Editing File

11

Glycolysis

Color index :

Main text

IMPORTANT

Extra Info

Drs Notes

Foundation Block - Biochemistry Team

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MED439

Objectives:

- Recognize glycolysis as the major oxidative pathway of glucose.
- List the main reactions of glycolytic pathway.
- Discuss the rate-limiting enzymes/Regulation.
- Assess the ATP production (aerobic/anaerobic).
- Define pyruvate kinase deficiency hemolytic anemia.
- Discuss the unique nature of glycolysis in RBCs.

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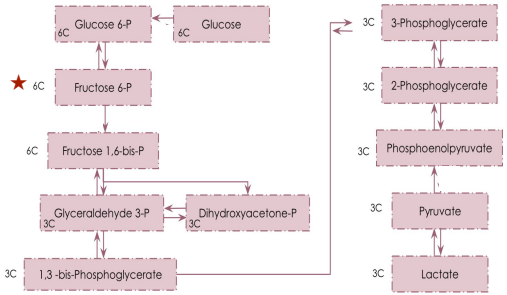
Glycolysis

- Glycolysis means breaks down glucose and forms pyruvate with the production of two molecules of ATP. The pyruvate is the end product of glycolysis.
- Glycolysis, the major pathway for glucose oxidation, occurs in the cytosol of all cells.
- It is unique, in that it can function either aerobically or anaerobically, depending on the availability of oxygen and intact mitochondria.
- It allows tissues to survive in presence or absence of oxygen, e.g., skeletal muscle.
- **RBCs**, which lack mitochondria, are completely reliant on glucose as their metabolic fuel, and metabolizes it by anaerobic glycolysis.

	Aerobic	Anaerobic
Presence of mitochondria and oxygen	Must have mitochondria and oxygen .	In absent of mitochondria or oxygen or both.
Number of steps	10 steps (reactions).	11 steps (reactions).
End product	End product is Pyruvate.	End product is Lactate or lactic acid .
Byproducts	Produce 2 ATP and 2 NADH = (8 ATP).	Produce 2 ATP



Glycolysis , an example of metabolic pathway

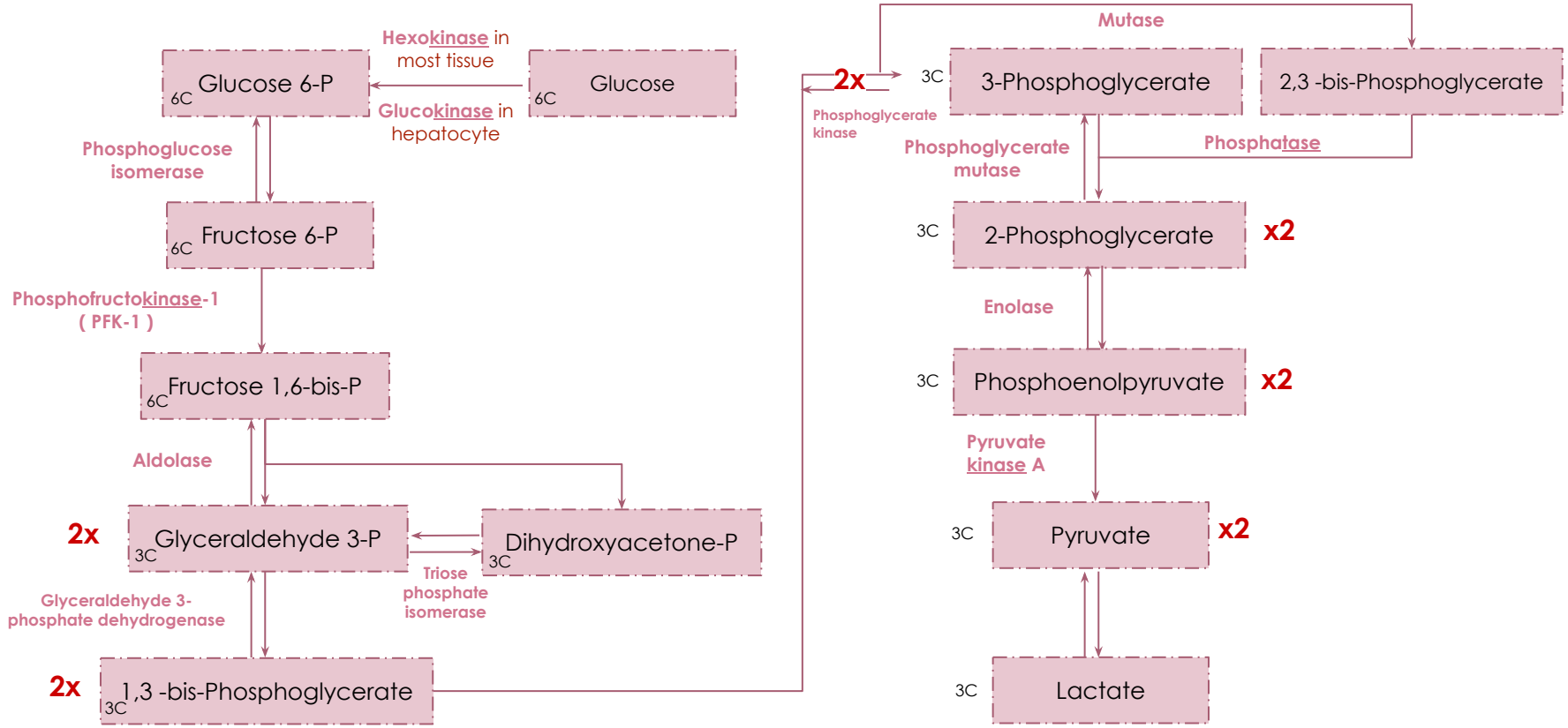
★ A product of first reaction becomes a substrate for second reaction.

