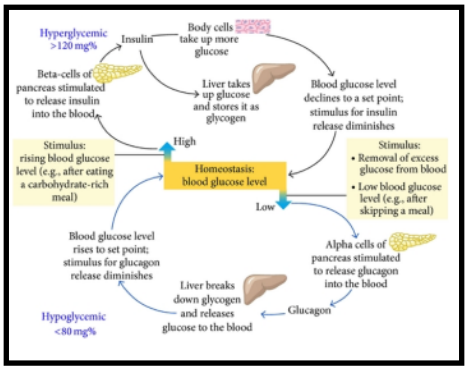
Regulation of Metabolism (W1)



Glucose Homeostasis

Introduction

Homeostasis

- Definition: The maintenance of a dynamic steady state by regulatory mechanisms that compensate for changes in external circumstances
- Organisms maintain homeostasis by keeping the concentrations of most metabolites at steady state
- In steady state, the rate of synthesis of metabolite equals the rate of breakdown of this metabolite
- Pathways are at steady state unless perturbed. After perturbation a NEW steady state will be established

Glucose Homeostasis

- The entry of glucose into the blood from various sources must be balanced by the uptake of glucose from the blood into various tissues such that the concentration of blood glucose is nearly constant at **5 mM**. This is homeostasis at the **molecular level**
- Failure of homeostatic mechanisms is often the root cause of human disease
- In diabetes, the regulation of blood glucose concentration is defective as a result of the **lack** of or **insensitivity** to **insulin**, leading to complications

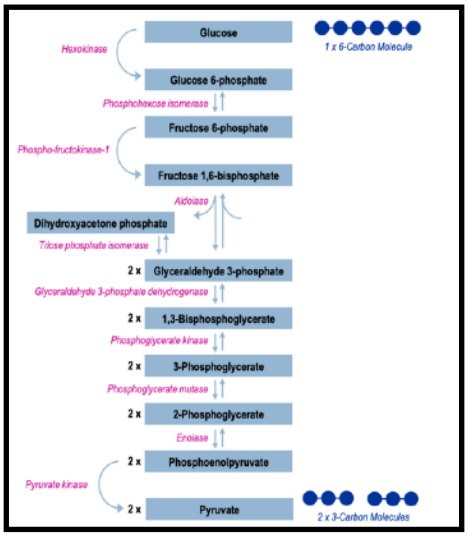
How does the body achieve glucose homeostasis?

Regulation of:
- Glycolysis/gluconeogenesis
- Glycogen metabolism

Intracellular control:
- Allosteric regulation
- Covalent modification

Intercellular control: role of hormones
- Insulin
- Glucagon
- Epinephrine

Glycolysis



Equation:

Glucose + 2 NAD^+ + 2 ADP + 2 P_i →

2 Pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O

- The **breakdown** of **1** molecule of glucose into **2 molecules of pyruvate**, while releasing **2 ATP** and **2 NADH** (energy)
- Occurs in the **cytosol** of most eukaryotic cells
- Involves 10 enzyme catalysed reactions
- Does NOT require oxygen (**anaerobic**)
- Fate of pyruvate is dependent on anaerobic or aerobic conditions
- Aerobic: used in citric acid cycle and onto electron transport chain
- Anaerobic: goes through fermentation
- There are 3 irrevisble steps in glycolysis

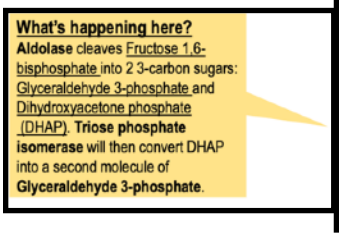
The Reaction

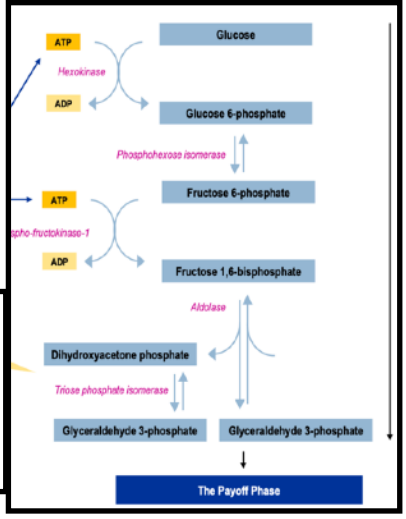
- First 5 phases are called the **Preparatory Phase**
  - Phosphorylation of glucose and conversion to **2** molecules of **glyceraldehyde-3-phosphate**
  - Requires energy - **2 ATP**
- Final 5 phases are called the **Payoff Phase**
  - Oxidative conversion of **glyceraldehyde-3-phosphate** to **pyruvate** and the **coupled** formation of **ATP** and **NADH**
  - Produces energy

The Preparatory Phase

- **Phosphorylation** of **glucose** and conversion to **2** molecules of **glyceraldehyde-3-phosphate**
- **2** molecules of **ATP** are considered the investment

