Glycogen Metabolism

Daniel Seifu

UGHE, March, 2024

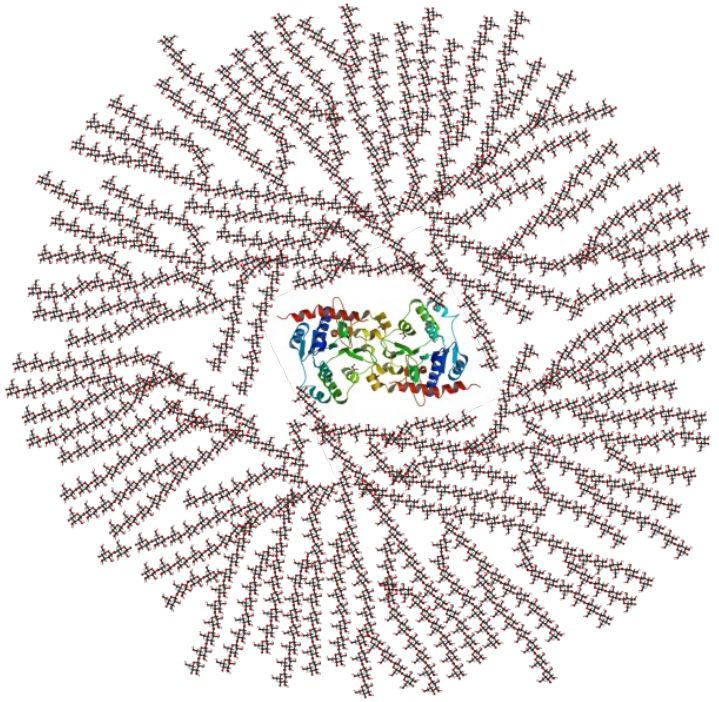
SESSION LEARNING OBJECTIVES

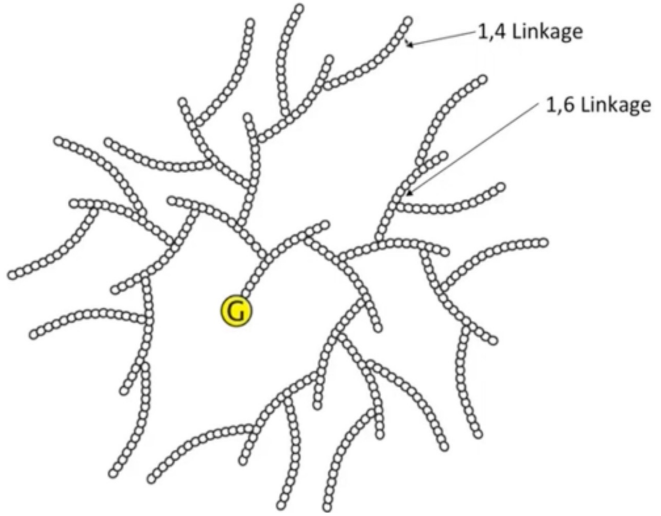
- Describe the importance of glycogenesis and glycogenolysis in liver and skeletal muscle
- Identify the rate -limiting enzymes of both pathways
- Describe the role of hormonal regulation of glycogen metabolism
- Explain the role of muscle glycogen during fed/fasting and exercises
- Explain the role of liver glycogen metabolism in blood glucose homeostasis
- Explain the allosteric regulation of glycogen metabolism in liver and skeletal muscle
- Understand the role of enzyme defects in glycogen metabolism and its association with glycogen storage diseases

Glycogen structure

Glycogen: A highly branched homopolysaccharide

- Formed of α-glucose units linked by (α1➞4)-glucosidic linkage along the main chain and (α1➞6)-glucosidic linkage at the branching point
- The gross structure of glycogen is dendritic in nature: Expanding from a core sequence bound to glycogenin. developing into a final structure resembling a head of cauliflower
- Creates many ends (non-reducing) of the molecule allowing for the fast release of glucose for energy conversion





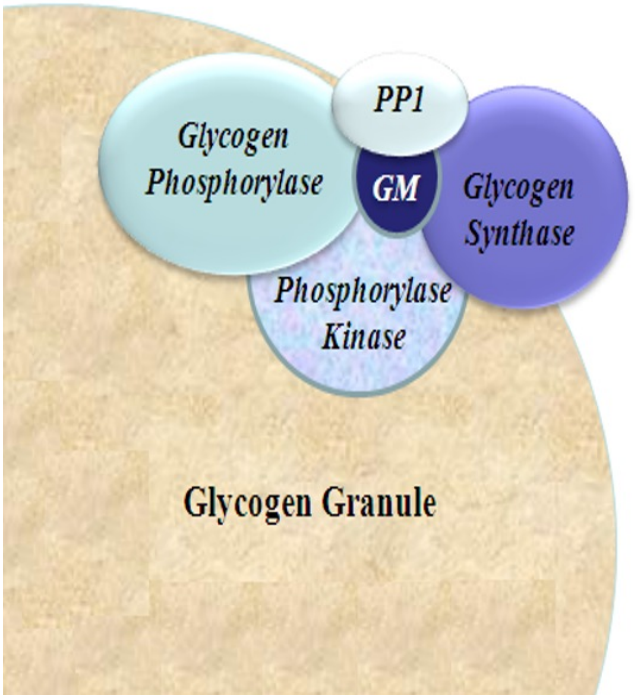
Glycogen storage

In vertebrates, glycogen is found primarily in the **Liver & skeletal muscle**

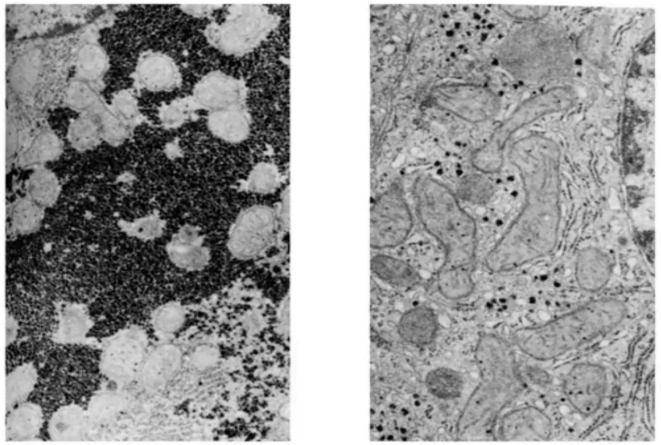
It may represent up to:

- 7-10% of the weight of liver
- 1% of the weight of muscle

- Glycogen is stored in large cytosolic granules
- Glycogen granules are complex aggregates of glycogen
- Enzymes that synthesize & degrades it as well as the *machinery for regulating these enzymes are closely associated*



Glycogen Storage in the Liver in the Fed and Fasted States



Fed State

24 Hour Fast

Functions of glycogen storage

In muscle:

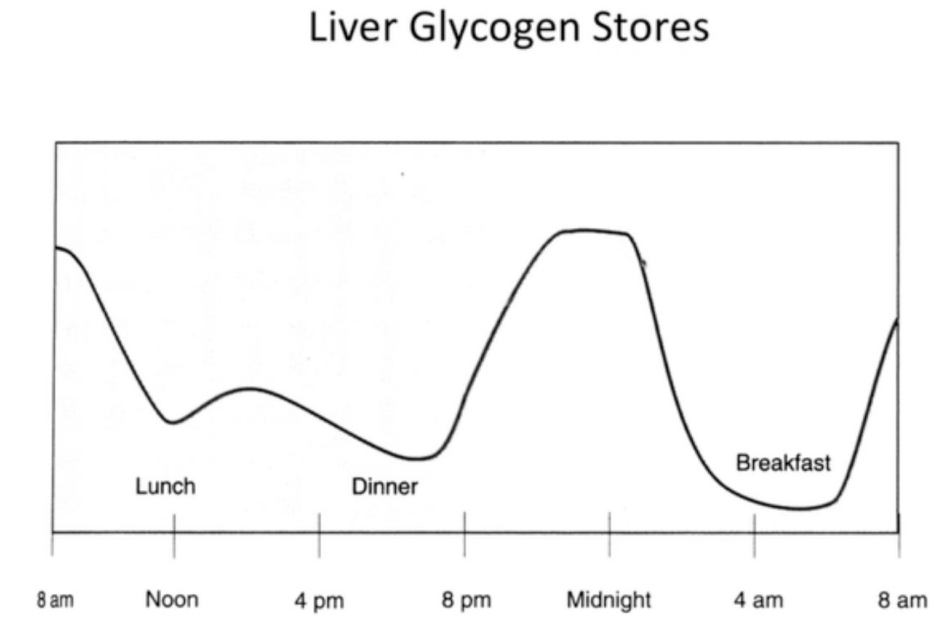
- A quick source of energy for anaerobic metabolism
- Can be exhausted in less than an hour during vigorous activity

In Liver:

- Serves as a reservoir of glucose for other tissues during fasting or between the meals.
- Releasing glucose to maintain blood glucose conc.
- Liver glycogen can be depleted in 12 to 24 hr's