A helpful video

Aerobic glycolysis (overview)

reversible

irreversible



More details in the next slides

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Aerobic glycolysis (1)

1 D- Glucose(6C) —(**Hexokinase** in most tissue / **Glucokinase** in hepatocyte)→ Glucose 6-phosphate (6C)

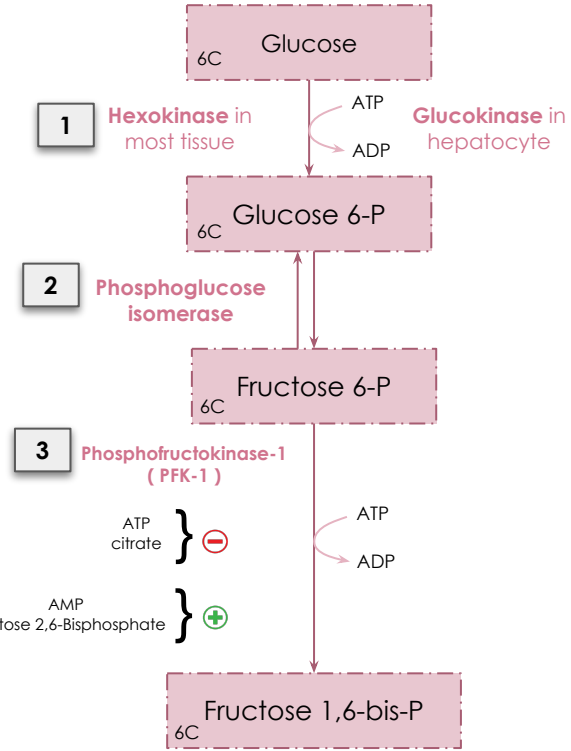
- Glucokinase is a hexokinase isozyme.
- ATP In.
- ADP Out.
- **Phosphorylation** of glucose (Adding a phosphate group) with the help of **Hexokinase/Glucokinase**.
- Regulation of hexokinase and glucokinase, click here .

2 Glucose 6-phosphate (6C) ⇌ (**Phosphoglucose isomerase**) Fructose 6-phosphate (6C)

- isomerase means it changes the configuration.
- **Isomerization** from aldose form to ketos form with the help of **Phosphoglucose isomerase.**

3 Fructose 6-phosphate (6C) —(**Phosphofructokinase-1 (PFK-1)**)→ Fructose 1,6-bisphosphate (6C)

- Bisphosphate means 2 phosphate groups not close to each others while Biphosphate means 2 phosphate groups close to each others.
- ATP in.
- ADP out.
- **Phosphorylation** of Fructose 6-phosphate (Adding a phosphate group) with the help of **Phosphofructokinase-1**
- Regulation of PFK-1 enzyme (can beinhibited or activated), more details click here :
  - ⊕ AMP and Fructose 2,6-bisphosphate.
  - ⊖ ATP and citrate.



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Aerobic glycolysis (2)

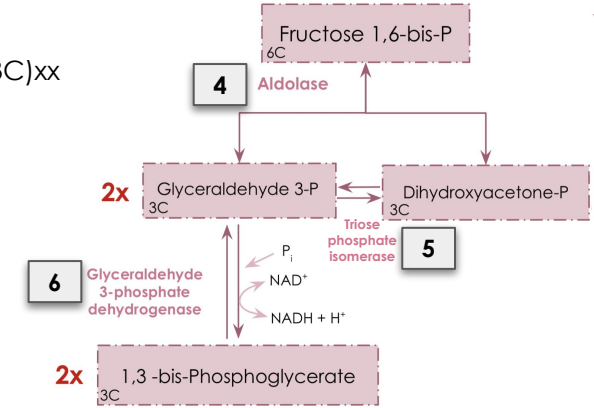
Reactions 1-5 consume 2 molecules of ATP

4 Fructose 1,6-bisphosphate (6C) $\xrightarrow[\text{Aldolase}]{}$ Dihydroxyacetone phosphate (3C) + Glyceraldehyde 3-phosphate (3C)

- Breaking down carbon-carbon bond with the help of **Aldolase** enzyme (split the molecule).