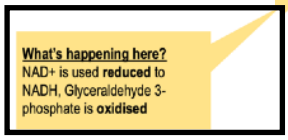
Hexokinase: Glu -> G-6-P
Pyruvate kinase: Phosphoenolpyruvate -> Pyruvate

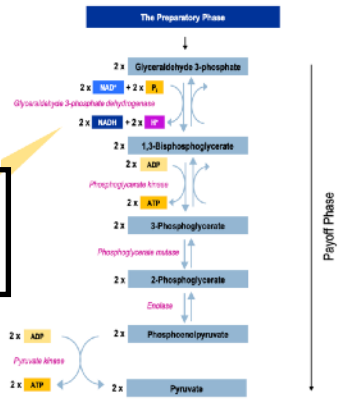




The Payoff Phase

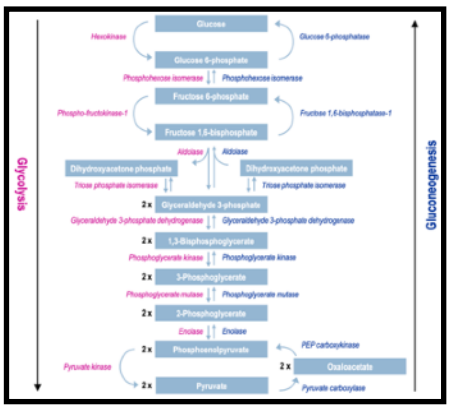
- **Oxidative** conversion of glyceraldehyde-3-phosphate to **pyruvate** and the coupled formation of **ATP** and **NADH**
- Releases **4** molecules of **ATP** and **2** molecules of **NADH**





Glycolysis Summary

- 10 enzymatic reactions that occur in the cytosol
- 2 distinct phases
- Highly regulated process
- Occurs when cells need energy
- **Active** when:
  - **[ATP]** is **low**
  - **[ADP]** is **high** (up to 5-fold)
  - **[AMP]** is relatively **high** (up to 20-fold)
  - **[NADH]** to [NAD+] ratio is **low (high NAD+)**
- Net gain: **2 ATP**, **2 NADH** & **2H+**, **2 pyruvate**



Gluconeogenesis

- Pathway active in the **liver** producing glucose for other cells to use
- Used to increase glucose levels in the blood when levels drop
- The generation of glucose from non-carbohydrate precursors such as **pyruvate** or **oxaloacetate**
- Not just the reverse of glycolysis
- When glycolysis is turned on, gluconeogenesis is turned off
- Turned off in fed state, but during fasting state (inbetween meals or vigorous exercise) it is turned on as glucose levels will be lower.
- Under hormonal control

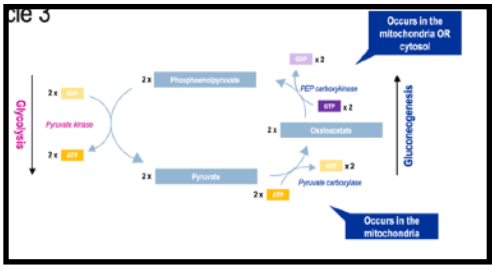
Net Reaction:

$2\ \text{Pyruvate} + 4\ \text{ATP} + 2\ \text{GTP} + 2\ \text{NADH} + 2\ \text{H}^+ + 4\ \text{H}_2\text{O} \rightarrow$
$\text{Glucose} + 4\ \text{ADP} + 2\ \text{GDP} + 6\ \text{P}_i + 2\ \text{NAD}^+$

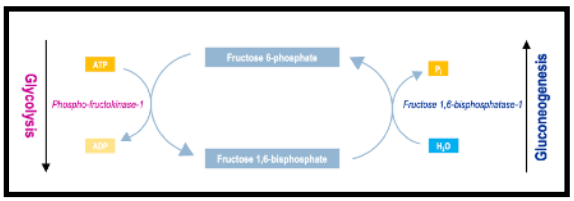
- 7 reversible reactions
- 3 **glycolytic enzymes** are **exergonic** and therefore **irreversible**
- **Bypassed** by **gluconeogenic enzymes**
- At these points, if both reactions are allowed to take place simultaneously ATP will be consumed without any work done: **futile cycle**
- Futile cycles provide an opportunity for regulation of pathways
- **1st**, **9th** and **10th** pathway of gluconeogenesis (bypass reactions)

Bypass Reaction 1: Futile Cycle 3

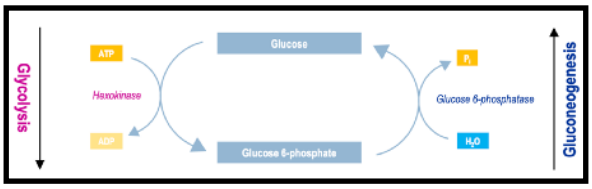
- 2 reactions required to convert **Pyruvate** to **Phosphoenolpyruvate**
- The first reaction occurs in the **mitochondria**
- The second can occur either in the **cytosol** OR the **mitochondria**
- Pyruvate —> Oxaloacetate —> Phosphoenolpyruvate



