DOCTOR 2020 | JU

METABOLISM

WRITER : LANA KHABBAS

CORRECTOR : ALI ALMAHROOK

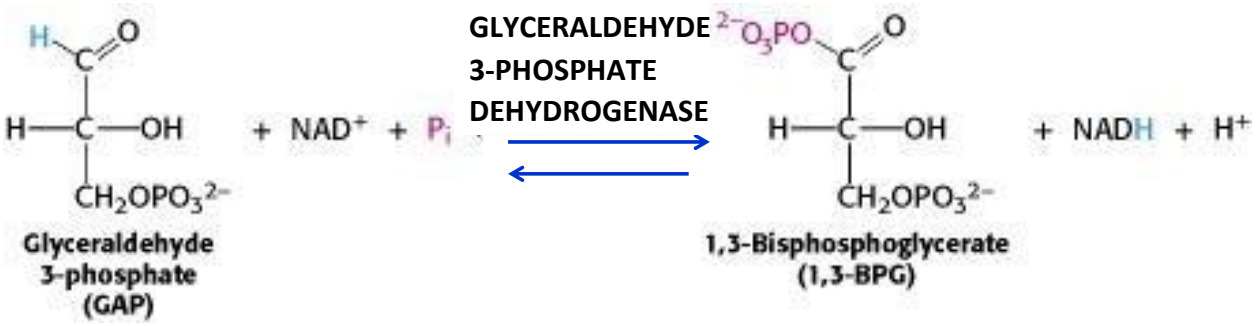
DOCTOR: DIALA ABU-HASAN

ملاحظة تحفيزية:الشيت قد يبدو طويل و ممل لكنه سهل "جدا"

In this sheet, we're going to continue glycolysis steps, regarding the last step we talked about (step 5) in which (Fructose 1,6- bisphosphate) is cleaved to two 3-carbon molecules: DHAP&GAP, and we said that these two covert to the other frequently, but the one needed to complete this glycolytic catabolic pathway is (GAP) Glyceraldehyde 3-phosphate.

- **Note from the last lecture: DHAP&GAP will be equally produced, and we already know that GAP will proceed in the pathway, thus it'll get depleted, so equilibrium will be shifted from DHAP to GAP, meaning that everything will covert to GAP.**

STEP 6

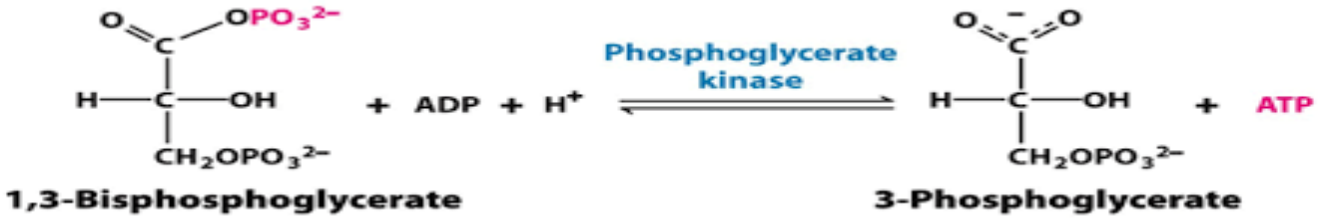


- **Here GAP gets oxidized by losing the carbonyl hydrogen that will be replaced by a phosphate group.**
- **NOTE HERE THAT WE DIDN'T USE ATP, we used Pi (inorganic phosphate) that's present as a free group in the cytosol.**
- **Further speaking, oxidation doesn't occur without reduction, so NAD^+ will get reduced to form NADH.**

✓ **WHAT WE SHOULD KNOW FROM THIS STEP!**

- **1.GAP forms 1,3 Bisphosphoglycerate after acquiring a phosphate group on C1**
- **2.A redox reaction happened, by losing the carbonyl hydrogen to NAD^+ that became NADH.**
- **3.Thus the enzyme catalyzing the rxn is named GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE.**
- **4.This step is reversible.**

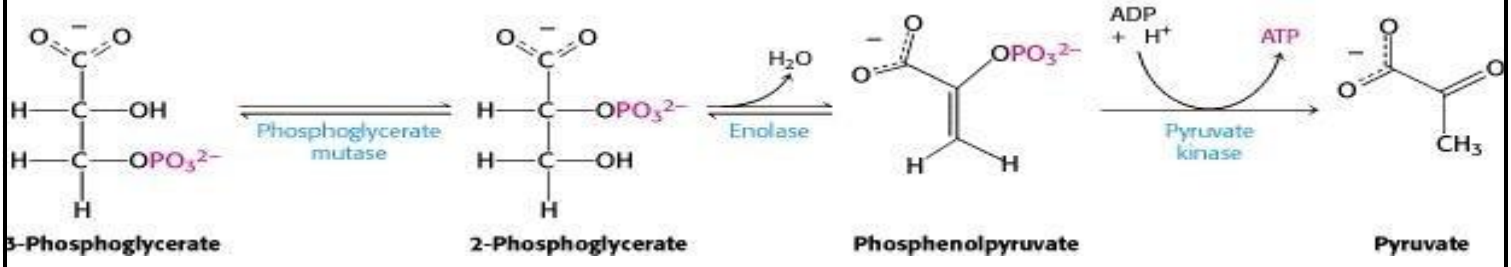
STEP 7



Next, we want to get out the phosphate group attached to C1 in (1,3-bisphosphoglycerate) of course, it was attached without energy consumption, because the bond containing it is high-energy (between Pi&C1), so we extract this energy by the help of the enzyme GLYCERATE KINASE, which catalyzes Pi attachment to an ADP molecule producing ATP&(3-phosphoglycerate.)