5 Dihydroxyacetone phosphate (3C) $\xrightarrow[\text{Triose phosphate isomerase}]{}$ Glyceraldehyde 3-phosphate (3C)xx

- isomerase means it changes the configuration.
- **Isomerization** from ketos form to aldose from with the help of **Triose phosphate isomerase**
- When the body needs fats it will convert glyceraldehyde 3-phosphate to dihydroxyacetone phosphate but in glycolysis the body needs energy so it convert dihydroxyacetone phosphate to glyceraldehyde 3-phosphate, By the end of reaction 5 we will have 2 molecule of glyceraldehyde 3-phosphate one from reaction 4 and the other from reaction 5 .



6 **2x** Glyceraldehyde 3-phosphate (3C) $\xrightarrow[\text{Glyceraldehyde 3-phosphate dehydrogenase}]{}$ **2x** 1,3 -bis-Phosphoglycerate (3C)

- **2x** P_i and NAD^+ in.
- **2x** NADH and H+ out.
- **Oxidation** of Glyceraldehyde 3-phosphate to 1,3 -bis-Phosphoglycerate with the help of **Glyceraldehyde 3-phosphate dehydrogenase.**
  "About NAD^+ and NADH , keep in mind that the reverse can happen "

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Aerobic glycolysis (3)

Reactions 6-10 produce two molecules in each reaction.

7 **2x** 1,3 -bis-Phosphoglycerate (3C) ⟶ (**Phosphoglycerate kinase**) ⟵ **2x** 3-Phosphoglycerate (3C)

- **2x** ADP in.
- **2x** ATP out.
- Adding a phosphate group to ADP turning it to ATP and forming 3-Phosphoglycerate with the help of **Phosphoglycerate kinase**

"Keep in mind that the reverse can happen "

8 **2x** 3-Phosphoglycerate (3C) ⟶ (**Phosphoglycerate mutase**) ⟵ **2x** 2-Phosphoglycerate (3C)

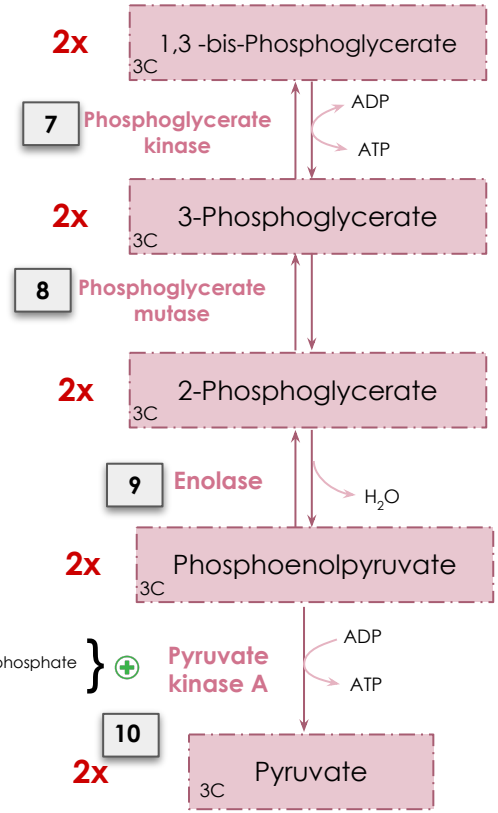
- **Isomerization** of 3-Phosphoglycerate to 2-Phosphoglycerate with the help of **Phosphoglycerate mutase**

9 **2x** 2-Phosphoglycerate (3C) ⟶ (**Enolase**) ⟵ **2x** Phosphoenolpyruvate (PEP) (3C)

- **2x** H_2O out.
- 2-Phosphoglycerate loses a molecule of water turning into (PEP) with the help of **Enolase**

10 **2x** Phosphoenolpyruvate (3C) ⟶ (**Pyruvate kinase A**) **2x** Pyruvate

- **2x** ADP in.
- **2x** ATP out.
- Adding a phosphate group to ADP turning it to ATP and forming Pyruvate with the help of **Pyruvate kinase A**.
- Regulation of Pyruvate kinase A (can be activated) :
  ⊕ Fructose 1,6-Bisphosphate .
- Regulation of Pyruvate kinase A , click here .



