Glycolysis

MED441
KING SAUD UNIVERSITY

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V1

Foundation Block - KSU

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- Boys slides'
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Editing File

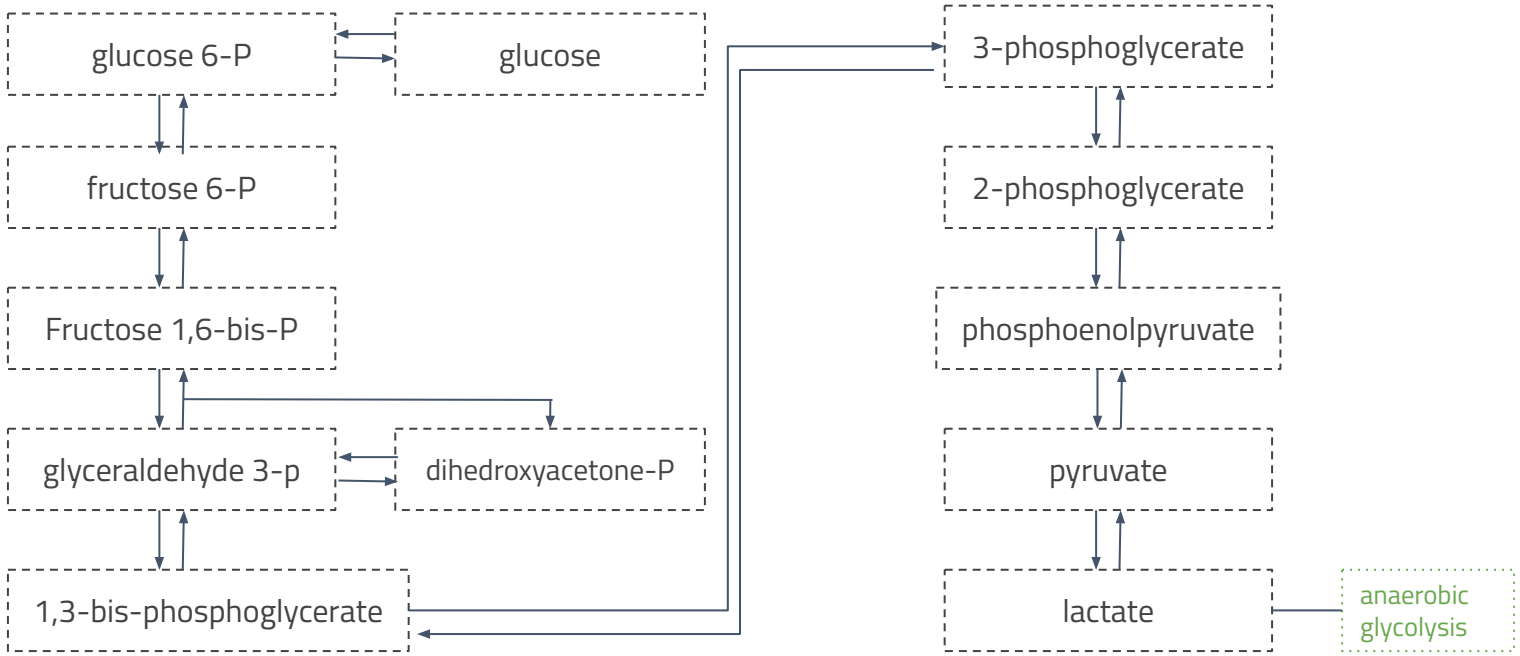
Objectives

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

Glycolysis

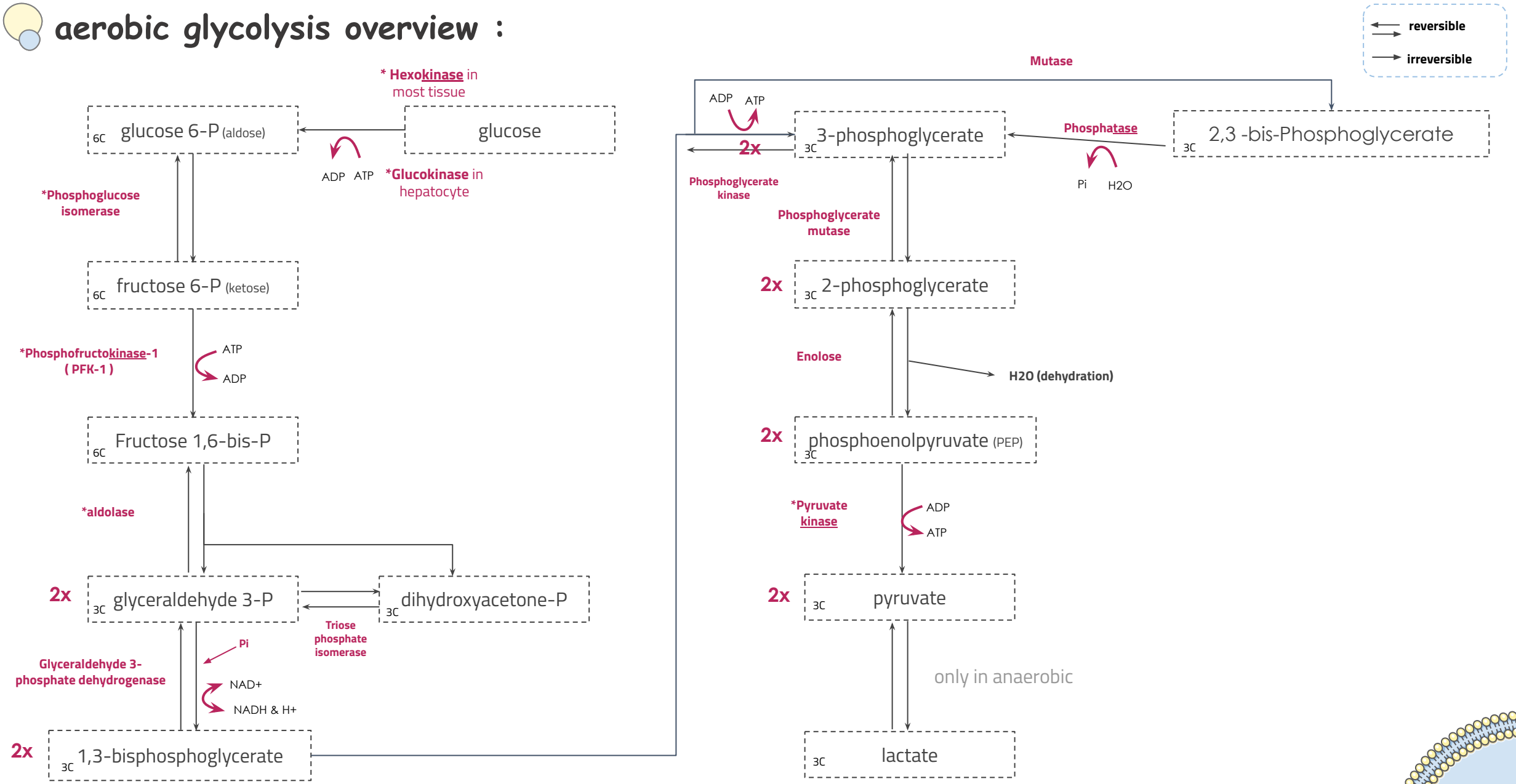
- Glycolysis, the major pathway for glucose oxidation, occurs in the cytosol of all cells. (oxidative phosphorylation is in the mitochondria)
- It is unique, in that it can function either aerobically or anaerobically, depending on the availability of oxygen and intact mitochondria.
- It allows tissues 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