- **Pyruvate carboxylase** and **PEP carboxykinase**

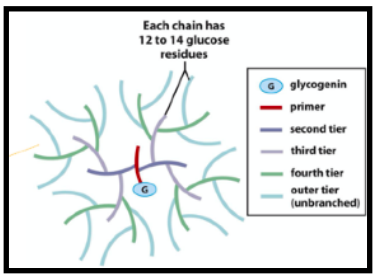


Bypass Reaction 2: Futile Cycle 2
- In **Glycolysis**: Fructose-6-phosphate is **phosphorylated** to Fructose-1,6-bisphosphate by **phosphofructokinase-1**
- In **Gluconeogenesis**: Fructose-1,6-bisphosphate is **hydrolysed** by **Fructose-1,6-bisphosphatase-1**, generating Fructose-6-phosphate



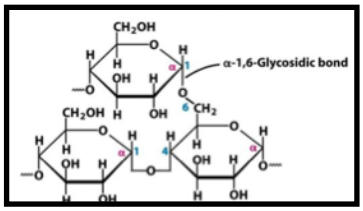
Bypass Reaction 3: Futile Cycle 1
- In **Glycolysis**: Glucose is **phosphorylated** to Glucose-6-phosphate by **Hexokinase**
- In **Gluconeogenesis**: Glucose-6-phosphate is **hydrolysed** by **Glucose-6-phosphatase**, generating Glucose

Gluconeogenesis Summary
- 7 enzymatic reactions in common with glycolysis
- Produces glucose from non-carbohydrate precursors
- Highly regulated process
- Takes place predominantly in the **liver**
- Occurs when glucose levels in the blood are low and glycogen stores are low
- Uses ATP (energy)
- Active when:
  - **[ATP]** is **high**
  - **[ADP]** is **low**
  - **[AMP]** is **low**
  - [NADH] to [NAD+] ratio is **high**



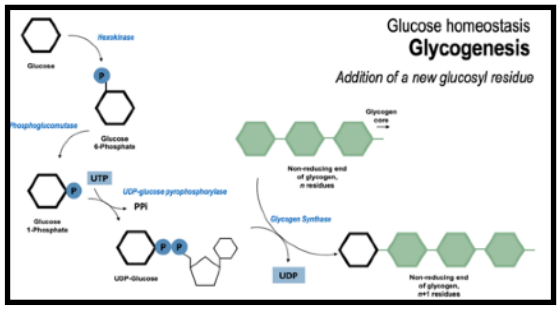
Glycogenesis and Glycogenolysis

Glycogen
- Primarily stored in the liver
- Occurs when glucose levels are high
- Branched molecule made up of glucose-residue chains
- Protein core- **glycogenin**
- End of each brand is referred to as the **nonreducing end**
- Each **branch extension** consists of long chain of glucosyl residues joined by α1-4 glycosidic linkages

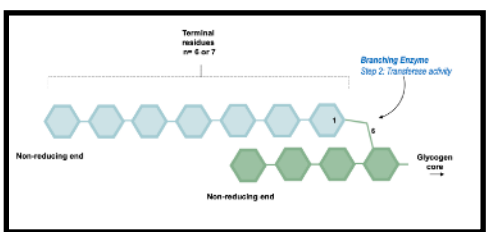


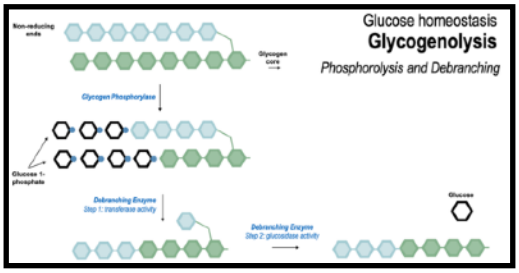
Types of linkages
- Residues **within** a branch are joined by **α1-4** linkages
- Residues at **branch points** are joined by **α1-6** linkages

1) Two processes Glycogenesis: **Elongation**
- Glucose is added to glycogen chains in the form of **UDP glucose**
- **UDP-glucose pyrophosphorylase**: also known as uridyl transferase, glucose-1-phosphate uridylytransferase
- **Pyrophosphate** = PPi, a molecule consisting of two phosphate groups



2) Two processes Glycogenesis: **Branching**
- Branching enzyme
- Begins by cleaving α1-4 linkage
- Chain must be at least **11** residues long
- Cleaves at **6-7 residues from the nonreducing end**





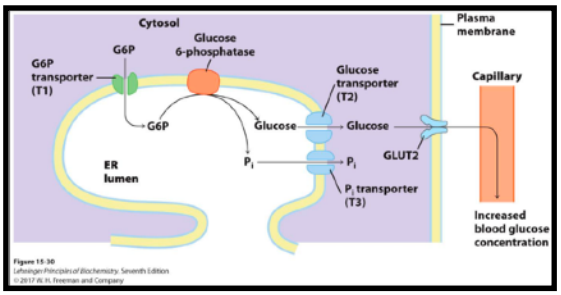
and transfer the chain to a glucosyl group and forms a new α1-6 linkage **closer** to the glycogen core

