less energy production by aerobic respiration, so it's more dependent on anaerobic respiration to produce energy.**

- **Decreasing of pyruvate carboxylase: which converts pyruvate to oxalacetate, so when it is reduced, more pyruvate accumulates, so it's going to head towards anaerobic respiration.**

Regulation of Glycolysis

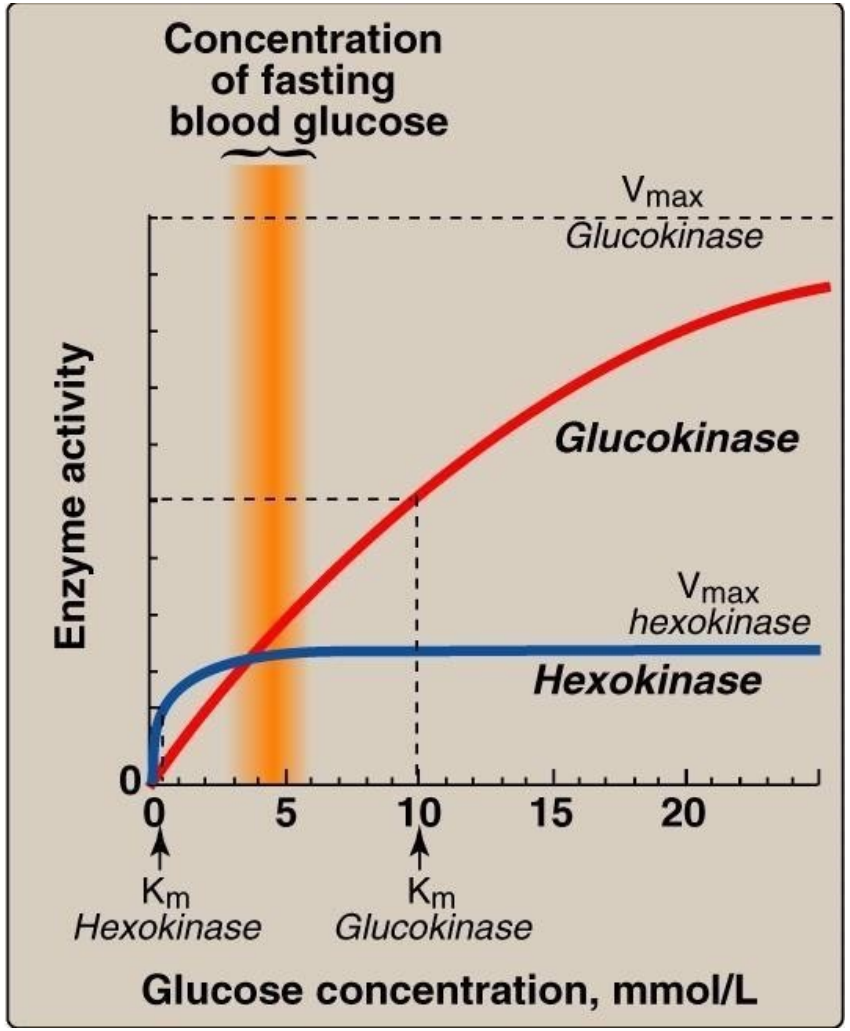


- **NOTE:** in this photo we see glycolysis and its opposite process gluconeogenesis

- **NOTE:** regulation of glycolysis occurs at the three irreversible steps) the steps that include hexokinase or glucokinase, phosphofructokinase-1 and pyruvate kinase.)

- **NOTE:** you will generally notice that the activator of a certian enzyme in glycolysis will be an inhibitor for the opposite enzyme in gluconeogenisis ,this ensures that these two opposite pathways dont operate at the same time.

Regulators of PFK and PK



Glucokinase and Hexokinase Activity

- **NOTE:** the X-axis represents glucose concentration while the Y-axis represents the enzymatic activity

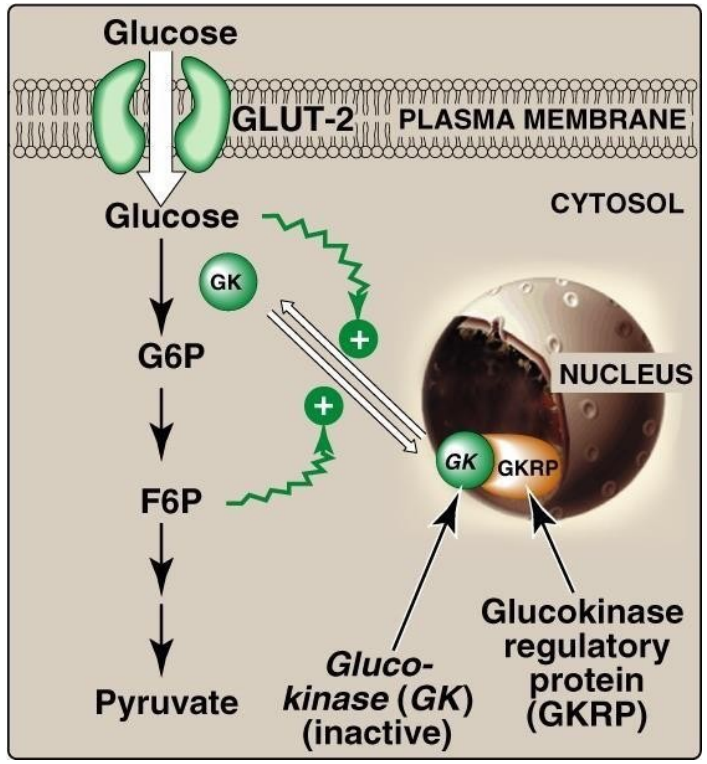
- **NOTE:** notice that the km of hexokinase is much lower than that of glucokinase

- **NOTE:** the orange region represent the fasting blood sugar , which means that when we eat a meal , the blood sugar increases alot and in two hours time insulin will be secreted in a sufficient amount to get most of this sugar into the cells and keeping a minimum level which is the fasting blood sugar

- **The complement in this slide:** so if you were fasting for two hours or have last eaten 10 hours ago or even 15 hours ago , once you measure your blood sugar level, it must fall within the range of the fasting blood sugar