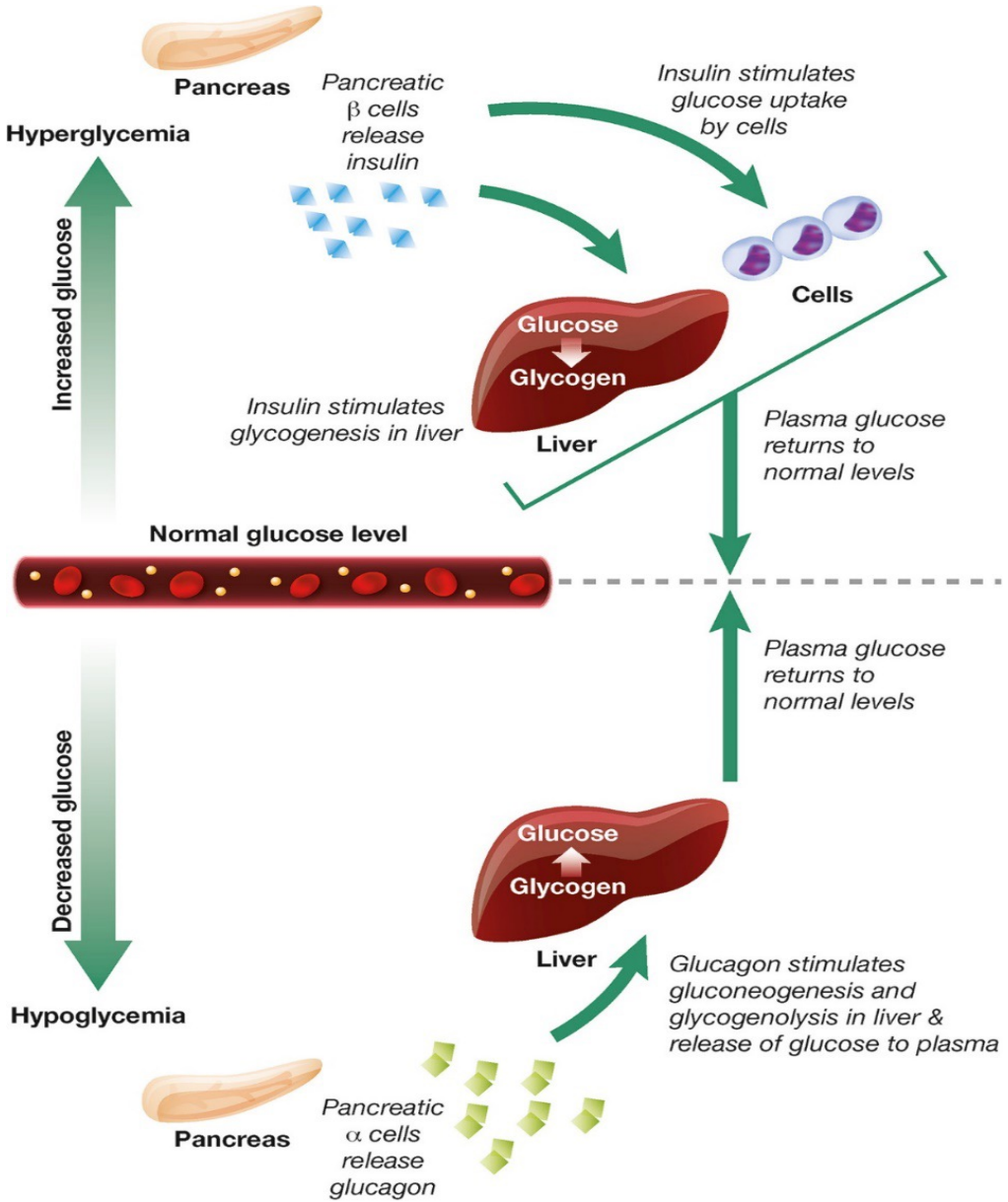
Mechanisms for storing & mobilizing glycogen is the same in muscle & liver; but the enzymes differ in subtle yet important ways . Reflect the different roles of glycogen in the two tissues

- In humans, the total amount of energy stored as glycogen is far less than the amount stored as fat (TAGs),
- Glucose cannot be stored as such within the cells, b/c: it is osmotically active, at equivalent amounts to glycogen it will induce osmotic lysis



Role of liver and pancreas in glycogen metabolism

- Maintain glucose homeostasis in the blood
- Insulin is secreted by the pancreas in response to high blood glucose levels
- Glucagon is secreted by the pancreas in response to low blood glucose levels



Glycogen synthesis

Glycogenesis

- Takes place in virtually all animal tissues but is especially prominent in the liver & skeletal muscles (cytosol)
- Very small amount of glycogen synthesis & storage in the CNS , this is why it is completely dependent on blood glucose

Sources of glucose

- For liver glycogen: Blood glucose & Other hexoses
- For muscle glycogen: Blood glucose only

Glycogen synthesis

The following important enzymes and proteins are required;

- Activated sugar
- Primer
- Glycogen synthase
- Branching enzymes

Glycogen synthesis

Activation of Glc-1-P to UDP-Glc

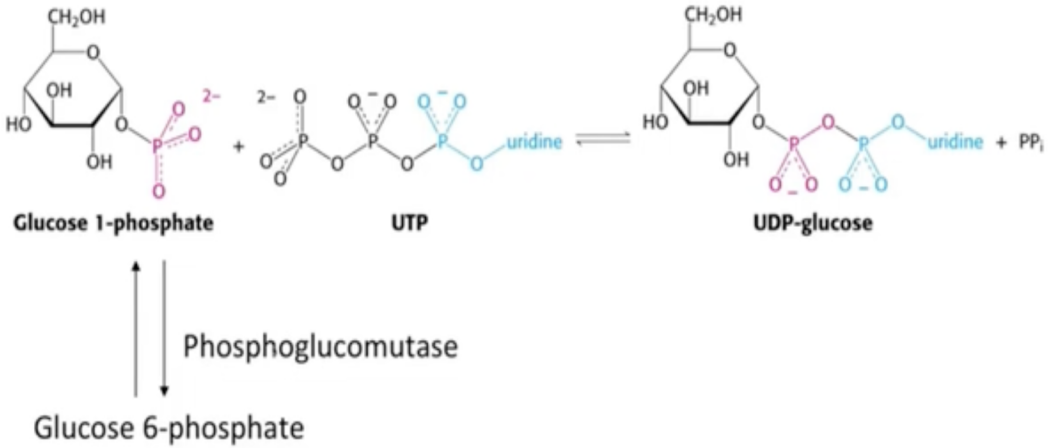
UDP-glucose is formed from glucose-1-phosphate: by **UDP-Glc pyrophosphorylase & pyrophosphatase**

Glucose-1-phosphate + UTP → UDP-glucose + PP_i

- **PP_i + H_2O → 2 P_i**

Overall: Glucose-1-phosphate + UTP → UDP-glucose + 2 P_i

- Spontaneous hydrolysis of the ~P bond in PP_i drives the overall rxn
- Cleavage of PP_i is the only energy cost for glycogen synthesis one ~P bond per glucose residue



- **Uridine diphosphate glucose (UDP-Glc)**

  Is active form of Glc for glycogen synthesis

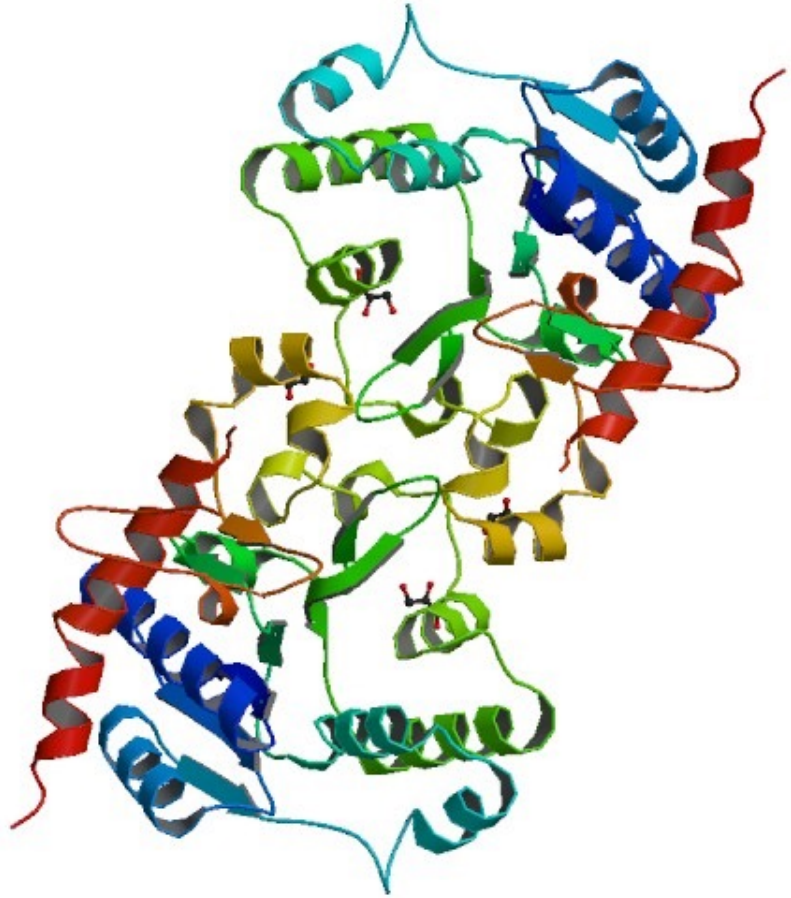
  As glucose residue precursors are added to glycogen, UDP-Glc is the substrate & UDP is released as a rxn product

- Nucleotide diphosphate sugars are precursors also for synthesis of other complex carbohydrates; Including oligosaccharide chains of glycoproteins, etc.

Glycogen synthesis

Glycogenin- primer synthesis

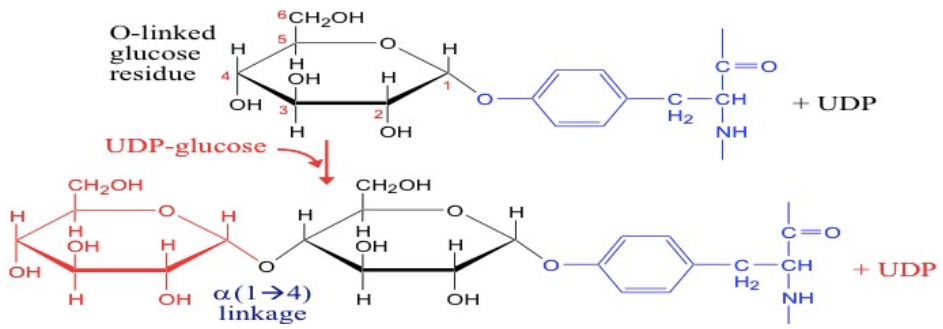
- Small protein that acts as a glycogen synthase primer
- It achieves this by catalyzing the addition of glucose to itself at Tyr-194.
- Once sufficient residues have been added, glycogen synthase takes overextending the chain.
- Glycogenin remains covalently attached to the reducing end of the glycogen molecule.