★ extra explanation

Summary of the aerobic glycolysis In order for you to gain a better understanding In tables

Reaction 1	
Reactant	D-Glucose
Product	Glucose 6-phosphate
Enzyme	1-**Hexokinase** in most tissue 2-**glucokinase** in hepatocyte
Action	adding phosphate
Consume	1 ATP

Reaction 2	
Reactant	Glucose 6-phosphate
Product	Fructose 6-phosphate
Enzyme	**Phosphoglucose isomerase**
Action	Isomerization from aldose form to ketos form
Consume	-

Reaction 3	
Reactant	Fructose 6-phosphate
Product	Fructose 1,6-bisphosphate
Enzyme	**Phosphofructokinase-1 (PFK-1)**
Action	adding phosphate
Consume	1 ATP

Reaction 4	
Reactant	Fructose 1,6-bisphosphate
Product	Dihydroxyacetone phosphate **and** Glyceraldehyde 3-phosphate
Enzyme	**Aldolase**
Action	Break down carbon-carbon bond (split the molecule)
Consume	-

Reaction 5	
Reactant	Dihydroxyacetone phosphate
Product	Glyceraldehyde 3-phosphate
Enzyme	**Triose phosphate isomerase**
Action	Isomerization from ketos form to aldose from
Consume	-

Reaction 6	
Reactant	**2x** Glyceraldehyde 3-phosphate
Product	**2x** 1,3 Bisphosphoglycerate
Enzyme	**Glyceraldehyde 3-phosphate Dehydrogenase.**
Action	Adding Phosphate group to the molecule
Byproduct	NADH

★ extra explanation

Summary of the aerobic glycolysis In order for you to gain a better understanding In tables ... contd

Reaction 7	
Reactant	**2x**1,3 Bisphosphoglycerate
Product	**2x** 3-Phosphoglycerate
Enzyme	**Phosphoglycerate kinase**
Action	Phosphate group add to ADP to become ATP.
Produce	**2x** ATP

Reaction 8	
Reactant	**2x** 3-Phosphoglycerate
Product	**2x** 2-Phosphoglycerate
Enzyme	**Phosphoglycerate mutase**
Action	The P group change position from O in carbon-3 to O in carbon-2
Consume	-

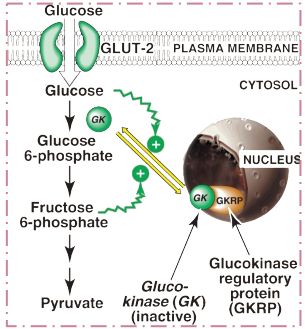
Reaction 9	
Reactant	**2x** 2-Phosphoglycerate
Product	**2x** Phosphoenolpyruvate
Enzyme	**Enolase**
Action	Remove water
Consume	-

Reaction 10	
Reactant	**2x** Phosopheonolpyruvate
Product	**2x** Pyruvate
Enzyme	**Pyruvate kinase A**
Action	Phosphate group add to ADP to become ATP
Produce	**2x** ATP

Regulation of enzymes
" Hexokinase / Glucokinase "

- Regulation of hexokinase (in most cells) and glucokinase (in liver or we can say hepatocyte " hepato means liver and cyte means cell).
- **Hexokinase** : when Glucose 6-phosphate is abundant it will indicate to the cell that it doesn't need hexokinase anymore and it will be inhibited directly .
- **Glucokinase** : is an enzyme that facilitates phosphorylation of glucose to glucose-6- phosphate. It is is an isozyme of hexokinase.
    1. In the presence of high Fructose-6- phosphate, glucokinase translocates and binds tightly to GKRP (Glucokinase regulatory protein) in the nucleus, making it **inactive** " it will not work " .
    2. When glucose levels are high in blood and hepatocytes (GLUT-2), glucokinase is released from GKRP (Glucokinase regulatory protein) and enters the cytosol .

	Hexokinase	Glucokinase
Site	All tissues (Hexokinase have 4 isoforms all of them in all tissues except glucokinase)	Hepatocytes and pancreas cells
Regulation by: (**inhibition**)	Glucose 6-phosphate	Fructose-6- phosphate
Regulation by: (**stimulation**)	-	Glucose
Function	Phosphorylates " adds a phosphate group on " glucose inside the body cells thus glucose concentration in blood is higher than glucose concentration in the cell.	After meals, it removes sugar " glucose " coming in the portal circulation, converting it into Glucose 6-phosphate