- **NOTE:** at fasting conditions , hexokinase is active, working and reaching it's vmax despite low blood sugar levels while glucokinase has a much lower activity at these same conditions , however when we have a much higher concentration of glucose , glucokinase takes over (but note that hexokianse remains active too at these high glucose concentrations)

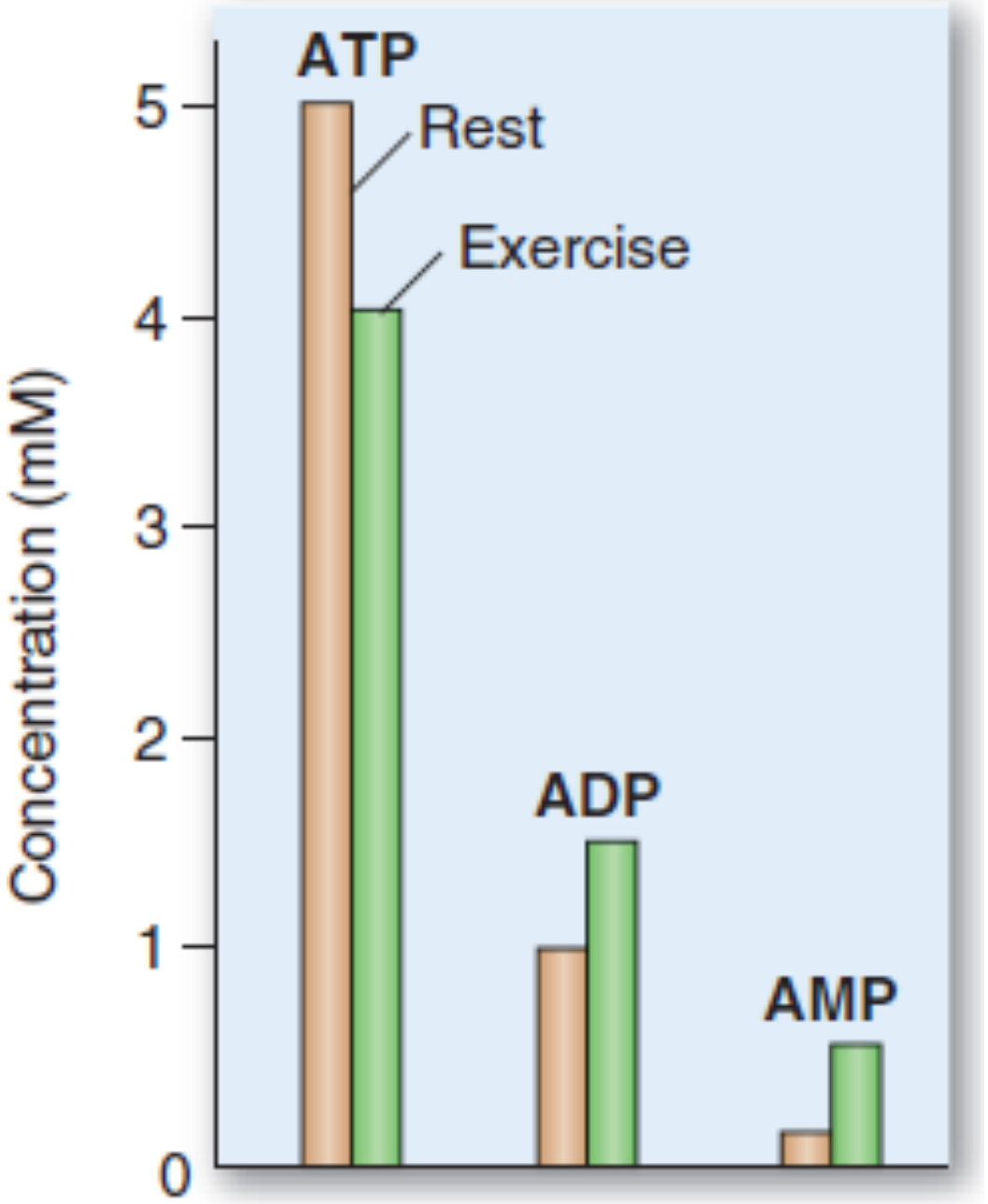
Glucokinase Regulation



- **NOTE:** glucokinase is regulated differently, when it's inactive (in cases of low glucose concentrations), it's going to be found in the nucleus bound to it's regulatory protien called glucokinase regulatory protien (GKRP) which captures glucokinase and traps it so that it will be sequestered in the nucleus

- **The complement in this slide:** now once glucose concentrations increase after eating a meal and it gets digested and absorped so it can be uptaken after insulin secretion by GLUTs , it will enter the cell resluting in increased glucose concentrations within this cell, now this glucose will activate the seperation of glucokinase from GKRP, and once it's seperated , it can move out of the nucleus to start phospholyrating glucose to glucose-6-phosphate resulting in further activation of glycolysis

- **NOTE:** once the concentration of fructose-6-phosohate becomes really high due to glycolysis which means that the concentration of glucose becomes low, the sequestration will be activated resulting in inhibiton of glucokinase and its transfer back to the nucleus