Glycogen synthesis

Glycogen primer: Usually a preformed ($\alpha 1 \rightarrow 4$) polyglucose chain, or branch having at least eight glucose residues, **Glycogenin**

- This intriguing protein is both: Primer & Enzyme that catalyzes their assembly
- The 1st step in the synthesis of a new glycogen molecule is the transfer of a Glc residue from: UDP-Glc to the -OH group of serine/Tyrosine[194] of glycogenin, catalyzed by the intrinsic Glucosyltransferase
    - Initiate's glycogen synthesis
    - Is also an enzyme that catalyzes attachment of a glucose molecule to one of its own tyrosine residues.
    - Is a dimer, and evidence indicates that the 2 copies of the enzyme glucosylate one another



- At C_4 of the attached glucose (UDP-glucose again the donor), to yield an O-linked disaccharide with $\alpha(1 \rightarrow 4)$ glycosidic linkage
- This is repeated until a short linear glucose polymer with $\alpha(1 \rightarrow 4)$ glycosidic linkages is built up on Glycogenin.

Glycogen synthesis

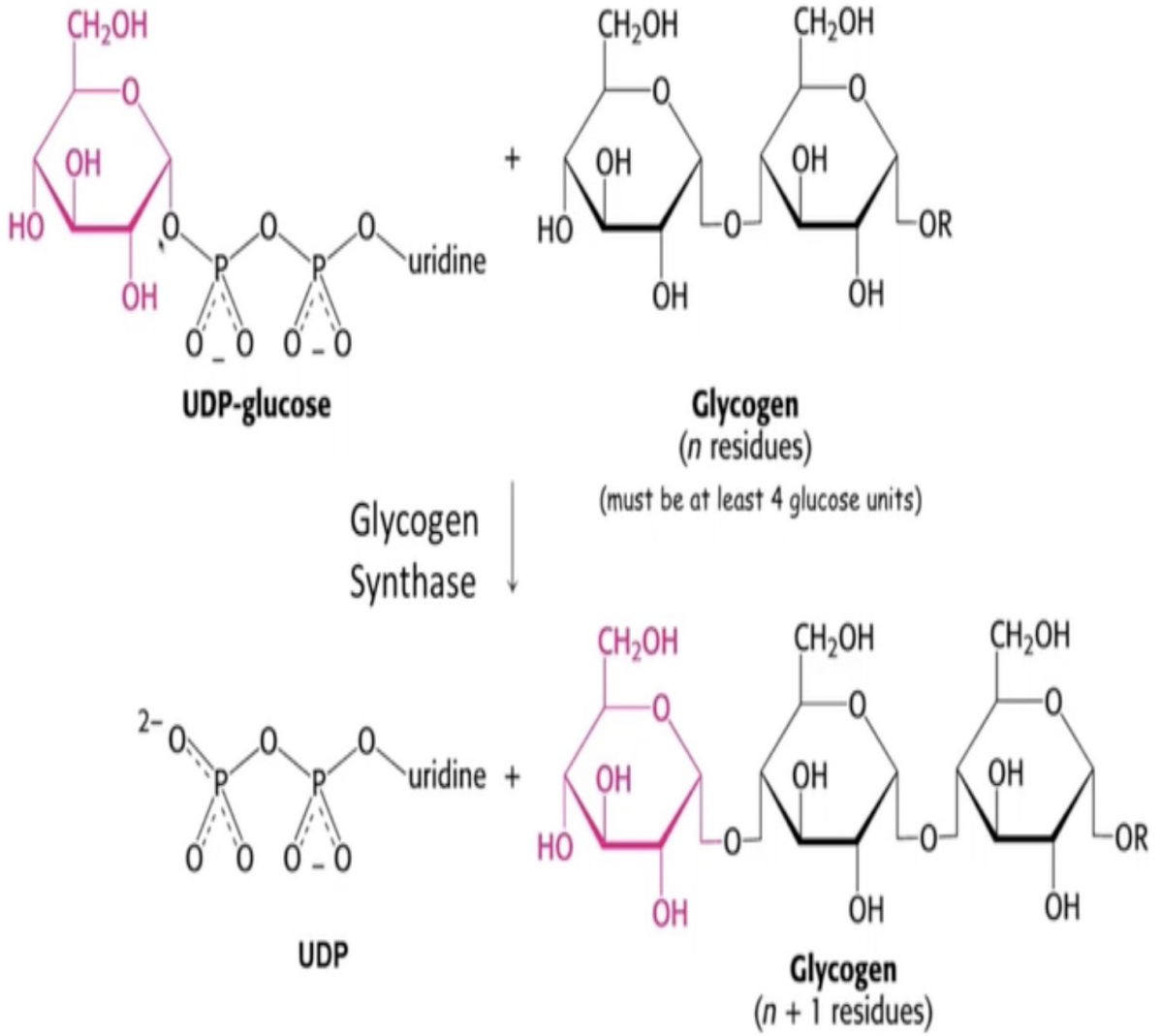
Glycogen synthase

- Is an enzyme that plays a crucial role in glycogenesis, the process of converting glucose into glycogen for storage in the liver, muscles, and other tissues.
- It is a glycosyltransferase enzyme that catalyzes the reaction between UDP-glucose and glycogen, forming a new glycosidic bond and lengthening the glycogen chain.
- Glycogen synthase activity is tightly regulated to ensure that glycogen synthesis is balanced with glycogen breakdown (glycogenolysis).
- The enzyme is **inactivated by phosphorylation** by protein kinases and activated by dephosphorylation by protein phosphatases.
- In addition, glycogen synthase is allosterically activated by glucose 6-phosphate, a metabolite that indicates high blood sugar levels.

There are two main isoforms of glycogen synthase in humans:

•Glycogen synthase 1 (GS1), which is found primarily in skeletal muscle

•Glycogen synthase 2 (GS2), which is found primarily in the liver



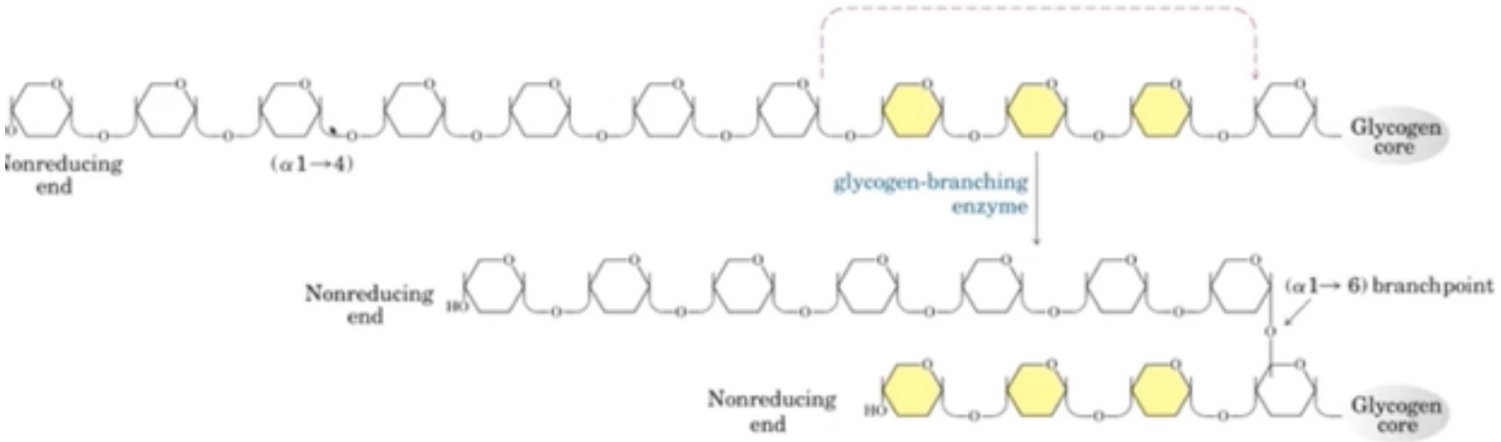
Glycogen synthesis

A branching enzyme

- Transfers a segment from the end of a glycogen chain to the C_6 hydroxyl of a glucose residue of glycogen to yield a branch with an ($\alpha 1 \rightarrow 6$) linkage also called: **Amylo (1→4) to (1→6) transglycosylase or Glycosyl-(4→6)-transferase**

Makes branch points of glycogen

- Catalyzes transfer of a terminal fragment of **6 or 7 Glc residues** .
- From the none reducing end of a glycogen branch having at least 11 residues to the C_6 -OH group of a Glc residue at a more interior position of the same or another glycogen chain.
- Branch point should be at least 4 residues from the previous branch point in the same chain



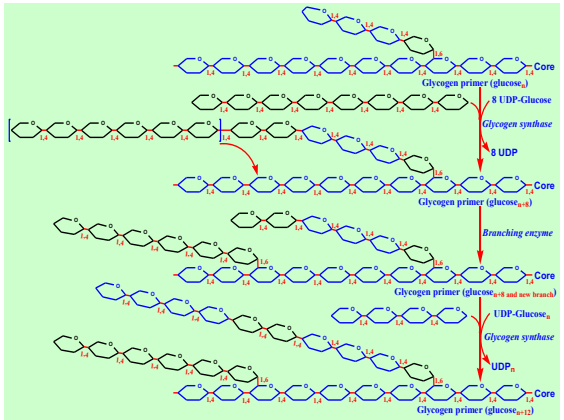
Glycogen synthesis

Branching of glycogen serves two major roles;

- Increased sites for synthesis &degradation, permitting rapid release of glucose 1-phosphate for muscle activity
- Enhancing the solubility of the molecule

Endurance athletes require a slower, more sustained release of Glc 1-P

- Some endurance athletes practice carbohydrate loading: Exercise to exhaustion (when muscle glycogen in largely depleted) followed by a high carbohydrate meal, which results in: Rapid glycogen synthesis, with fewer branch points than normal.



