Glycolysis
Embden-Meyerhof Pathway

By:

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Objectives:

- ❑ understanding aspects of glucose digestion and transport
- ❑ Understand concepts of Glycolysis under aerobic and anaerobic conditions.
- ❑ Calculate energetics of glycolysis.
- ❑ Discuss function of glycolysis with emphasis of RBCs.
- ❑ Describe regulation of glycolysis.
- ❑ Discuss disease related to glycolysis.
- ❑ Answer questions about pyruvate dehydrogenase

CARBOHYDRATE DIGESTION:

- Most of the carbohydrates in foods are in complex forms, a very small amount are monosaccharides.
- In the mouth, salivary amylase hydrolyzes starch polymers to dextrins (<8–10 glucoses).
- the stomach acidity destroys the salivary amylase.
- In the intestine, the dextrins are hydrolyzed to the disaccharides to be further digested as follow:

– Maltase cleaves maltose to 2 glucoses

– Isomaltase cleaves isomaltose to 2 glucoses

– Lactase cleaves lactose to glucose and galactose

– Sucrase cleaves sucrose to glucose and fructose

- Uptake of glucose into the mucosal cells is performed by the sodium/glucose transporter, an active transport system.

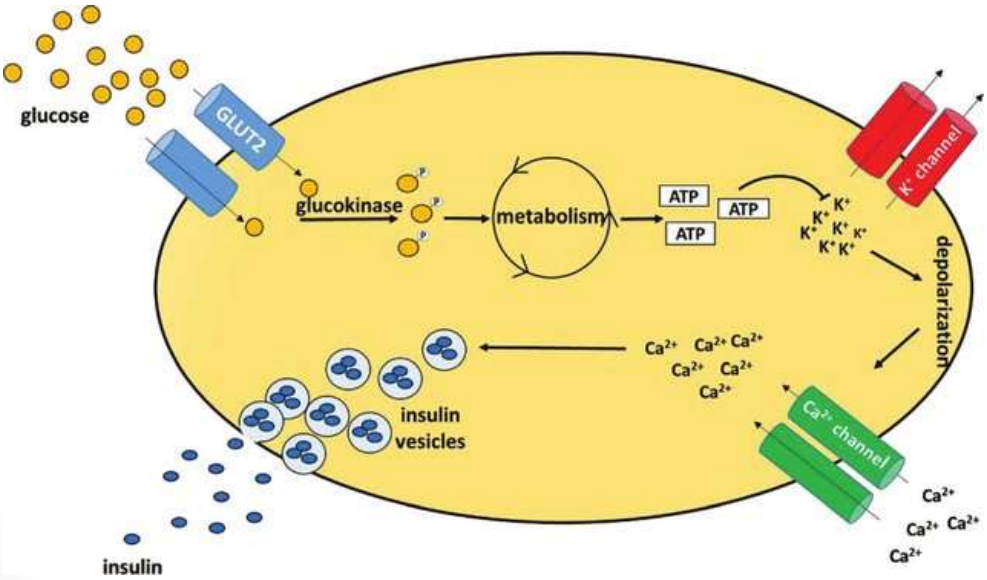
Glucose Transport into cells:

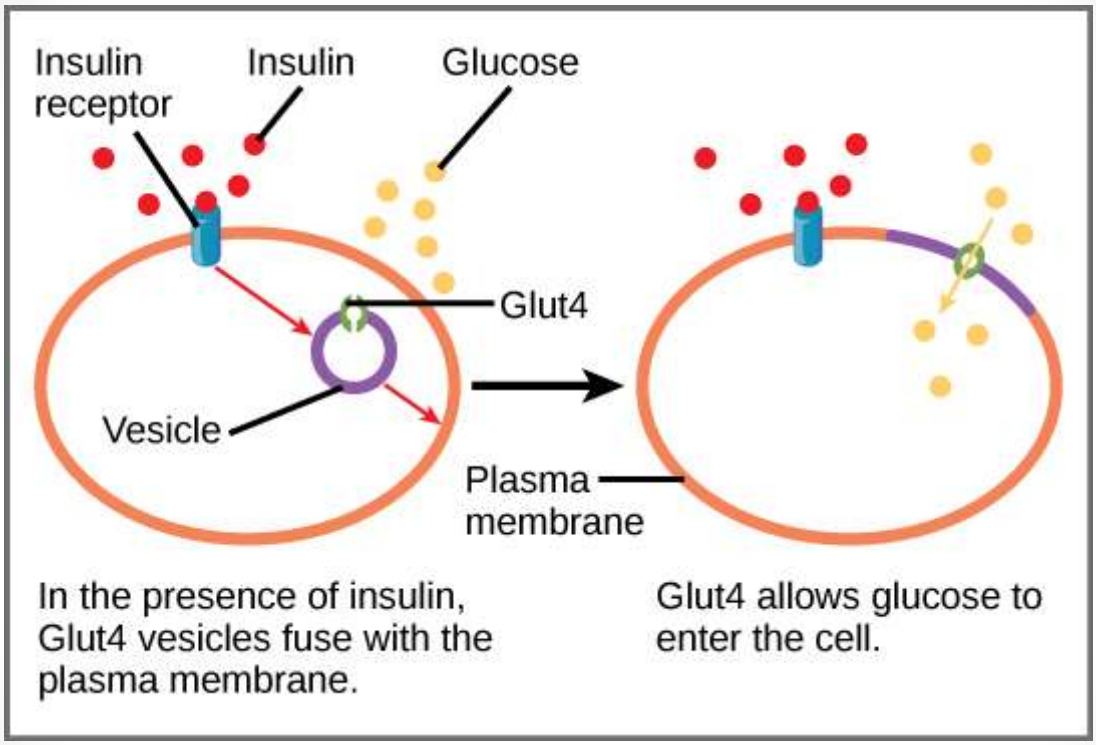
Glucose enters the cells by one of two transport mechanisms:

- **Na+ independent facilitated diffusion transport system:** Include glucose transporters in cell membranes designated GLUT-1 to GLUT-14. They don't utilize ATP.
- **Na+ dependent active co- transporter system:** as **SGLT1**. It absorbs glucose against their concentration gradient. It utilizes ATP. This system is active in intestine and kidney.
- **NB:** (Normal glucose concentration in peripheral blood is 70–110 mg/dL).

Properties of Selected Members of Human Glucose Transporters:

Type of GLUT	Tissues	Specific character	Functions
GLUT-1 GLUT-3	Most tissues (brain, red cells)	Low Km (High affinity) i.e. act on low concentration of blood glucose.	Basal uptake of glucose (insulin independent)
GLUT-2	• Liver, • pancreatic beta cells	High Km (low affinity) i.e. act only on high concentration of blood glucose.	*Storage. *Beta cell sensitization to produce insulin.
GLUT-4	• Skeletal muscle, • adipose tissue • Heart.	GLUT-4 is present intracellular and translocate to cell membrane in presence of insulin only. responds to glucose conc in peripheral blood	Insulin stimulate glucose uptake in skeletal muscles and adipose tissue. (Insulin dependent)





Major pathway of Glucose oxidation:

Glucose —**Glycolysis** (Cytoplasm, no O2 need)→ **Pyruvate** —**Oxidative Decarboxyl.** (Mitochondria, Presence of O2)→ **Acetyl CoA** —**Krebs' Cycle** (Mitochondria, Presence of O2)→ **CO2 + H2O + ATP**

Pyruvate —**Anaerobic Glycolysis** (Cytoplasm, no O2 need)→ **Lactate**

Definition of Glycolysis:

- Glycolysis is a cytoplasmic pathway that converts glucose into 2 pyruvates or 2 lactate, releasing a modest amount of energy in 2 substrate-level phosphorylations and 1 oxidation reaction.
- If a cell has mitochondria and oxygen, glycolysis is aerobic, but if (no mitochondria or oxygen) , glycolysis occur anaerobically (erythrocytes, exercising skeletal muscle), Glycolysis also provides intermediates for other pathways (Fatty acid synthesis).
- It is also utilized in its opposite direction in gluconeogenesis.
- It is also a route of metabolism for fructose, galactose, and other dietary carbohydrates, but they enter glycolysis through specific intermediates.

Intracellular site and tissue distribution of Glycolysis:

in the cell cytoplasm of all tissues but especially important in :

- **RBCs** because they are devoid of mitochondria.
- Tissue with limited blood supply and lack mitochondria as cornea
- Tissue with relatively few mitochondria as **leukocytes**
- **Contracting muscles** due to occlusion of blood vessels by the muscular contraction that decreases oxygen.
- **Brain:** because fatty acids can't pass blood brain barrier, so brain takes most of its energy from glycolysis.

Steps of glycolysis:

It consists of 10 steps:

- The first five steps:

glucose $\xrightarrow{-2ATP}$ 2 glyceraldehyde-3-phosphate

- The second five steps: + 4 ATP molecules + 2 NADH
- The net production is 2 ATP & 2 NADH molecules per one molecule of glucose.

1- Glucose into glucose 6-phosphate, with the conversion of ATP into ADP by **hexokinase enzyme** in all tissues and **glucokinase** in the liver. *Glucose entering the cell is trapped by phosphorylation using ATP*

2- Glucose 6-phosphate is isomerized to fructose 6-phosphate by **phosphohexose isomerase enzyme**.

3- Fructose 6-phosphate is phosphorylated by ATP, forming fructose 1,6-bisphosphate and ADP by **phosphofructokinase-1 (PFK-1) enzyme**. This is the first committed step of glycolysis.

4- Fructose 1,6-bisphosphate is cleaved—**with aldolase enzyme** —to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP).

5- Dihydroxyacetone phosphate is isomerized—by **phosphotriose isomerase enzyme**—to glyceraldehyde 3-phosphate, i.e Two moles of glyceraldehyde 3-phosphate are formed from 1 mole of glucose.