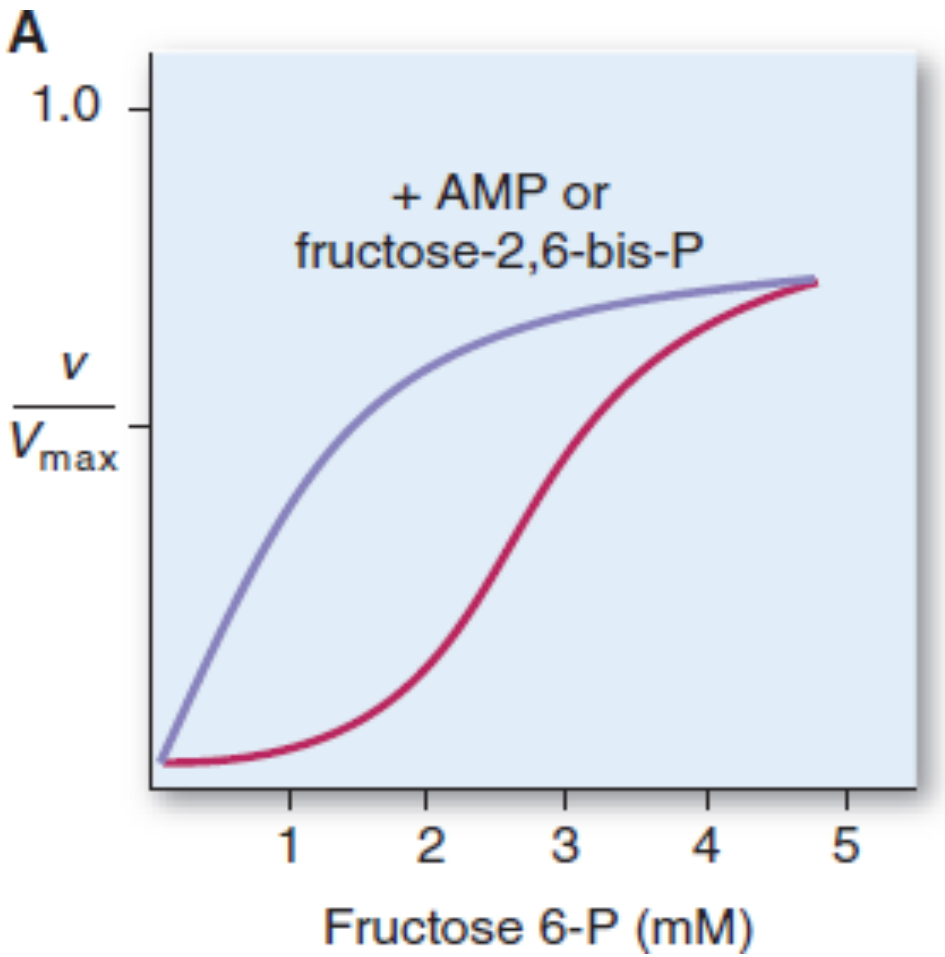
Regulation by ATP and AMP

- **NOTE:** if we want to compare the levels of ATP with AMP or ADP under different conditions , rest vs exercise , we will notice that ATP levels in the rest state are much higher than its levels in the exercise state whereas ADP and AMP are the opposite as they increase in concentration in cases of exercise

- **NOTE:** both ADP and AMP reflect a very low energy state , and that's why they activate the enzymes of glycolysis while ATP reflects a high energy state thus inhibiting these enzymes

$$ADP + ADP \rightleftharpoons ATP + AMP$$

Regulation of PFK by Fructose 2,6- bisphosphate



- **NOTE:** **phosphofructokinase-1 is activated by allosteric regulators like:**
  1) **Fructose-2,6-bisphosphate**
  2) **AMP**

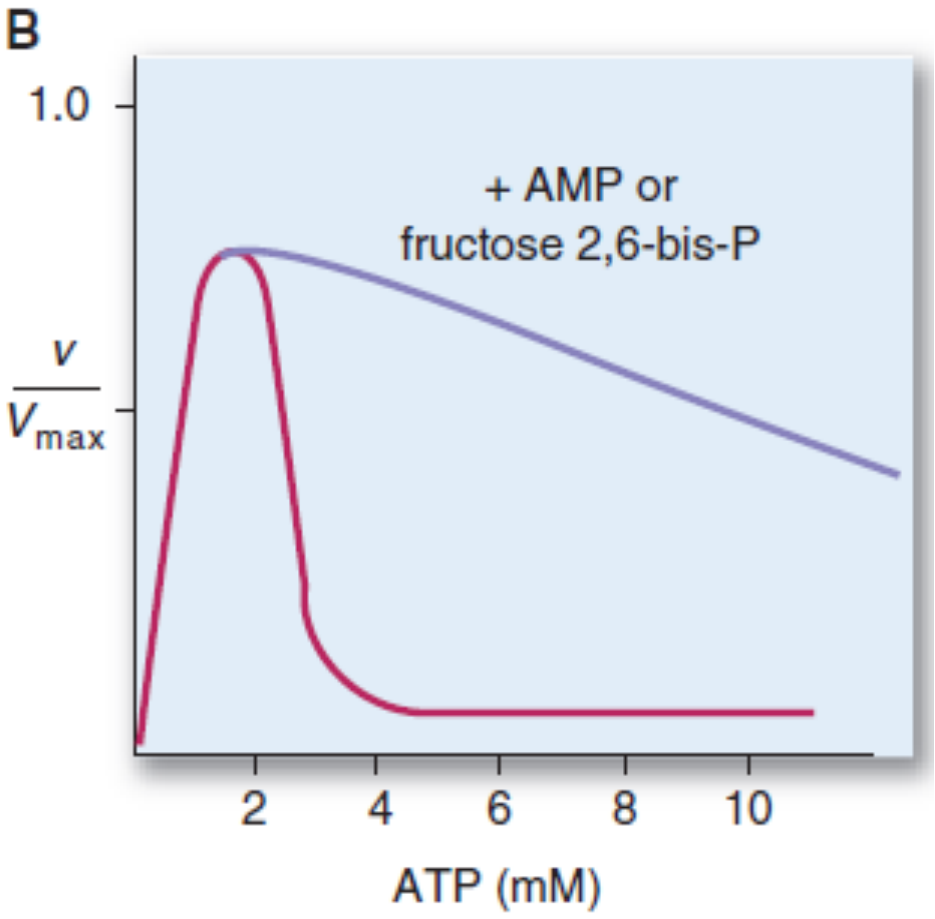
- **NOTE:** **AMP is an indicator of a low energy state because it represents increased breakdown of ATP ,so it will activate glycolysis to produce ATP**

Fruc. 6-phosphate + ATP ⟶ Fruc. 2,6 bisphosphate + ADP

- **The complement in this slide:** look at the graph in the previous slide :if you wanted to blot the substrate concentration vs velocity , you will get the red curve , but once you add AMP or fructose-2,6-bisphosphate , the curve was shifted to the left meaning that at a lower concentration you could achieve a much higher velocity
- For example if you took 1mM fructose-6-phosphate the velocity of PFK would be very low without the presence of an activator whereas it reaches vmax/2 with the activator at the same concentration

- **NOTE:** You don't need both AMP and fructose-2,6-bisphosphate in order to increase the velocity , meaning that you don't have you add both activators to cause the acceleration as either of them will give you the same effect once added

How about the other substrate?