- ✓ **WHAT WE SHOULD KNOW FROM THIS STEP!**
  - **1,3-bisphosphoglycerate gets converted to 3-phosphoglycerate, by extracting the Pi on C1.**
  - **2.This phosphate group reacts with ADP forming ATP**
  - **3.Thus the name of the enzyme is glycerate kinase.**
  - **4.This step is reversible.**

STEP 8-10



In these steps, we're trying to extract the remaining phosphate group in 3-phosphoglycerate, but it can't be directly withdrawn, so it needs to be isomerized to 2-phosphoglycerate by phosphoglycerate mutase (a type of isomerase), then 2-phosphoglycerate is converted to phosphoenolpyruvate by the action of ENOLASE (so-called because it introduces a double bond in the structure, by extracting a water molecule from 2-phosphoglycerate).

Then the modification that will happen is removing the phosphate group and moving the double bond from between the carbons to between the carbon and the oxygen that was attached to the phosphate (C=O carbonyl), by the enzyme PYRUVATE KINASE. However, ADP gets phosphorylated to form ATP, thus named kinase, with its substrates being ADP&phosphoenolpyruvate at the same time. Although phosphoenolpyruvate lost its phosphate, but ADP gained it.

PHOSPHOENOLPYRUVATE:

PHOSPHO-BECAUSE OF THE PHOSPHATE GROUP

ENOL: BECAUSE OF THE DOUBLE BOND.

- This enzyme is one of the few enzymes that consider ADP the substrate, not the other compound.
- NOTE: The last step (phosphoenolpyruvate to pyruvate) is IRREVERSIBLE!!

✓ WHAT WE SHOULD KNOW FROM THIS STEP!

- 1.We can't remove the phosphate from 3-phsophoglycerate directly, so we should isomerize it to 2-phosphoglycerate, by PHOSPHOGLYCERATE MUTASE.
- 2.To form pyruvate, we should introduce a double bond in the compound, and this is done by ENOLASE! Which withdraws water from the previous compound producing PHOSPHOENOLPYRUVATE.
- 3.We are only a step far! The phosphate group now can be removed and gained by ADP to form ATP,by the action of PYRUVATE KINASE, and pyruvate is simultaneously generated by translocating the double bond.

As you remember, in the FIRST PHASE of glycolysis we consumed 2ATP during phosphorylation of intermediates. In the SECOND PHASE,2ATP were produced (step 7&10), in addition to 1NADH from oxidation-reduction rxns(step 6)
NOTE THAT THIS HAPPENS TO 1 GAP!

But we are talking about glucose(6C), 2GAP (3C) are produced, so everything must be doubled! {IN STEPS 6-10}
2ATP become 4ATP
1NADH becomes 2NADH

As a result, a net of 2ATP is formed in glycolysis (after subtracting the 2ATP consumed) + 2NADH (which -after entering ETC- generate approx. 6ATP). Thus, 8ATP is the amount of energy produced by glycolysis. Though being less than the amount generated from Krebs and ETC, but it's considered important.

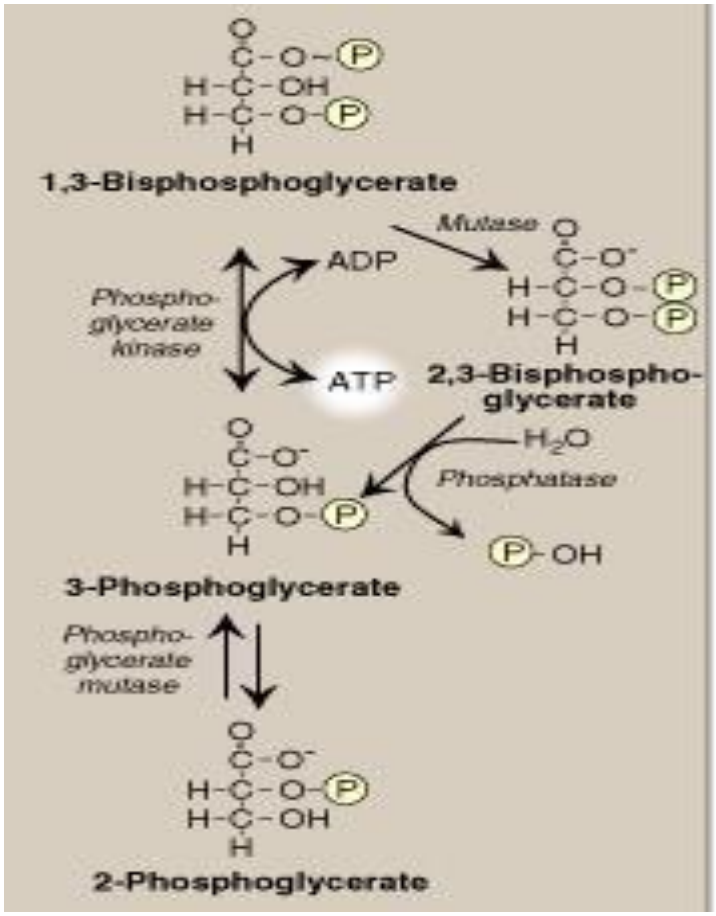
- **The main progress of ATP production is initiated after glycolysis by pyruvate(3C) conversion to Acetyl-CoA(2C) that gets into Krebs cycle.**

Glycolysis in RBCs

Glycolysis somehow gets modified in RBCs.

