# "Introduction to Metabolism and Glycolysis"

## I. METABOLISM OVERVIEW

- In cells, enzyme reactions reactions rarely occur in isolation.
- Instead, they are organized into multistep sequences called pathways, such as that of glycolysis.
- In a pathway, the product of one reaction serves as the substrate of the subsequent reaction.
- Most pathways can be classified as either:
    - Catabolic (degradative)
    - Anabolic (synthetic)
- Catabolic pathways:
    - Break down complex molecules, such as:
        - Proteins
        - Polysaccharides
        - Lipids
    - To a few simple molecules, e.g.:
        - Carbon dioxide
        - Ammonia
        - Water

- Anabolic pathways:
    - Form complex end products from simple precursors.
    - Example: Synthesis of the polysaccharide glycogen from glucose.

- Different pathways can intersect, forming an integrated and purposeful network of chemical reactions.

- Metabolism is the sum of all the chemical changes occurring in a cell, a tissue, or the body.

- Metabolites are intermediate products of metabolism.

- Central metabolic pathways are involved in:
    - Synthesizing and degrading:
        - Carbohydrates
        - Lipids
        - Amino acids

## A. Metabolic Map

- Metabolism is best understood by examining its component pathways.
- Each pathway is composed of multienzyme sequences.
- Each enzyme, in turn, may exhibit:
    - Important catalytic features
    - Important regulatory features

## B. Catabolic Pathways

- Catabolic reactions serve to capture chemical energy in the form of ATP from the degradation of energy-rich fuel molecules.
- ATP generation by degradation of complex molecules occurs in three stages.
- Note: Catabolic pathways are typically oxidative and require oxidized coenzymes such as:
    - Nicotinamide adenine dinucleotide [$NAD^+$]

- Catabolism also allows:
    - Molecules in the diet
    - Or nutrient molecules stored in cells
    - To be converted into basic building blocks needed for the synthesis of complex molecules.

- Catabolism is described as a convergent process in which:
    - A wide variety of molecules are transformed into a few common end products.

## Stages of Catabolism

### 1. Hydrolysis of Complex Molecules

- In the first stage, complex molecules are broken down into their component building blocks.

- Examples:
    - Proteins → Amino acids
    - Polysaccharides → Monosaccharides
    - Fats (triacylglycerols) → Free fatty acids and glycerol

2. Conversion of Building Blocks to Simple Intermediates

- In the second stage, these diverse building blocks are further degraded to:
    - Acetyl coenzyme A (CoA)
    - And a few other simple molecules
- Some energy is captured as ATP, but:
    - The amount is small compared with the energy produced during the third stage of catabolism.

3. Oxidation of Acetyl CoA

- The tricarboxylic acid (TCA) cycle (see Chapter 9) is the final common pathway in the oxidation of fuel molecules that produce acetyl CoA.
- Oxidation of acetyl CoA generates large amounts of ATP via:
    - Oxidative phosphorylation

- This occurs as electrons flow from:
    - NADH and
    - Flavin adenine dinucleotide ($FADH_2$)
    - To oxygen ($O_2$)

C. Anabolic Pathways

Definition and Characteristics

- In contrast to catabolism, anabolism is a divergent process in which:
    - A few biosynthetic precursors (such as amino acids)
    - Form a wide variety of polymeric, or complex, products (such as proteins)

Energy Requirement

- Anabolic reactions require energy (are endergonic).

- Energy is generally provided by:
    - Hydrolysis of ATP to:
        - Adenosine diphosphate (ADP)
        - Inorganic phosphate (Pi)

Note: Catabolic reactions generate energy (are exergonic).

Reducing Power in Anabolism

- Anabolic reactions often involve chemical reductions.
- The reducing power is most frequently provided by the electron donor:
    - NADPH (phosphorylated NADH)

II. METABOLISM REGULATION

Overview

- The pathways of metabolism must be coordinated so that:
    - Production of energy or
    - Synthesis of end products
    - Meets the needs of the cell

- Individual cells function as part of a community of interacting tissues, not in isolation.

- A sophisticated communication system has evolved to coordinate the functions of the body.

Regulatory Signals

- Regulatory signals that inform a cell of the metabolic state of the body as a whole include:

    - Hormones
    - Neurotransmitters
    - Availability of nutrients

- These signals, in turn, influence signals generated within the cell

A. Intracellular Communication

Regulation Within the Cell

- The rate of a metabolic pathway can respond to regulatory signals arising from within the cell.

- Influencing factors include:
    - Availability of substrates
    - Product inhibition
    - Alterations in levels of allosteric activators or inhibitors
- These intracellular signals typically:
    - Elicit rapid responses
    - Are important for moment-to-moment regulation of metabolism

B. Intercellular Communication

Essential Role

- The ability to respond to intercellular signals is essential for the development and survival of organisms.