- **NOTE:** ATP acts as an inhibitor for PFK because it indicates a high energy state so there's no need to break down glucose to produce energy

- **NOTE:** citrate is also an inhibitor because increased concentrations of it indicates a very active krebs cycle so a lot of energy is being produced and there's no need to activate glycolysis

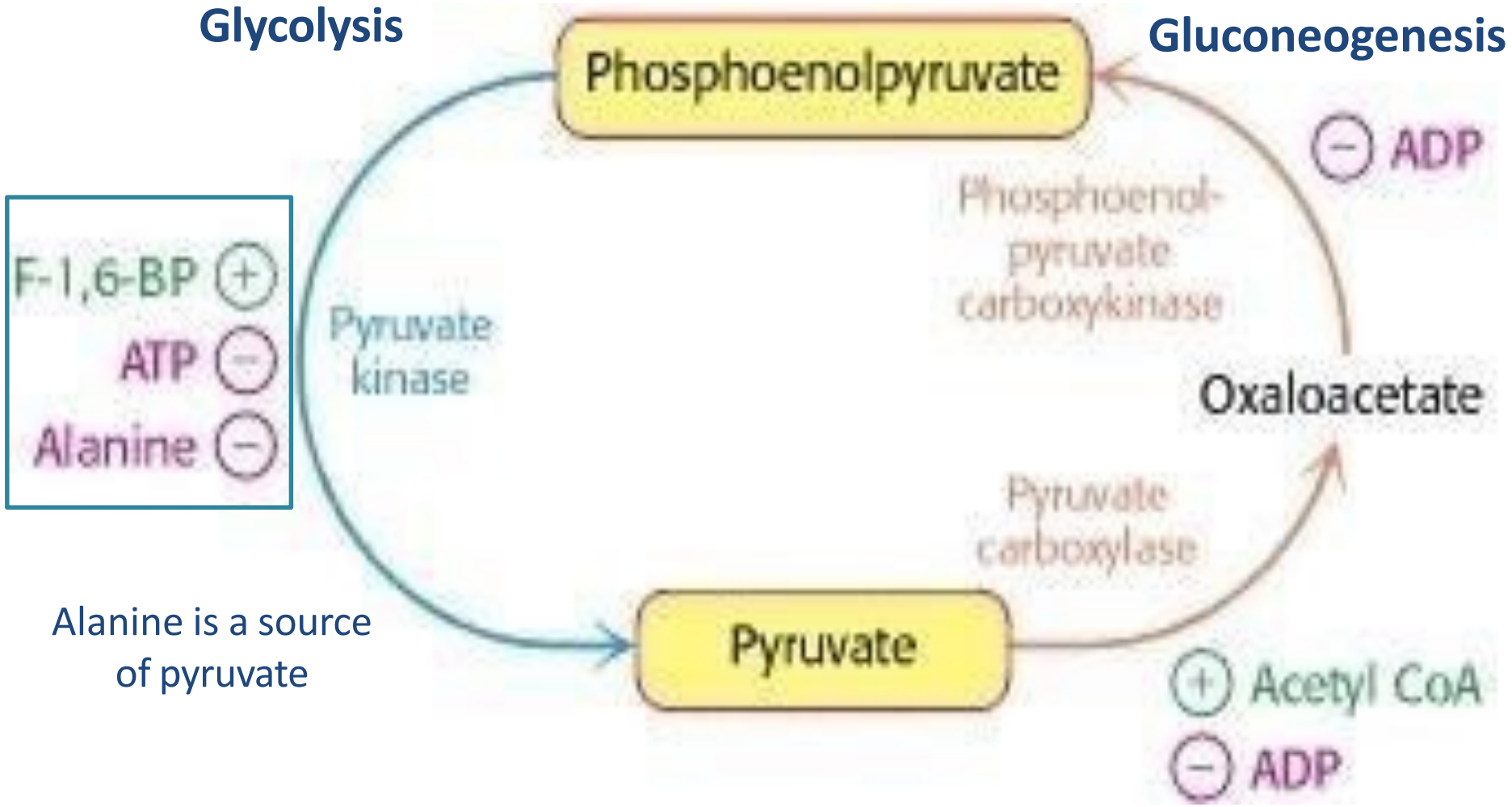
- **NOTE:** protons are also inhibitors because once we have high concentrations of them ,it means that the ETC is working with high energy production so we don't need to activate glycolysis

- **NOTE:** now if you blot the same reaction) which is the phospholyration of fructose-6-phosphate, (but you look at the concentration of the second substrate which is ATP as we want to take a phospate from it) look at the next slide , (you will notice in the beginning at low concentrations the velocity increases
- Now note that ATP has a dual function in this case ,meaning that at first it can as substarte at low concentrations increasing the velocity of the reaction , but at a certain point , the concentration of ATP becomes really high and thus now ATP will act as an allosteric inhibitor inhibiting PFK

- **NOTE:** so ATP is both an inhibtor and a substrate for PFK

- **NOTE:** in the presene of AMP or fructose-2,6-bisphosphate , the decrease in the velocity is slower , meaning that the enzyme is capable of tolerating high concentrations of ATP without reducing the velocity by much