- A product of one reaction is the substrate of the subsequent reaction



-helpful video

aerobic glycolysis overview :



Aerobic glycolysis (1)

1

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

- Kinase means phosphorylation enzyme " an enzyme that adds a phosphate group "
- Glucokinase is a hexokinase isozymes (isoforms)
- **Irreversible**
- ATP In. (energy consuming)
- ADP Out
- Regulation of hexokinase and glucokinase

2

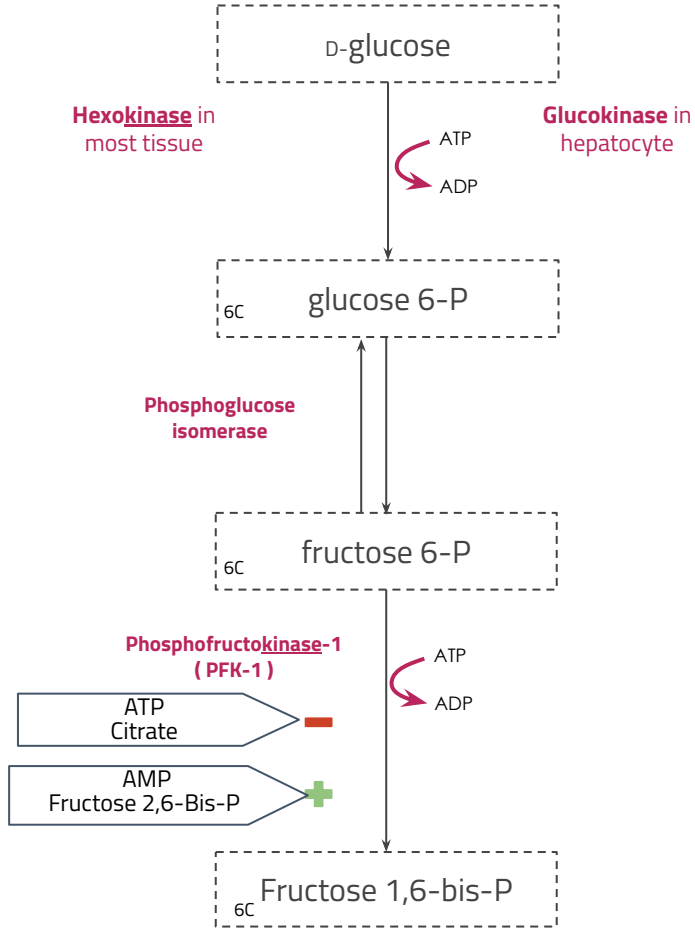
Glucose 6-phosphate (6C) ⇄ (Phosphoglucose isomerase) **Fructose 6-phosphate (6C)**

- isomerase means it changes the configuration and no energy lost
- reversible
- Isomerization from aldose form to ketos form

3

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

- The rate limiting step of glycolysis
- ATP in (energy consuming)
- **Irreversible**
- ADP out
- can be regulated :
  - activated ✚ AMP and Fructose 2,6-bisphosphate.
  - inhibited ▬ ATP and citrate.



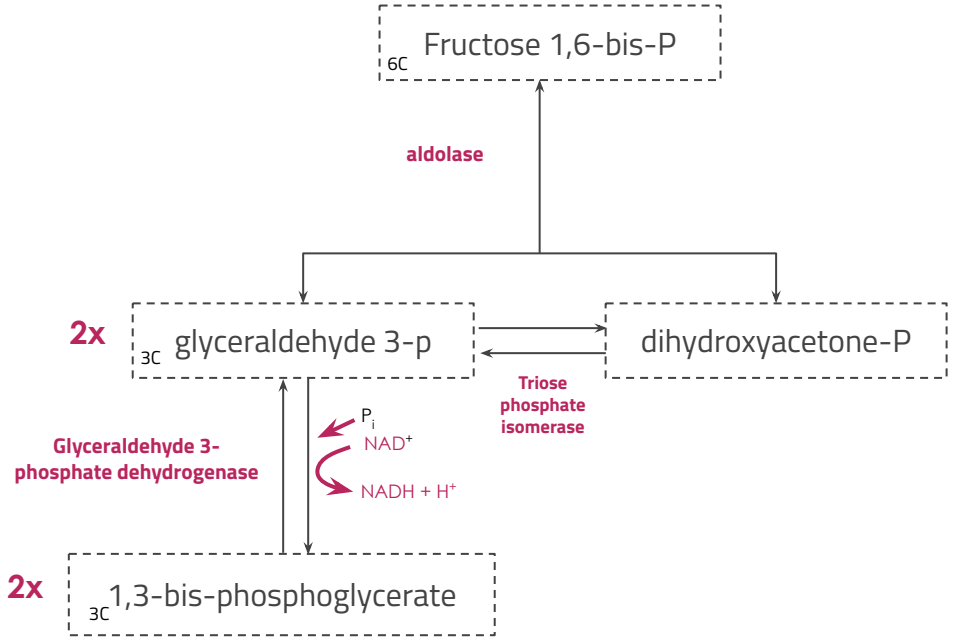
Aerobic glycolysis (2)

note that Reactions 1 - 5 consumed 2 molecules of ATP

4

Fructose 1,6-bisphosphate (6C) $\xrightleftharpoons{\text{Aldolase}}$ **Dihydroxyacetone phosphate (3C) + Glyceraldehyde 3-phosphate (3C)**