Glycogenolysis

- Occurs when blood sugar **drops**
- Stored residues are cleaved from glycogen branches and subsequently converted to glucose
- **Two** enzymes required to break two bonds

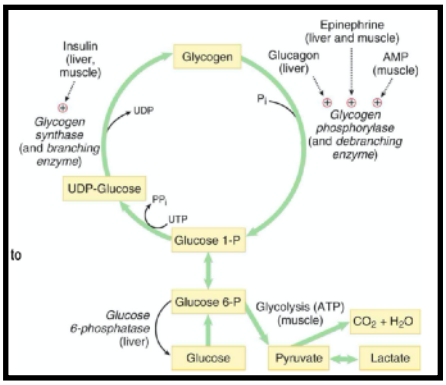
The fate of glucose-1-phosphate

- Glucose-1-phosphate —> Glucose-6-phosphate —> Glucose
- **Final** reaction takes place in **Endoplasmic Reticulum** (active site)
- Enzyme is **sequestered** to **ER**. If the enzyme was present in the cytosol the **first step of glycolysis would be reversed** and **glycolysis** would **not** proceed. Important type of enzymatic regulation
- Muscle cells do **not** have **glucose-6-phosphatase** so cannot convert the glucose residues broken down from glycogen to glucose and therefore cannot contribute to blood glucose concentrations. Instead, they are used **directly** for glycolysis in the muscle cells



Glycogenesis and Glycogenolysis Summary

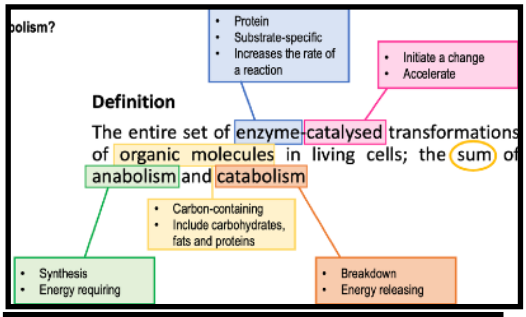
- Glycogen:
  - Branched molecule
  - Polymeric Glucose reserves
- Glycogenesis:
  - Occurs when blood glucose is **high**
  - Conversion of glucose to UDP-glucose prior to glycogen formation
- Glycogenolysis:
  - Occurs when blood glucose concentrations **drop**



Introduction to Metabolic Regulation

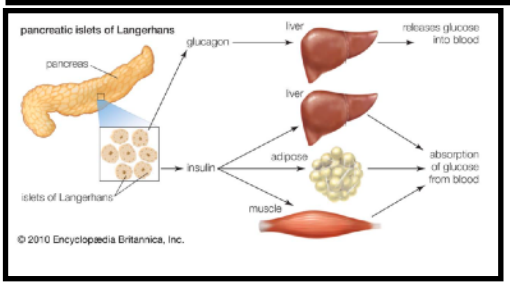
What is metabolism?

The entire set of enzyme-catalysed transformations or organic molecules in living cells; the sum of anabolism and catabolism



Hormonal Regulation

- **Insulin**, **glucagon** and **epinephrine**:
- Chemical signals released into blood
- Only “target” cells respond to a given hormone
- Hormones combine with specific receptor proteins
- Hormones are quickly eliminated from the blood
- Insulin = released when glucose is **high**
- Glucagon = released when glucose is **low**
- Epineprhine = released when action is needed



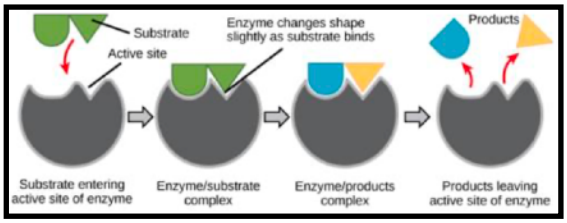
Hormonal Regulation: Can control both the amount and the activity of an Enzyme

Hormonal vs Intracellular Regulation

- Primary determinants of metabolic activity of **liver**, **muscle** and **adipose** tissue

- **Hormonal** control (intercellular) is **slower** than regulation by allosteric activation/inhibition and covalent modification (intracellular)
- Effects can result in 1-20 fold increases in enzyme activity

When are hormones released?
- **Insulin**: [glucose] too **high**
- **Glucagon**: [glucose] too **low**
- **Epinephrine**: preparation of muscles, lints and heart for a burst of activity - activate glucagon



Amount of Enzyme
Enzymes
- Essential component of metabolic processes
- **Catabolic** - breaking down molecules
- **Anabolic** - forming larger molecules
- **Active site**: site where substrate binds to enzyme
- **Regulatory site**: binding site for regulatory molecules