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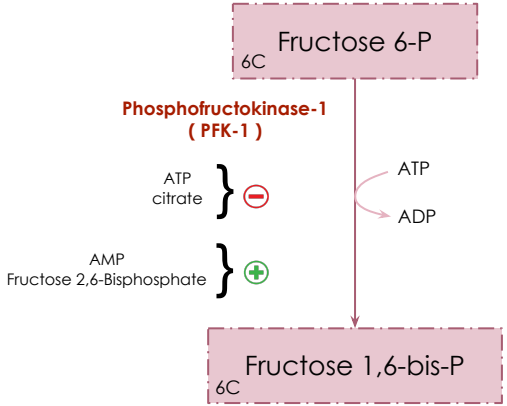
Regulation of enzymes
" PFK-1 / Pyruvate kinase A "

Phosphofructokinase-1 (PFK-1) "Enzyme" :

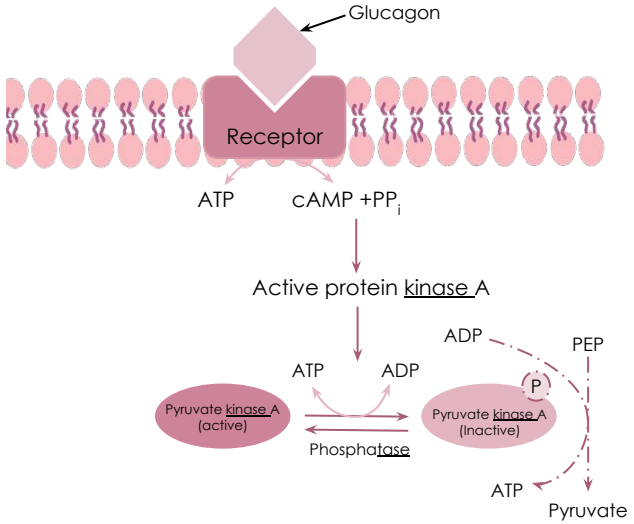
Rate limiting enzyme

" يعني يأثر بالـ glyclusis كله "



- Reaction number 3 in glycolysis it's irreversible reaction
  - ⊕ stimulated by AMP and Fructose 2,6-bisphosphate .
  - ⊖ inhibited by ATP and citrate.
- They are not involved in the chemical reaction, they have an allosteric effect.

Pyruvate kinase A Covalent Modification"Enzyme" :



- Reaction number 10 in glycolysis it's irreversible reaction. once glucagon (hormone) bind to the receptor it convert the enzyme pyruvate kinase A from active from to an inactive form by phosphorylation .
  - ⊕ Activation of enzyme can be done by phosphatase (removes a phosphate group)
- When it's in the active form It catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP.

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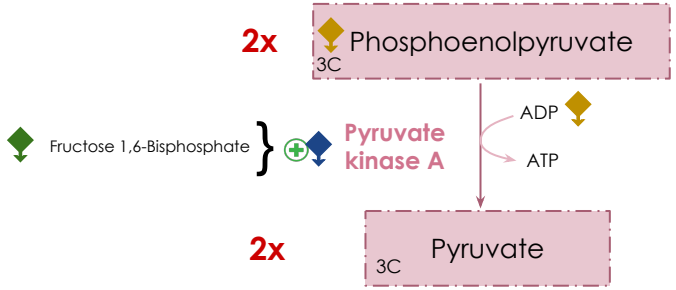
Pyruvate kinase deficiency: hemolytic anemia

- Pyruvate kinase deficiency is due to genetic mutation (it affects the survival of red blood cells).
- The enzyme may show an abnormal K_m or V_{max} for substrates or coenzymes.
- The enzyme may show an abnormal response to the activator fructose 1,6 - bisphosphate.
- Enzyme activity or stability may be altered , or the amount of enzyme may be decreased .



- Pyruvate kinase mutation may lead to :
  1. Altered enzyme Kinetics . (mutation in the allosteric binding site that will inhibit enzyme activity, since the F-1,6-P activate the enzyme so in this case altered allosteric site = inhibit/lower the activity of the enzyme).
  2. Altered response to activator . (mutation in the active site that will stop the enzyme activity, since the enzyme activity depend on its substrate mainly).
  3. Altered enzyme stability.
  4. Decreased amount of the enzyme.