Notice that even RBCs –which lack nuclei and mitochondria- can carry out glycolysis,which indicates that this pathway is universal.

The shunt happens in the step where 1,3-Bisphosphoglycerate converts to 3-phosphoglycerate.This step isn't performed by RBCs.Instead,2 steps occur,ending up with the production of 3-phosphoglycerate,then the regular pathway proceeds.



Actually what happens is that 1,3-Bisphosphoglycerate gets converted by a mutase to 2,3-Bisphosphoglycerate.

By transferring the phosphate of C1 to C2

Then the phosphate on C2 leaves as a free group by a phosphatase-WITHOUT PHOSPHORYLATION OF ADP TO FORM ATP,producing 3-phosphoglycerate.

- **Note here that we produced the same product as the ordinary glycolysis,but we lost an ATP molecule (we didn't form it).**

- **THE VERY IMPORTANT QUESTION here is how can RBCs that lack mitochondria-the energy factory of any cell-,thus can't depend on ETC to produce ATP,do without ATP produced from this pathway? -though not having enough alternatives to produce energy.**

  Before answering this question,let's analyze the scenario of glycolysis in RBCs.

  According to the alternative pathway RBCs underwent to produce 3-phosphoglycerate,there is no energy produced (the NET ATP of glycolysis is 2 ATP(2 phosphoenolpyruvate to 2 pyruvate) minus 2ATP consumed in the FIRST PHASE in phosphorylation rxns,which equals zero).Although there are 2 NADH in the outcome,but they aren't utilized by ETC to produce energy.So glycolysis is nothing to RBCs,in terms of energy!!!

- **NOW,LET'S ANSWER OUR QUESTION!WHAT'S THE REASON OF THIS SHUNT?**

  The situation is that RBCs contain hemoglobin that has an essential function of high-affinity binding to oxygen in oxygen-rich tissues(e.x:lungs,where frequent gas change occurs),then it's going to travel through the bloodstream reaching capillaries,where the affinity of oxygen has to change(decrease) in order to release oxygen to low-oxygen tissues.So when oxygen is released the affinity should be low,especially when transforming to deoxyhemoglobin –where it doesn't bind oxygen-

  Broadly speaking,2,3-Bisphosphoglycerate binds to deoxyhemoglobin decreasing its affinity to oxygen and preventing it from binding again.Thus being released to tissues.

  This is one factor that contibutes to the reduction of affinity of hemoglobin to oxygen close to tissues.Remember other factors related to the structure of the heme itself,containing Iron ions(ferrous ions) that induce conformational changes affecting oxygen affinity.

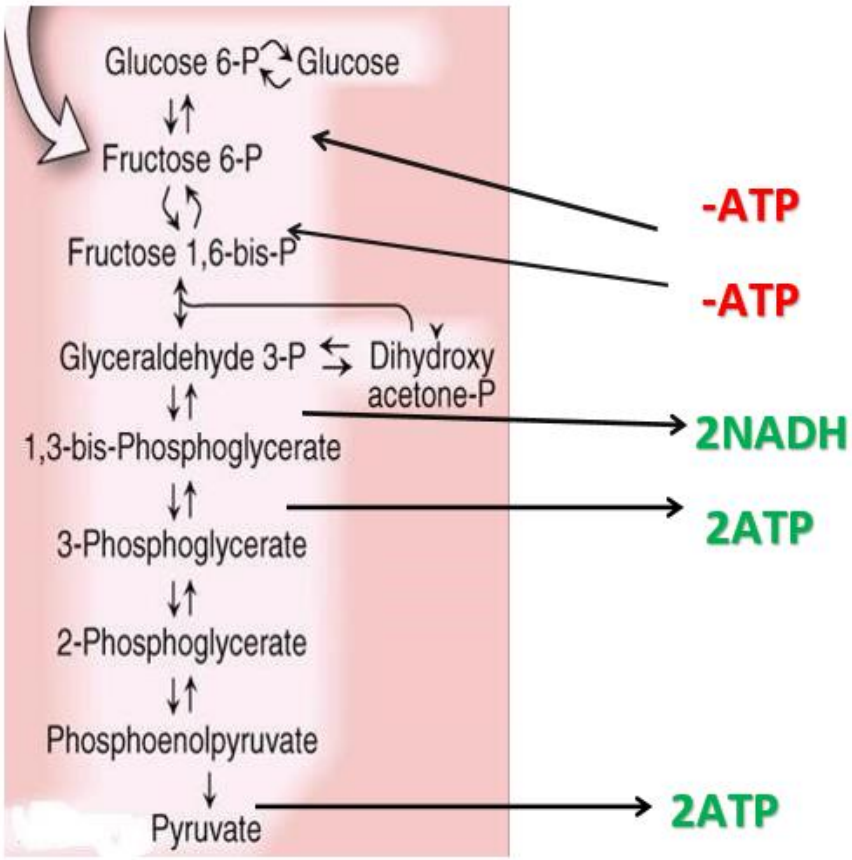
- **Still!! We have partially answered our main question,now how do RBCs gain their energy needs?provided that glycolysis doesn't result in a benefit,so what provides energy??**

  ANAEROBIC RESPIRATION.Since RBCs are low-energy demanding cells,having a low metabolic rate,so they don't need large amount of energy,thus it's fine by this pathway.