Summary of glycogen synthesis

Steps: The pathway involves the following steps:

- Conversion of Glc-6-P into Glc-1-P;**Phosphoglucomutase**
- Activation of Glc-1-P to the sugar nucleotide **UDP-Glc; UDP-glucose pyrophosphorylase & pyrophosphatase**
- Glycogen primer formation : Primes the initial sugar residues in glycogenin
- Transfer of glucose to glycogen chain in α1→4 linkage; **glycogen synthase**
- when the α1→4 chain exceeds eight residues in length, synthesis of branches by "branching enzyme", also called amylo-α(1→4)→**α(1→6)-transglucosidase(transglycosylase) or glycosyl-(4:6)-transferase**

This branching enzyme : transfers some of the α1→4-linked sugars to an α1→6 branch, setting the stage for continued elongation of both α1→4 chains until they, in turn, become long enough for transfer by branching enzyme

Regulation of glycogen synthesis

Activation of Glycogen Synthase

1.Inhibition of Glycogen Synthase Kinase (GSK)

- A key step is the inhibition of glycogen synthase kinase (GSK), specifically GSK-3.
- Insulin signaling leads to the phosphorylation of GSK, making it inactive.

2. Dephosphorylation of Glycogen Synthase

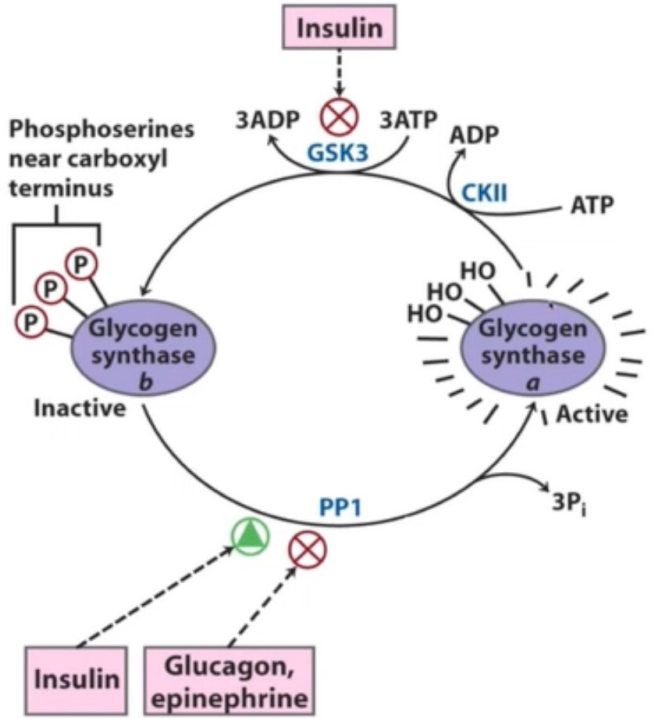
- When GSK is inactive, it can't phosphorylate glycogen synthase, which normally inactivates it.
- This allows protein phosphatases to remove phosphate groups from glycogen synthase, activating it.

Additional Factors

3. Glucose 6-phosphate (G6P):

- Insulin also promotes glucose uptake into cells.
- This increases the concentration of G6P, an allosteric activator of glycogen synthase, further enhancing its activity.

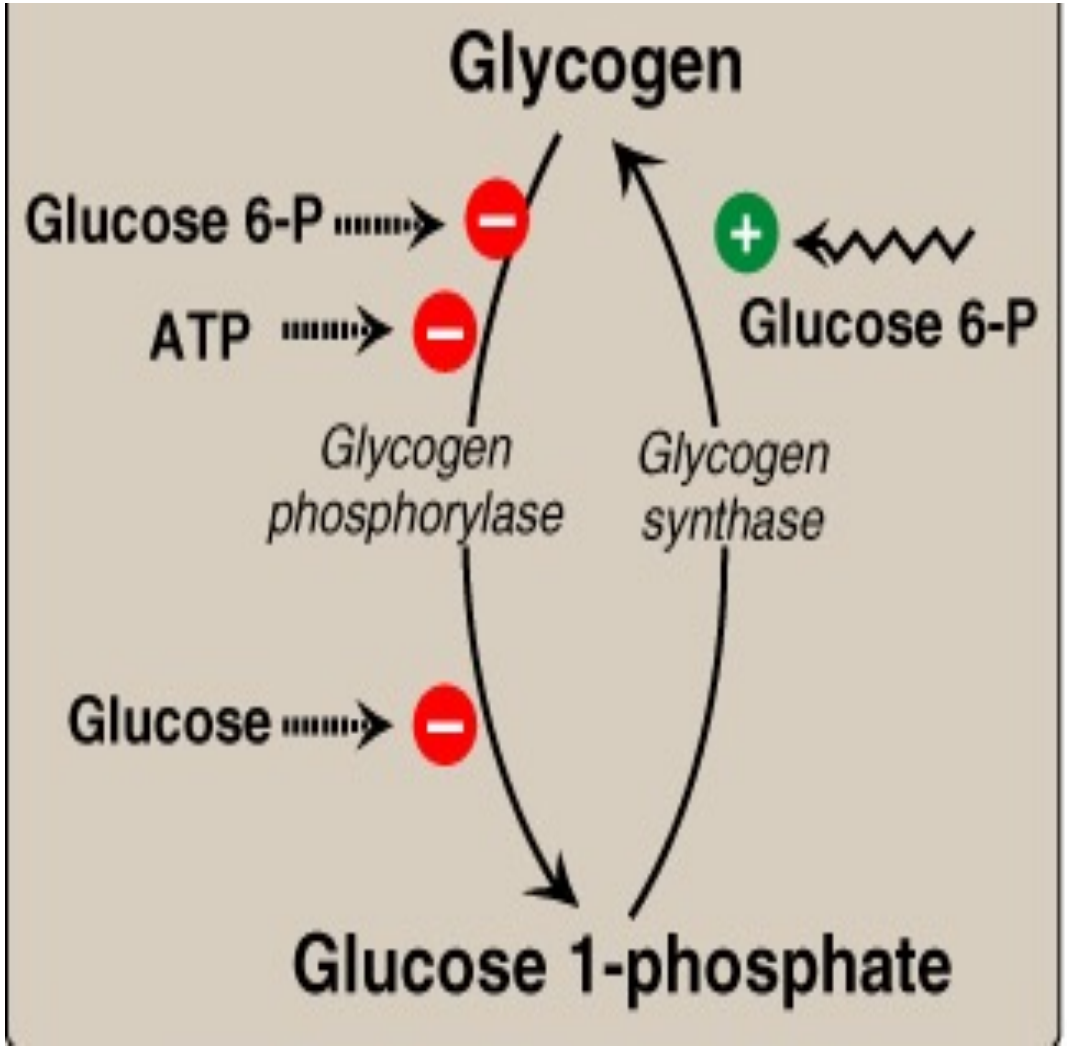
How Insulin Promotes Glycogen Synthesis



Allosteric regulation of glycogen synthesis in liver

Glycogen Synthase is allosterically activated by; **Glc-6-P** (opposite of effect on Phosphorylase)

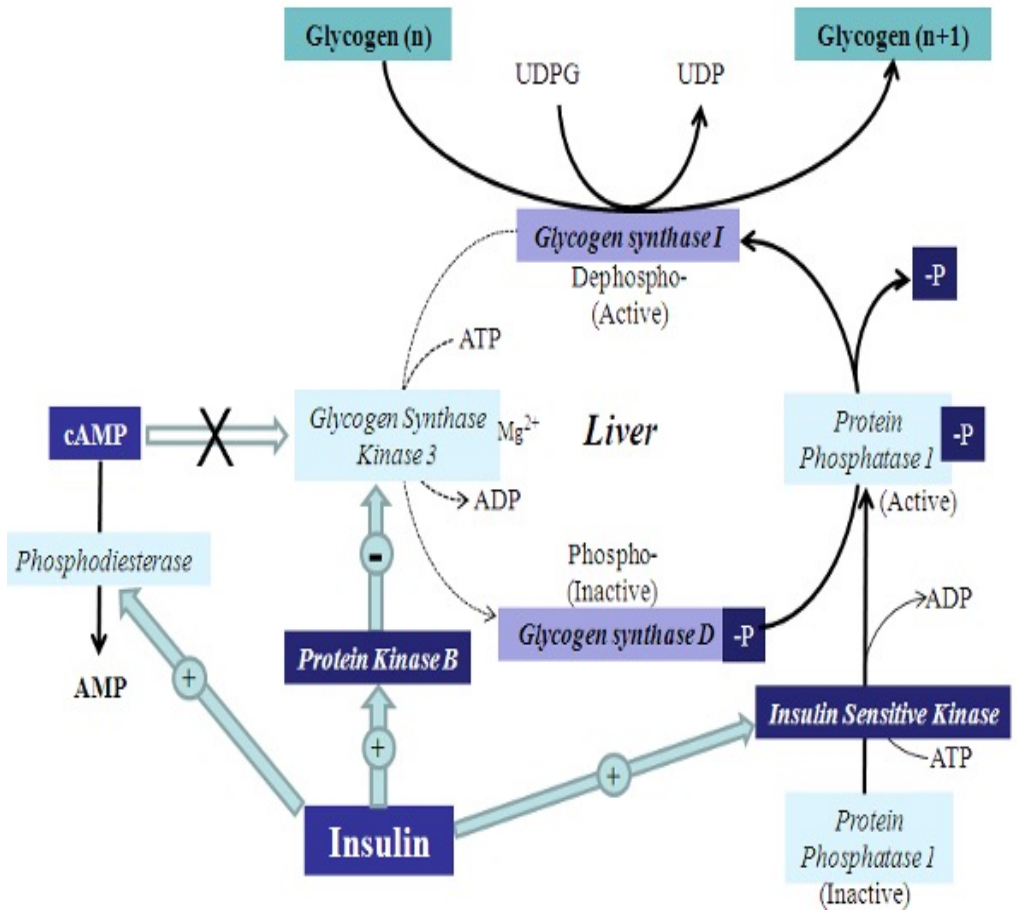
- Thus, **Glycogen Synthase is active** when high blood Glc leads to elevated intracellular Glc-6-P
- **High cytosolic Glc-6-phosphate**; which would result when blood glucose is high, turns off the signal with regard to glycogen synthesis
- The **conformation of Glycogen Synthase** induced by the allosteric activator Glc-6-phosphate is susceptible to dephosphorylation by Protein Phosphatase