Regulation of Pyruvate Kinase



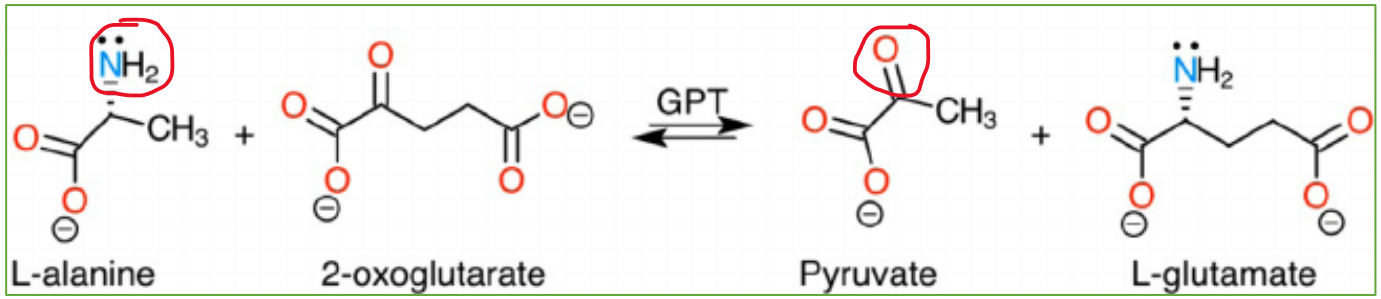
Alanine is a source of pyruvate

- **The complement in this slide:** pyruvate kinase is activated by the product of the third step of glycolysis which is fructose –1,6-bisphosphate .
- Note that the product of the third step activates an enzyme in the last step , this is what we refer to as **feed forward activation**

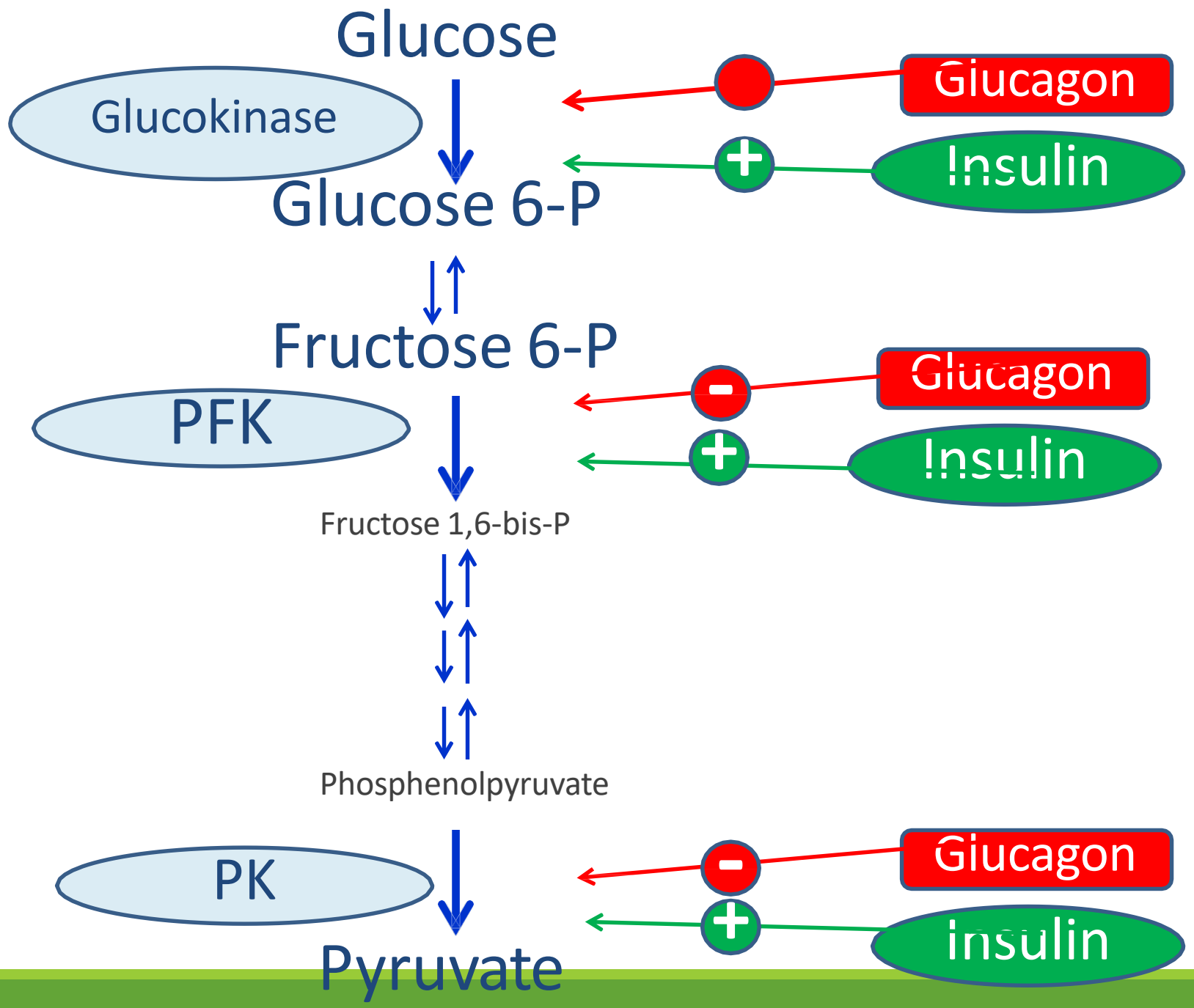
- **NOTE:** ATP indicates high energy state so it inhibits this enzyme