Long-Range Integration

- Signaling between cells provides for long-range integration of metabolism

- Usually results in a response such as a change in gene expression
    - Slower than responses seen with intracellular signals

## Mechanisms of Cell-to-Cell Communication

- Communication between cells can be mediated by:
    - Surface-to-surface contact
    - In some tissues, formation of gap junctions, allowing:
        - Direct communication between the cytoplasms of adjacent cells

## Chemical Signaling (Main Mechanism for Energy Metabolism)

- For energy metabolism, the most important communication route is:
    - Chemical signaling between cells via:
        - Bloodborne hormones
        - Neurotransmitters

## C. G Protein-Linked Receptors and Second Messenger Systems

### Hormones and Neurotransmitters as Signals

- Hormones and neurotransmitters act as signals
- Receptors act as signal detectors
- Receptors are proteins embedded in the plasma membranes of their target cells

### Receptor Activation

- Receptors respond to a ligand bound to them by:
    - Initiating a series of reactions
    - Resulting in specific intracellular responses

### G Protein-Coupled Receptors (GPCRs)

- Many receptors that regulate metabolism are:
    - Linked to intracellular GTP-binding proteins called G proteins
    - Known as G protein-coupled receptors (GPCRs)

## Second Messengers

- GPCRs regulate production of second messengers
    - So named because they intervene between:
        - The original extracellular messenger (hormone or neurotransmitter)
        - The ultimate intracellular effect
- Second messengers are part of the cascade of events that:
    - Converts (transduces) ligand binding into a response

## Key Second Messenger Systems Regulated by G Proteins

1. Phospholipase C system
    - Involves calcium and the phosphatidylinositol system
2. Adenylyl cyclase (adenylate cyclase) system
    - Particularly important in regulating pathways of intermediary metabolism

## Initiation of Second Messenger Systems

- Both systems are initiated by hormone ligands (e.g., epinephrine, glucagon) binding to:
    - Specific GPCRs embedded in the plasma membrane of the target cell
    - These cells will then respond to the hormone

## Structural Features of GPCRs

- GPCRs are characterized by:
    - An extracellular ligand-binding domain
    - Seven transmembrane α helices
    - An intracellular domain that interacts with heterotrimeric G proteins composed of:
        - α subunit
        - β subunit
        - γ subunit

## Note on Insulin Signaling

- Insulin, another key regulator of metabolism, does not signal via GPCRs

- Instead, insulin acts via a receptor with tyrosine kinase activity

D. Adenylyl Cyclase

I. Hormone Binding to GPCR and Activation of Adenylyl Cyclase

- Hormone ligands bind to GPCRs (e.g., β-adrenergic and $\alpha_2$-adrenergic receptors)

- This triggers either:

    - An increase or decrease in the activity of adenylyl cyclase

- Adenylyl cyclase:

    - A membrane-bound enzyme
    - Converts ATP → cyclic AMP (cAMP), also called 3′,5′-adenosine monophosphate, when activated

## GTP-Dependent Regulatory Proteins (G Proteins)

### Structure and Inactive Form

- Heterotrimeric G proteins:
    - Composed of α, β, and γ subunits
    - Located on the inner face of the plasma membrane
- Named because α subunit binds GTP when active
- In inactive form, the α subunit is bound to GDP

### Activation Mechanism

- Ligand binding to GPCR causes:
    - A conformational change in the receptor
    - GDP is replaced with GTP on the α subunit
- The GTP-bound α subunit:
    - Dissociates from the βγ dimer
    - Moves to adenylyl cyclase, modulating its activity
- Amplification: One activated GPCR can activate many Gα subunits

## Types of Gα Subunits and Their Effects

- Gαs ("s" for stimulatory):
    - Stimulates adenylyl cyclase → ↑ cAMP
- Gαi ("i" for inhibitory):
    - Inhibits adenylyl cyclase → ↓ cAMP

## Formation and Role of cAMP

- Activated adenylyl cyclase converts:
    - ATP → cAMP (cyclic adenosine monophosphate)
- cAMP acts as a second messenger
- It activates:
    - Protein kinase A (PKA) → a serine/threonine kinase

## Inactivation of the G Protein Signal

- Gα subunit has inherent GTPase activity
- This causes:
  - Hydrolysis of GTP → GDP
  - Inactivation of Gα
  - Dissociation from adenylyl cyclase
  - Reassociation with βγ subunit → G protein returns to inactive state

## Clinical Relevance: Toxins Affecting G Proteins

### Cholera Toxin (Vibrio cholerae)

- Causes ADP-ribosylation of Gαs
- Inhibits GTPase activity of Gαs in intestinal cells
- Result: Persistent activation of adenylyl cyclase → ↑↑ cAMP

Pertussis Toxin (Bordetella pertussis)

- Causes ADP-ribosylation of Gαi
- Inactivates Gαi in respiratory tract cells
- Result: Loss of inhibition on adenylyl cyclase → ↑↑ cAMP

2. Protein Kinases

A. Activation of cAMP-Dependent Protein Kinases

- cAMP activates a family of enzymes:
    - Called cAMP-dependent protein kinases, including Protein Kinase A (PKA)
- Mechanism of PKA activation:
    - PKA structure: 2 regulatory subunits + 2 catalytic subunits
    - cAMP binds to the regulatory subunits
    - This causes release of the catalytic subunits, activating PKA

B. Function of Active PKA

- PKA is a serine/threonine kinase
    - Transfers phosphate from ATP → specific serine or threonine residues of target proteins
- Effect of phosphorylation:
    - If the substrate is an ion channel protein → may directly alter cell function
    - If the substrate is an enzyme → may become activated or inhibited

Note: Not all protein kinases are cAMP-dependent

- Example: Protein Kinase C (PKC)
- Activated via phospholipase C signaling
- Calcium-dependent, not cAMP-dependent

3. Protein Phosphatases

- Phosphorylated proteins are reversed by phosphoprotein phosphatases
    - These enzymes hydrolytically cleave phosphate esters
- Function: Ensure that phosphorylation effects are not permanent

4. cAMP Hydrolysis

- cAMP is rapidly degraded by:
    - cAMP phosphodiesterase
    - This enzyme cleaves the cyclic 3′,5′-phosphodiester bond
- Product: 5′-AMP
    - Not an intracellular signaling molecule
- Effect: Ensures that cAMP signaling ends rapidly when the extracellular signal (hormone/neurotransmitter) is gone