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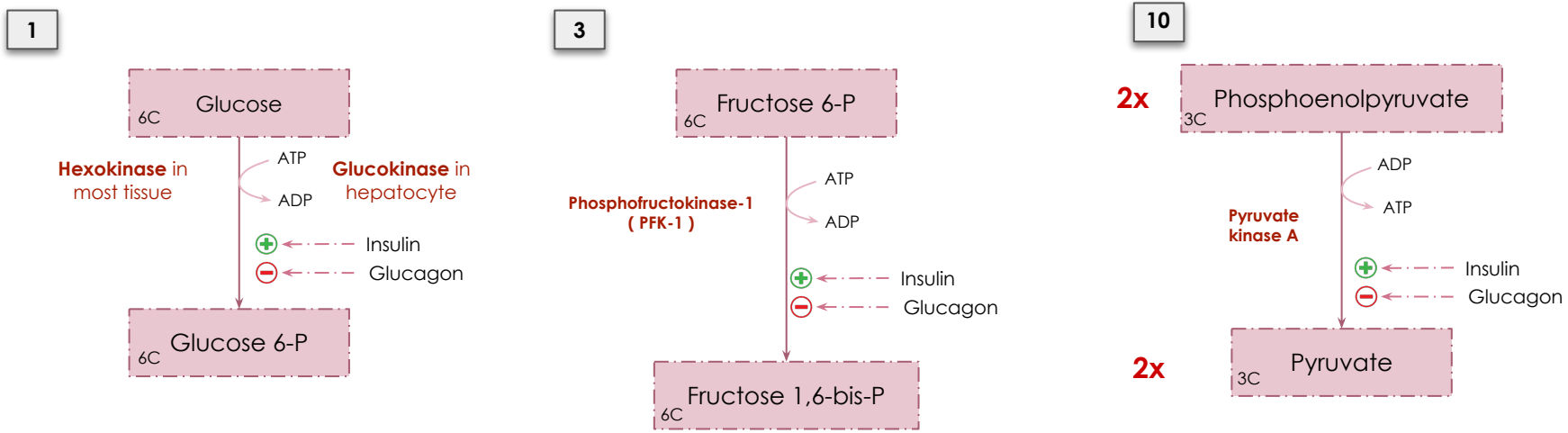
Regulation of enzymes

" long term regulation (hormonal) "



- Long term regulation of glycolysis **in reactions 1 , 3 and 10** :

1. Glucagon: **Repression** .
   - inhibits the glycolysis .

2. Insulin: **Induction** .
   - Activities the uptake of glucose (GLUT 4).

- Regulatory Mechanisms:
  1. Rapid and short-term: Allosteric and Covalent modifications are the enzymes regulation in the three irreversible steps.
  2. Slow and long-term: Induction/repression (insulin and glucagon respectively).

Substrate-level phosphorylation Vs. Oxidative phosphorylation

- Phosphorylation is the metabolic reaction of introducing a phosphate group into an organic molecule.

Oxidative phosphorylation	Substrate-level phosphorylation
"Production of ATP molecules from the redox reactions of an electron transport chain". Formation of high-energy phosphate bonds by phosphorylation ADP to ATP.	" Production of ATP molecules via transfer of a phosphate group from an intermediate high-energy substrate directly to ADP". Formation of high-energy phosphate bonds by phosphorylation ADP to ATP (or GDP to GTP).
Coupled to the transfer of electrons from reduced coenzyme to molecular oxygen via ETC	Coupled to cleavage of a high-energy metabolic intermediate (substrate)
In mitochondria (ETC).	In cytosol or mitochondria (glycolysis and Krebs cycle).

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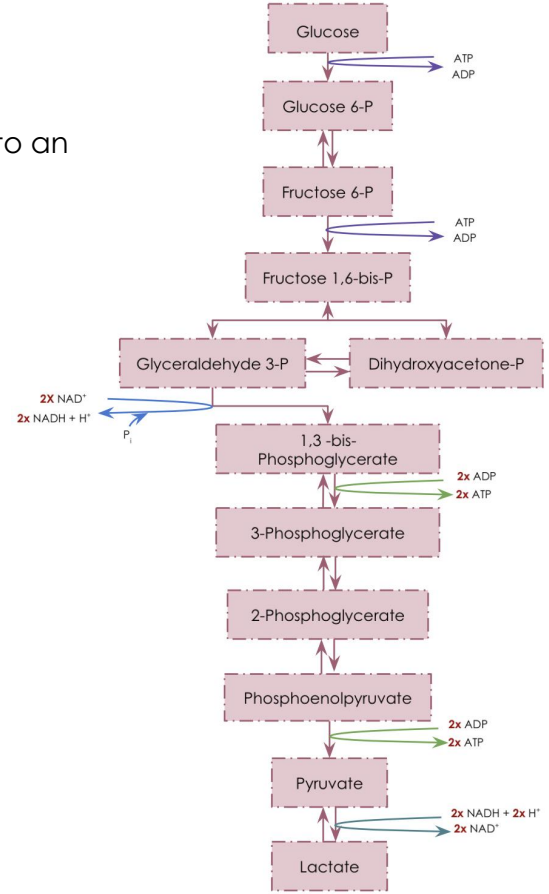
Aerobic glycolysis : ATP production

- Phosphorylation is the metabolic reaction of introducing a phosphate group into an organic molecule.