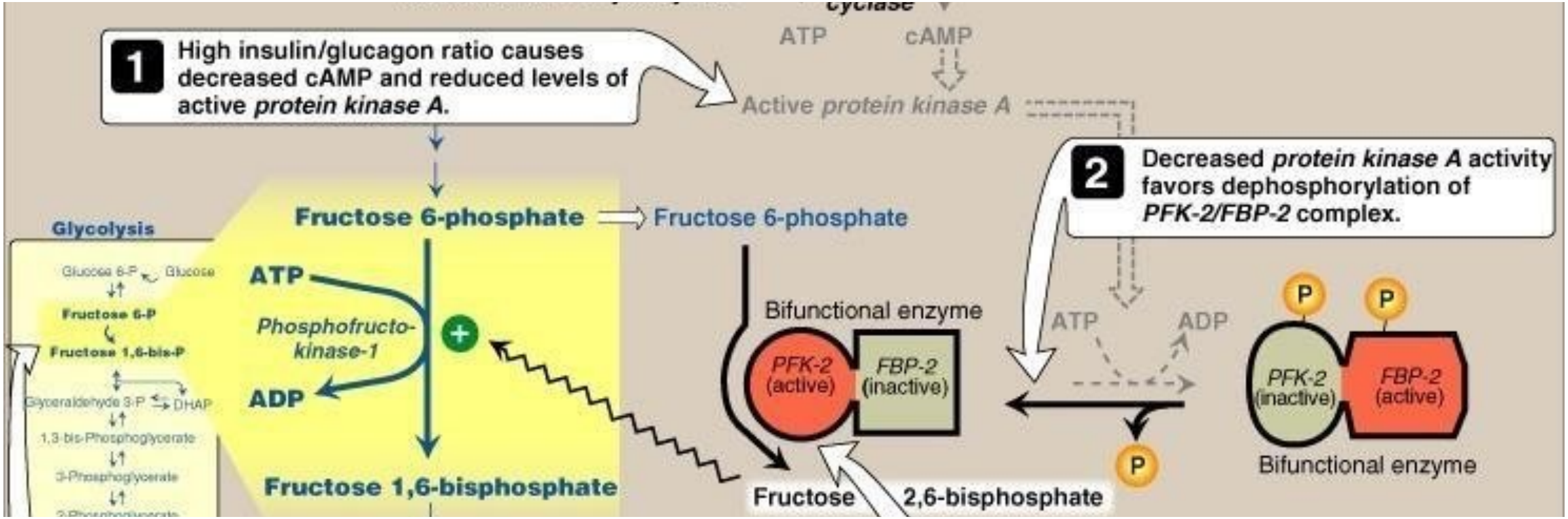
- **NOTE:** if you look at the structure of alanine and you removed the amino group from it , it gives us pyruvate , so pyruvate is the alpha keto acid of alanine which means that alanine is a source of pyruvate so increased concentrations of alanine give us more pyruvate so there's no need to activate further pyruvate production by glycolysis , so alanine inhibits the pyruvate kinase enzyme

- **NOTE:** this photo is only for clarification of the interconversions between alanine and pyruvate



Hormonal Regulation





Hormonal Regulation of Phosphofructokinase

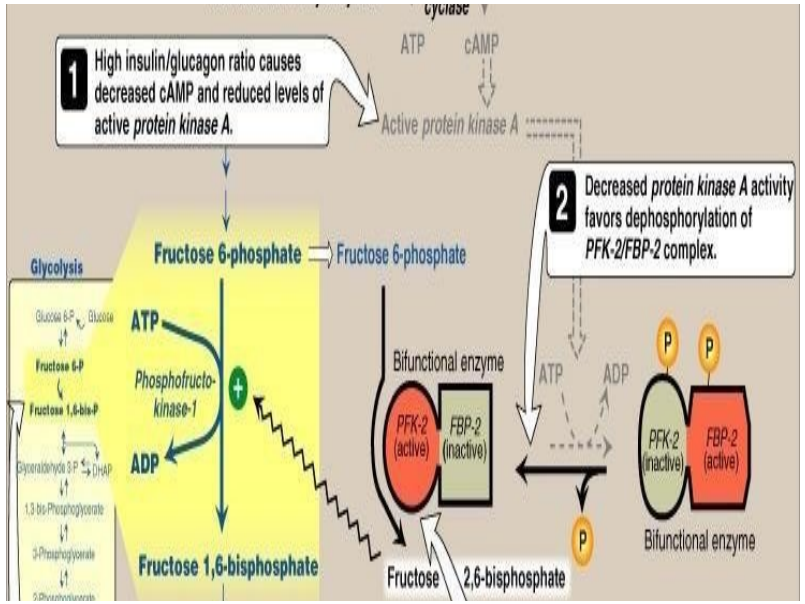
- **The complement in this slide:** the hormones that regulate sugar levels are insulin and glucagon

- **NOTE:** how does insulin affect glycolysis ?
  - It increases it , because insulin is secreted in cases of high blood sugar levels to increase sugars uptake into the cells , therefore we will have high concentrations of sugars in the cells so they will start using these sugars by directing them to glycolysis

- **NOTE:** as long as we have sugar in our cells , it's going to be the major source of energy whatever the type of cell

- **NOTE:** generally speaking , insulin activates the three irreversible steps , but don't think that insulin will directly bind to hexokinase or glucokinase or phosphofructo kinase or even pyruvate kinase to activate them , instead the regulatory mode occurs through signaling pathways

- **The complement in this slide:** now lets see how the signaling happens during regualtion



- **NOTE:** in this photo , the concentration of insulin is high while the concentration of glucagon is low , so we are at a well fed state
- Insulin will bind to it's receptor which is receptor tyrosine kinase(RTK (glucogon's receptor) which is not present in the current state that we are discussing and that's why the funtions that it preforms is present faintly in the photo (is G-protien coupled receptor (GPCR) , GPCR stimulates CAMP production that activates protien kinase A which will start phospholyration it's target protiens.
- In the photo we have there will be no phospholyration of the target protiens