Note:

- Caffeine (a methylxanthine derivative) inhibits cAMP phosphodiesterase
- Result: Prolonged cAMP activity

## III. GLYCOLYSIS OVERVIEW

### A. Universal Importance of Glycolysis

- Used by all tissues for:
    - Oxidation of glucose
    - ATP production (energy)
    - Generating intermediates for other metabolic pathways
- Central role in carbohydrate metabolism:
    - Virtually all dietary or endogenous sugars can be converted to glucose
    - Glucose is then funneled into the glycolytic pathway

## B. End Product and Oxygen Availability

- In aerobic conditions ($O_2$ available):
    - End product = Pyruvate
    - Pyruvate undergoes oxidative decarboxylation → Acetyl CoA
        - Acetyl CoA enters TCA cycle (major fuel)
    - Requires NADH reoxidation via electron transport chain
    - This 10-reaction sequence is called aerobic glycolysis

- In anaerobic conditions ($O_2$ limited or absent):
    - Pyruvate → Lactate
    - NADH is oxidized to $NAD^+$ (needed to continue glycolysis)
    - This pathway is called anaerobic glycolysis

C. Significance of Anaerobic Glycolysis

- Occurs in:
    - Tissues lacking mitochondria
        - e.g., Red Blood Cells (RBCs), parts of the eye
    - Tissues with insufficient oxygen supply (e.g., during hypoxia)
- Allows ATP generation even without oxygen

IV. GLUCOSE TRANSPORT INTO CELLS

A. Glucose Cannot Passively Diffuse

- Requires transport systems for entry into cells

B. Two Main Glucose Transport Mechanisms

1. $Na^+$- and ATP-independent transport system:
    - Facilitated diffusion
    - Involves GLUT transporters
    - Found in most tissues

2. $Na^+$- and ATP-dependent cotransport system:

    - Known as SGLT (sodium-glucose linked transporters)
    - Actively transports glucose against concentration gradient
    - Found in intestinal epithelial cells and renal tubules

A. Sodium- and ATP-Independent Transport System (Facilitated Diffusion)

1. Mediated by GLUT Transporters

- GLUT = Glucose Transporters (Family of 14 isoforms: GLUT-1 to GLUT-14)

- Passive transport system: Does not require sodium or ATP

- Transport mechanism:

    - Monomeric transmembrane proteins
    - Exist in two conformational states
    - Glucose binds extracellularly → conformational shift → glucose enters cell
    - Transport is via facilitated diffusion

- GLUTs are uniporters:
    - Transport only one molecule at a time
    - Movement is down concentration gradient
    - Energy-independent

2. Tissue-Specific Expression of GLUT Isoforms

| GLUT Isoform | Primary Tissue Distribution | Key Function |
|---|---|---|
| GLUT-1 | Most tissues | Basal glucose uptake |
| GLUT-2 | Liver, kidney, pancreatic β cells | Bidirectional glucose transport |
| GLUT-3 | Neurons, placenta | High-affinity glucose uptake |
| GLUT-4 | Muscle and adipose tissue | Insulin-regulated glucose uptake |
| GLUT-5 | Small intestine, testes | Fructose transport (not glucose) |

B. Recommended Name

- GLUT-4 is insulin-dependent:
    - Insulin increases the number of active GLUT-4 transporters
    - Important for postprandial glucose uptake

3. Specialized Transport Functions

- Facilitated diffusion:
    - Occurs down glucose concentration gradient
    - No ATP required

a. Glucose Uptake Transporters

- GLUT-1, GLUT-3, and GLUT-4:
    - Primarily responsible for glucose uptake from blood

b. Bidirectional Glucose Transporters

- GLUT-2:
    - In liver and kidneys
    - Can import glucose (when blood glucose is high)
    - Can export glucose (when blood glucose is low, e.g., fasting)

c. Fructose-Specific Transporter

- GLUT-5:
    - Located in small intestine and testes
    - Transports fructose, not glucose

# Tissue Distribution of Selected GLUT Isoforms

| GLUT Isoform | Location | Key Function | Km (mM) |
|---|---|---|---|
| GLUT-1 | Most tissues | Basal glucose uptake | 1 |
| GLUT-2 | Liver, kidney, pancreatic β cells | Bidirectional glucose transport | 15–20 |
| GLUT-3 | Neurons, placenta | High-affinity glucose uptake | 1 |
| GLUT-4 | Muscle and adipose tissue | Insulin-regulated glucose uptake | 5 |
| GLUT-5 | Small intestine, testes | Fructose transport (not glucose) | 10 |

- Km indicates affinity:

  -> Lower Km = higher affinity for glucose
  -> Higher Km = lower affinity (responds at higher glucose concentrations)

B. Sodium- and ATP-Dependent Cotransport of Glucose

1. Location of $Na^+$-Glucose Cotransport

- Present in:
  - Intestinal epithelial cells
  - Renal tubules
  - Choroid plexus (part of blood-brain barrier)

2. Mechanism of Sodium-Dependent Glucose Cotransport

- Type of transport: Secondary active transport
- Symport system: Glucose and $Na^+$ transported together
- Glucose moves against its concentration gradient (low → high)

- $Na^+$ moves down its electrochemical gradient (high → low)

Energy Source

- ATP hydrolysis is indirect:
    - Powers $Na^+/K^+$ ATPase, which:
        - Pumps 3 $Na^+$ out, 2 $K^+$ into the cell
        - Maintains high extracellular $Na^+$ concentration
- The $Na^+$ gradient provides the driving force for glucose uptake

