GLYCOLYSIS

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BIOMEDICAL IMPORTANCE

- Most tissues have at least some need for glucose.
- In the brain, the need is serious, and even in prolonged fasting the brain can meet no more than about 20% of its energy needs from ketone bodies.
- **Glycolysis**, the major pathway for glucose metabolism, **occurs in the cytosol of all cells** to provide energy and intermediates for other metabolic pathways.
- **Glycolysis is the principal route for carbohydrate metabolism**.
- Erythrocytes (red blood cells), which lack mitochondria, are completely dependent on glucose as their metabolic fuel, and metabolize it by anaerobic glycolysis.
- However, to oxidize glucose beyond pyruvate (the end product of glycolysis) requires both oxygen and mitochondrial enzyme systems: the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.

DIGESTION & ABSORPTION OF CARBOHYDRATES

- The digestion of carbohydrates is by hydrolysis to liberate oligosaccharides, then disaccharides and free monosaccharides.
- The increase in blood glucose after a test dose of a carbohydrate compared with that after an equivalent amount of glucose is known as the **glycemic index**.
- Foods that have a low glycemic index are considered to be more beneficial because they cause less fluctuation in insulin secretion.

DIGESTION & ABSORPTION OF CARBOHYDRATES

- **Amylases Catalyze the Hydrolysis of Starch**
- The hydrolysis of starch is catalyzed by **salivary** and **pancreatic amylases**, which catalyze random hydrolysis of a(1 → 4) glycoside bonds, yielding dextrins, then a mixture of glucose, maltose, and maltotriose and small branched dextrins (from the branchpoints in amylopectin).
- **Disaccharidases Are Brush Border Enzymes**
- The disaccharidases, maltase, sucrase-isomaltase (a bifunctional enzyme catalyzing hydrolysis of sucrose and isomaltose), lactase, and trehalase are located on the brush border of the intestinal mucosal cells (enterocytes).
- Then the resultant monosaccharides and those arising from the diet are absorbed in the small intestine.

There Are Two Separate Mechanisms for the Absorption of Monosaccharides in the Small Intestine

- The SGLT1 transporter is coupled to the Na^+-K^+ pump, allowing glucose and galactose to be transported against their concentration gradients.
- The GLUT5 Na^+-independent facilitative transporter allows fructose, as well as glucose and galactose, to be transported down their concentration gradients.
- Exit from the cell for all monosaccharides is via the GLUT2 facilitative transporter.

SGLT: sodium-glucose linked transporter

GLUT: glucose transporter

TRANSPORT OF GLUCOSE INTO CELLS

- Glucose cannot diffuse directly into cells, but enters by one of two transport mechanisms:
  - Na^+-independent, facilitated diffusion transport system (GLUT 1 – 14)
  - Na^+-monosaccharide cotransporter system

TABLE 12.1 Properties of Selected Members of Human Glucose Transporters

Transporters	Major Tissue Distribution	Properties
GLUT1	Brain, microvessels, red blood cells, placenta, kidney, and many other cells	Low K_m (about 1 mM), ubiquitous basal transporter
GLUT2	Liver, pancreatic β-cell, small intestine	High K_m (15–20 mM)
GLUT3	Brain, placenta, fetal muscle	Low K_m, provides glucose for tissue cells metabolically dependent on glucose
GLUT4	Skeletal and heart muscle, fat tissue (adipocytes)	K_m of 5 mM, insulin responsive transporter
GLUT5	Small intestine, testes	Exhibits high affinity for fructose
SGLT1	Small intestine and renal tubules	Low K_m (0.1–1.0 mM)
SGLT2	Renal tubules	Low K_m (1.6 mM)