- **DON'T MISTAKENLY SAY THIS CAUSES FATIGUE!IT'S NOT A MUSCLE!!**
  - **The situation is different in muscles,because when they depend on anaerobic respiration,they produce big amounts of lactate,thus fatigue occurs.But here,RBCs don't have this pathway aggressively activated.In fact,lactate accumulation and fatigue don't occur in any muscle,but exercising muscles!**

- **AT THIS POINT,LET'S SUMMARIZE WHAT WE'VE TAKEN!**
  - **We have 2ATP consumed due to phsophorylations.**
  - **We have 2NADH from oxidation-reduction rxns.**
  - **We have 2ATP produced per one GAP (from 1,3-Bisphosphoglycerate to 3-phosphoglycerate&from phosphoenolpyruvate to pyruvate),thus 4ATP are produced from one glucose.**
  - **So we have a NET PRODUCTION OF ATP (4-2=2).**
  - **Notice that oxygen hasn't shown up in any step! Emphasizing that glycolysis can take place all the time,regardless of oxygen presence.**

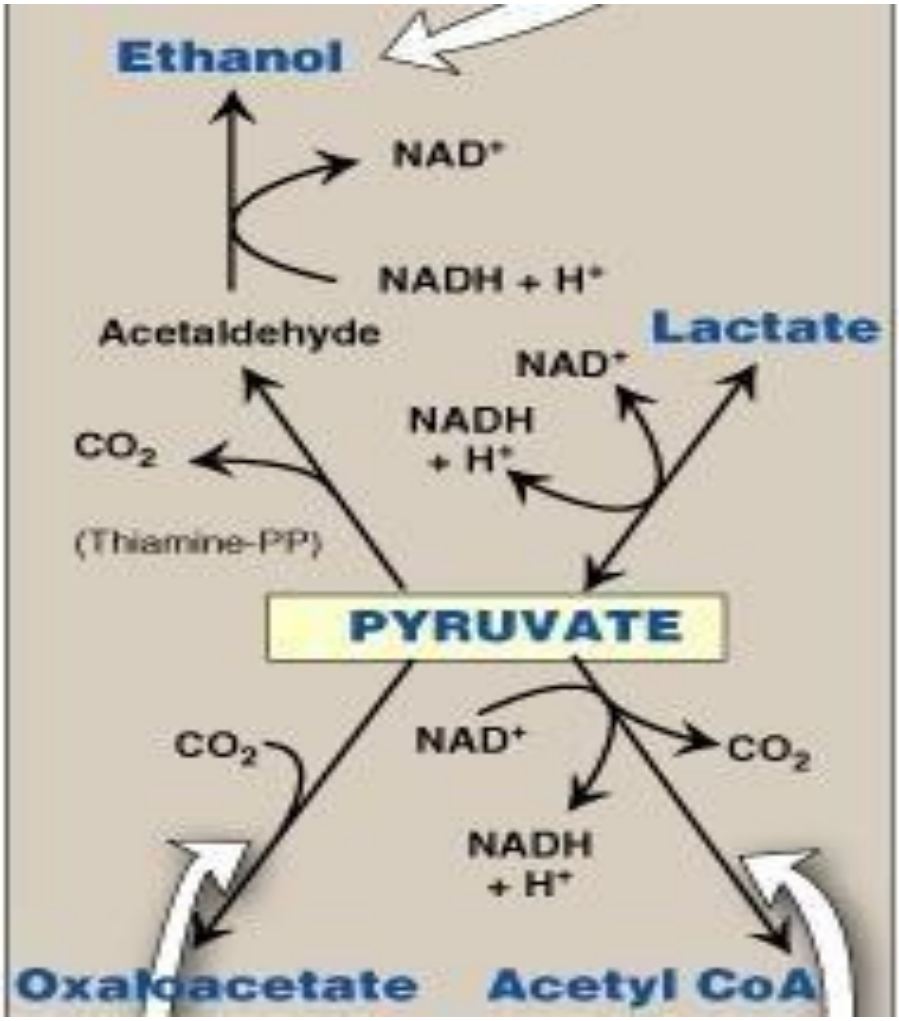
Energy need and production.



Pyruvate Fates:

We know that pyruvate under aerobic conditions will undergo oxidative decarboxylation producing Acetyl CoA and NADH, then it gets into Krebs cycle.

The second route is what occurs in RBCs or exercising muscles, when a big amount of energy is needed in emergency situations, especially in anaerobic conditions, using pyruvate to generate lactate.