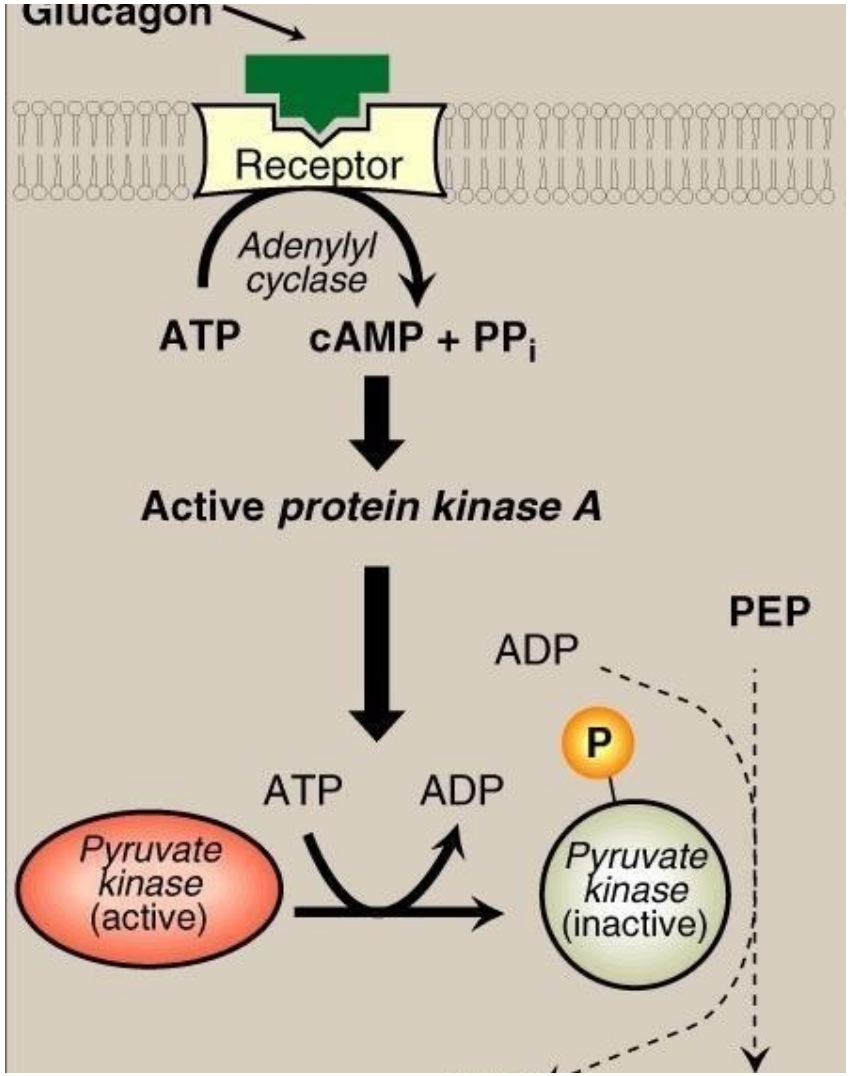
- In the photo we have one of protien kinas A target protiens , its called a bifuntional enzyme which an enzyme that has two sides ,one side acts as a kinase (phosphofructokinase-2), while the other side acts as a phosphatase(fructose-2,6-bisphosphate phosphatase(

- **The complement in this slide:** the kinase side will phospholyrate fructose-6-phosphate to fructose-2,6-bisphosphate which is a regulatory molecule and not a glycolytic intermediate , while the phosphatase side will remove the phosphate from fructose-2,6-bisphosphate converting it back to fructose-6-phosohate

- **NOTE:** logically , one of the two sides or enzymes must be active at a time so that we don't have phospholyration and dephospholyration happening at the same time !

- **NOTE:** now let's go back to discussing our case in which we don't have a phospholyration of the target protien by protien kinase A , here the phosphatse is inactive while the kinase is active , meaning that we are producing fructose-2,6-bisphosphate which is a regulator of phosphofructokianse-1 , so it will bind to it and activate it increasing the activity of glycolysis in cases of high insulin concentrations

- **NOTE:** now if we assume the opposite state) opposite to the photo : (
  - We will have low insulin and high glucagon levels) fasting conditions, (the pathway that is faint in the photo is now active leading to the phsopholyration of the bifunctional enzyme so now the kinase is inactive and the phosphatase is active so it dephospholyrates fructose-two,six-bisphosphate to fructose-six-phosphate and in this case the activator of glycolysis is absent so there's no activation of glycolysis.



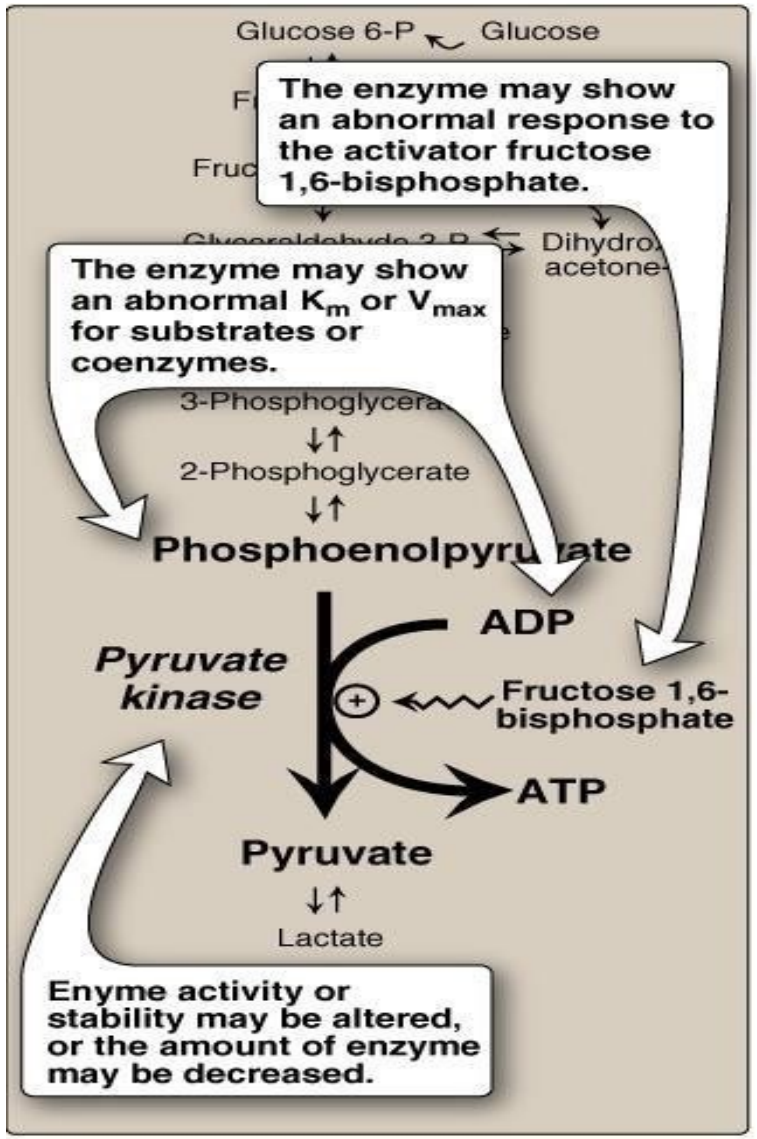
Hormonal Regulation of Pyruvate Kinase

- **The complement in this slide:** another target of protien kinase A is pyruvate kinase