Hormonal Regulation of glycogen synthesis

Insulin, produced in response to high blood glucose, triggers a separate signal cascade that leads to activation of **Phosphoprotein Phosphatase-1**

- This phosphatase catalyzes: Removal of regulatory phosphate residues from: **Phosphorylase, Phosphorylase Kinase, & Glycogen Synthase**
- Thus, insulin antagonizes effects of the cAMP cascade induced by glucagon & epinephrine
- Mechanisms of insulin action; Regulatory effects of insulin on: Hepatic & muscle carbohydrate metabolism

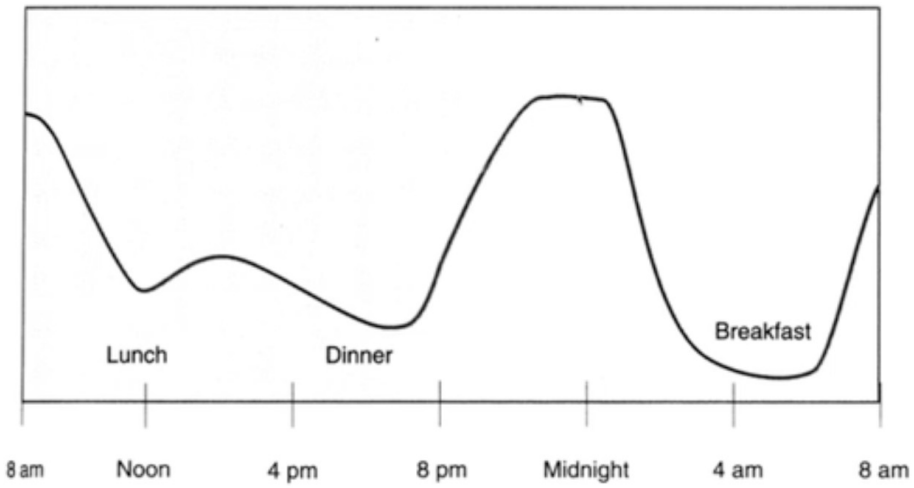


Glycogen breakdown

Glycogenolysis: It is the process of glycogen catabolism (or breakdown) into:

- Glucose $\Rightarrow$ to blood, in the *liver* Or,
- Glucose-6-phosphate $\Rightarrow$ in the *skeletal muscles*

- It is not the reverse of glycogenesis, but it is a separate pathway
- In skeletal muscle and liver, Glucose units of the outer branches of glycogen enter; Glycolytic pathway or to blood through the action of three enzymes:
  - Glycogen phosphorylase
  - Glycogen debranching enzyme
  - Phosphoglucomutase



Pathway of Glycogenolysis

Steps: Sequential removal of terminal glucose residues by **glycogen phosphorylase**

Debranching by bifunctional debranching enzyme

- **Transferase activity &(α1→6) glucosidase activity**

- Once branches are transferred & the glucosyl residue at C-6 is hydrolyzed and Glycogen phosphorylase activity can continue
- Glucose 1-phosphate is converted to glucose 6-phosphate by **Phosphoglucomutase**

Glycogenolysis

Glycogen Phosphorylase catalyzes:

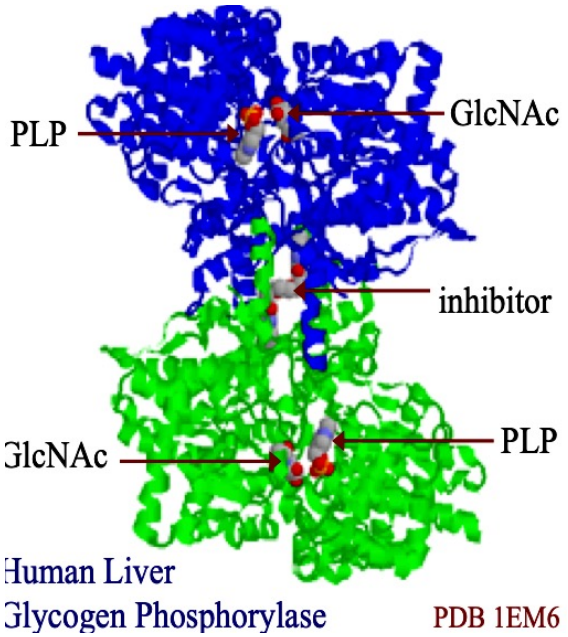
- Phosphorolytic cleavage of the (a1→4) glycosidic linkages of glycogen release glucose-1-phosphate as reaction product

$$\text{Glycogen}_{(n\ \text{residues})} + P_i \rightarrow \text{Glycogen}_{(n-1\ \text{residues})} + \textbf{Glucose-1-phosphate}$$

Glycogen Phosphorylase:

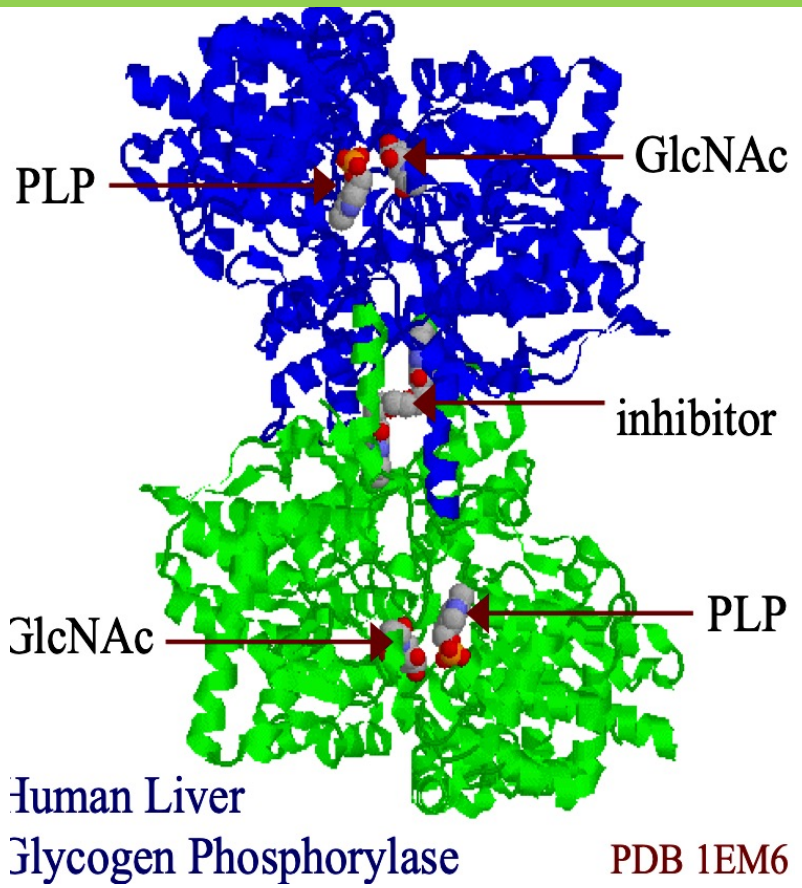
- Homodimeric enzyme
- Subject to allosteric control;
- Its transitions between Relaxed (active) & Tense (inactive) conformations

Pyridoxal phosphate (PLP); a derivative of vitamin B_6, serves as prosthetic group for Glycogen Phosphorylase



Glycogenolysis

- A class of drugs developed for treating the hyperglycemia of diabetes: Chloroindole-carboxamides; Inhibit liver Phosphorylase allosterically
  - These inhibitors bind at the dimer interface stabilizing the inactive (tense) conformation



Why would an inhibitor of Glycogen Phosphorylase be a suitable treatment for diabetes?

Debranching enzymes

Bifunctional enzyme known as oligo (α1→4) to (α1→6) glucantransferase

Has 2 independent active sites

- **Transferase activity:**
  - Transfers 3 Glc residues from a 4-residue limit branch to the end of another branch
  - Diminishing the limit branch to a single Glc residue
- **(a1→6) Glucosidase activity:**
  - Catalyzes hydrolysis of the (a1→6) linkage, yielding free glucose

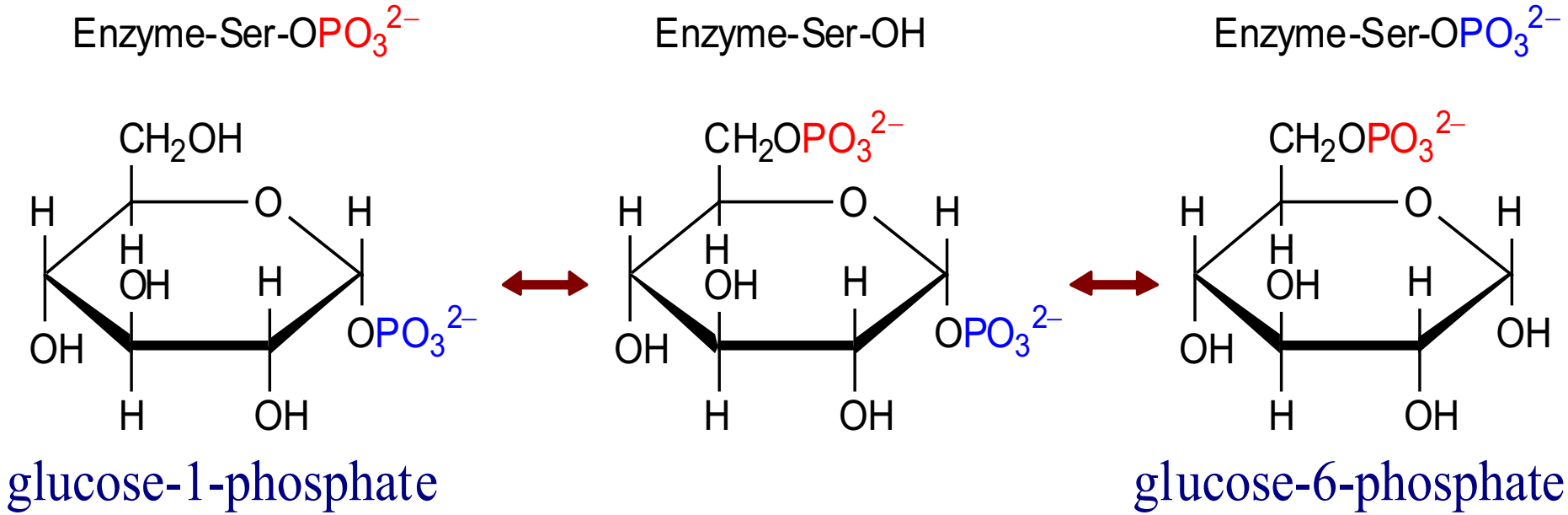
The major product of glycogen breakdown is **Glucose-1-phosphate**, from Phosphorylase activity

Glycogenolysis

Phosphoglucomutase catalyzes the reversible rxn:

Glc-1-phosphate ←→ Glc-6-phosphate

- A serine OH at the active site donates & accepts P_i.
- The bisphosphate is not released.