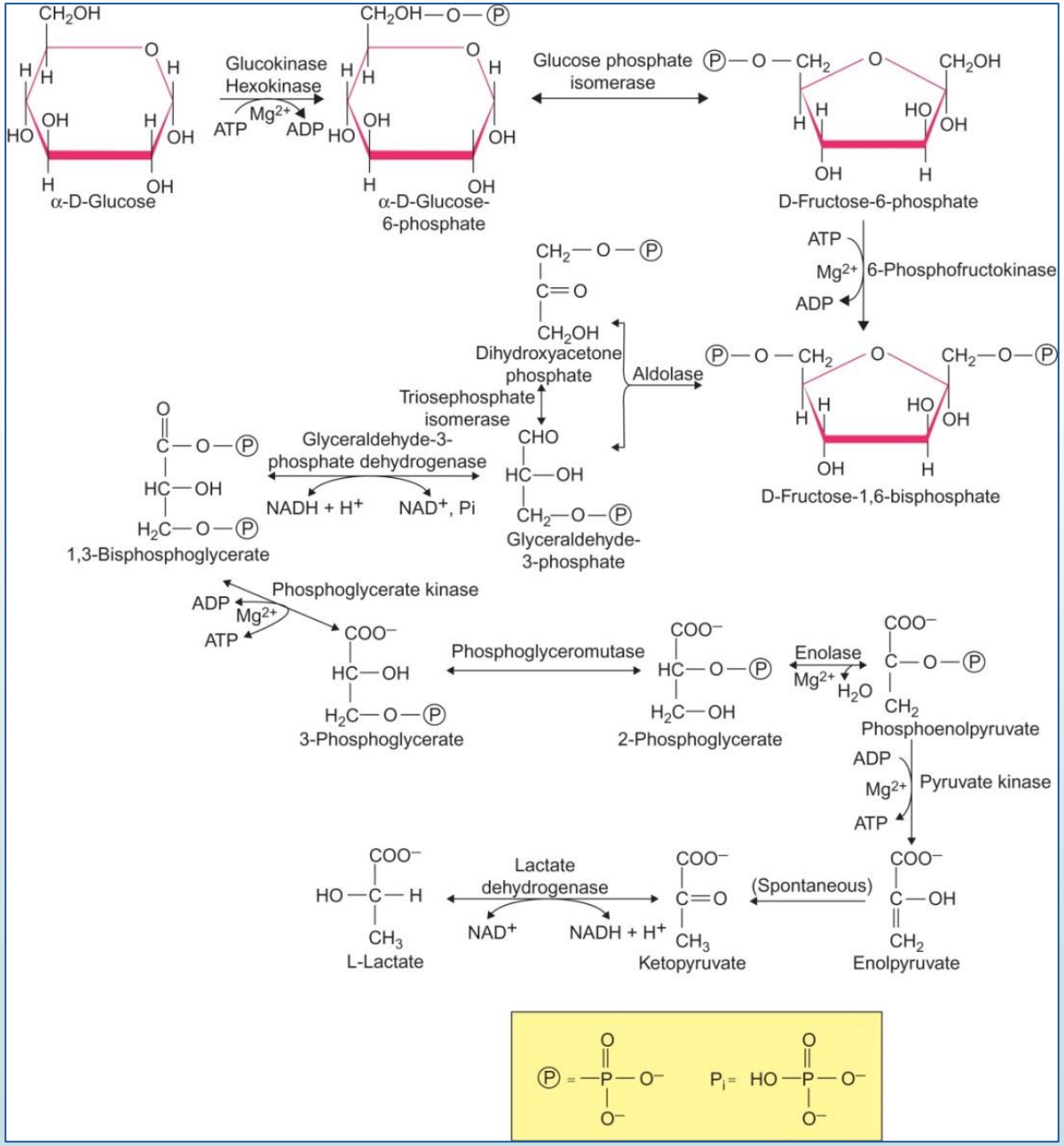
Reactions of Glycolysis

- The overall equation for glycolysis from glucose to lactate is as follows:

  Glucose + 2 ADP + 2 P_i → 2 Lactate + 2 ATP + 2 H_2O

- Glucose enters glycolysis by phosphorylation to glucose-6-phosphate, catalyzed by **hexokinase,** using ATP as the phosphate donor.
- Under physiological conditions, the phosphorylation of glucose to glucose-6-phosphate can be regarded as irreversible.
- Hexokinase is inhibited allosterically by its product, glucose-6-phosphate.
- Liver parenchymal cells and β cells of the pancreas also contain an isoenzyme of hexokinase, known as **glucokinase (*hexokinase D, or type IV*)**, which has a K_m very much higher than the normal intracellular concentration of glucose.