- Aldolase breaks down carbon-carbon bond (split the molecule).



5

Dihydroxyacetone phosphate (3C) $\xrightleftharpoons{\text{Triose phosphate isomerase}}$ **Glyceraldehyde 3-phosphate (3C)**

- isomerase means it changes the configuration.
- Isomerization from ketos form to aldose from.
- Glyceraldehyde 3 P and Dihydroxyacetone P have different pathway (glycolysis and glycerol pathway respectively)
- note from 439 : When the body needs fats it will convert glyceraldehyde 3-phosphate to dihydroxyacetone phosphate but in glycolysis the body needs energy so it will 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.

"About NAD^+ and NADH , keep in mind that the reverse can happen "

Aerobic glycolysis (3)

-note that Reactions 6-10 produce 2 molecules in each reaction

7

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

- **2x** ADP in.
- **2x ATP** out.
- produce energy
- reversible
- substrate level phosphorylation

8

2x 3-Phosphoglycerate (3C) —Phosphoglycerate mutase→ **2x 2-Phosphoglycerate (3C)**

- position of phosphate group is changed

9

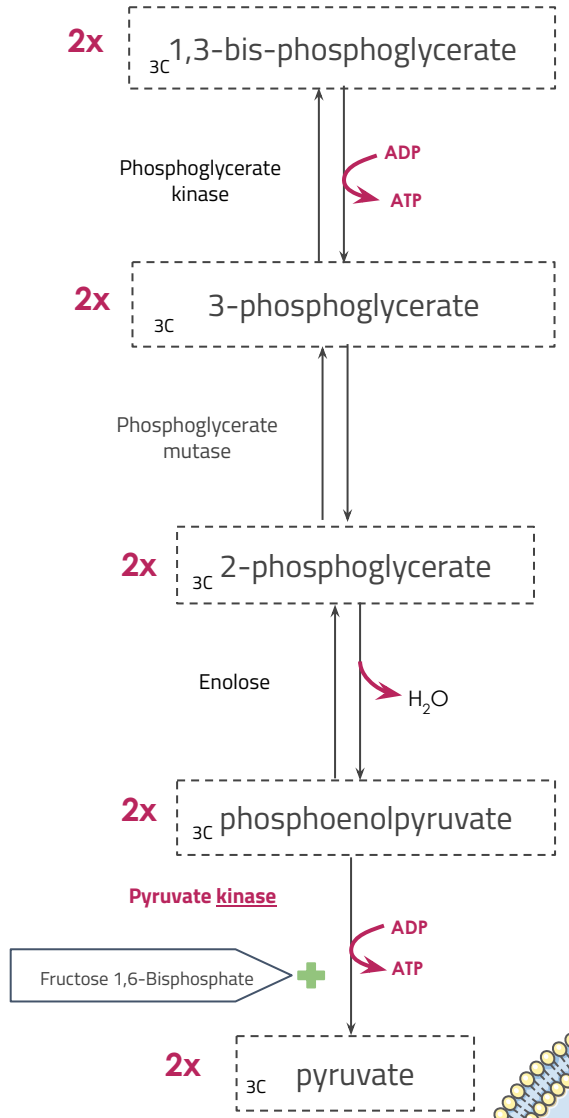
2x 2-Phosphoglycerate (3C) —Enolase→ **2x Phosphoenolpyruvate (3C)**

- **2x** H_2O out.

10

2x Phosphoenolpyruvate (3C) —Pyruvate kinase→ **2x Pyruvate**

- **2x** ADP in.
- **2x ATP** out.
- produce energy
- can be regulated (activated) :
  Fructose 1,6-Bisphosphate .
- Regulation of pyruvate kinase A
- substrate level phosphorylation

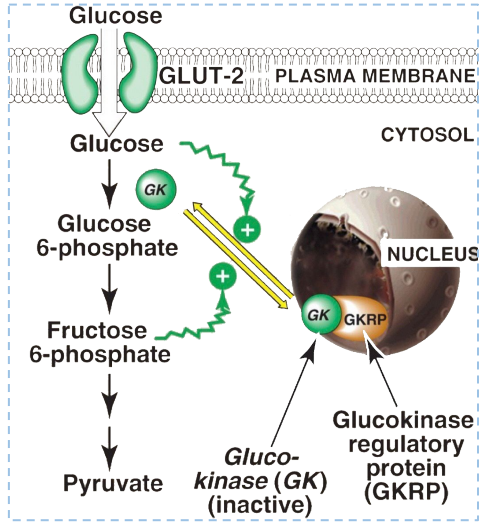


Regulation of enzymes: Glucokinase/Hexokinase

Regulation of : hexokinase (in most cells) and glucokinase (in liver or we can say hepatocyte)

- **Hexokinase :** it is inhibited by the reaction product, glucose-6-P (1st reaction) which accumulates when further metabolism of this hexose is reduced
- **Glucokinase (GK) :** It is inhibited indirectly by Fructose-6-P (2nd reaction) and is indirectly stimulated by glucose .
    - In the presence of high fructose-6-phosphate, Glucokinase (GK) translocates and binds tightly to **GKRP** (glucokinase regulatory protein) in the nucleus, making it **inactive** (by translocation into the nucleus) .
    - When glucose levels are high in blood and hepatocytes (**GLUT-2**), GK is released from GKRP and enters the cytosol