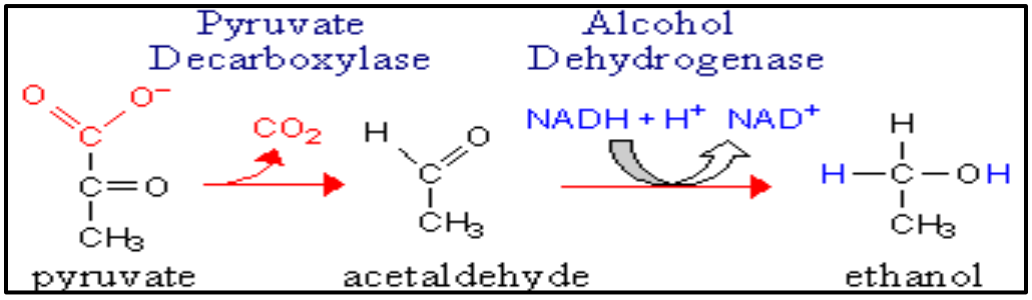


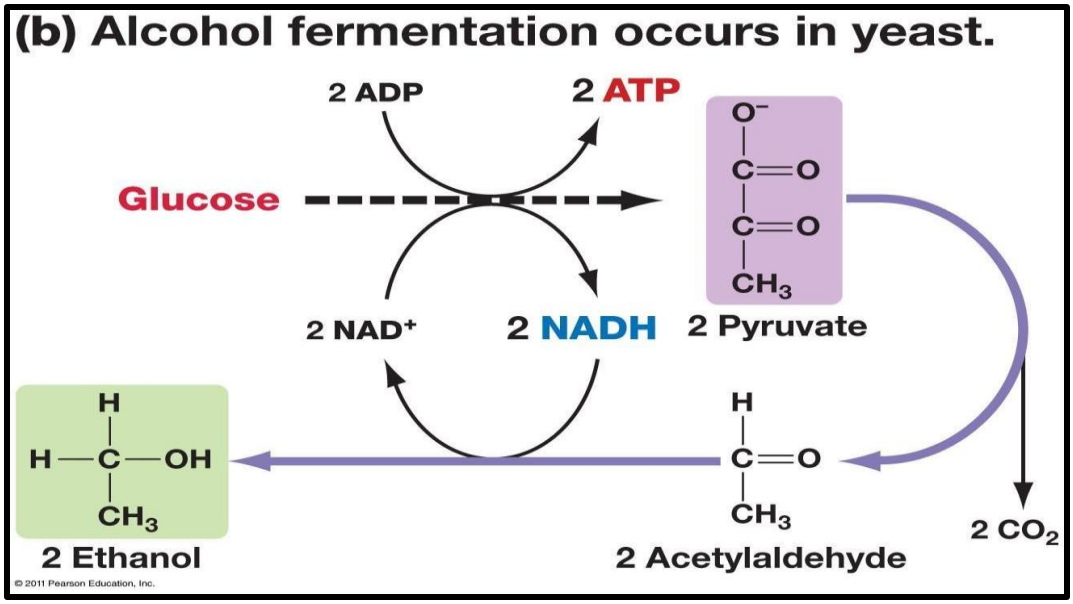
This figure shows us how pyruvate is utilized for energy production (pyruvate →lactate or pyruvate →Acetyl-CoA)
Pyruvate also can undergo other pathways, such as being carboxylated (adding CO2), which produces Oxaloacetate(4C).

- **THE QUESTION HERE IS:DO THE 2 PATHWAYS(Acetyl-CoA&Oxaloacetate) occur simultaneously?**
  - **No, because when pyruvate is destined to from either Acetyl-CoA or lactate, it's used to generate energy. But when it's directed towards oxaloacetate formation, this indicates low glucose concentration that increases the need to produce it (here conversion of pyruvate to oxaloacetate aids in glucose synthesis, not breaking down).**

- **Note here that we are building up glucose from non-carbohydrate molecules, like oxaloacetate(GLUCONEOGENESIS).**
  - **We might ask ourselves: if this is the case, from where did pyruvate come if we need glucose?**
  - **Pyruvate can be synthesized from other precursors, such as amino acids.**
  - **So it doesn't make sense to make glucose and degrade it at the same time!!**

- **Another fate of pyruvate is conversion to acetaldehyde.**
  - **If you remember, acetaldehyde is a two-carbon compound, thus pyruvate gets decarboxylated, then acetaldehyde is reduced forming ethanol. This mostly occurs in yeast cells(fermentation).**
  - **Decarboxylation of pyruvate generates CO_2 (gas).(هون بنشوف سبب انتفاخ العجينة و تخمرها)**
  - **When acetaldehyde is reduced to ethanol (primary alcohol), oxidation of NADH to NAD^+ must take place. This reaction is reversible (can happen in the backward direction, producing more NADH.**