GLYCOLYSIS



Phosphorylation of glucose

- Phosphorylated sugar molecules do not readily penetrate cell membranes by two reasons:
  - there are no specific transmembrane carriers for these compounds,
  - they are too polar to diffuse through the lipid core of membranes.
- The irreversible phosphorylation of glucose, therefore, effectively traps the sugar as cytosolic glucose 6-phosphate, thus committing it to further metabolism in the cell.
- Humans have several isozymes of the enzyme hexokinase (I-IV) that catalyze the phosphorylation of glucose to glucose 6-phosphate.

Hexokinase vs. Glucokinase

- Hexokinase is inhibited allosterically by glucose-6-phosphate whereas glucokinase is not.
- Hexokinase has a low K_m (and, therefore, a high affinity) for glucose. On the other hand, glucokinase has a high K_m (a low affinity) for glucose.
- Therefore, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated, such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein.
- Glucokinase has also a high V_{max}, allowing the liver to effectively remove the flood of glucose delivered by the portal blood.

- Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases, but rather is indirectly inhibited by fructose 6-phosphate.
- Glucokinase regulatory protein (GKRP) in the liver regulates the activity of glucokinase through reversible binding. In the presence of fructose 6-phosphate, glucokinase is translocated into the nucleus and binds tightly to the regulatory protein, thus rendering the enzyme inactive. When glucose levels in the blood (and also in the hepatocyte) increase, glucokinase is released from the regulatory protein, and the enzyme re-enters the cytosol where it phosphorylates glucose to glucose 6-phosphate.

Phosphorylation of fructose 6-phosphate

- The irreversible phosphorylation reaction catalyzed by **phosphofructokinase-1 (PFK-1)** is the most important control point and the rate-limiting and committed step of glycolysis.
- PFK-1 is inhibited allosterically by elevated levels of ATP, which act as an “energyrich” signal indicating an abundance of high-energy compounds. Elevated levels of citrate, an intermediate in the Krebs cycle, also inhibit PFK-1.
- Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell’s energy stores are depleted.

Regulation of PFK-1 by fructose 2,6-bisphosphate

- **Fructose 2,6-bisphosphate** is the most potent activator of PFK-1 in liver and is able to activate the enzyme even when ATP levels are high.
- Fructose 2,6-bisphosphate is formed by phosphofructokinase-2 (PFK-2), an enzyme different than PFK-1. PFK-2 is a bifunctional protein that has both the kinase activity that produces fructose 2,6-bisphosphate and a phosphatase activity that dephosphorylates fructose 2,6-bisphosphate back to fructose 6-phosphate.
- In liver, the kinase domain is active if dephosphorylated and is inactive if phosphorylated.

Cleavage of fructose 1,6-bisphosphate & Isomerization of dihydroxyacetone phosphate

- Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.
- Triose phosphate isomerase interconverts dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Dihydroxyacetone phosphate must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway.
- This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate.

Oxidation of glyceraldehyde 3-phosphate

- The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by **glyceraldehyde 3-phosphate dehydrogenase** is the first oxidation-reduction reaction of glycolysis.
- The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of P_i to the carboxyl group.
- The high-energy phosphate group at carbon 1 of 1,3-BPG conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate.
- The energy of this high-energy phosphate drives the synthesis of ATP in the next reaction of glycolysis.
- The enzyme is inhibited by **iodoacetate**, which is thus able to inhibit glycolysis.
- Arsenate can prevent net ATP and NADH production by glycolysis by competing with inorganic phosphate as a substrate, without inhibiting the pathway itself.

Synthesis of 3-phosphoglycerate producing ATP

- When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP.
- This reaction is catalyzed by phosphoglycerate kinase which is physiologically reversible.
- This reaction is an example of **substrate-level phosphorylation**.

Synthesis of 2,3-bisphosphoglycerate (2,3-BPG) in red blood cells

- Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase.
- 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in red blood cells (*it increases O_2 delivery*).
- 2,3-BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis.
- In the red blood cell, glycolysis is modified by formation of these shunt reactions.