6- Glyceraldehyde 3-phosphate is oxidized by NAD+ and with inorganic phosphate (Pi) to form 1,3-bisphosphoglycerate (contain high energy phosphate) and NADH + H+. by **glyceraldehyde 3-phosphate dehydrogenase enzyme**.

7- 1,3-Bisphosphoglycerate reacts with ADP—using **phosphoglycerate kinase enzyme**—to produce 3-phosphoglycerate and ATP. **(Substrate level phosphorylation)**

8- 3-Phosphoglycerate is converted—via **phosphoglycero mutase enzyme**—to 2-phosphoglycerate.

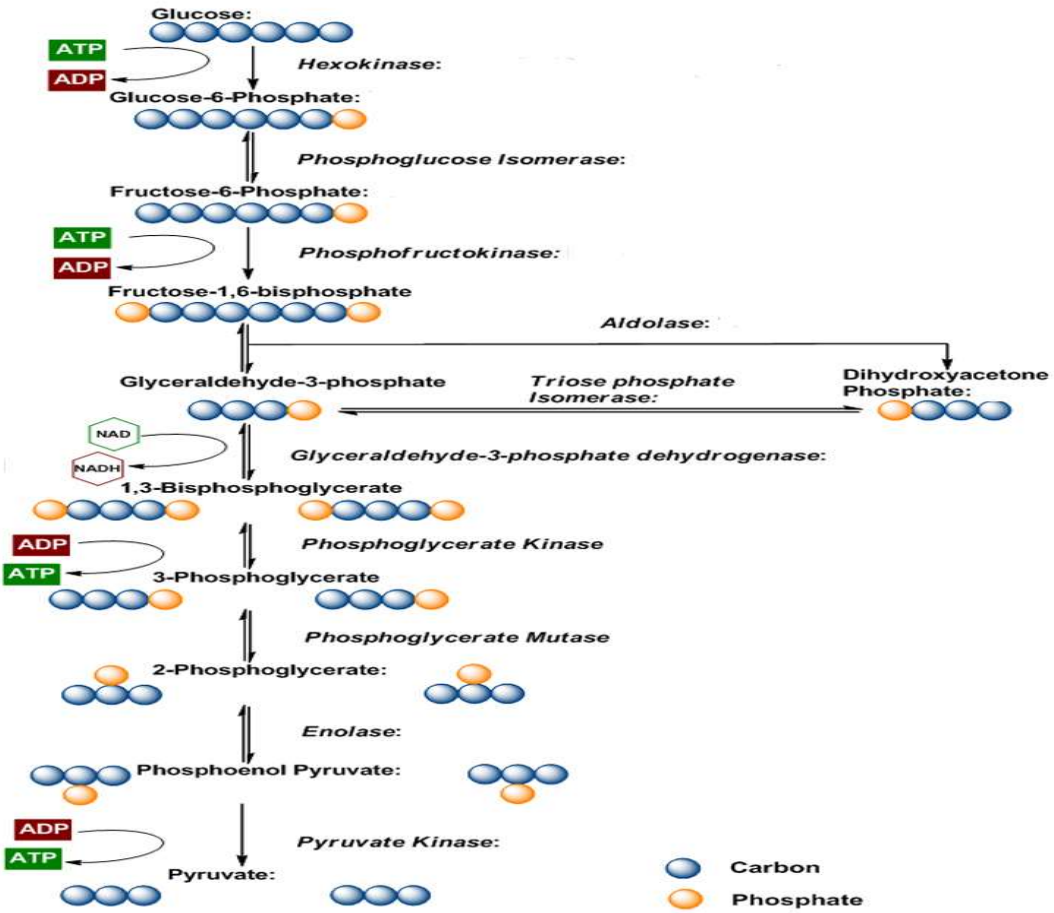
9- 2-Phosphoglycerate is dehydrated using **enolase enzyme**—to phosphoenolpyruvate (PEP), contains a high-energy phosphate.