ATP Consumption	Reaction 1 + Reaction 3 " From glycolysis steps" **2 ATP**
NADH Production	Reaction 6 " From glycolysis steps" 2 NADH " 1 NADH means 3 ATP" **2 X 3 = 6 ATP** " From ETC (oxidative level)"
ATP Production	AND Reaction 7 + Reaction 9 " From substrate level (glycolysis steps)" Keep in mind these 2 reactions was multiplied by 2 **2 ATP X 2 = 4 ATP**
Net	10 - 2 = 8 ATPs



★ To summarize

Aerobic glycolysis : ATP production

ATP consumed	2 ATP	
ATP produced	Substrate-level (directly)	**2 ATP x 2 = 4 ATP**
	Oxidative-level (in ETC)	**2 NADH X 3 = 6 ATP**
Total	10	
Net	**10** " produced " **- 2** " consumed " **= 8 ATP**	

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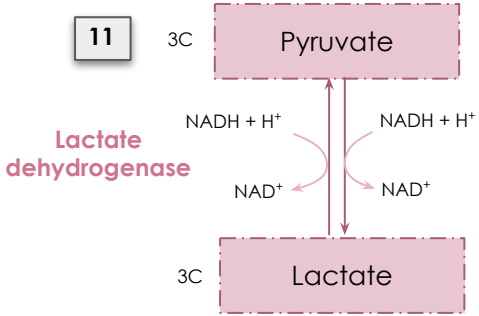
Anaerobic glycolysis

- Anaerobic glycolysis is the transformation of glucose to lactate when limited amounts of oxygen (O_2) are available. Anaerobic glycolysis is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes.
- NADH is produced cannot be used by ETC for ATP production (No O_2 and/ or no mitochondria).
- Less ATP production, as compared to aerobic glycolysis.
- The end product of anaerobic glycolysis is lactate "obligatory output". (why?) Because if it is not formed, All cellular NAD^+ will be converted to NADH, with no means to replenish (fill again) the cellular NAD^+ Glycolysis stops death of the cell.
- Anaerobic Glycolysis is important in RBCs because they don't have mitochondria .

Anaerobic enzyme : Lactate dehydrogenase

11 **2x** Pyruvate (3C) ⇌ (Lactate dehydrogenase) **2x** Lactate

- Reversible reaction.
- Regeneration of NAD^+ (Fermentation) with the help of **Lactate dehydrogenase**
- **Lactate dehydrogenase** enzyme is used for both direction .
- **2x** NADH + H^+ in .
- **2x** NAD+ out .



★ To summarize

Anaerobic glycolysis : ATP production

ATP consumed	2 ATP	
ATP produced	Substrate-level (directly)	**2 ATP x 2 = 4 ATP**
	Oxidative-level (in ETC) ماقدروا يطلّعون من الـ NADH طاقة لانه مادخل الـ ETC NADH can NOT be used by ETC (oxidative-level) because: there is no O_2 and/or no mitochondria.	**2 NADH X 3 = 6 ATP**
Total	4	
Net	**4** " produced " **- 2** " consumed " **= 2 ATP**	

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Anaerobic glycolysis in RBC (2,3 BPG shunt)

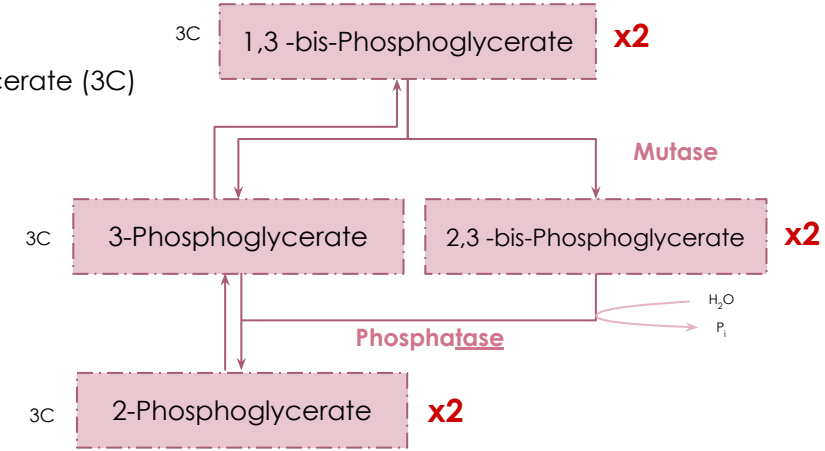
***1** **2x** 1,3 -bis-Phosphoglycerate (3C) —**Mutase**→ **2x** 2,3 -bis-Phosphoglycerate (3C)

- Mutase enzyme It is important for association and dissociation between O_2 and hemoglobin.
- Increase in "2,3-BPG" will help the loss of association between O_2 and hemoglobin and will release more O_2.
- It usually occurs with people who live in high altitude.