3. Transporters Involved

- Sodium-Dependent Glucose Transporters = SGLTs
    - SGLT = Sodium-Glucose Linked Transporter
    - Require $Na^+$ for function ($Na^+$ is co-transported with glucose)

SGLT2

- Location: Proximal tubules of kidney
- Function: Major glucose reabsorption transporter from filtrate back into blood

## 4. Clinical Correlation: SGLT2 Inhibitors (Gliflozins)

- Mechanism:
    - Inhibit SGLT2 in kidneys
    - Reduce glucose reabsorption
    - Increase urinary glucose excretion
    - Lower blood glucose levels
- Used to treat:
    - Type II Diabetes Mellitus
    - Particularly helpful in hyperglycemia

# V. GLYCOLYSIS REACTIONS

## Overview of Glycolysis

- Glycolysis = Metabolic pathway converting glucose (6C) to pyruvate (3C)
- Occurs in cytosol
- Does not require oxygen (anaerobic)

- Divided into two phases:

1. Energy-Investment Phase (First 5 reactions)

    - ATP is consumed to phosphorylate intermediates
    - Prepares glucose for cleavage and further metabolism

2. Energy-Generation Phase (Last 5 reactions)

    - ATP is produced via substrate-level phosphorylation
    - Net gain:
        - +2 ATP per glucose (4 ATP made - 2 ATP used)
        - +2 NADH formed
        - 2 Pyruvate produced

A. Glucose Phosphorylation

Purpose of Phosphorylation

- Glucose → Glucose-6-phosphate (G6P)

- Catalyzed by: Hexokinase or Glucokinase (Hexokinase IV)

Why phosphorylation?

- Prevents glucose from leaving the cell:
    - Phosphorylated sugars cannot cross cell membranes
        - Too polar
        - No transporters for phosphorylated sugars
- Irreversible step → commits glucose to intracellular metabolism
- G6P is a branch point for glycolysis, glycogenesis, pentose phosphate pathway

Hexokinase Isozymes (I–IV)

1. Hexokinases I–III

- Found in most tissues
- Regulatory enzyme of glycolysis
- Km: Low → High affinity for glucose
- Efficient even at low glucose concentrations

- Vmax: Low → Prevents excessive trapping of phosphate as G6P
- Inhibited by G6P (product inhibition)
- Broad substrate specificity:
    - Can phosphorylate other hexoses (e.g., fructose, mannose)
- Hexokinase is one of three key regulatory enzymes:
    - Hexokinase (Glucose → G6P)
    - Phosphofructokinase-1 (PFK-1)
    - Pyruvate kinase (PK)

2. Hexokinase IV (Glucokinase)

A. Location & Function

- Present in:
    - Liver parenchymal cells
    - Pancreatic β-cells
    - Hypothalamic neurons

- Functions:
    - In β-cells:
        - Acts as a glucose sensor
        - Helps set the threshold for insulin secretion
    - In liver:
        - Facilitates glucose phosphorylation during hyperglycemia
        - Prevents postprandial hyperglycemia
    - In hypothalamus:
        - Participates in glucose sensing during hypoglycemia
        - Affects adrenergic response to low blood sugar
- Despite the misleading name "glucokinase":
    - It has broad sugar specificity, like other hexokinases

B. Kinetics of Glucokinase

- Km: High (~10 mM)

  → Low affinity for glucose
  → Only active when glucose concentration is high (e.g., after a carb-rich meal)

- Vmax: High

  → Allows liver to quickly phosphorylate large amounts of glucose
  → Prevents glucose spillover into systemic circulation
  → Minimizes postprandial hyperglycemia

- GLUT-2:

  - Bidirectional transporter
  - Ensures rapid equilibration of glucose across hepatocyte membrane

C. Regulation of Glucokinase Activity

- Not directly inhibited by glucose-6-phosphate

  → Unlike Hexokinase I-III

## C. Regulation of Glucokinase Activity

- Not directly inhibited by glucose-6-phosphate

  → Unlike Hexokinase I-III

- Regulated indirectly by:

  - Inhibitor: Fructose 6-phosphate
    - Promotes binding to GKRP → glucokinase becomes inactive
    - Enzyme is translocated to nucleus

  - Stimulator: Glucose
    - Promotes release from GKRP
    - Glucokinase returns to cytosol → becomes active

- GKRP (Glucokinase Regulatory Protein):

  - Mediates reversible nuclear-cytoplasmic shuttling
  - Acts as competitive inhibitor of glucose usage by glucokinase
  - Acts only in hepatocytes

# Glucokinase and Glycolytic Reactions

## A. Glucokinase as a Glucose Sensor

- Role: Maintains blood glucose homeostasis
- Acts as glucose sensor in:
  - Pancreatic β-cells → regulates insulin secretion
- Clinical correlation:
  - Inactivating mutations in glucokinase → cause Maturity-Onset Diabetes of the Young Type 2 (MODY 2)
  - Characterized by:
    - Impaired insulin secretion
    - Persistent hyperglycemia

B. Glucose 6-Phosphate Isomerization

- Enzyme: Phosphoglucose isomerase
- Reaction:

  Glucose 6-phosphate $\rightleftharpoons$ Fructose 6-phosphate

- Nature of Reaction:
  - Readily reversible
  - Not rate-limiting
  - Not regulated
- Purpose: Rearrangement allows next phosphorylation step at C1 of fructose

C. Fructose 6-Phosphate Phosphorylation

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

Fructose 6-phosphate + ATP $\rightarrow$ Fructose 1,6-bisphosphate + ADP

- Type of reaction:
    - Irreversible
    - Highly regulated
    - Rate-limiting step of glycolysis
    - Committed step: Once passed, glucose is committed to glycolysis