Glycogenolysis

Glucose-6-phosphate:

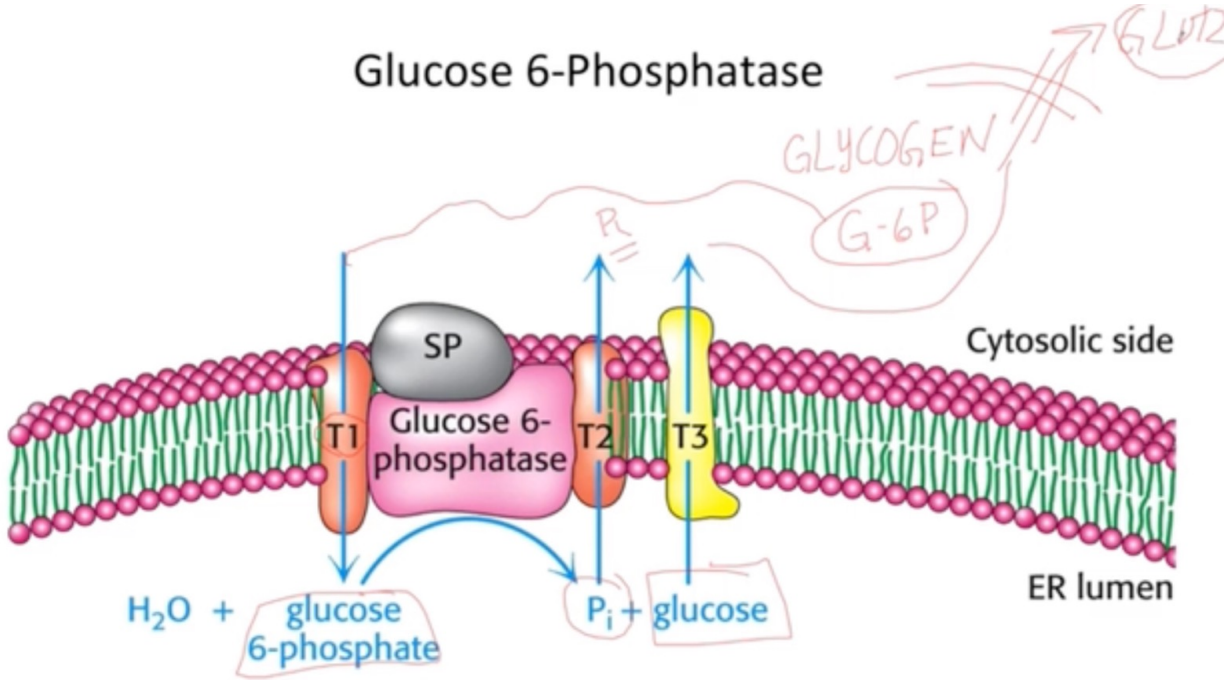
- May enter Glycolysis or
- Mainly in liver be dephosphorylated for release to the blood

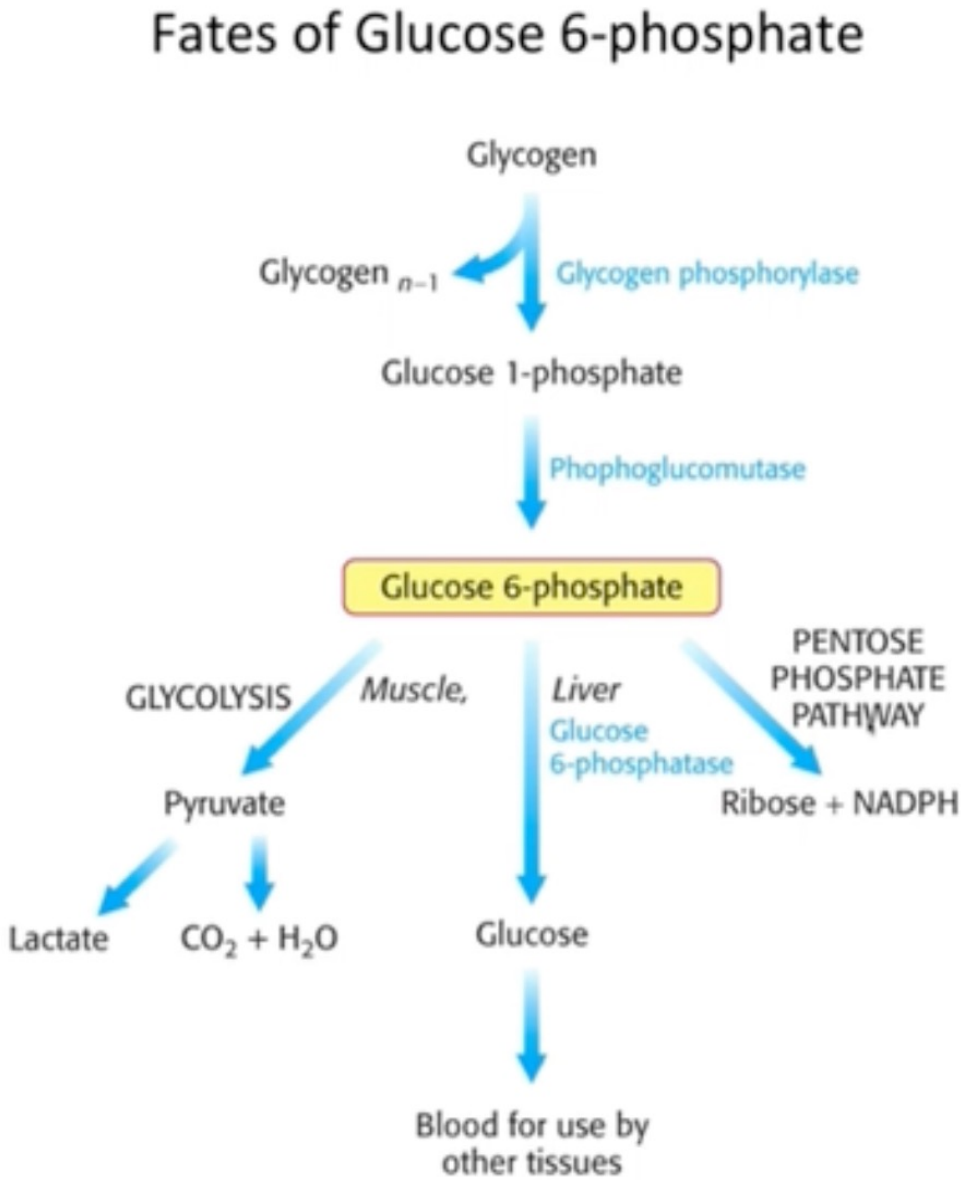
Liver Glucose-6-phosphatase catalyzes the following,

Glc-6-phosphate + H_2O → Glucose + P_i

Essential to the liver's role in maintaining blood glucose:

Most other tissues lack this enzyme





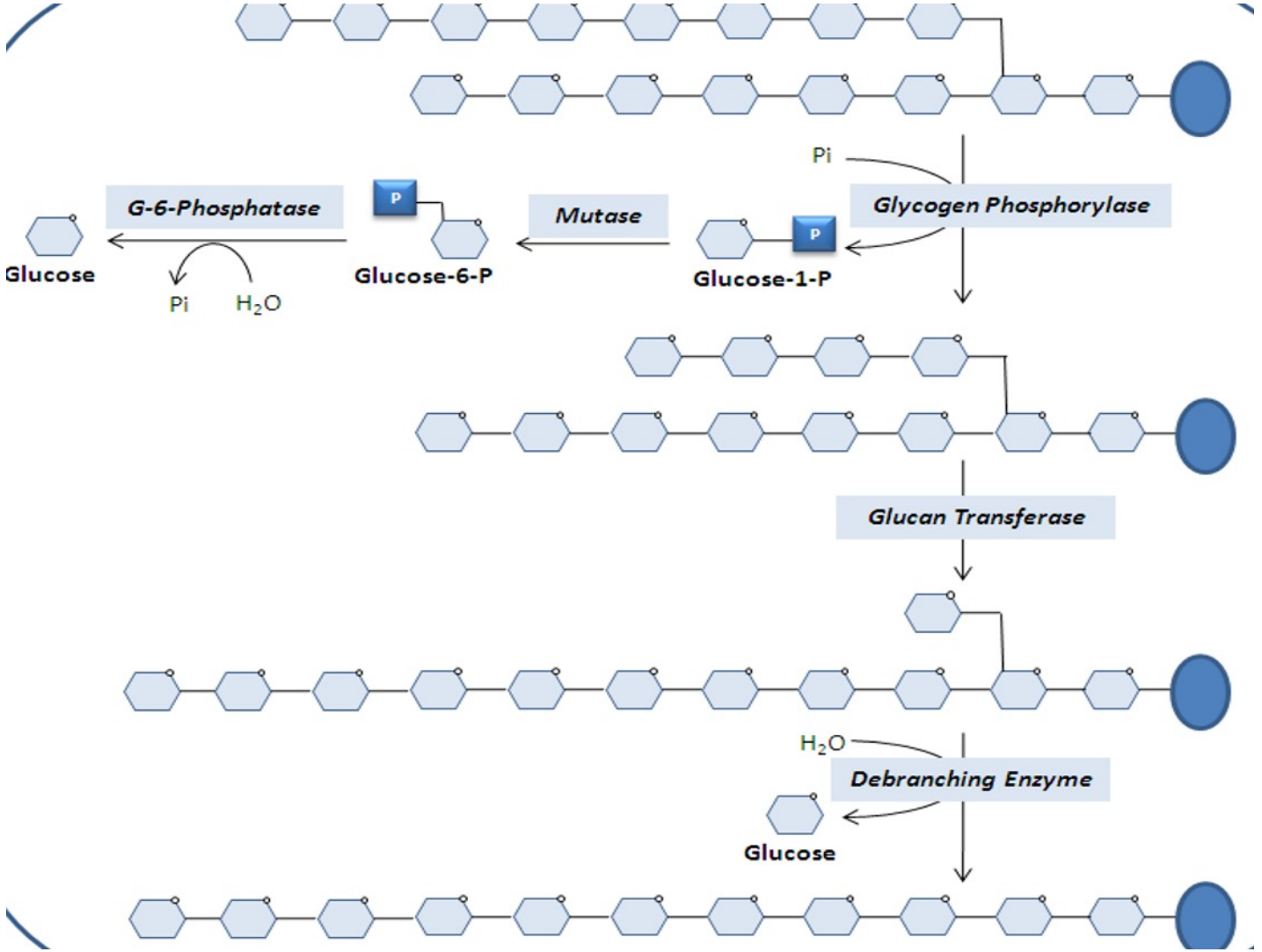
Steps in glycogenolysis

A glycogen storage site

- On the surface of the Phosphorylase enzyme
- Binds the glycogen particle

Given the distance between storage & active sites,

- Phosphorylase can cleave a(1→4) linkages only to within 4 residues of an a(1→6) branch point (limit branch)



Regulation of Glycogenolysis & Glycogenesis

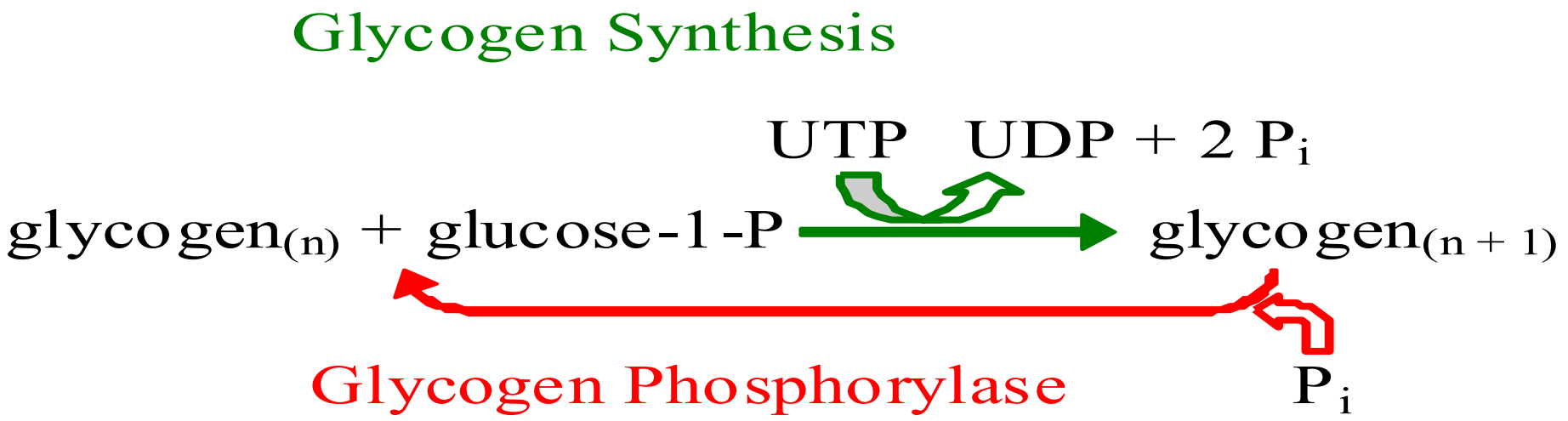
The principal enzymes controlling glycogen metabolism: **Glycogen phosphorylase & Glycogen synthase**

Regulated by:

- Allosteric mechanisms
- Covalent modifications due to its reversible nature : Phosphorylation & dephosphorylation of enzyme protein In response to hormone action

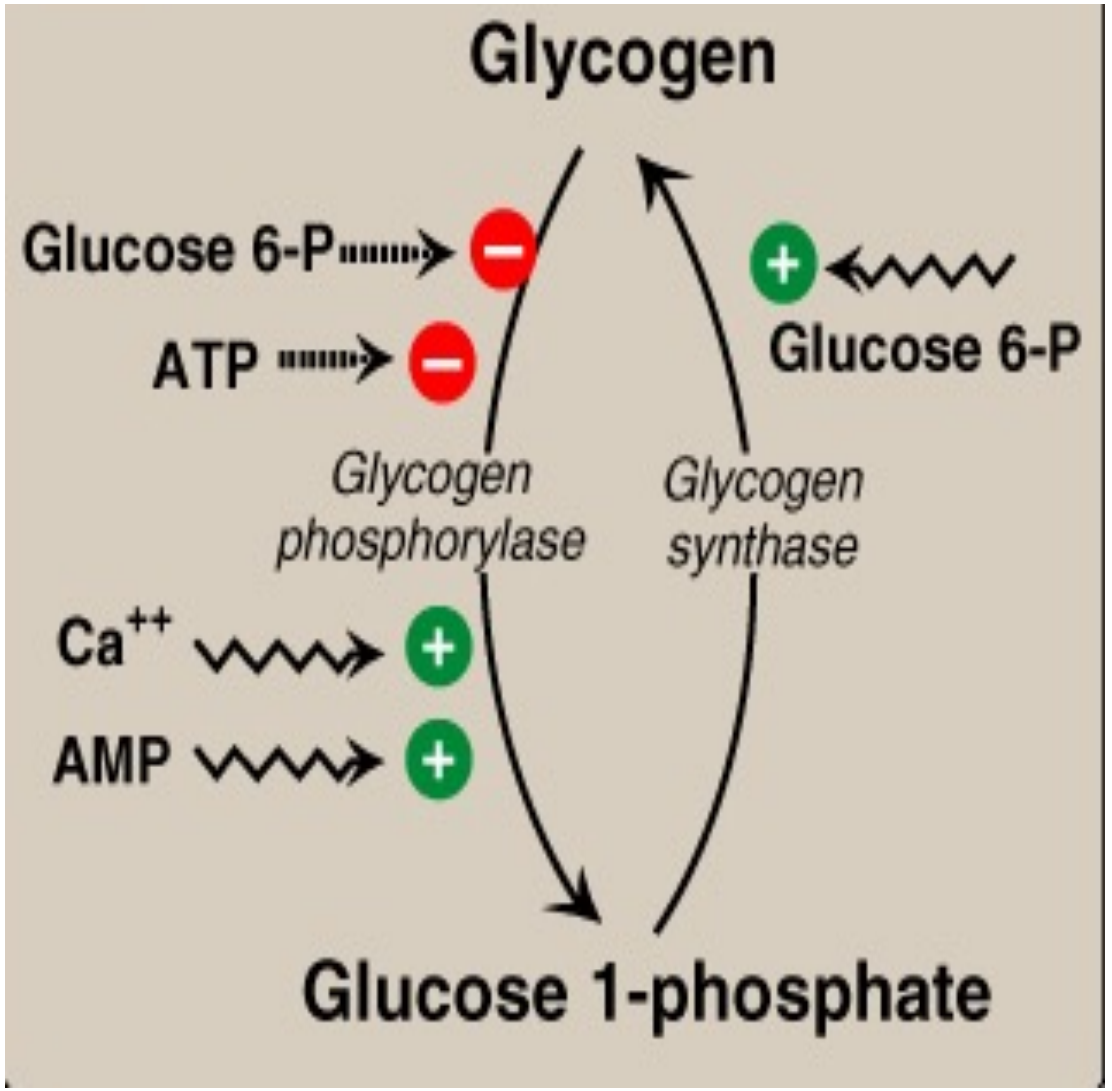
Regulation of Glycogenolysis & Glycogenesis

- If both pathways were active simultaneously in a cell, there would be a
  - Futile Cycle
  - With cleavage of one ~P bond per cycle (in forming UDP-glucose).
- To prevent such a futile cycle, **Glycogen Synthase and Glycogen Phosphorylase** are ,**Reciprocally regulated by: Allosteric effectors & Phosphorylation**