Why do biochemical reaction need to be regulated?
- Rates of biochemical reactions depend on many factors:
- The flow of metabolites through the pathways is regulated to maintain **homeostasis**
- Homeostasis occurs when concentrations of metabolites are kept at a **steady state** in the body
- When perturbed, a **new steady state** is **achieved**
- Sometimes, the levels of required metabolites must be **altered** very **rapidly**
  - Need to increase the capacity of glycolysis during action
  - Need to reduce the capacity of glycolysis after the action
  - Need to increase the capacity of gluconeogenesis after successful action

AMOUNT of Enzyme
1. Lifespan of Enzyme
- All proteins have finite lifespans
- Different proteins in the same tissue have very different half-lives
  - Less than an hour to about a week for liver enzymes
  - Stability correlates with the sequence at N-terminus
  - Some proteins are as old as you are eg. Crystallins in the eye lens

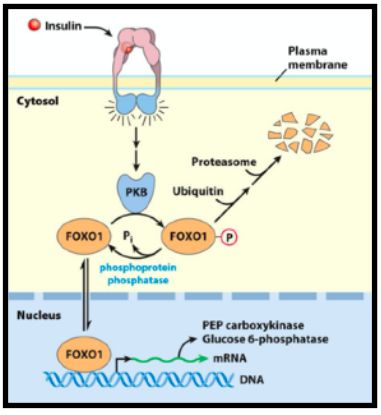
TABLE 15-1 Average Half-Life of Proteins in Mammalian Tissues

Tissue	Average half-life (days)
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Constitutive enzymes
- **Long** lifespans: days to months
- Rate of synthesis = rate of degradation
- Are required in **constant concentrations**
- Examples: **Glycolytic** enzymes, **citric acid cycle** enzymes

Inducible/Repressible enzymes
- **Short** lifespans, synthesised only when required
- Rate of synthesis and degradation can be **increased** or **decreased**
- Examples: **Hormones**, **growth factors**

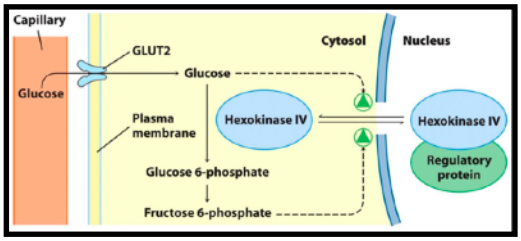
2. Induced or repressed synthesis
- The synthesis of an enzyme can be controlled, either increasing its expression or decreasing it
- Example: **Induced** by **insulin**
  - **Hexokinase II** & **glucokinase** (Glu->G-6-P)
  - Liver **phosphofructokinase** (PFK-1)
  - **INCREASE** Glycolysis
- Example: **Repressed** by **insulin**
  - **PEP Carboxykinase** (for gluconeogenesis)
  - **Glucose-6-phosphatase**



- Gluconeogenic enzymes therefore **DECREASED**

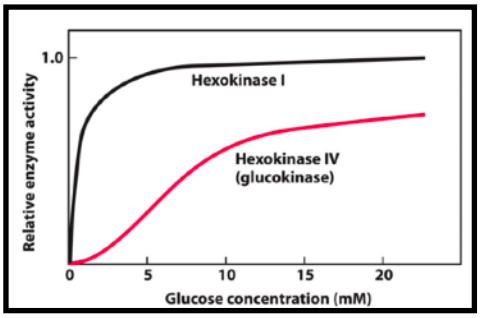
3. **Compartmentalised**

- **Glucose-6-phosphatase** (organ compartmentalised)
  - G-6-P -> Glucose + Pi (last stage of gluconeogenesis)
  - In **gluconeogenic tissues** (liver, kidney)
  - But **not** in **glycolytic tissues** (muscles, brain, fat)
- Red blood cells do not contain mitochondria, therefore do not have the enzymes for the:
  - Citric acid cycle
  - Electron transport chain
- **Hexokinase** (organelle compartmentalised)
  - **Sequestered** to the **nucleus until conditions favour glycolysis** in the liver (high glucose)



4. Exist as **isozymes**

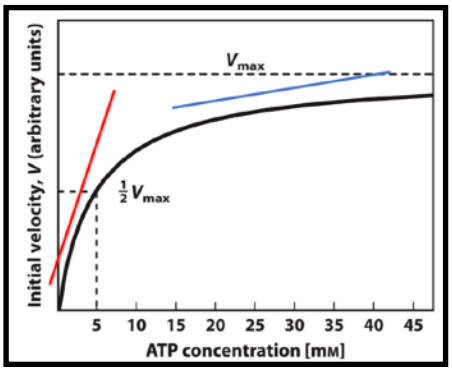
- Multiple forms of an enzyme that catalyse the same reaction but differ in amino acid sequence, substrate affinity, Vmax and/or regulatory properties
- May exist within a single cell/tissue
- Are often the products of different genes
- Catalyse the same reaction but have different primary structures
- Example: Hexokinase
  - Reaction: phosphorylation of glucose, first step of Glycolysis
  - ATP + glucose —> Glu-6-P + ADP
  - Four iso-enzymes of hexokinase:
  - **Hexokinase I, II and III (Muscles)**
  - Km for glucose = 0.04mM (**low**) for I, II, III
  - **Hexokinase IV (Glucokinase) (Liver)**
  - Km for glucose ~10mM (**high**) (lower affinity) -
- if Km was lower, then a small amount of glucose would be used up in **glycolysis and not stored as glycogen**
- If Km was lower, glucose **released** from gluconeogenesis would be **metabolised very quickly**
- Km = concentration of substrate which permits the enzyme to achieve half Vmax