***2** **2x** 2,3 -bis-Phosphoglycerate (3C) ⇄ (**Phosphatase**) **2x** 2-Phosphoglycerate (3C)

- H_2O in .
- P_i out .
- Removing a phosphate group from 2,3 -bis-Phosphoglycerate turning to 2-Phosphoglycerate with help of **Phosphatase**.

- Shunt = diversion (تحويلة):
  - 2,3-BPG " 2,3-bis-phosphoglycerate " Shunt is responsible for loading and unloading of oxygen .
  - No ATP production .
  - Present in both fetal and adult hemoglobin .
  - in fetal hemoglobin, it interacts less with hemoglobin .
  - Presence of 2,3-BPG shunt reduces hemoglobin's oxygen affinity **i.e.** fetal hemoglobin has higher affinity to oxygen than adult hemoglobin.



★ To summarize

Anaerobic glycolysis in RBC (2,3 BPG shunt) : ATP production

ATP consumed	2 ATP	
ATP produced	Substrate-level (directly)	**2 ATP x 2 = 4 ATP** (without shunt) **1 ATP x 2 = 2 ATP** (with shunt)
	Oxidative-level (in ETC) ماقدروا يطلّعون من الـ NADH طاقة لانه مادخل الـ ETC NADH can NOT be used by ETC (oxidative-level) because: there is no O_2 and/or no mitochondria.	**2 NADH X 3 = 6 ATP**
Total	4 or 2	
Net	**4** " produced " **- 2** " consumed " **= 2 ATP** (without shunt) **2** " produced " **- 2** " consumed " **= 0 ATP** (with shunt)	

Anaerobic glycolysis in RBC (2,3 BPG shunt)

" summary "

End product	1. Lactate . 2. No net production or consumption of NADH .
Energy yield	- If no shunt is formed = 2 ATP . - If shunt occurs = 0 ATP .
PK deficiency hemolytic anemia depends on	- Degree of PK deficiency. - Compensation 2,3-BPG

Take home messages

- Glycolysis is the major oxidative pathway for glucose.
- Glycolysis is employed by all tissues.
- Glycolysis is a tightly-regulated pathway.
- PFK-1 is the rate-limiting regulatory enzyme.
- Glycolysis is mainly a catabolic pathway for ATP production, but it has some anabolic features (amphibolic).
- Pyruvate kinase deficiency in RBCs results in hemolytic anemia.

Quiz

Q1 : Oxidative phosphorylation happens in			
A) Nucleus	B) Cytosol	C) Mitochondria	D) Rough ER
Q2 : The net ATP production in Aerobic Glycolysis is			
A) 8 ATP	B) 4 ATP	C) 7 ATP	D) 5 ATP
Q3 : Which of the following biochemical pathways does NOT require oxygen ?			
A) Krebs cycle	B) Glycolysis	C) ETC	D) Calvin cycle
Q4 : The final product of Anaerobic Glycolysis is			
A) Pyruvate	B) Lactate	C) Citrate	D) Acetyl coenzyme A
Q5 : There are ..... steps reaction in aerobic glycolysis			
A) 13	B) 20	C) 10	D) 15
Q6 : The first 3 steps in glycolysis are			
A) Irreversible	B) Reversible	C) Both of them	D) None of them

SAQs :

Q1: What is the function of enolase enzyme ?

Q2: How many NADH are produced by glycolysis per glucose ?

★ **MCQs Answer key:**

1) C 2) A 3) B 4) B 5) c 6) A

★ **SAQs Answer key:**

1) Remove water, so it will convert phosphoglycerat to Phosopheonolpyruvate
2) 2 NADH

Alia Zawawi
Nada Babilli
Rania Aqil
Reem alamri
Reema Alomar
Reem Alqahtani
Renad Alhumaidi
Shaden Alobaid
Noura Alsalem
Lama Alahmadi
Sadem Alhazmi
Somow Abdulrahman
Budoor Almubarak
Samar Almohammedi
Nuha Alkudsi
Norah Alsheikh
Muneerah Alssdhan
Mayasem Alhazmi
Noura alshathri
Duaa Alhumoudi

Mansour albawardi
Hassan alshuraf
Abdulrahman almbki
Mohammed alsayari
Abdullaziz alomar
Abdulaziz alrabiah
Saud alrasheed
Abdullah almazro
Hamad almousa
Ahmad alkhayat

A goal should scare you a little and excite you a lot!.

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