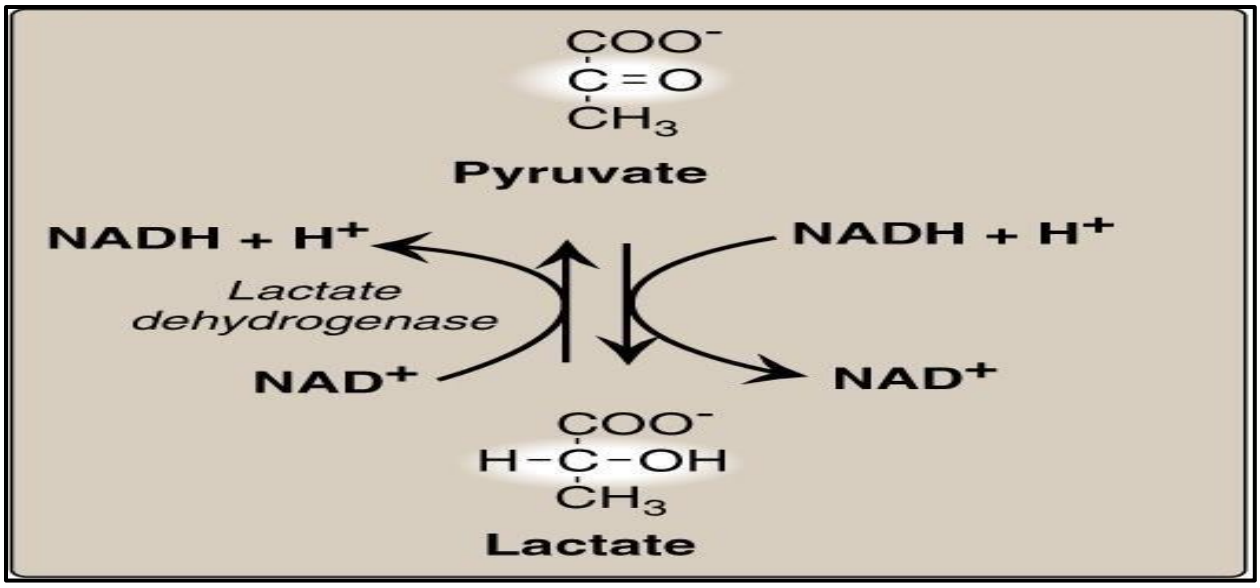




Now let's see how pyruvate gets converted to lactate (anaerobic respiration)

- Notice the difference between them, pyruvate is actually reduced to form lactate (carbonyl group is transformed to alcohol, by the action of lactate dehydrogenase), also NADH gets oxidized to form NAD^+.
- Additionally, the rxn is reversible.

بعرف إنّك زهقت بس الحياة صعبة لازم تكمّل ٨-٨



Pyruvate+NADH ⇌ Lactate + NAD^+

Look carefully at the structure of lactate, you're definitely seeing a carboxylic group(COO^-) and OH group, which contribute to its acidity, consequently when there is overproduction of lactate, metabolic acidosis occurs (metabolic because it is a product from a metabolic pathway).

- When does overproduction of lactate (as a metabolic product) happen?
  - In situations of high-energy demand, for example in exercising muscle –NOT RESTING- which carry out aerobic respiration but the amount of energy produced by oxygen isn't enough, so they initiate anaerobic respiration producing excess amount of lactate, which eventually result in fatigue.
  - Thus, you shouldn't wear a mask if you want to play sports, because of metabolic acidosis in muscle cells (as a consequence of anaerobic respiration), in addition to decreasing oxygen levels when wearing the mask. Not forgetting that you're exhaling so much CO_2 and inspiring it back, so some type of acid accumulation will further happen.

- Lactate is also produced -to a less extent- in RBCs (low-energy demand cells that depend on anaerobic respiration, thus produce lactate)
- Another case of lactate production is hypoxia, either general such as acute or chronic shortness of breath, or local hypoxia (in a certain tissue, such as ischemia or stroke, when blood vessel occlusions occurs, thus blood and oxygen can't reach a specific location, and this causes hypoxia)

To sum up:

➢ **When is lactate produced?**

1. **Cells with low energy demand such as RBCs.**
2. **To cope with increased energy demand in rigorously exercising muscle, lactate level is increased 5-10 folds.**
3. **Hypoxia (to survive brief episodes of hypoxia).**

Lactic acidosis: -

- **Decreased plasma pH**
- **The most common cause of metabolic acidosis**
  - **High production of lactic acid**
  - **Low degradation(utilization/metabolism) of lactic acid even if its normally produced.**

- **Most common cause: Impairment of oxidative metabolism due to collapse of circulatory system.**
  - **Impaired O_2 transport**
  - **Respiratory failure**
    - **E.g. asthma, COPD, lung cancer, COVID-19. These disorders present with extremely low oxygen levels impeding respiration, thus the cell will activate anaerobic respiration, which results in lactate accumulation that will develop into lactic acidosis.**
  - **Uncontrolled hemorrhage**

- **Here we're talking about losing excessive amount of blood, which puts the patient is a seriously life-threatening situation, progressing into hypovolemic shock, which leads to disturbances in fluid homeostasis and osmotic pressure. With increased amount of blood lost, we lose more and more RBCs, thus we lose more and more hemoglobin, and finally: losing oxygen. So lactic accumulation takes place.**

- **Direct inhibition of oxidative phosphorylation**
  E.g. when there is a mutation in any of the complexes(I-IV)
  Generally, when any condition directly affects Krebs cycle, or downstream reactions, the cell will try to find an alternative pathway to generate energy, one example is anaerobic respiration, thus producing excessive amounts of lactic acid.
- **Hypoxia in any tissue**
  Either general or localized in certain tissues
- **Alcohol intoxication (high NADH/NAD^+)**
  The first step in ethanol metabolism is converting ethanol to acetaldehyde (the reverse step of that in anaerobic respiration). Ethanol gets oxidized to acetaldehyde thus NAD^+ will get reduced forming NADH
  - **When having such a high conc. of NADH, what do you think will happen?**

  You're right! Krebs cycle will be directly inhibited, because Krebs cycle required high conc. of NAD^+ to proceed. Actually, NADH and NAD^+ exist as a pool, alternating to each other with a fixed ratio. This ratio here is disturbed with high reduced form: oxidized form, which results in Krebs cycle inhibition, switching to anaerobic respiration that produces more lactate.
- **Decreased gluconeogenesis**
  Gluconeogenesis is the production of glucose from non-carbohydrate sources, happens when there is no external carbohydrate source (no nutrition), or when sugar reservoirs(e.g:glycogen) are depleted.
  One of the substrates used to synthesize glucose in gluconeogenesis is oxaloacetate, so when oxaloacetate is continuously consumed, Krebs cycle will stop, because oxaloacetate is the initiator and terminator of Krebs cycle. Thus, lactic acidosis occurs.

- **Decreased TCA cycle activity**

Can be a result of any condition, such as genetic mutations affecting any of the cycle enzymes, or cofactors.