1. Regulation of PFK-1 by Intracellular Energy Status

- Inhibitors:
    - ATP (allosteric inhibition)
        - Signals high energy state
        - Prevents excess breakdown of glucose
    - Citrate
        - TCA cycle intermediate
        - Signals abundance of biosynthetic precursors
        - Promotes glycogen synthesis instead of glycolysis
- Activators:
    - AMP (allosteric activation)
        - Signals low energy state
        - Stimulates glycolysis to generate more ATP

# Regulation of PFK-1 by Fructose 2,6-Bisphosphate

## 1. Fructose 2,6-Bisphosphate (F-2,6-BP)

- Most potent activator of PFK-1—can override ATP inhibition.
- Formed from fructose 6-phosphate by PFK-2.

## 2. PFK-2: A Bifunctional Enzyme

| Domain | Reaction | Product |
|---|---|---|
| Kinase | Fructose 6-P → F-2,6-BP | Activates PFK-1 |
| Phosphatase | F-2,6-BP → Fructose 6-P | Removes activator |

- PFK-2 isozyme-specific regulation
    - Liver PFK-2: Phosphorylation → kinase OFF / phosphatase ON.
    - Cardiac PFK-2: Phosphorylation → kinase ON / phosphatase OFF.
    - Skeletal-muscle PFK-2: Not covalently regulated.

3. Reciprocal Control of Pathways

- F-2,6-BP activates glycolysis (↑ PFK-1).
- F-2,6-BP inhibits fructose 1,6-bisphosphatase (key gluconeogenic enzyme).
- Ensures glycolysis and gluconeogenesis are not maximally active simultaneously—prevents a futile cycle.

4. Hormonal / Nutritional States

a. Well-fed (High insulin, Low glucagon)

- PFK-2 is dephosphorylated in liver.
- Kinase domain active → ↑ F-2,6-BP → ↑ PFK-1 → ↑ Glycolysis.
- F-2,6-BP acts as an intracellular signal of glucose abundance.

b. Fasting (High glucagon, Low insulin)

- PFK-2 is phosphorylated in liver.
- Phosphatase domain active → ↓ F-2,6-BP → ↓ PFK-1 → ↓ Glycolysis, ↑ Gluconeogenesis.

D. Fructose 1,6-Bisphosphate Cleavage

- Enzyme: Aldolase
- Reaction:

Fructose 1,6-bisphosphate → DHAP + Glyceraldehyde 3-phosphate (G3P)

- Features:
  - Reversible
  - Not regulated
  - Aldolase B (liver isoform) also cleaves fructose 1-phosphate in dietary fructose metabolism

## E. DHAP Isomerization

- Enzyme: Triose phosphate isomerase
- Reaction:

$$\text{DHAP} \rightleftharpoons \text{Glyceraldehyde 3-phosphate (G3P)}$$

- Significance:
  - Only G3P continues in glycolysis
  - Net yield: 2 G3P molecules per glucose
  - DHAP can also be used in triacylglycerol synthesis

## F. G3P Oxidation to 1,3-Bisphosphoglycerate

- Enzyme: Glyceraldehyde 3-phosphate dehydrogenase
- Reaction:

$$\text{G3P} + \text{Pi} + \text{NAD}^+ \rightarrow \text{1,3-BPG} + \text{NADH} + \text{H}^+$$

- Key point: First oxidation-reduction step in glycolysis

1. 1,3-BPG Synthesis

- High-energy compound
- Energy conservation:
    - Oxidation of aldehyde → carboxylic acid
    - Coupled with Pi addition to form high-energy 1,3-BPG
- Purpose: High-energy phosphate of 1,3-BPG used in ATP generation in next glycolytic step

Clinical Insight: Arsenic Poisoning

Trivalent Arsenic (Arsenite)

- Inhibits enzymes requiring lipoic acid (e.g., pyruvate dehydrogenase complex)
- Interferes with aerobic energy metabolism

Pentavalent Arsenic (Arsenate)

- Competes with Pi at glyceraldehyde 3-phosphate dehydrogenase

- Forms 3-phosphoglycerate without forming 1,3-BPG
- Bypasses ATP + NADH production
- Glycolysis continues, but no net energy gain

Additional Effect:

- Arsenate interferes with ATP synthase ($F_1$ domain)
  → Forms ADP-arsenate, rapidly hydrolyzed → further energy loss

2. 2,3-Bisphosphoglycerate (2,3-BPG) Synthesis in RBCs

- Enzyme: Bisphosphoglycerate mutase
- Reaction:

  1,3-BPG → 2,3-BPG (in red blood cells)

- Significance of 2,3-BPG:
  - Present in high concentrations only in RBCs
  - Increases $O_2$ delivery by decreasing hemoglobin's affinity for oxygen

- Fate:
    - 2,3-BPG is hydrolyzed to 3-phosphoglycerate by a phosphatase
    - 3-phosphoglycerate re-enters glycolysis
- Shunt pathway:
    - This RBC-specific detour reduces net ATP gain but enhances oxygen delivery

G. 3-Phosphoglycerate Synthesis & ATP Production

- Enzyme: Phosphoglycerate kinase
- Reaction:

    1,3-BPG + ADP → 3-phosphoglycerate + ATP

- Features:
    - Reversible kinase reaction (unlike most kinases)
    - Substrate-level phosphorylation (ATP generated without ETC involvement)