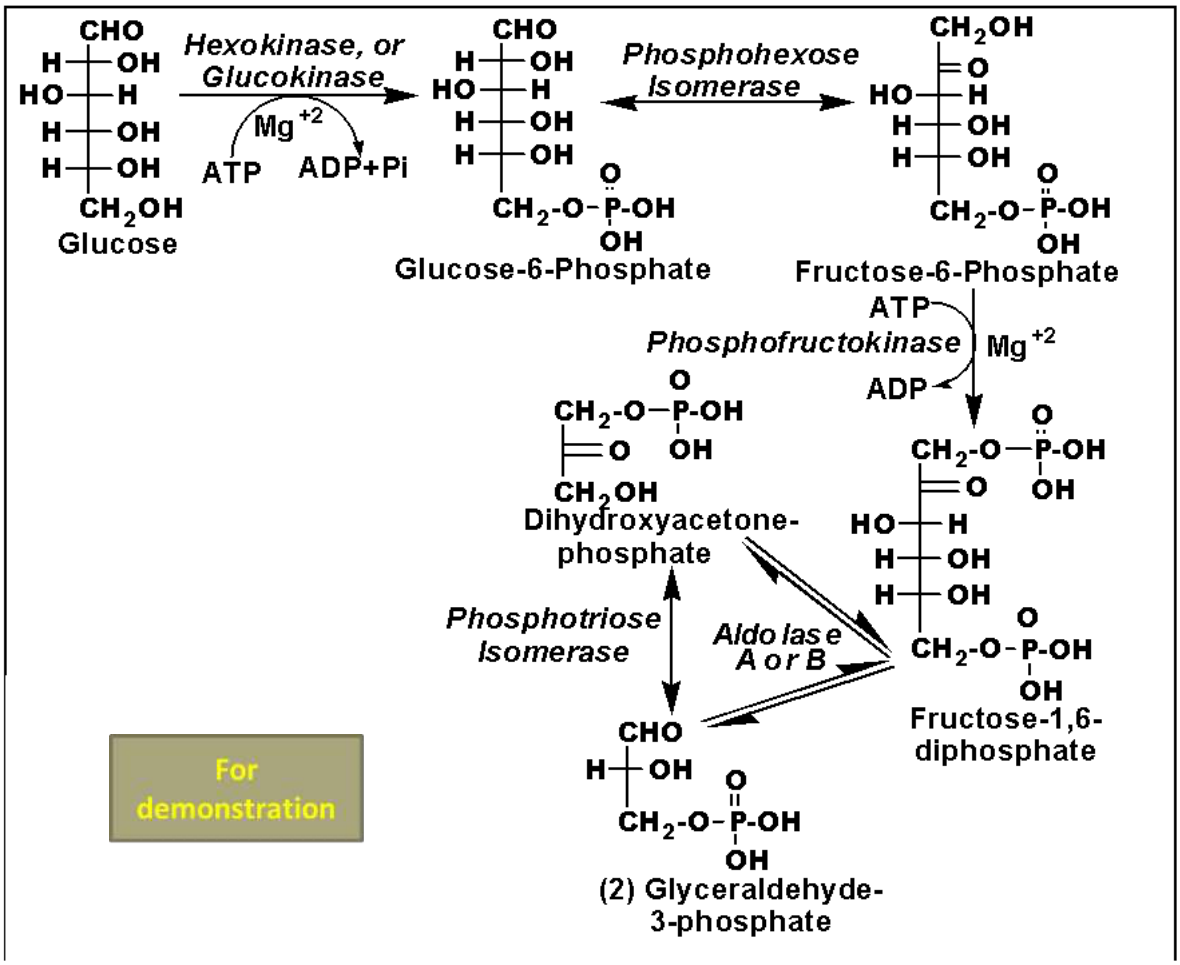
10- Phosphoenolpyruvate reacts with ADP to form pyruvate and ATP by **pyruvate kinase enzyme**. **(Substrate level phosphorylation)**

- **Under aerobic conditions** glycolysis end at this point and pyruvate is oxidatively decarboxylated into acetyl-CoA that enters Krebs' cycle for further oxidation. NADH.H+ produced enters ETC in mitochondria to produce ATP and get reoxidized into NAD.

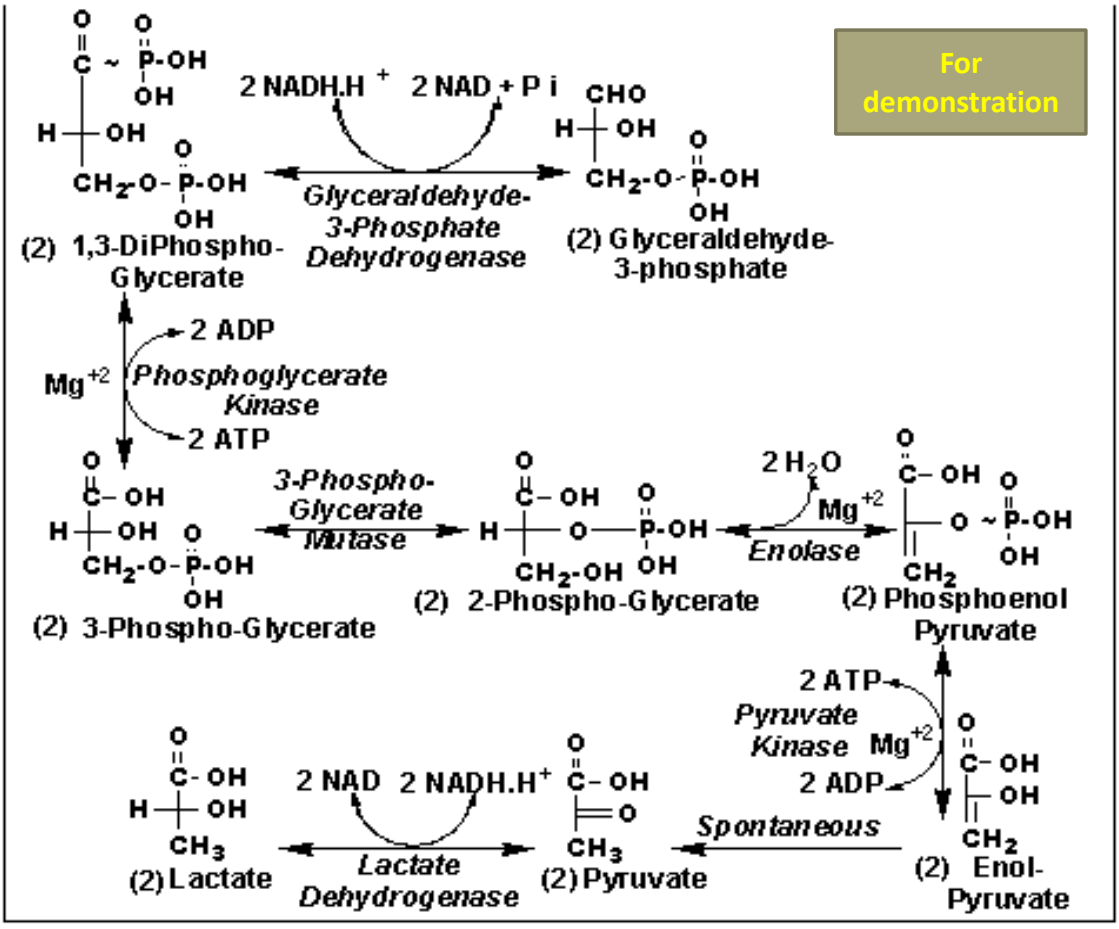
- **Under anaerobic conditions**; $NADH.H^+$ must be reoxidized to NAD to allow glycolysis to continue. This is done by lactate dehydrogenase (LDH) that uses NADH.H+ and regenerate NAD.