- **Decreased Pyruvate carboxylase**

Pyruvate carboxylase is the enzyme responsible for conversion of pyruvate to oxaloacetate. If it gets defected, low or no amount of oxaloacetate will be produced, thus Krebs cycle will be inhibited.

- **Decreased Pyruvate dehydrogenase**

Pyruvate dehydrogenase is responsible for pyruvate conversion to Acetyl-CoA. Regarding this, decreased Pyruvate dehydrogenase leads to decreased production of Acetyl-CoA, which can't proceed towards Krebs cycle. Hence, anaerobic respiration gets activated producing more and more lactate.

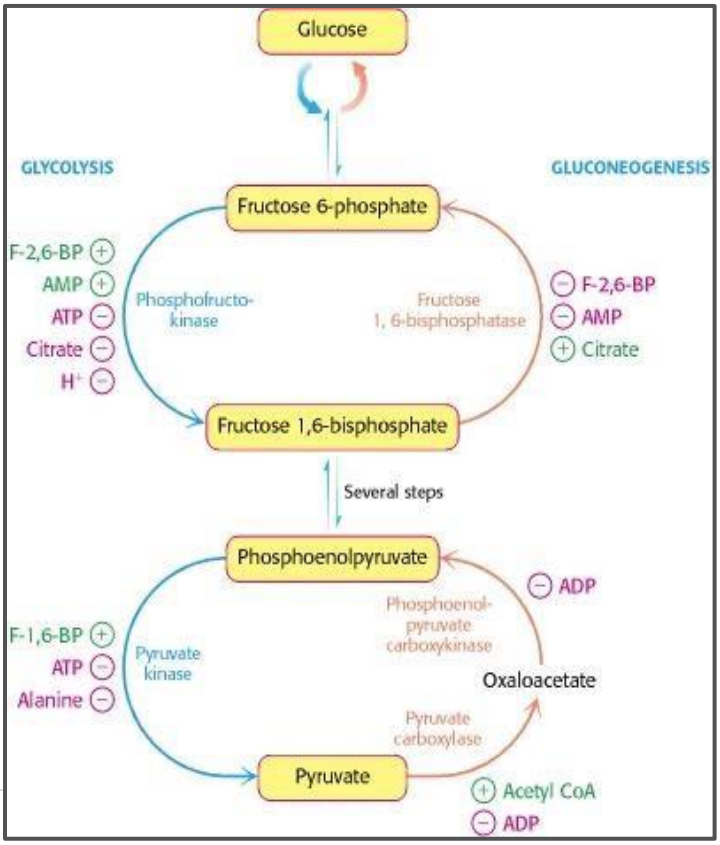
A nice way to distinguish btw Pyruvate carboxylase and dehydrogenase:
CARBOXYLASE---OXALOACETATE (BOTH HAVE 'X')
DEHYDROGENASE—Acetyl-CoA (ONE OF THE Cs is lost 'de')

Regulation of glycolysis: -

Glycolysis is highly regulated at the three irreversible steps in (step 1, step 3 a step 10).

The blue arrows on the left are the ones which are regulated as a part of glycolysis, while the arrows at the opposite side represent gluconeogenesis(the synthetic pathway that somehow is the reverse of glycolysis), which will be discussed later.

☑ The activators and inhibitors for these steps are required.



Notice the step that is activated by **Phosphofructokinase** (Fructose 6-phosphate to Fructose 1,6-Bisphosphate) , activators are **Fructose 2,6-Bisphosphate** (NOT THE PRODUCT) and **AMP**. **AMP** is a marker that senses the low energy state of the cell, so it activates glycolysis more and more to compensate for energy. Opposed to **ATP**, which if produced abundantly, we won't need more energy.

- **Citrate is another inhibitor. HOW??**

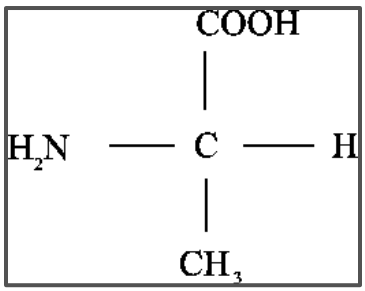
- **Remember that citrate is the first product of Krebs cycle, and it does regulate the own step of its synthesis (Oxaloacetate + acetyl-CoA to Citrate) by feedback inhibition. Thus, when citrate is present in high conc. this will inhibit Krebs cycle at some point as well as glycolysis because the cell no longer needs Acetyl -CoA, so it doesn't need more pyruvate. Hence, glycolysis is inhibited, as an indicator of the high-energy state in the cell.**