Shift of the phosphate group from carbon 3 to carbon 2

- The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase is freely reversible.

Dehydration of 2-phosphoglycerate

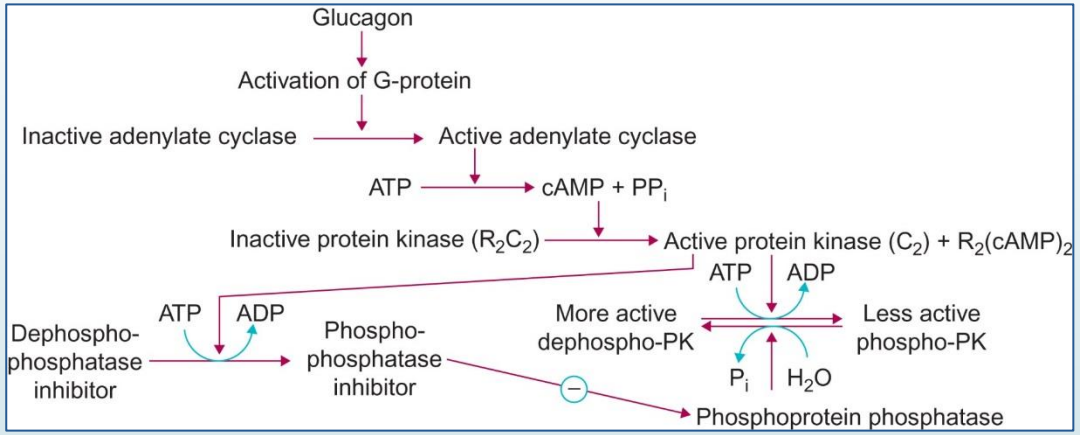
- The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the 2-phosphoglycerate molecule, resulting in the formation of phosphoenolpyruvate (PEP), which contains a high energy enol phosphate.
- Enolase is inhibited by **fluoride,** and when blood samples are taken for measurement of glucose, glycolysis is inhibited by taking the sample into tubes containing fluoride.
- Enolase is also dependent on the presence of either Mg^{2+} or Mn^{2+} ions.

Formation of pyruvate producing ATP

- The conversion of PEP to pyruvate is catalyzed by pyruvate kinase, the third irreversible reaction of glycolysis.
- The equilibrium of the pyruvate kinase reaction favors the formation of ATP.
- This is another example of **substrate-level phosphorylation**.
- **In liver, pyruvate kinase is activated by fructose 1,6-bisphosphate**, the product of the phosphofructokinase-1 reaction.
- This feed-forward regulation has the effect of linking the two kinase activities: increased phosphofructokinase-1 activity results in elevated levels of fructose 1,6-bisphosphate, which activates pyruvate kinase.

Covalent modification of pyruvate kinase

- Phosphorylation by a cAMP-dependent protein kinase leads to inactivation of pyruvate kinase in the liver.
- When blood glucose levels are low, elevated **glucagon** increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of pyruvate kinase.
- Therefore, PEP is unable to continue in glycolysis, but instead enters the gluconeogenesis pathway.
- This, in part, explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon.
- Dephosphorylation of pyruvate kinase by a phosphoprotein phosphatase results in reactivation of the enzyme.



Pyruvate is reduced to lactate in anaerobic glycolysis

- Lactate, formed by the action of **lactate dehydrogenase**, is the final product of anaerobic glycolysis in eukaryotic cells.
- The formation of lactate is the major fate for pyruvate in **lens** and **cornea of the eye**, **kidney medulla**, **testes**, **leukocytes** and **red blood cells**, because these are all poorly vascularized and/or lack mitochondria.