$$\text{Pyruvate} + NADH.H^+ \longleftrightarrow \text{Lactate} + NAD$$





For demonstration



Hexokinase	Glucokinase
Site: Most tissues.	Liver and pancreatic islet cells.
Affinity for glucose: It has a high affinity (low Km) i.e. acts at very low glucose concentration).	It has a low affinity (a high Km). It acts at high blood glucose concentration (> 90 mg/dl)
Allosteric inhibition by glucose-6-phosphate): Yes.	No.
Effect of feeding, glucose and insulin: No change in activity	Increased rate of activity (induction).
Effect of fasting or diabetes: No change in activity.	Decreased rate of activity (repressed).
Function: for energy production irrespective of glucose blood concentration.	remove glucose from the blood following a meal for storage.

Bioenergetics of glycolysis:

Under anaerobic conditions of one mole of glucose:

1- Total ATP lost = 2 ATP;

- One ATP in the activation of glucose to glucose-6-p.
- One ATP in the activation of fructose-6-p to fructose1,6 diphosphate.

2- Total ATP gained = 4 ATP;

- 2 ATP by substrate level phosphorylation from 1,3 diphosphoglycerate
- 2 ATP by substrate level phosphorylation from phosphoenol pyruvate.

3- Net ATP gained = 4 ATP gained - 2 ATP lost = 2 ATP.

Bioenergetics of glycolysis:

Under aerobic conditions of one mole of glucose:

1. Total ATP lost = 2 ATP, as anaerobic conditions.

2. Total ATP gained = 8 or 10 ATP

- 4 ATP (obtained by substrate level phosphorylation)
- **2 $NADH.H^+$** (produced from oxidation of glyceraldehyde-3-phosphate) $\Rightarrow$ **2 X 2 or 3 ATP = 4 or 6 ATP**, after oxidation in the ETC, depending on the transporting shuttle used. Thus, 8 or 10 ATP are generated.

3. Net ATP gained = 6 or 8 ATP as follows,

- 8 ATP or 10 ATP – 2 ATP = 6 or 8 ATP

Shuttles for NADH.H+:

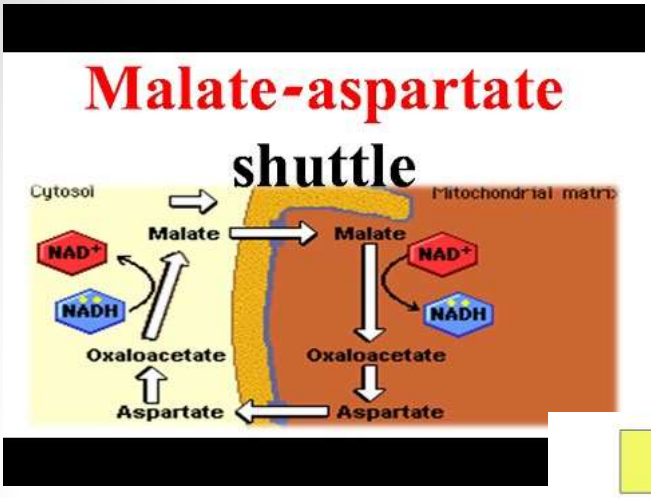
NADH, produced in glycolysis, can be utilized for ATP production in the mitochondria; however the inner mitochondrial membrane is impermeable to NADH. Therefore, the electrons are passed to the mitochondrial electron transport chain by two shuttle systems:

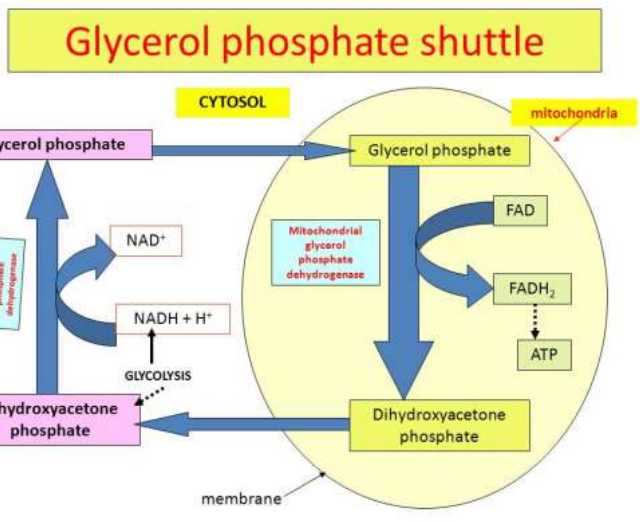
A. Malate Aspratate shuttle:

- Using this shuttle, the 2 cytoplasmic NADH.H$^+$ gives 2 mitochondrial NADH.H$^+$ $\Rightarrow$ 2 X 3 ATP = 6 ATP.

B) Glycerophosphate shuttle:

- Using this shuttle the 2 cytoplasmic NADH.H$^+$ gives 2 mitochondrial $FADH_2$ $\Rightarrow$ 2 X 2ATP = 4 ATP.





For demonstration

Differences between aerobic and anaerobic glycolysis:

	Aerobic	Anaerobic
1- End product	pyruvate	Lactate
2- Energy	6-8 ATP	2 ATP
3-Regeneration of NAD^+	Through ETC in mitochondria	Through lactate formation
4- Availability to TCA in mitochondria	Available	Not available as pyruvate is converted into lactate.

Clinical Correlates:

- The **enzyme enolase** is inhibited by **fluoride.** for glucose estimation tests, blood is collected in tubes containing fluoride to prevent continuing glycolysis in a patient's blood samples
- **Maturity-onset diabetes of the young (MODY) type 2** is an autosomal dominant disorder involving mutations in the **glucokinase gene** with deficiency of glucokinase activity (in pancreas) result in hypoinsulinemia and hyperglycemia that is usually managed with diet alone.
- **Arsenate toxin:** inhibits the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by mimicking phosphate in the reaction. The arsenate-containing product is water labile, enabling glycolysis to proceed but resulting in two ATPs less.
- **Iodoacetate,** an SH poison inhibits **glyceraldehyde-3-phosphate dehydrogenase** which contains this group at its active site.

Regulation of Glycolysis:

A. ***Key regulatory enzymes:*** These Three enzymes in the pathway catalyze reactions that are irreversible. When the liver produces glucose, different reactions and thus different enzymes must be used at these 3 points: Hexokinase (or Glucokinase), phosphofructokinase-1 (PFK-1) and pyruvate kinase.

I) Hexokinase & Glucokinase:

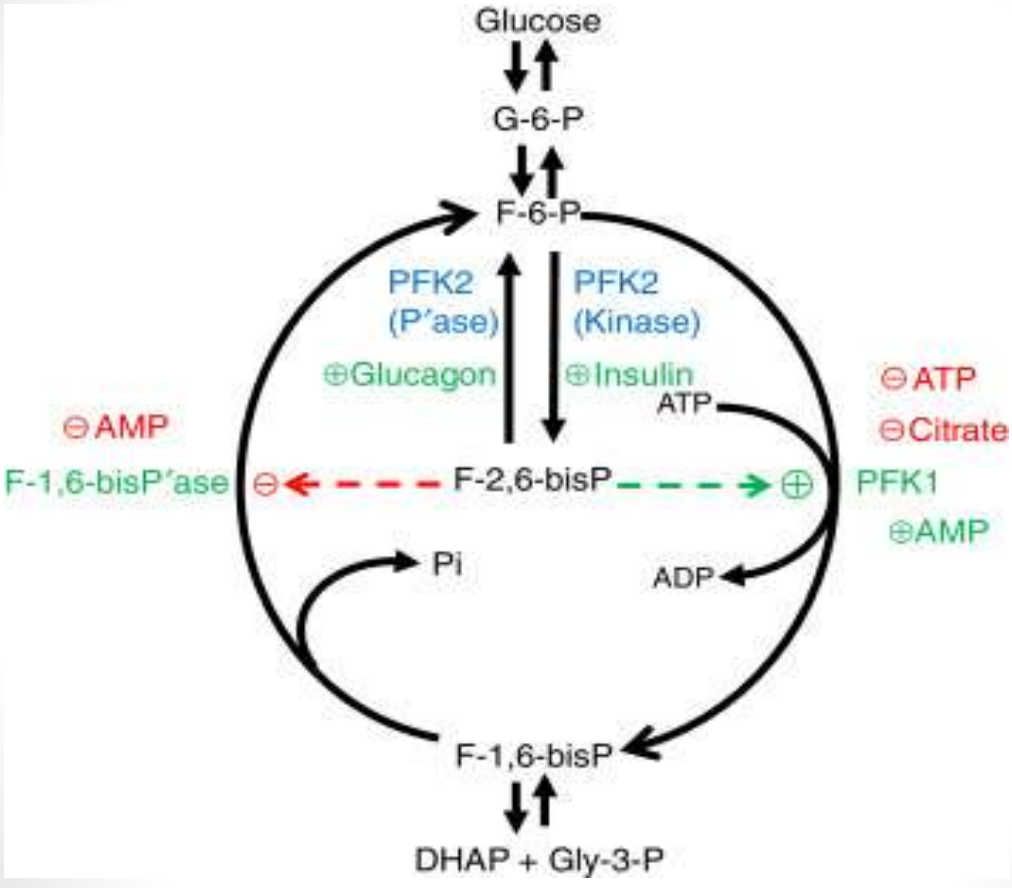
- Hexokinase: inhibits allosterically by glucose-6-p.
- Glucokinase: is stimulated by feeding and insulin & inhibited by fasting and diabetes.