- **HOW DO PROTONS INHIBIT THIS STEP (step 3)?**

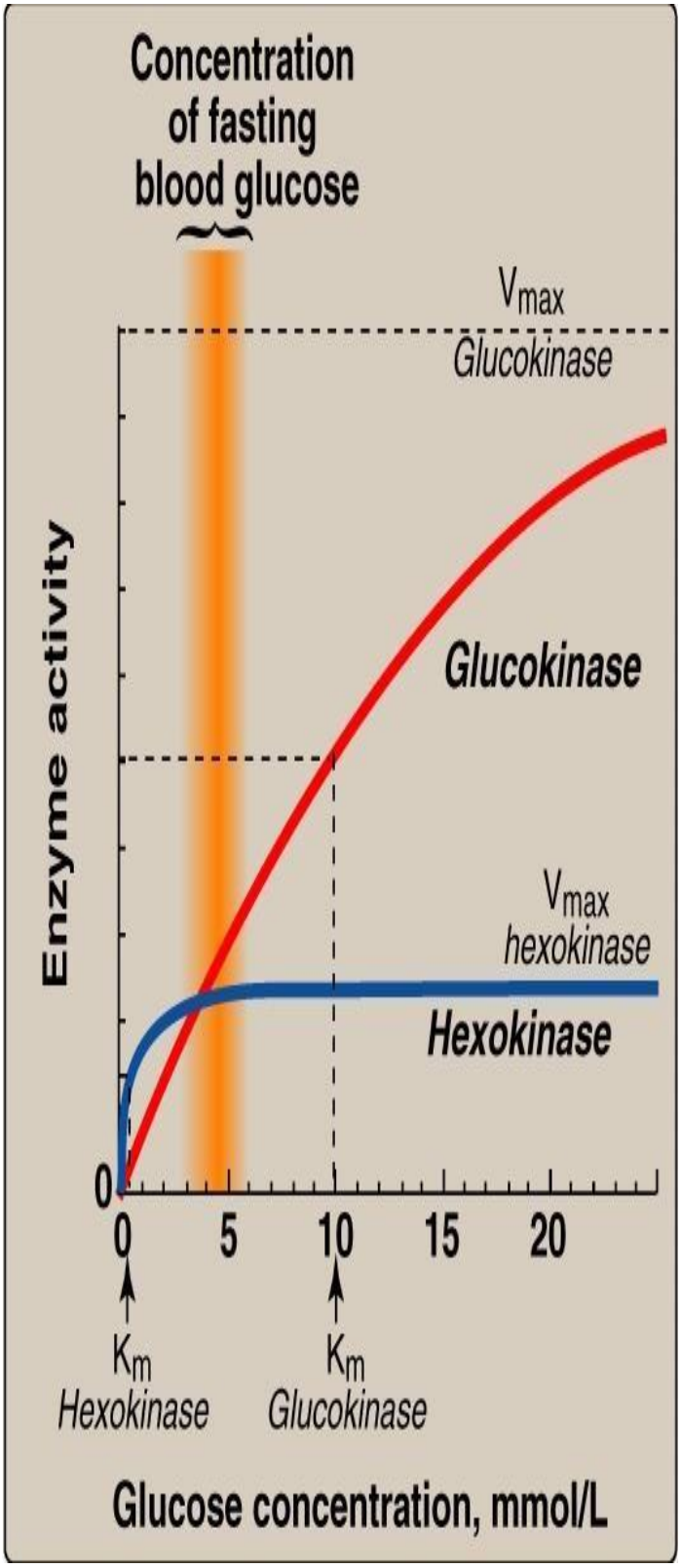
- **We know that protons form when redox reactions happen, producing NADH, meaning that a high proton concentration is an indicator of an abundant amount of NADH, which inhibits Krebs cycle as well as glycolysis for the same reason above.**

- **NOW LET'S TALK ABOUT THE LAST STEP (Phosphoenolpyruvate to Pyruvate).**
  Pyruvate kinase in this step is activated by high conc. of Fructose 1,6-bisphosphate, which is the product of the previous step we talked about, its high presence will stimulate the enzyme to continue synthesizing pyruvate, not to stop in the middle of the pathway.
  As mentioned above,ATP is a marker of high-energy state, that's why it acts as an inhibitor.

- **You noticed Alanine, right? How does Alanine inhibit this step?**
  The right figure shows Alanine structure.
  Generally speaking, when removing the amino group (deamination/transamination), the resultant compound is alpha keto-acid.
  So the alpha keto-acid for Alanine is Pyruvate, that definitely means Alanine is a source of Pyruvate (the final product). Therefore, when present in high amounts it will build up more pyruvate, which leads –at some point- to the inhibition of this pathway.



Glucokinase&Hexokinase Activity:the first step in glycolysis



We all agree that hexokinase is the enzyme that's always functional,at any time,in any tissue and at any glucose conc. Capturing any glucose present to initiate glycolysis.Notice its enzymatic activity,its Vmax is much lower than glucokinase,and that makes sense! Because it's constantly active,so it has to keep working at a moderate speed. On the contrary,glucokinase has a much higher Vmax,because it selectively works under certain conditions(high amount of glucose).

In terms of Km and affinity.Hexokinase of course has a lower Km (higher affinity) for glucose since it binds it all the time.Whereas gluco-kinase has a higher Km(lower affinity) for glucose because it only binds it at certain conditions.Also note the difference in the curves,hexokinase reaches Vmax at a faster rate compared to glucokinase.

The orange line represents sugar levels at fasting conditions (or 2 hours after eating a meal) , a situation in which blood sugar gradually declines to a certain level called fasting blood sugar,and there is no more supply of blood sugar,all sugar will be stored in your cells,either from what you've eaten or what cells previously reserved.

FASTING BLOOD SUGAR: At this level of sugar, the majority of the enzymatic activity comes from hexokinase. Refer to the figure, hexokinase works at a significantly higher rate at this conc. compared to glucokinase, reaching Vmax effectively.

يعطيك العافية دكتور،بنتمنّى إنّه الشيت فادتك وسهّلنا عليك،إن أصبنا فمن الله وإن أخطأنا فمن أنفسنا،وأي سؤال بخطر عبالك نحن بالخدمة في أي وقت إن شاء الله

تم بحمد الله