	Hexokinase	Glucokinase
Site	All tissues	hepatocytes
Inhibited by	glucose-6-Phosphate (1st reaction)	Fructose-6-Phosphate (2nd reaction) (indirectly)
Stimulated by	-	Glucose (indirectly)

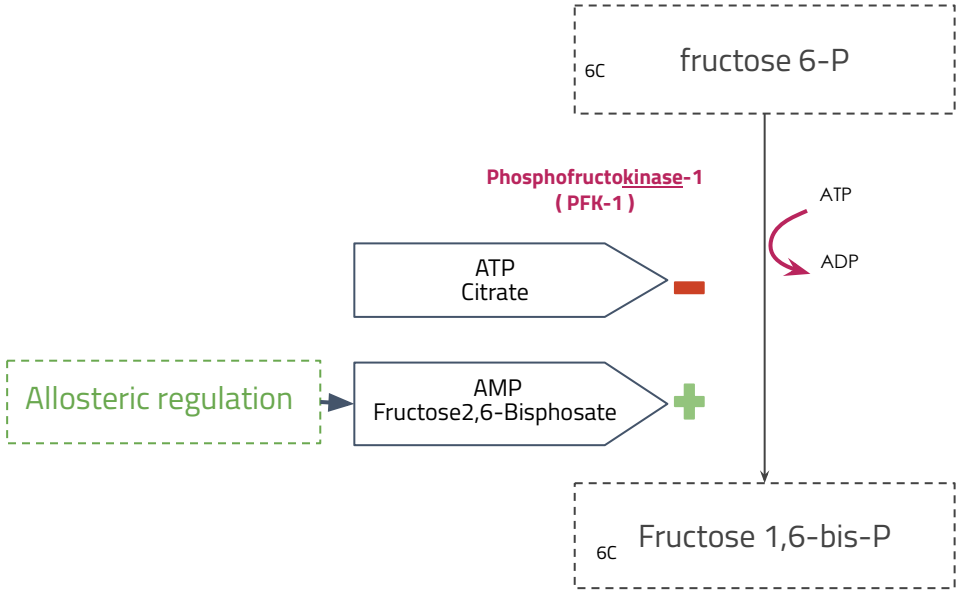


-helpful video

Regulation of enzymes: PFK-1 & Pyruvate Kinase

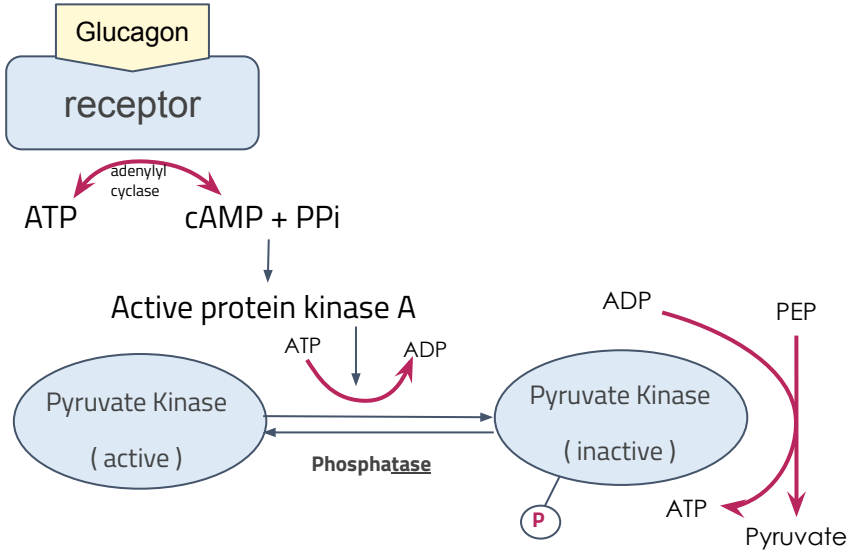
Phosphofructokinase-1 (PFK-1) enzyme :

- Rate limiting enzyme
- Reaction number 3 in glycolysis , is Irreversible reaction
- can regulate glycolysis through allosteric regulation
- ✚ activated by AMP and Fructose 2,6-bisphosphate.
- ▬ inhibited by ATP and citrate.
- regulatory mechanism : rabid , short term