<u>Activity of Enzyme</u>

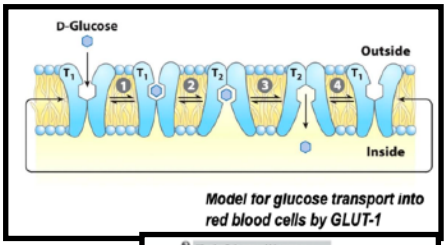
1. **Concentration** of Substrate(s)

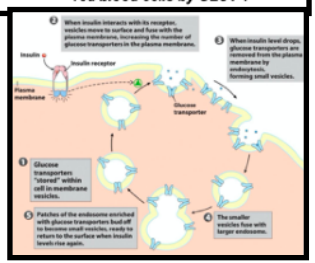
- **Rate of** a **reaction** depends on the concentration of substrates
- The rate is more sensitive to concentration at low concentrations
  - **Chemical kinetics**: Frequency of substrate meeting the enzyme matters
- The rate becomes insensitive at high substrate concentrations
  - The enzyme is nearly **saturated** with substrate

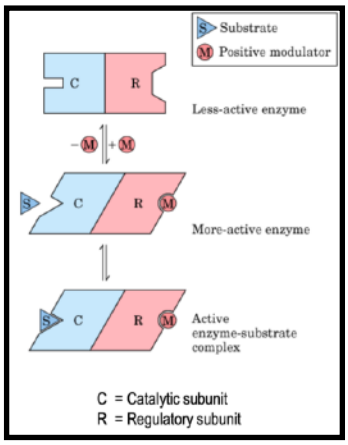


Example: Cellular transport of glucose across membranes

- **Facilitated transport**: Glucose transporter GLUT1-5
- Concentrations of glucose in **blood** plasma ~ **4.5mM**
- Concentration of glucose in **cytoplasm** is **much lower**
- Glucose enters cells through specific **transporters**
- Glucose uptake by **brain** and **red blood cells** is **insulin-independent**
- Glucose uptake by **muscle** and **adipose** tissue is **insulin-dependent (required)**

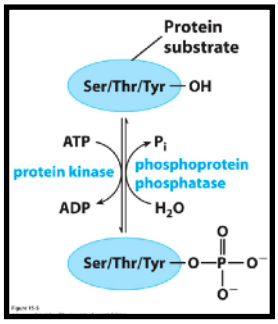




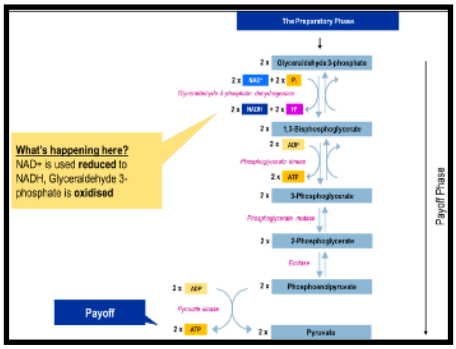


Glucose uptake by **muscles** and **adipose** tissue is insulin-dependent
- Insulin **stimulates** translocation of **GLUT-4** glucose transporters to the **surface** of **myocytes** (synthesising glycogen) and **adipocytes** (synthesising triacylglycerols)
- Results in **increase** of **glucose uptake** to 15 fold or more
- In type **I diabetes**: there is **no insulin** released and therefore **no mobilisation** of **GLUT-4**

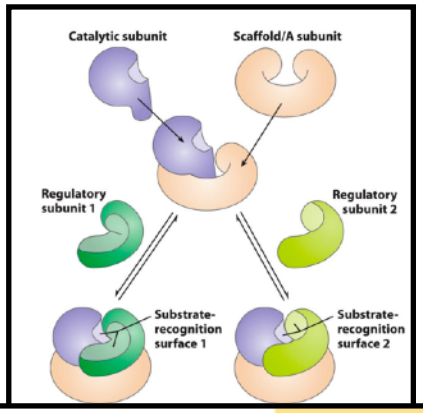
2. **Allosteric Modulation**
- REVERSIBLE, NON-COVALENT binding of a modulator at a site other than the active site
- Allosteric enzymes:
  - Have **separate binding** site for their modulators (inhibitors or activators)
  - Have quaternary structure and are composed of subunits
  - The subunits can adopt more than one conformation
  - Binding of substrate occurs **more readily** to **one conformation**
  - Binding of a modulator brings about a conformational change in the enzyme