ALTERNATIVE ROUTES OF PYRUVATE

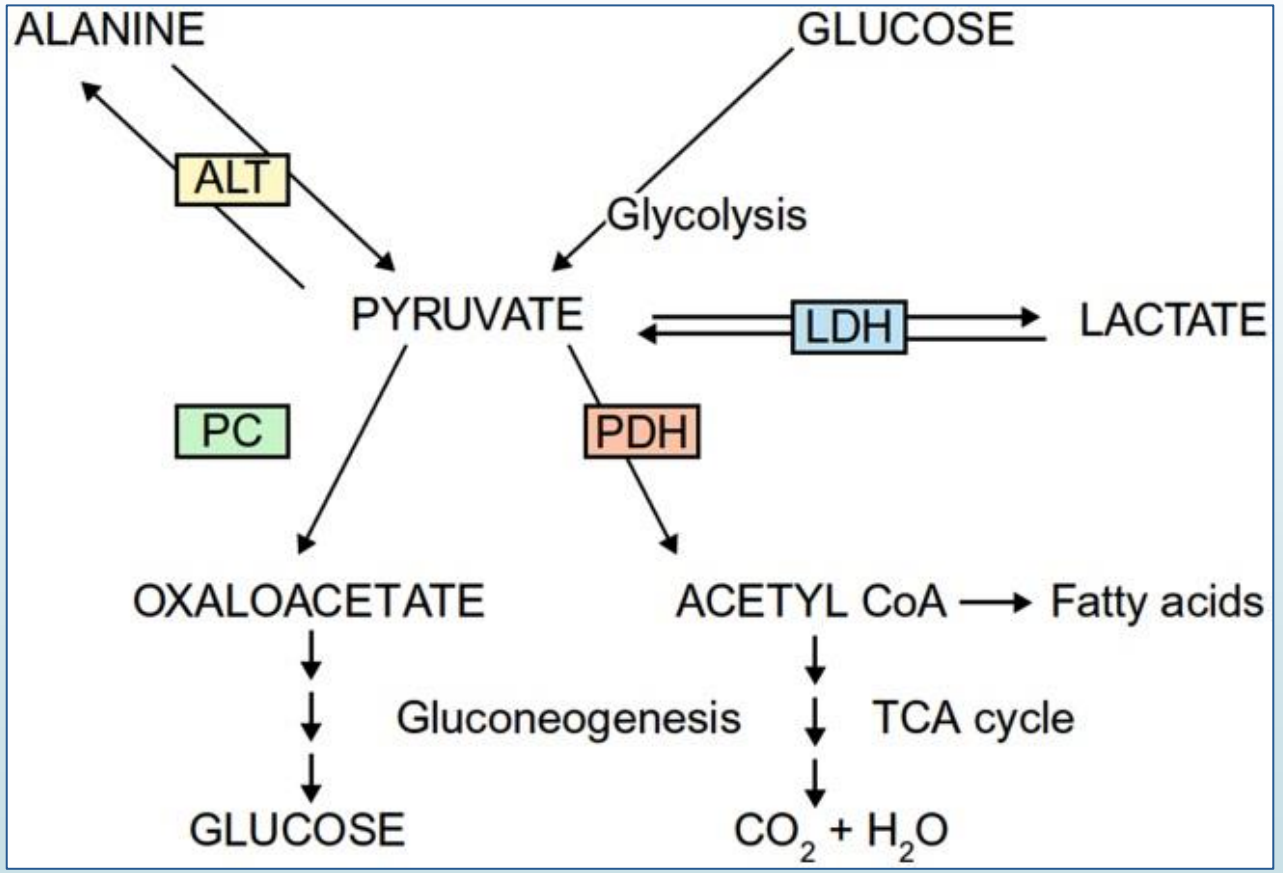


TABLE 12.2 Properties of Enzymes of Glycolysis

Enzyme	Coenzymes and Cofactors	Allosteric Modulators		Equilibrium Constant at pH 7.0 (K'_{eq})	$\Delta G^{\circ\prime}$ kcal/mol (kJ/mole)
		Positive	Negative		
Hexokinase	Mg^{2+}	ATP, P_i	Glucose-6-phosphate	650	−4.0 (−16.7) (nonequilibrium)*
Glucokinase	Mg^{2+}	–	–	–	(nonequilibrium)
Glucose-phosphate isomerase	Mg^{2+}	–	–	0.5	+4.0 (+1.7) (near-equilibrium)†
6-phosphofructokinase	Mg^{2+}	Fructose-2,6-bisphosphate, ADP, AMP, P_i, K^+, NH_4^+	ATP, citrate	220	−3.4 (−14.2) (nonequilibrium)*
Fructose-bisphosphate aldolase		–	–	0.001	+5.7 (+23.8) (near-equilibrium)‡
Triose-phosphate isomerase	Mg^{2+}		–	0.075	+1.8 (+7.5) (near-equilibrium)†
Glyceraldehyde-3-phosphate dehydrogenase	NAD	–	–	0.08	+1.5 (+6.3) (near-equilibrium)†
Phosphoglycerate kinase	Mg^{2+}	–	–	1500	−4.5 (−18.8) (near-equilibrium)†
Phosphoglyceromutase	Mg^{2+}, 2,3-bisphosphoglycerate	–	–	0.02	+1.1 (+4.6) (near-equilibrium)†
Enolase	Mg^{2+}	–	–	0.5	+0.4 (+1.7) (near-equilibrium)†
Pyruvate kinase§	Mg^{2+}, K^+	Fructose-1,6-bisphosphate	ATP, alanine, acetyl-CoA	200,000	−7.5 (−31.4) (nonequilibrium)*
Lactate dehydrogenase	NAD	–	–	16,000	−6.0 (−25.1) (near-equilibrium)†

*Physiologically irreversible reactions.
†Physiologically reversible reactions.
‡This reaction, despite a high positive $\Delta G^{\circ\prime}$ value, is reversible under in vivo conditions.
§Pyruvate kinase is also regulated by cAMP-dependent phosphorylation. The dephosphorylated form is more active, and the phosphorylated form is less active.

HORMONAL REGULATION OF GLYCOLYSIS

- Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of *glucokinase, phosphofructokinase, and pyruvate kinase* in liver.
- These changes reflect an increase in gene transcription, resulting in increased enzyme synthesis.
- High activity of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the well-fed state.