- ATP Yield:
    - 2 molecules of ATP (1 per 1,3-BPG), compensating for the 2 ATP consumed earlier
    - Net ATP at this point: 0 (break-even)

H. Phosphate Group Shift

- Enzyme: Phosphoglycerate mutase
- Reaction: 3-phosphoglycerate → 2-phosphoglycerate
- Features:
    - Freely reversible isomerization
    - Prepares substrate for dehydration

I. 2-Phosphoglycerate Dehydration

- Enzyme: Enolase
- Reaction: 2-phosphoglycerate → Phosphoenolpyruvate (PEP) + $H_2O$
- Product:
    - PEP: High-energy enol phosphate compound

- Features:
    - Reversible despite high-energy product
    - Energy redistribution makes PEP suitable for ATP production in next step
- Clinical Note:
    - Fluoride inhibits enolase
    - Used in water fluoridation to reduce lactate production by oral bacteria → ↓ dental caries

J. Pyruvate Synthesis and ATP Production

1. Final Step of Glycolysis

- Enzyme: Pyruvate kinase (PK)
- Reaction: Phosphoenolpyruvate (PEP) + ADP → Pyruvate + ATP
- Type of Reaction:
    - Irreversible (third irreversible step of glycolysis)
    - Substrate-level phosphorylation

1. Final Step of Glycolysis

- Enzyme: Pyruvate kinase (PK)
- Reaction: Phosphoenolpyruvate (PEP) + ADP → Pyruvate + ATP
- Type of Reaction:
    - Irreversible (third irreversible step of glycolysis)
    - Substrate-level phosphorylation
- Energy Source:
    - High-energy enol phosphate bond in PEP drives ATP synthesis
- Net Gain:
    - 2 ATP (1 per PEP, 2 PEP per glucose)

2. Regulation of Pyruvate Kinase (PK)

A. Feedforward Regulation

- Activator: Fructose 1,6-bisphosphate (product of PFK-1 reaction)

- Mechanism:
    - Links upstream kinase (PFK-1) to downstream kinase (PK)
    - Increased PFK-1 activity → ↑ Fructose 1,6-BP → Activates PK
- Inhibitor: ATP (indicates high energy state)

B. Covalent Regulation (in Liver Only)

- Inactivator: cAMP-dependent protein kinase A (PKA)
- Mechanism:
    - ↓ Blood glucose → ↑ Glucagon → ↑ cAMP
    - cAMP activates PKA → Phosphorylates & inactivates PK (liver isozyme only)
- Effect:
    - PEP diverted to gluconeogenesis, not glycolysis
    - Explains glucagon's inhibition of hepatic glycolysis

- Reactivation:
    - Dephosphorylation by a phosphatase restores PK activity

3. Clinical Relevance: Pyruvate Kinase (PK) Deficiency

A. Dependence of RBCs on Glycolysis

- RBCs lack mitochondria → depend solely on glycolysis for ATP
- ATP needed for:
    - Metabolic functions
    - Ion pumps (maintain cell shape and flexibility for capillary passage)

B. Consequences of PK Deficiency

- ↓ PK activity → ↓ Glycolysis → ↓ ATP → ↓ Membrane integrity

- Effects on RBCs:
    - Membrane deformity → Phagocytosis by splenic macrophages
    - Hemolysis → Mild to severe hemolytic anemia
- Severe cases: Require regular transfusions

C. Genetic Notes

- PK gene is shared between liver and RBC isozyme
    - Liver unaffected due to:
        - Higher PK synthesis capacity
        - Ability to make ATP via oxidative phosphorylation
- Severity depends on:
    - Degree of deficiency (5%–35% of normal PK activity)
    - RBC compensation via ↑ 2,3-BPG levels
- Enzyme Defect: Mutant PK has altered kinetics or reduced stability

D. Evolutionary Advantage

- Heterozygous individuals show resistance to severe malaria

Tissue-Specific Expression of Pyruvate Kinase (PK)

- Gene Expression:
    - Same gene encodes PK in RBCs and liver
    - Different transcription start sites used in each tissue
- Result:
    - Tissue-specific PK isozymes
    - Explains differing regulation and expression in liver vs RBC

K. Pyruvate Reduction to Lactate (Anaerobic Glycolysis)

1. Enzyme: Lactate Dehydrogenase (LDH)

- Reaction: Pyruvate + NADH + $H^+$ → Lactate + $NAD^+$
- Purpose: Regenerates $NAD^+$ required for glycolysis to continue

- Occurs in:
    - Anaerobic conditions
    - Cells/tissues lacking mitochondria or with limited oxygen supply
- Final Product: Lactate (under anaerobic conditions in eukaryotes)

2. Major Sites of Anaerobic Lactate Formation

- RBCs: Lack mitochondria → exclusively use lactate pathway
- Poorly vascularized tissues:
    - Lens and cornea of the eye
    - Renal medulla

3. Lactate Formation in Exercising Muscle

- Cause: NADH production > ETC capacity
    - From glycolysis (glyceraldehyde 3-phosphate DH)
    - From TCA cycle (3 $NAD^+$-linked dehydrogenases)

- Effect: ↑ NADH/NAD⁺ ratio → favors pyruvate → lactate conversion

- Outcome:
    - Lactate accumulation → ↓ intracellular pH
    - Can cause muscle cramps

- Lactate fate:
    - Diffuses into blood
    - Transported to liver for gluconeogenesis

4. Lactate Utilization in Other Tissues

A. Factors Affecting LDH Reaction Direction

- Depends on:
    - Pyruvate/lactate concentrations
    - NADH/NAD⁺ ratio

B. In Liver and Heart

- Low NADH/NAD⁺ ratio (compared to muscle)