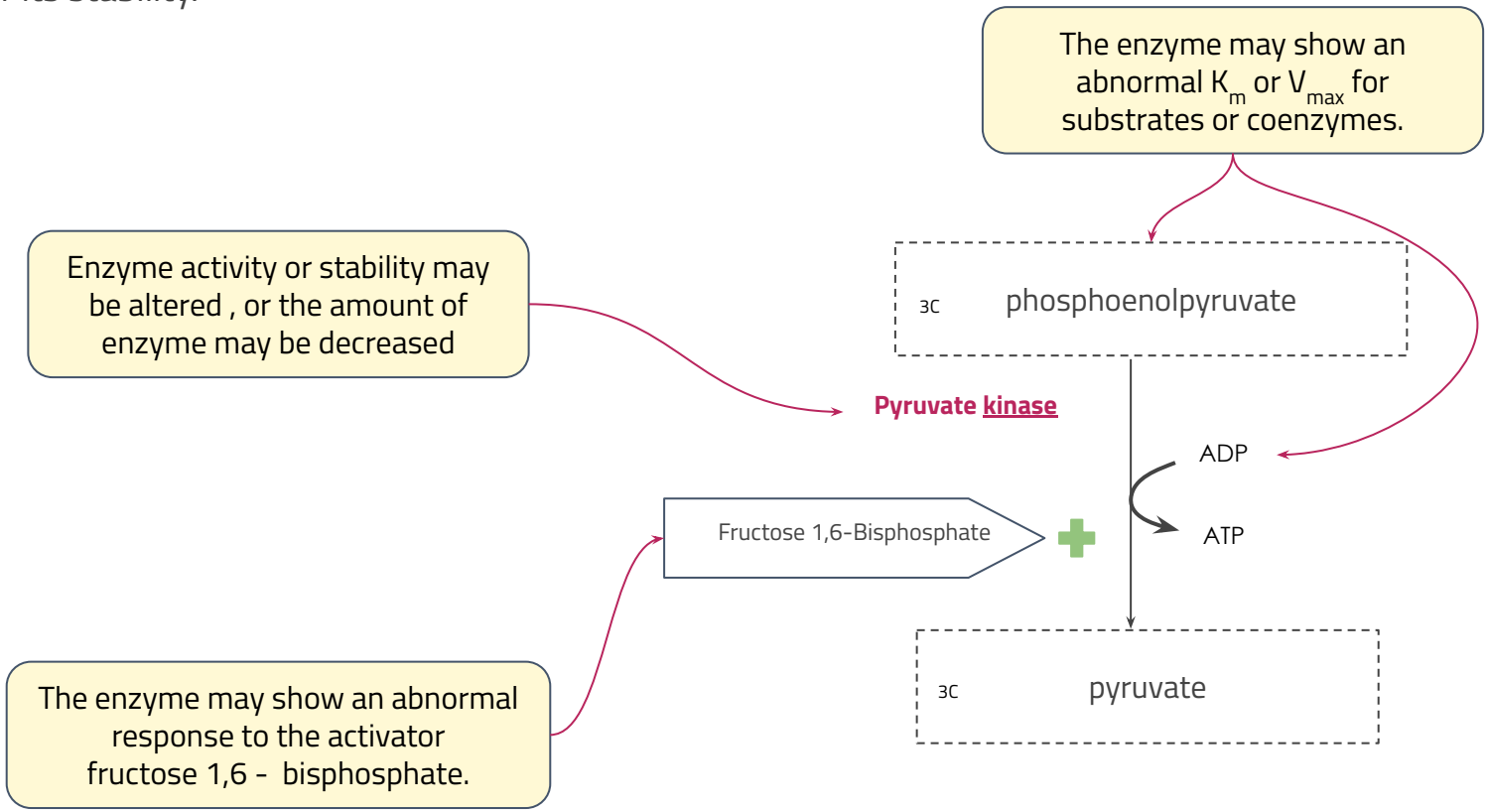
Pyruvate Kinase covalent modification:

- Reaction number 10 in glycolysis , Irreversible reaction
- Once glucagon (hormone) bind to the receptor it will activate the adenylyl cyclase that will produce cAMP which will activate protein kinase A. This protein will inhibit pyruvate kinase by adding P group to it (phosphorylation)
- ✚ Activation of enzyme can be done by phosphatase (removes a phosphate group)
- Protein kinase A is cAMP dependent
- 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.
- regulatory mechanism : rabid , short term

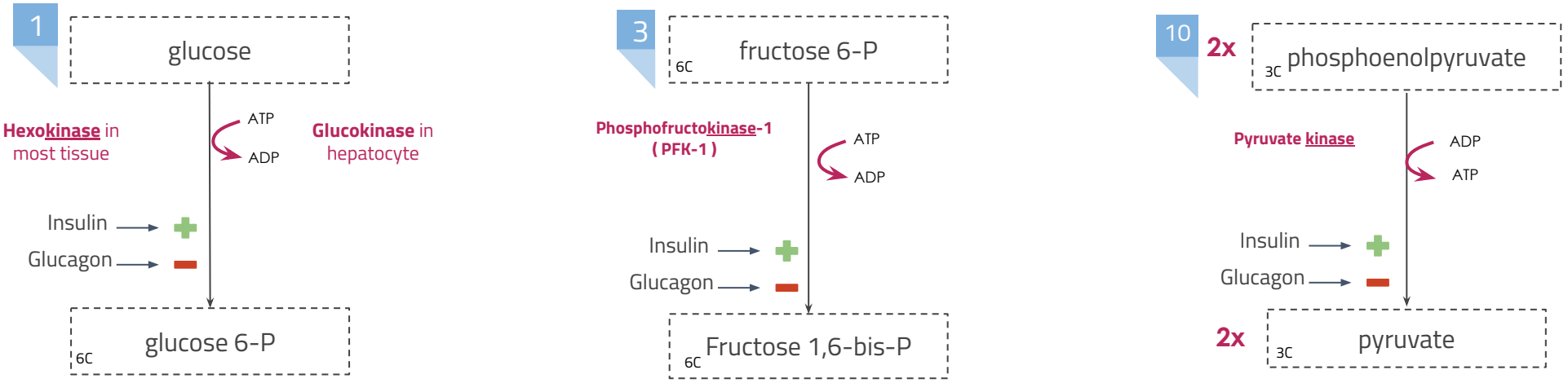


Pyruvate Kinase Deficiency Hemolytic Anemia

- Degree of deficiency is determined by mutation type (complete or mild/partial)
- Pyruvate kinase mutation may lead to :
  1. Altered enzyme Kinetics . (mutation in the allosteric binding site and its goal to inhibit enzyme activity) partial deficiency
  2. Altered response to activator . (mutation in the active site and its goal to stop enzyme activity) complete deficiency
  3. Decreased amount of the enzyme or its stability.