II) Phosphofructokinase-1: It is the most important enzyme because it is the first committed reaction in glycolysis:

- **Inhibited allosterically by** ATP, citrate (High energy signals).
- **activated allosterically by** AMP which signal depletion of energy stores.
- **It is also regulated by fructose 2,6 biphosphate:** It is the most potent activator of PFK-1 (glycolysis), and also an inhibitor of fructose 1,6 – biphosphatase (gluconeogenesis). Fructose 2,6 biphosphate is formed by phosphofructokinase-2 (PFK-2), which is activated by insulin and inhibited by glucagon.

III) Pyruvate kinase:

- It is inhibited by excess ATP
- It is stimulated by fructose-1,6-bisphosphate (feed-forward activation), ADP and AMP.
- ***B. Covalent regulation:*** phosphorylation-dephosphorylation mechanism. Its active form is the dephosphorylated form
- ***C. Hormonal regulation:***
- **Insulin (Fed state):** stimulates glycolysis, dephosphorylation.
- **Adrenaline and glucagon (Fasting state):** are inhibitory of glycolysis, phosphorylation.



Glycolysis in RBCs:

1- Red blood cells (RBCs) lack mitochondria, therefore they depend on glycolysis as a source of energy:

- RBCs need ATP mainly for Na-K ATP pump. so defect of glycolysis lead to accumulation of Na+ and hence water inside RBCs ending in hemolysis of RBCs.
- So deficiency of glycolytic enzymes is associated with hemolytic anemia
- The most common deficiency is pyruvate kinase (PK):

- pyruvate kinase (PK) deficiency:
- is the second most common genetic deficiency that causes a hemolytic anemia after G6PD
- Characteristics include:
- Chronic hemolysis
- Increased 2,3-BPG and therefore a lower-than-normal oxygen affinity of HbA
- Absence of Heinz bodies (Heinz bodies are more characteristic of G6PDH deficiency)
- In pyruvate kinase deficiency, the decrease in ATP causes the erythrocyte to lose its characteristic biconcave shape and its destruction in the spleen. In addition, decreased ion pumping by Na+/K+-ATPase results inloss of ion balance and causes osmotic fragility, leading to swelling and lysis.

2- Rapoport-Lubering cycle: It is a side-pathway from glycolysis in RBCs

- **It is formed of two steps:**

1,3-diphosphoglycerate is mutated into 2,3-diphosphoglycerate (2,3-DPG or BPG) by mutase: from high to low energy bond.

By 2,3-DBG phosphatase, producing 3-phosphoglycerate. That rejoins the pathway of glycolysis.

Importance of Rapoport-Lubering cycle:

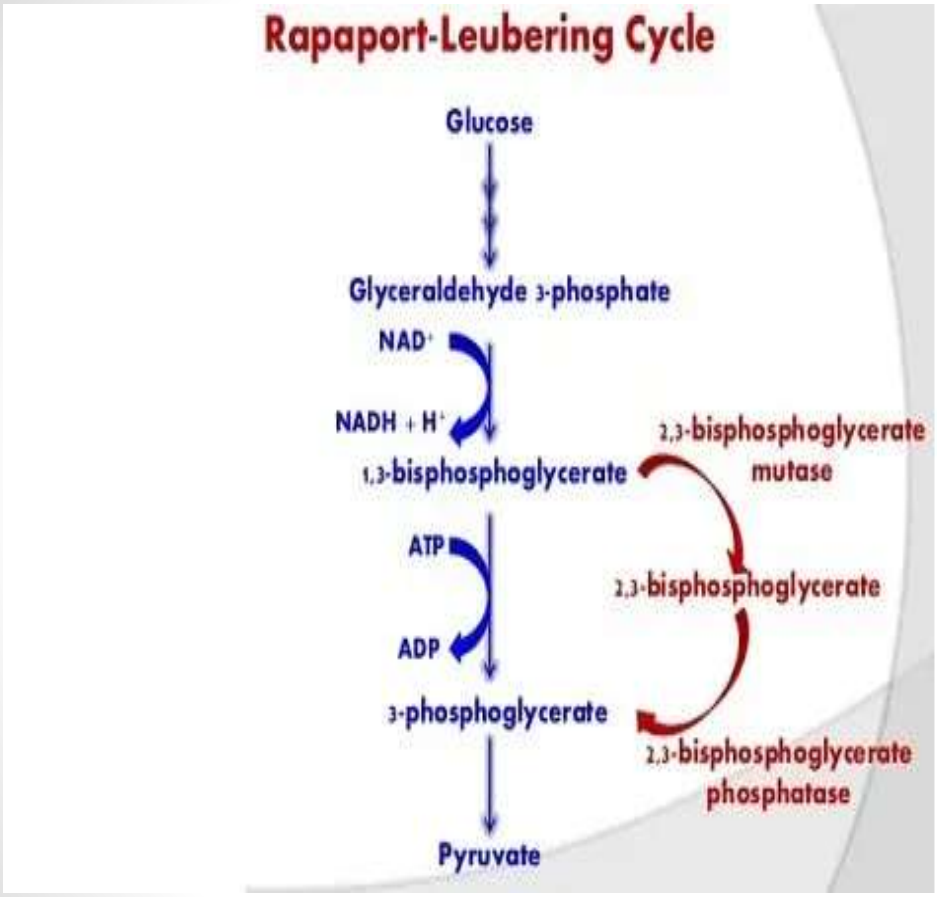
Production of 2,3 DPG (or BPG) that

A- It binds to the β-chains of hemoglobin A, decreases its affinity to oxygen, helping oxygen dissociation and unloads oxygen in tissues (shift of oxygen dissociation curve to the right) but still allows 100% saturation in the lungs. An abnormal increase in erythrocyte 2,3-BPG might shift the curve far enough so HbA is not fully saturated in the lungs.

B- Levels of 2,3-DPG increases markedly in peripheral hypoxic tissues and hypoxic conditions (high altitudes, anemia).

C- During storage of blood in blood banks, 2,3-DPG concentration decreases gradually to reach traces at ten days. So, Hb of this blood has a high affinity to O_2 and is not suitable for blood transfusion to hypoxic patients and severely ill patients.

D- Fetal hemoglobin (alpha2 gamma2) binds 2,3-DPG less strongly than adult hemoglobin and therefore has a higher O_2 affinity to extract O_2 from mother's blood.



Importance of Glycolysis:

1. It is the major source of energy in certain tissues, e.g. RBCs and skeletal muscles.
2. It provides pyruvate for Krebs' cycle.
3. Dihydroxyacetone phosphate (DHAP) is used in liver and adipose tissue for triglyceride synthesis.
4. 3-phopshoglycerate can be converted into AAs.
5. Production of 2,3-DPG for tissue oxygenation.
6. Glycolysis in anaerobic tissues is the major source of lactic acid that is used by the liver for gluconeogenesis.

Glycolysis in cancer cells:

Rapidly growing tumor cells exhibit a high rate of glycolysis with inadequate oxygen supply (hypoxia) and lactate accumulation. This produces acidic local pH in the tumor, a situation that was utilized to develop cancer therapy that could be locally activated by this acidic pH.

Lactic acidosis:

Blood lactate comes from glycolysis in RBCs and muscles.

Fates of blood lactate:

1- taken up by the heart to be oxidized into pyruvate then to acetyl CoA and Krebs' cycle

2- Uptake by liver and kidney to be converted into glucose by gluconeogenesis.

3- Excreted as in sweat and urine.

- **Causes of hyperlactemia:**

1. Sever exhaustive muscle exercise.
2. Anoxia that increases lactate production as in shock.
3. Hypoglycemic antidiabetic drugs that increase lactate production by anaerobic oxidation of glucose.
4. Liver diseases that decrease lactate utilization.

NB: If hyperlactemia occurred (blood lactate exceeds it normal level, this depletes the blood alkali reserve leading to lactic acidosis. Uncontrolled lactic acidosis could cause coma.

MCQs:

A 47-year-old obese man complains of having to get out of bed three times a night tourinate (polyuria), being constantly thirsty (polydipsia), and eating more often (polyphagia). The patient is diagnosed with insulin resistant diabetes mellitus (type 2). If the patient's symptoms are due to a problem at the level of the glucose transporter, which one of the tissues indicated below will be most affected?

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(B) Small intestine

(C) Muscle

(D) Brain

(E) Liver

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Compared with the resting state, vigorously contracting skeletal muscle shows:

A. decreased AMP/ATP ratio.

B. decreased levels of fructose 2,6-bisphosphate.

C. decreased NADH/NAD+ ratio.

D. increased oxygen availability.

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Thank You