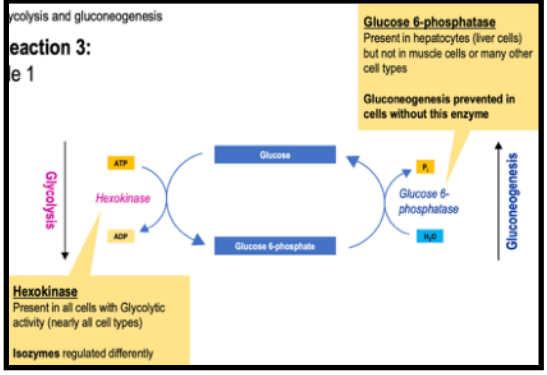


3. **Phosphorylation/Dephosphorylation**
- Addition or removal of a phosphate group
- Covalent modification
- **Phosphorylation** is catalysed by **protein kinases**
- **Dephosphorylation** is catalysed by **protein phosphatases**, or can be spontaneous
- Typically proteins are phosphorylated on the hydroxyl groups of **Ser**, **Thr**, or **Tyr**
- Phosphorylation may activate or inactivate an enzyme (like a switch!)



4. **Regulatory molecules** (Coenzymes/proteins)
- Cells contain limited concentrations of coenzymes such as:
  - NAD+ and NADH
  - NADP+ and NADPH
  - ATP, ADP and AMP
  - Acetyl-S-CoA
- In cells, [NAD+] + [NADH] = 0.5mM
- Binding of regulatory protein subunits affects specificity
- Two different regulatory subunits exist that can bind with each other
- Creates different substrate binding sites
- **Holoenzyme** = active form of enzyme





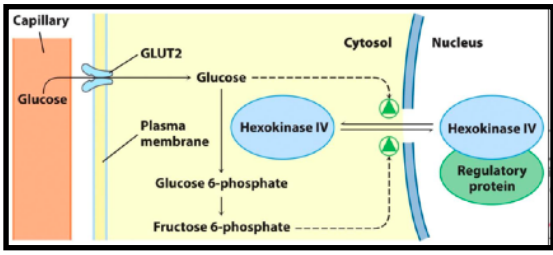
<u>Regulation of Glycolysis and Gluconeogenesis</u>
<u>Bypass Reaction 3: Futile Cycle 1:</u>
- Beginning of glycolysis, end of gluconeogenesis
- Catalysed by enzymes with have large -ve deltaG values (favours forward direction)
- **Hexokinase**: present in all cells with Glycolytic activity

- Isozymes regulated differently
- Glucose-6-phosphatase: Present in **hepatocytes** but **not in muscle cells** or many other cell types
  - **Gluconeogenesis prevented** in cells **without** this enzyme

Hexokinase
- Four **isozymes** for hexokinase exist (I to IV)
- Different isozymes exist in different tissues
- Isozymes perform the **same reaction** but can be **regulated differently**

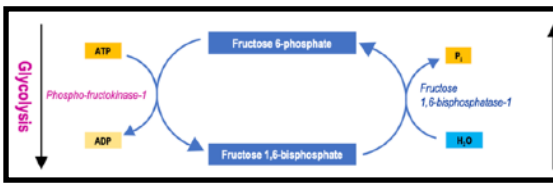
Hexokinase I, II, III
- Found in **muscle cells**
- Allosterically **inhibited** by their **product**, Glucose-6-phosphate
- **High affinity** for Glucose (Low Km ~ 0.1mM) —> **Favours glycolysis greatly** in muscle cells

Hexokinase IV (also called **glucokinase**)
- Found in the **liver**
- **Low affinity** for glucose (High Km ~ 10mM)
- Inhibited by a nuclear binding protein specific to the liver (not inhibited by product)
  - Binding protein draws Hexokinase IV into the **nucleus** when glucose is **low**, **preventing** it from catalysing the reaction
  - When glucose levels are **high**, regulatory protein **releases** Hexokinase IV back into the **cytosplasm** where it can perform its activity



- When blood glucose levels rise, glucose is transported to the **liver** cells and as a result of the low affinity of glucokinase, its activity continues to rise and **blood glucose levels continue to rise**
- Ensures glucokinase is only active when glucose levels are really high
- When blood glucose levels are low, glucose produced by gluconeogenesis will be protected because it won't be phosphorylated to G-6-P and hence can leave the liver and into the blood stream.

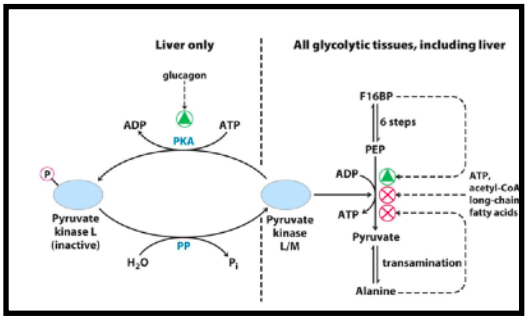
Bypass Reaction 2: Futile Cycle 2:
Phospho-fructokinase-1 (**PFK-1**) - glycolysis
- Allosterically regulated
- Both substrate and regulatory binding sites
- **ATP** can **negatively regulate** the activity of the enzyme
- Enzyme **inhibited** when **ATP is high** (high glucose level in blood)
- **AMP** and **ADP allosterically activate** the enzyme (low glucose levels in blood)
- **Citrate** is an **allosteric inhibitor**, signals that the cell reaches its required energy needs and glycolysis is not required. Increases the inhibitory action of ATP