Regulation of enzymes : Long term regulation "Hormonal" of Glycolysis



- Long term regulation of glycolysis **in reactions 1 , 3 and 10** : (irreversible , Rate limiting enzymes)
  - Insulin : **Induction**
  - Glucagon : **Repression** (Inhibits glycolysis)

SUMMARY (regulation of glycolysis) :

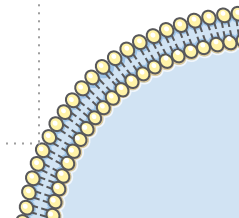
- Regulatory Enzymes (Irreversible reactions):
  - Glucokinase/Hexokinase
  - PFK-1
  - Pyruvate kinase
- Regulatory mechanism :
  - **Rapid, short-term:** Allosteric (Glucokinase/Hexokinase ,PFK-1) , Covalent modifications (Pyruvate kinase)
  - **Slow, long-term:** Induction/repression (insulin & Glucagon)

Substrate-level phosphorylation vs Oxidative phosphorylation

-helpful video

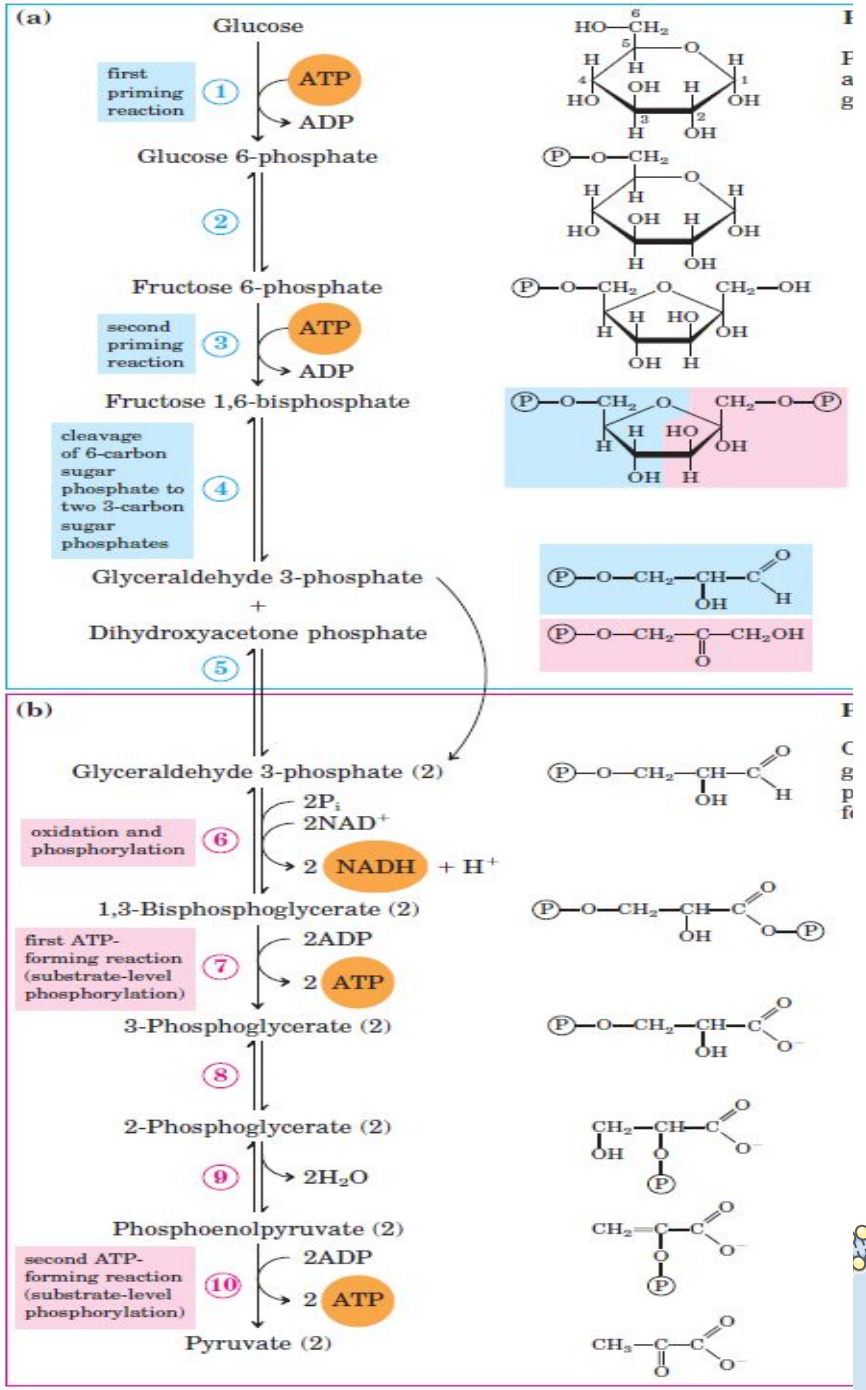
Phosphorylation: is the metabolic reaction of introducing a phosphate group into an organic molecule
*It's important in cellular process such as protein synthesis, cell division, signal transduction, cell growth.

Oxidative phosphorylation	Substrate-level phosphorylation
The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP **coupled to** the transfer of electrons from reduced coenzymes to molecular oxygen via the electron transport chain (ETC).	The formation of high-energy phosphate bonds by phosphorylation of ADP to ATP (or GDP to GTP) **coupled to** cleavage of high-energy metabolic intermediate (substrate)
Indirect ATP formation through redox reactions involving O2 as a final electron acceptor.	Direct ATP formation through phosphate transfer from substrate to ADP.
In mitochondria (in electron transport chain ETC)	In cytosol or mitochondria (It occurs in glycolysis & Kreb cycle)



Glycolysis: Aerobic glycolysis

ATP Consumption	2 ATP consumed in reaction 1 and 3
NADH Consumption	NADH was consumed when we convert pyruvate to lactate.
NADH Production	2 NADH was produced in reaction 6. (from glycolysis steps) 1 NADH = 3 ATP (Oxidative-level) for each NADH , 3ATP will be produced in ETC in the mitochondria.
ATP Production	2 ATP was produced in reaction 7 and 9 (from glycolysis steps) 2 ATP x 2= 4 ATP (substrate-level)
Net ATP produced	ATP consumed 2 ATP ATP produced 2x2 = 4 ATP (substrate-level) 2x3= 6 ATP (oxidative-level) total = 10 ATP 10-2 = 8 ATP



Anaerobic Glycolysis

Anaerobic glycolysis is the process by which the normal pathway of glycolysis is routed to produce lactate. It occurs at times when energy is required in the absence of oxygen.

NADH produced cannot be used ETC for ATP production, due to the lack of (O2 or/and no mitochondria)

Less ATP production, as compared to aerobic glycolysis.