- Action: Lactate → Pyruvate (oxidation)

i. Liver

- Pyruvate converted to:
    - Glucose (via gluconeogenesis)
    - Acetyl-CoA (oxidized in TCA cycle)

ii. Heart

- Exclusive pathway: Lactate → Pyruvate → $CO_2$ + $H_2O$ via TCA cycle

3. Lactic Acidosis

- Definition:
    - Elevated plasma lactate levels
    - A form of metabolic acidosis

- Causes (Circulatory Collapse):
    - Myocardial infarction
    - Pulmonary embolism
    - Uncontrolled hemorrhage
    - Shock (any form)

- Pathophysiology:
    - ↓ Oxygen delivery → impaired oxidative phosphorylation
    - ↓ ATP production via ETC
    - ↑ Anaerobic glycolysis for survival → ↑ lactic acid production
- Clinical Significance:
    - Even small ATP amounts from glycolysis can be life-saving temporarily
    - Oxygen debt = extra $O_2$ needed to restore metabolic balance after hypoxia
- Oxygen Debt Importance:
    - Strongly related to morbidity and mortality
    - Lactic acid levels in blood help in:
        - Early detection of $O_2$ debt
        - Monitoring recovery

## L. Energy Yield from Glycolysis

### 1. Anaerobic Glycolysis

- Net ATP Yield:
  - 2 ATP per glucose (via substrate-level phosphorylation)
  - No net NADH gain or loss
- End Product:
  - 2 Lactate per glucose
  - Occurs in absence of oxygen

### 2. Aerobic Glycolysis

- Net ATP Yield:
  - Same as anaerobic: 2 ATP per glucose
- Additional Yield:
  - 2 NADH per glucose (from glyceraldehyde 3-phosphate DH step)
  - Each NADH → ~3 ATP via ETC

- Important Note:
    - NADH cannot cross inner mitochondrial membrane
    - Shuttle systems (e.g., malate-aspartate shuttle) are needed to transfer reducing equivalents

## VI. HORMONAL REGULATION OF GLYCOLYSIS

### A. Short-Term Regulation

- Mechanism:
    - Allosteric activation/inhibition
    - Covalent modification: phosphorylation/dephosphorylation
- Time Scale:
    - Minutes to hours
- Target Enzymes (Irreversible steps):
    - Glucokinase
    - PFK-1 (Phosphofructokinase-1)
    - Pyruvate kinase (PK)

B. Long-Term Regulation

- Mechanism:
    - Changes in gene transcription → new enzyme synthesis
- Time Scale:
    - Hours to days
- Trigger:
    - High-carbohydrate meals
    - Insulin administration
- Effect:
    - ↑ Synthesis of glucokinase, PFK-1, and PK in the liver
    - Favors conversion of glucose to pyruvate (absorptive state)

C. Transcription Factors Involved

- SREBP-1c (Sterol regulatory element-binding protein-1c):
    - Activated by insulin
- ChREBP (Carbohydrate response element-binding protein):
    - Activated by glucose
- Function:
    - Both regulate transcription of:
        - Glycolytic enzymes
        - Fatty acid synthesis enzymes

D. Fasting/Diabetic State

- Hormonal Profile:
    - High glucagon, low insulin

- Effect:
    - ↓ Gene expression of:
        - Glucokinase
        - PFK-1
        - Pyruvate kinase
- Metabolic Consequence:
    - ↓ Glycolysis
    - Promotes glucose conservation

## VII. ALTERNATE FATES OF PYRUVATE

### A. Oxidative Decarboxylation to Acetyl CoA

- Enzyme:
    - Pyruvate dehydrogenase complex (PDHC)
- Nature of reaction:
    - Irreversible

- Tissues active in this pathway:
    - Tissues with high oxidative capacity (e.g., cardiac muscle)
- Function:
    - Converts pyruvate → acetyl CoA
    - Acetyl CoA enters the TCA cycle
    - Provides carbon source for fatty acid synthesis