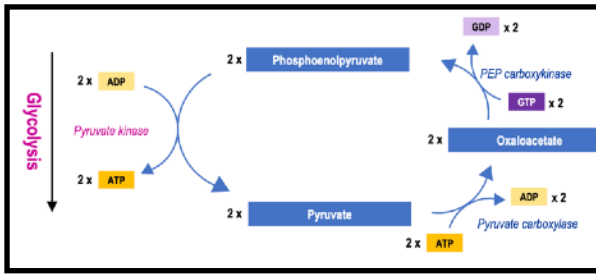
- **Fructose-2,6-bisphosphate** is an **allosteric activator**. It **increases** the **affinity of PFK-1** for Fructose-6-phosphate, decreases its affinity for ATP and citrate
  - NOT an intermediate in glycolysis. Produced **specifically** for regulation of glycolysis and gluconeogenesis

Fructose-1,6-bisphosphatase-1 - gluconeogenesis
- Allosterically regulated
- **Inhibited** by **increased AMP** — a by product of ATP consumption
- **ATP** is a **positive regulator**
- Inhibited by Fructose-2,6-bisphosphate - allosteric repressor

Pyruvate kinase **inactivated** by **phosphorylation.** Glucagon inv.

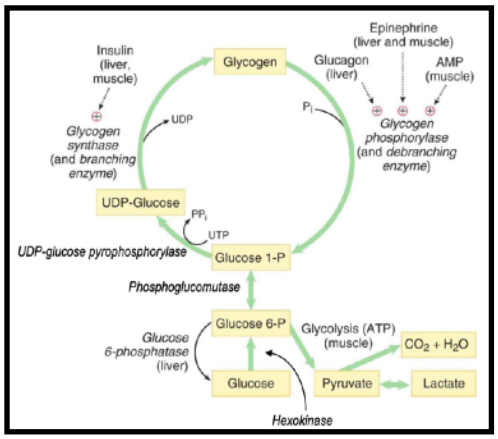


Bypass Reaction 1: Futile Cycle 3:
Pyruvate kinase



- Three **isozymes** (L=liver, M=muscle), also erythrocyte
- Allosterically **activated** by **fructose-1,6-bisphosphate**: **high flow** through glycolysis
- Allosterically **inhibited** by signs of abundant energy supply (all tissues)
  - **High ATP**
  - **Acetyl-CoA** and long-chain fatty acids
  - **Alanine** (enough amino acids)
- Isozyme present in **liver** but **not muscle** is **inhibited** by **phosphorylation** (response to glucose depletion by hormone **glucagon**)
- When glycogen is released due to low glucose levels, this results in a **higher** level of cyclic amp (**cAMP**), a signalling molecule that **activates** the **enzyme cAMP-dependent-kinase**, also known as **protein kinase A (inactivates pyruvate kinase)**
  - It **phosphorylates** the **isozyme** in the **liver** and inactivates it. This ensures **glucose in the liver can be delivered to the tissues**
- Isozyme of pyruvate kinase in the **muscle** is regulated by the **hormone epinephrine**, which is released when additional energy is required. It increases the amount of **cAMP** which activates glycolysis

Regulation of Glucogenesis and Glycogenolysis
Genesis = synthesis | Lysis = breakdown



Muscle Glycogen:
- Provides a **quick source** of energy for either aerobic or anaerobic metabolism
- Can be used up in less than one hour during vigorous activity

Liver Glycogen:
- Serves as a **reservoir of glucose** for other tissues when dietary glucose is not available.
- This is especially important for the brain
- Can be depleted in 12-24 hours
- The general mechanism for storing and mobilising glycogen are the same in the muscle and the liver, but the enzymes differ reflecting the different roles of glycogen in the two tissues

Regulation is controlled on multiple levels
- At the **hormonal** level
- At the **enzyme** level
- **Allosteric** regulation and
- **Covalent** regulation of **glycogen synthase** and **glycogen phosphorylase**

Controlled through regulating **glycogen synthase** (GS)
- **Insulin**-signalling pathway
  - Increase glucose import into muscle
  - Stimulates the activity of **muscle hexokinase (I,II,III)**
  - **Activates** glycogen synthase
- Increase hexokinase activity enables activation of glucose
- Glycogen synthase (**GS**) activity is **promoted** by **insulin**
- **Glycogen synthase** is **inactive** when **phosphorylated (3)**, and **active** when **dephosphorylated**
- In active form it is called **Glycogen synthase A** and the enzyme responsible for **dephosphorylating** GS-A is **phosphoprotein phosphatase-1** (**PP1**)
- When insulin binds to its receptor it leads to intracellular pathways that lead to activation of PP1