- **NOTE:** in the photo present in the previous slide we are at fasting conditions where glucagon levels are high
- The phospholyration of pyruvate kinase results in it's inhibtion , why?
  - At fasting conditions ,blood sugar levels are low so pyrvate production is lower so pyruvate kinase activity is inhibited

Clinical Hint: Pyruvate Kinase Deficiency

- The most common among glycolytic enzyme deficiencies
- RBCs are affected
- Mild to severe chronic hemolytic anemia
- ATP is needed for Na+/K+ pump➔ maintain the flexible shape of the cell
- Low ATP ➔ premature death of RBC
- Abnormal enzyme; mostly altered kinetic properties



Alterations observed with various mutant forms of pyruvate kinase

- **The compliment in this slide:** pyruvate kinase is the most commonly affected glycolytic enzyme by mutations

- **NOTE:** mutations leading to it's defeciency result in the production of zero ATP in the last step of glycolysis , energy may even be consumed

- **NOTE:** the most affected cells by this are RBCs

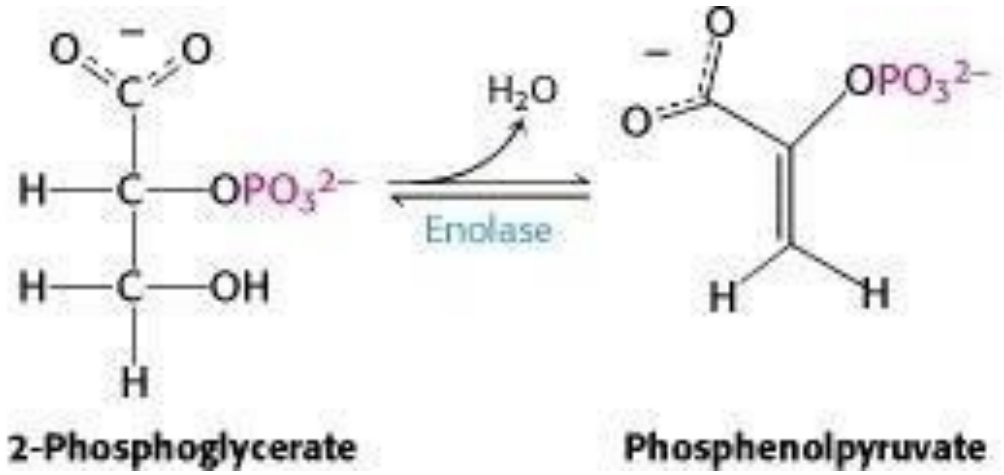
- **NOTE:** one of RBCs needs of energy is to regulate or preform the function of sodium-potassium pump , and sodium-potassium pump by doint its function will mantain the shape of the RBC , so ATP means that this pump won't be able to preform it's function in mantainig the shape of the RBC leading to the premature death of the RBC) before 120 days (as in the cases of sickle cell anemia

External Inhibitors of Glycolysis

Inorganic Inhibitors of Glycolysis Fluoride

NOTE: **fluoride may be present in toothpaste**

- Fluoride inhibits Enolase



Fluoridated water → ↓ bacterial enolase →

Prevention of Dental Carries

Inorganic Inhibitors of Glycolysis

Arsenic Poisoning

–Pentavalent Arsenic (Arsenate) competes with phosphate as as a substrate for GA3PDH

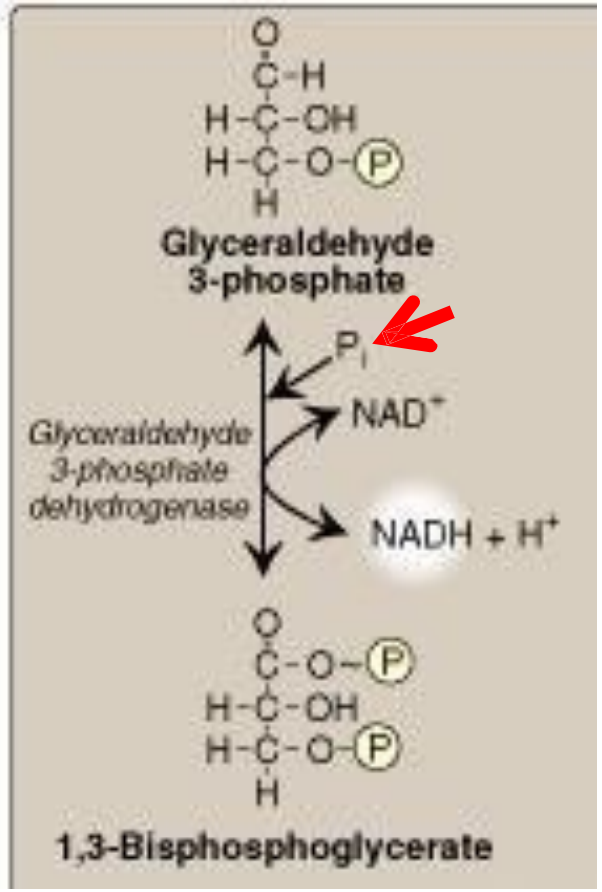
↓ ATP synthesis

–Trivalent Arsenic (Arsenite) Forms stable complex with -SH of lipoic acid

↓ Pyruvate Dehydrogenase

↓ α ketoglutarate Dehydrogenase

➔ Neurological disturbances…….**DEATH**



اللهم إنا نسألك أن تنصر أهل غزة، وتنجيهم من يد اليهود وسطوتهم واجعل خلاصهم قريبا ونجاتهم غير مستحيلة وانتقم شر الانتقام من أعدائهم .اللهم انصرهم وثبت اقدامهم وسدد رميهم يارب العالمين إنك على كل شيء قدير اللهم غزة وأهل غزة وأراضي وأطفال ورجال غزة.

اللهم الطف باخواننا المسلمين المستضعفين في فلسطين.

اللهم كن لهم مؤيدًا ونصيرًا ومعينًا وظهيرًا ،اللهم اجعل لهم من كل همٍّ فرجًا ومن كل ضيق مخرجًا ومن كل بلاء عافية