Lactate is an obligatory end product 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 enzyme: Lactate dehydrogenase

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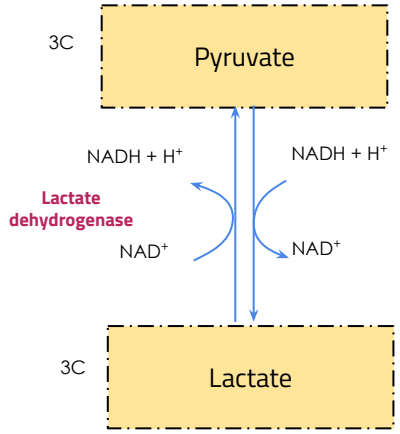
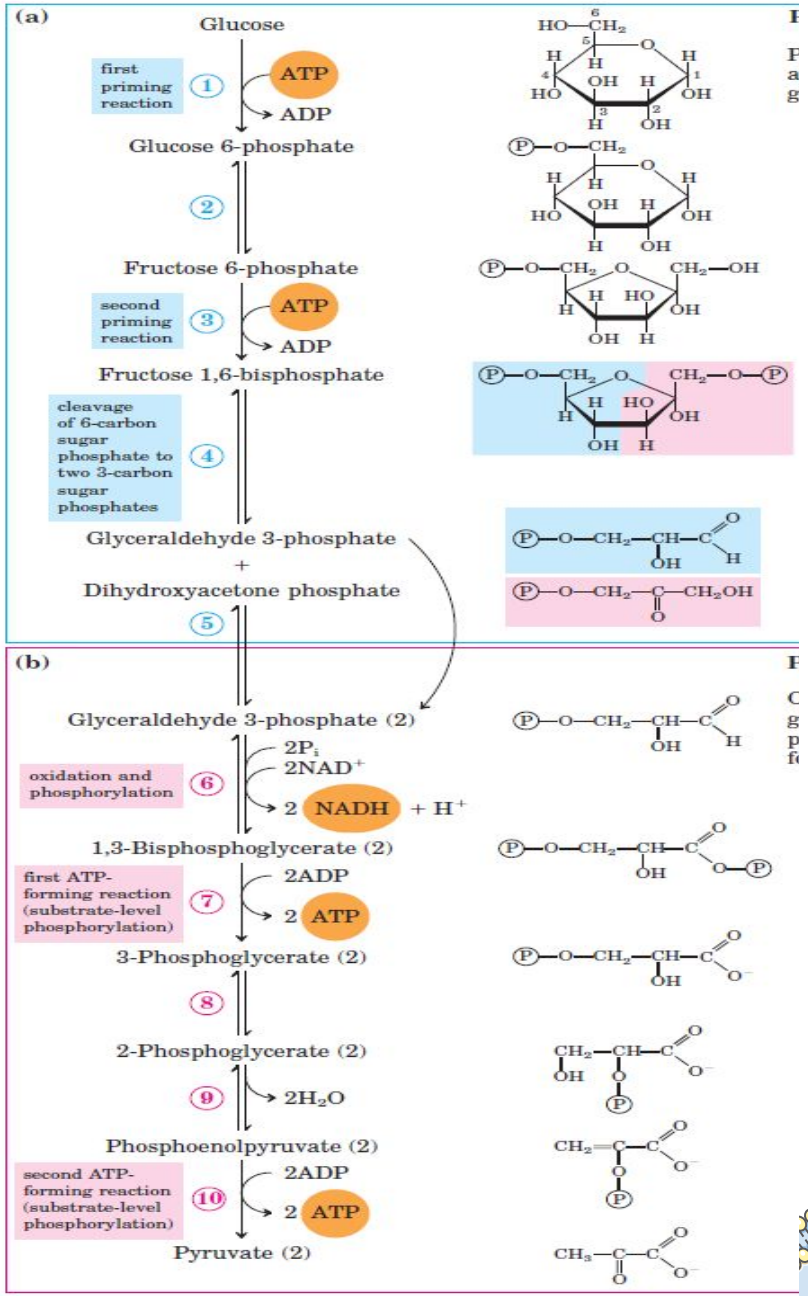
2x Pyruvate (3C) ⇌ **2x** Lactate

(Lactate dehydrogenase)



- Reversible reaction.
- **Lactate dehydrogenase** enzyme is used for both direction .
- **2x** NADH + H^+ in .
- **2x** NAD+ out .



Anaerobic Glycolysis : ATP Production

ATP Consumed	2 ATP	
ATP Produced	Substrate-level (Directly)	Oxidative-level
	2 ATP x 2= 4 ATP	2 NADH x 3= 6 ATP the hole energy will be consumed to produce lactic acid (0 ATP)
Total	4 ATP	
Net	4 -2 = 2 ATP	

Anaerobic Glycolysis in RBCs (2,3-BPG Shunt)

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

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

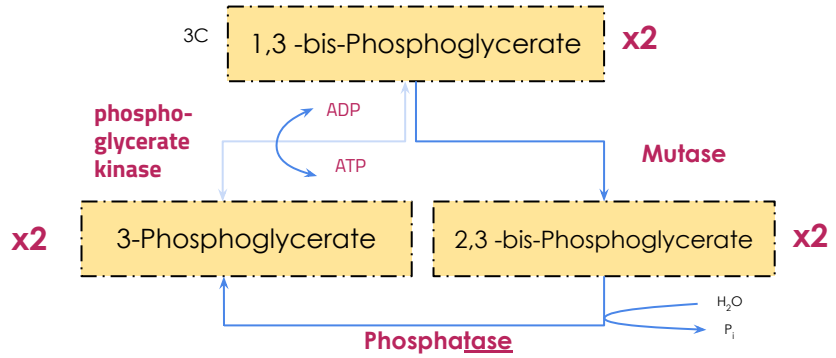
- H_2O in .
- P_i out .