B. Carboxylation to Oxaloacetate

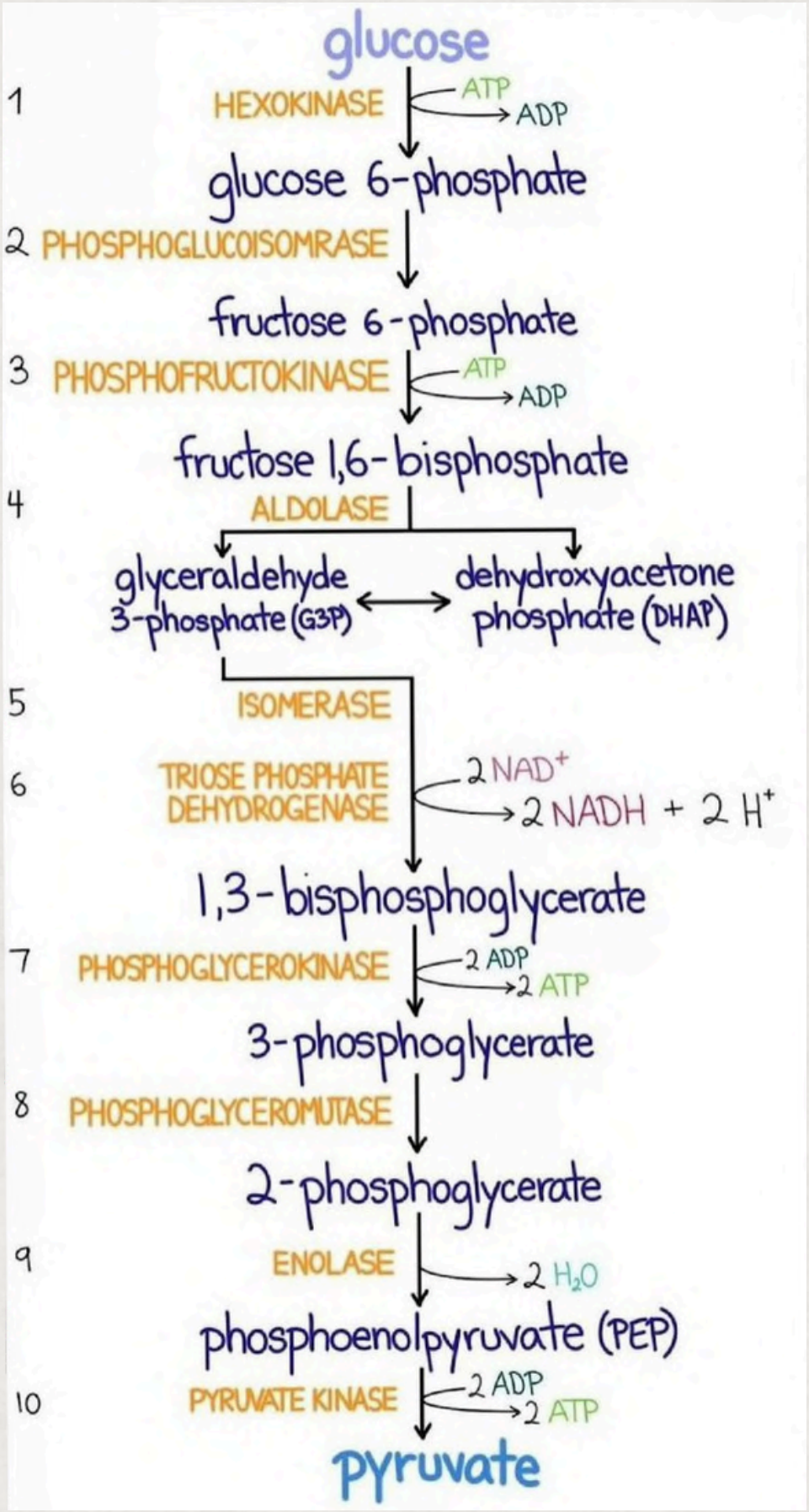
- Enzyme:
    - Pyruvate carboxylase
- Cofactor required:
    - Biotin
- Nature of reaction:
    - Irreversible

- Functions:
    - Anaplerotic (replenishes TCA cycle intermediates)
    - Provides substrate for gluconeogenesis

C. Reduction to Ethanol (in Microorganisms Only)

- Occurs in:
    - Yeast and certain microorganisms
    - Not in humans
- Step 1:
    - Enzyme: Pyruvate decarboxylase
    - Reaction: Pyruvate → Acetaldehyde
    - Cofactor: Thiamine (Vitamin B1)
- Step 2:
    - Acetaldehyde → Ethanol
    - Uses NADH to regenerate $NAD^+$

# Flowchart



# GLYCOLYSIS: QUICK REVIEW

10 Steps
Glucose (6C) → 2 Pyruvate (3C)
Occurs in the cytoplasm of all cells.
Net yield (aerobic): 2 ATP, 2 NADH

1. Glucose → Glucose-6-phosphate (G6P)

- Enzyme: Hexokinase (all cells) / Glucokinase (liver, pancreas)
- ATP used
- Promoters:
    - Glucokinase: Insulin
- Inhibitors:
    - Hexokinase: G6P (product inhibition)
    - Glucokinase: Fructose-6-P (via GKRP)

2. G6P → Fructose-6-phosphate (F6P)

- Enzyme: Phosphoglucose isomerase
- Promoters/Inhibitors: None significant

3. F6P → Fructose-1,6-bisphosphate (F1,6BP)

- Enzyme: Phosphofructokinase-1 (PFK-1)
- ATP used
- Key regulatory step (rate-limiting)
- Promoters:
    - AMP, Fructose-2,6-bisphosphate, Insulin
- Inhibitors:
    - ATP, Citrate, Glucagon (via ↓F2,6BP)

4. F1,6BP → Glyceraldehyde-3-phosphate (G3P) + Dihydroxyacetone phosphate (DHAP)

- Enzyme: Aldolase
- Promoters/Inhibitors: None significant

5. DHAP ⇌ G3P

- Enzyme: Triose phosphate isomerase
- Reversible step

6. G3P → 1,3-Bisphosphoglycerate (1,3-BPG)

- Enzyme: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
- Produces: 1 NADH per G3P
- Requires: Pi
- Inhibited by: Arsenate

7. 1,3-BPG → 3-Phosphoglycerate (3PG)

- Enzyme: Phosphoglycerate kinase
- Produces: 1 ATP per G3P (substrate-level phosphorylation)
- Promoters/Inhibitors: None significant

8. 3PG → 2-Phosphoglycerate (2PG)

- Enzyme: Phosphoglycerate mutase
- Promoters/Inhibitors: None significant

9. 2PG → Phosphoenolpyruvate (PEP)

- Enzyme: Enolase
- Produces: $H_2O$ (dehydration step)
- Inhibitors: Fluoride ($F^-$)

10. PEP → Pyruvate

- Enzyme: Pyruvate kinase (PK)
- Produces: 1 ATP per G3P
- Promoters:
    - F1,6BP (feedforward activation), Insulin
- Inhibitors:
    - ATP, Alanine, Glucagon (via phosphorylation by PKA)