- Conversely, gene transcription and synthesis of glucokinase, phosphofructokinase, and pyruvate kinase are decreased when plasma glucagon is high and insulin is low, for example, as seen in fasting or diabetes.

Energy output from glycolysis

- Despite the production of some ATP during glycolysis, the end products, pyruvate or lactate, still contain most of the energy originally contained in glucose.
- The TCA (Krebs) cycle is required to release that energy completely.
- **Anaerobic glycolysis:**
  - Two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate.
  - There is no net production or consumption of NADH.
- **Aerobic glycolysis:**
  - The direct consumption and formation of ATP is the same as in anaerobic glycolysis—that is, a net gain of two ATP per molecule of glucose.
  - Two molecules of NADH are also produced per molecule of glucose.

Glycolytic enzyme deficiencies in erythrocytes are important

- The most common deficiency is that of pyruvate kinase (PK).
- PK deficiency is limited to the erythrocytes, and produces mild to severe chronic hemolytic anemia (erythrocyte destruction), with the severe form requiring regular cell transfusions.
- Almost all individuals with PK deficiency have a mutant enzyme that shows abnormal properties—most often altered kinetics.
- *Pyruvate kinase deficiency is the second most common cause (after **glucose 6-phosphate dehydrogenase [G6PD] deficiency**) of enzyme deficiency related nonspherocytic hemolytic anemia.*

REFERENCES

- *Lippincott's Illustrated Reviews Biochemistry, 5th Edition. Harvey RA, Ferrier DR. Lippincott Williams & Wilkins, 2011; Chapter 8.*
- *Essentials of Medical Biochemistry with Clinical Cases, Second Edition. Bhagavan NV, Ha C-E, Academic Press, 2015; Chapter 12.*
- *Harper's Illustrated Biochemistry, 30th Edition. Rodwell VW, Bender DA, Botham KM, Kennely PJ, Weil PA. Lange, 2015; Chapter 17.*
- *Lehninger Principles of Biochemistry, Sixth Edition. Nelson DL, Cox MM. W.H. Freeman and Company 2013; Chapter 14.*