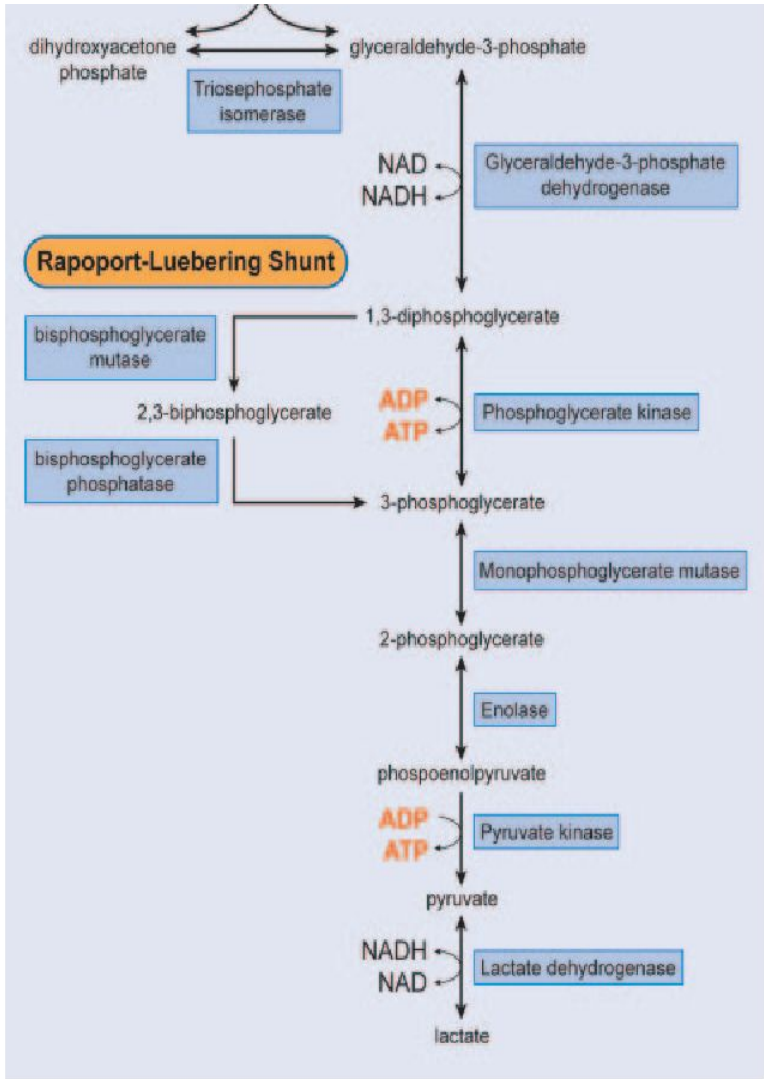
***3** **2x** 1,3 -bis-Phosphoglycerate (3C) —phospho- glycerate kinase→ **2x** 3-Phosphoglycerate (3C)

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

2,3-BPG shunt help us in loading and unloading of oxygen from hemoglobin.

No ATP production.





Glycolysis in RBCs (Net ATP production)

ATP consumed	2 ATP	
ATP produced	Substrate-level (directly)	Oxidative-level
	2 ATP x 2 = 4 ATP (without shunt) **1 ATP x 2 = 2 ATP** (with shunt)	**2 NADH X 3 = 6 ATP** the hole energy will be consumed to produce lactic acid (0 ATP)
Total	4 or 2 ATP (depends if there is shunt or not)	
Net	4 - 2 = 2 ATP (without shunt) 2-2 = 0 ATP (with shunt)	

Glycolysis in RBCs (Summary)

End product	-Lactate -No net production or consumption of NADH
Energy yield	-If no 2,3-BPG is formed : 2 ATP -If 2,3-BPG shunt occurs: 0 ATP
PK Deficiency hemolytic anemia depends on	-Degree of PK Deficiency → Mutation -Compensation by 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 occurs in :							
A	Nucleus	B	Mitochondria	C	Cytosol	D	Rough ER
Q2: PFK-1 is activated by ……. and inhibited by ……. ?							
A	AMP - ATP	B	ATP - Citrate	C	AMP - Fructose	D	cAMP - GKRP
Q3:During glycolysis, Glucose will be converted into glucose-6-phosphate in the liver by which enzymes?							
A	Glucokinase	B	Hexokinase	C	Glucose-6-p dehydrogenase	D	Phosphoglucose isomerase
Q4: How many net ATPs produced during aerobic glycolysis							
A	4 ATP	B	10 ATP	C	2 ATP	D	8 ATP
Q5: which of the following is considered as rate limiting enzyme ?							
A	Pyruvate kinase A	B	Lactate dehydrogenase	C	PFK-1	D	A and C

Answer Key: 1) B 2) A 3) A 4) D 5) C

Q6:what is the difference between Substrate-level phosphorylation vs. Oxidative phosphorylation ?

Q7: Mention net Yield in Glycolysis of RBCs?

Q8:What is the end product of anaerobic glycolysis ?

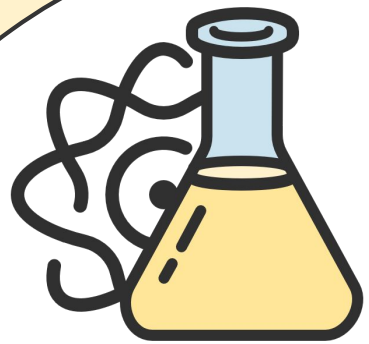
Q9:How many NADH are produced glycolysis per glucose?

Q6: Slide 12

Q7:
-2 ATP if 2,3-BPG is not formed.
-0 ATP if 2,3-BPG is formed.

Q8: Lactate

Q9: 2 NADH



Biochemistry 441

Girls

Boys

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Yara Almufleh
Reema Alrashedi
Wareef Almousa
Joud Alangari
Fay Alluhaidan
Sarah Alhamlan
Arwa Almobeirek
Jumana AL-qahtani
Latifa Alkhdiri
Alanoud Alhaider
Futoon Almotairi
Manal Aldhirgham
Raaoum Jabor
Norah alawlah
Shahad Helmi
Rand Aldajani

Khalid Alhamdi - Leader

Ahmed Alayban
Sultan Alosaimi
Abdullah Alomran
Bassam Alghizzi
Ibrahim Aljurayyan
Mohammed Almutairi
Turki Alkhalifa
Malik Alshaya
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