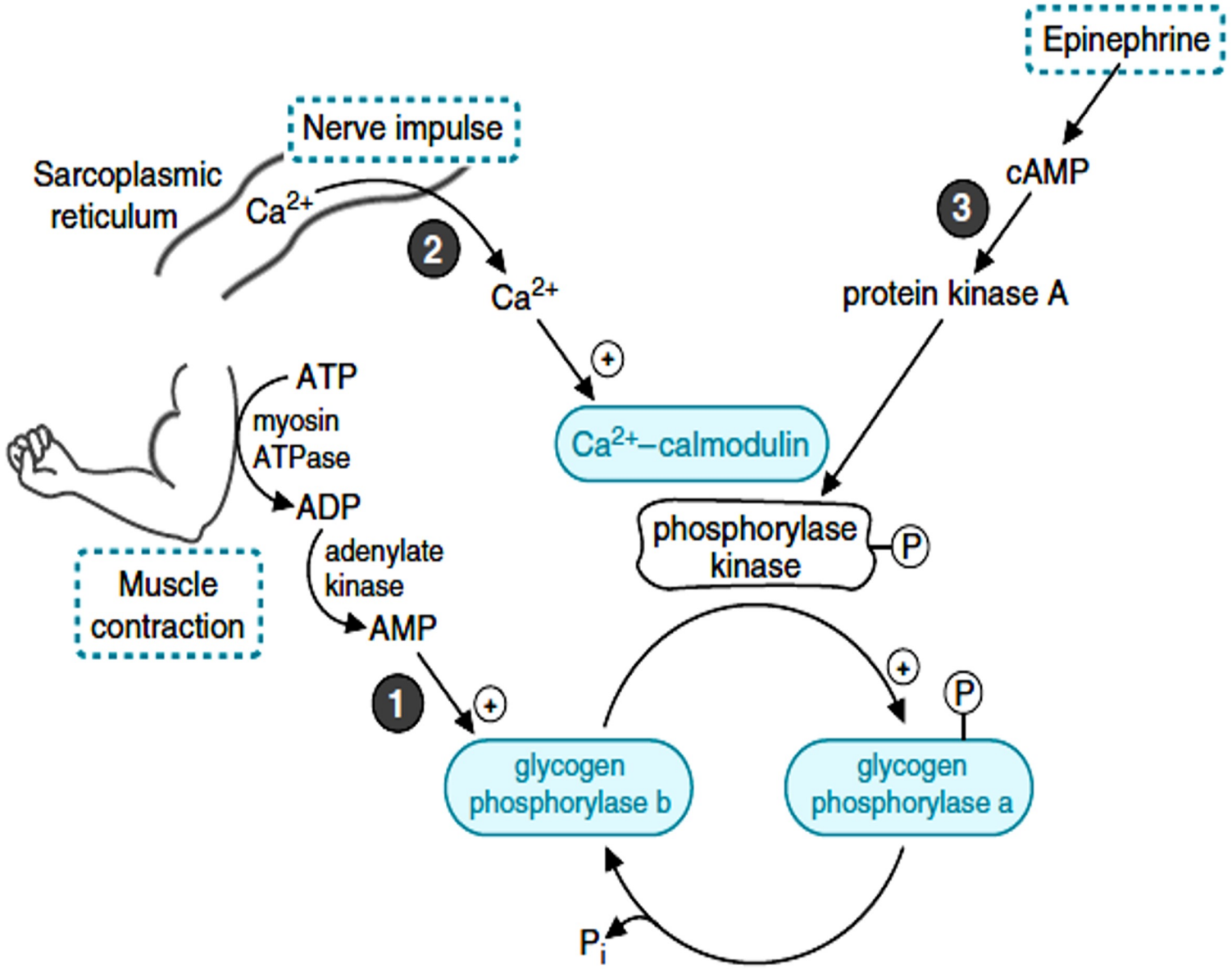


Allosteric regulation of glycogen breaks down in muscle

- **Glycogen Phosphorylase** in **muscle** is subject to allosteric regulation by **AMP, ATP**, & **Glc-6-phosphate**
- A separate **isozyme of Phosphorylase expressed** in **liver** is **Less sensitive** to these allosteric controls
  - **AMP activates Phosphorylase, Promoting the relaxed conformation**
  - **ATP & glucose-6-phosphate,** which both have **binding sites that overlap with that of AMP**, **inhibit Phosphorylase, Promoting the tense conformation**
- **Thus, glycogen breakdown is inhibited when ATP & Glc-6-phosphate** are **plentiful**



Activation of muscle glycogen phosphorylase during exercise

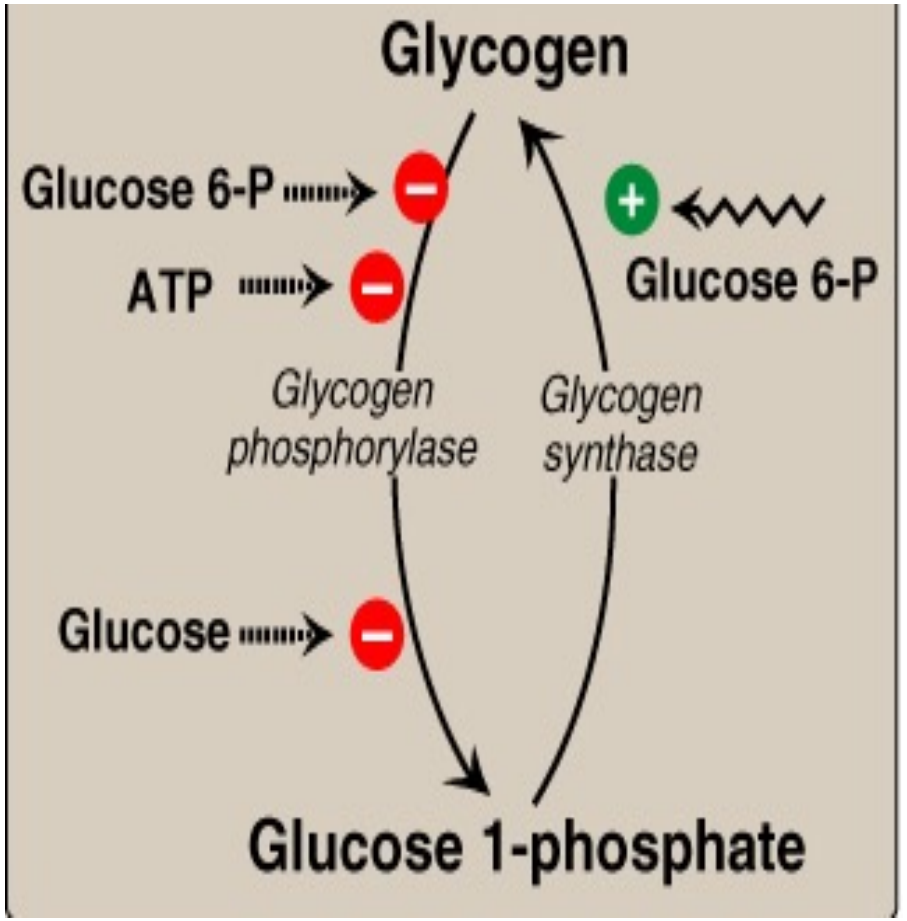


Ca^{++} regulates glycogen breakdown in muscle: during activation of contraction in skeletal muscle,

- Ca^{++} is released from the sarcoplasmic reticulum to cytosol to promote actin/myosin interactions
  - The released Ca^{++} also activates Phosphorylase Kinase, which in muscle includes calmodulin as its δ–subunit
  - Phosphorylase Kinase is partly activated by binding of Ca^{++} to this subunit

Allosteric regulation of glycogen breaks down in liver

- Glycogen Synthase is allosterically activated by; Glc-6-P (opposite of effect on Phosphorylase)
- Thus, Glycogen Synthase is active when high blood Glc leads to elevated intracellular Glc-6-P
- High cytosolic Glc-6-phosphate; which would result when blood glucose is high, urns off the signal with regard to glycogen synthesis
- The conformation of Glycogen Synthas induced by the allosteric activator Glc-6- phosphate is susceptible to dephosphorylation by Protein Phosphatase



Glucagon Signaling

- **Primary target sites:**
  - **Liver** – Purpose to release glucose into the bloodstream
    - Downregulates **Glycogenesis**
    - Downregulates ***Glycolysis*** (breakdown of glucose/hexoses to make energy)
    - Upregulates Glycogenolysis (breakdown of glycogen into glucose monomers)
    - Upregulates ***Gluconeogenesis*** (biosynthesis of glucose from organic precursors)
    - Upregulates ***Lipolysis*** and ***Ketogenesis***

Glucagon Signaling

In skeletal muscles glycogen: a reservoir of glucosyl units for the generation of ATP from glycolysis & glucose oxidation

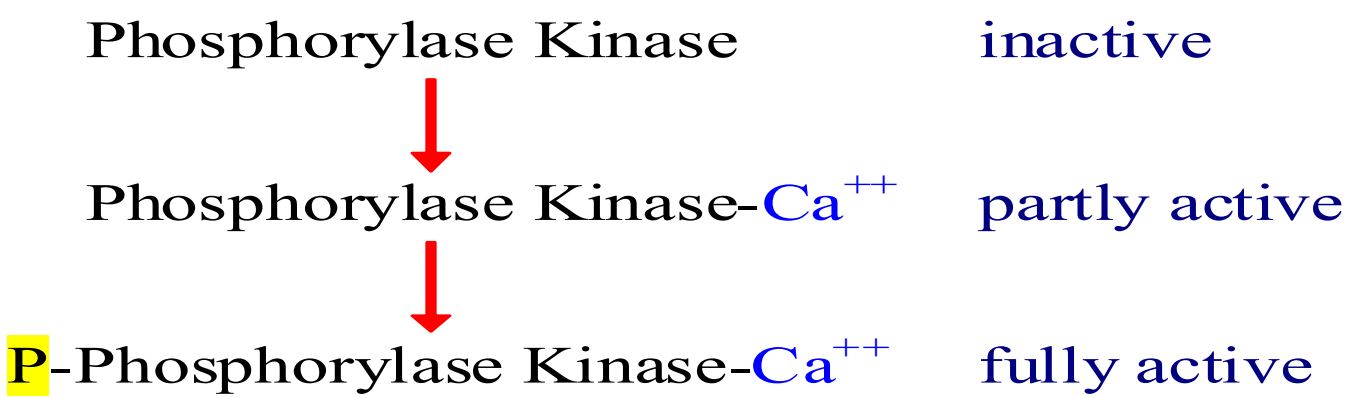
- Muscle glycogenolysis is regulated principally by;
  - AMP which signals a lack of ATP
  - Ca^{2+} released during contraction
  - Epinephrine which is released in response to exercise & other stress situations
- Epinephrine through the β-adrenergic receptor (cAMP-mediated),providing a supply of carbohydrate for the energy needs of muscle
- This occurs not only during 'fight or flight' situations, but also during prolonged exercise

There are also two important hormone-independent mechanisms: for activation of glycogenolysis in muscle

Glucagon Signaling

- First, the influx of Ca^{2+} into the muscle cytoplasm; in response to nerve stimulation
    - Activates the basal, unphosphorylated form of phosphorylase kinase
    - By action of the Ca^{2+}-calmodulin complex
- A second mechanism for activation of muscle glycogenolysis involves; direct allosteric activation of phosphorylase by AMP

Activation of muscle glycogen phosphorylase during exercise



Glucagon/Epinephrine Signaling

Regulation by covalent modification (phosphorylation): the hormones Glucagon & Epinephrine

- Activate G-protein coupled receptors to trigger cAMP cascades
- Both hormones are produced in response to low blood Glc

Glucagon: is synthesized by α-cells of the pancreas,

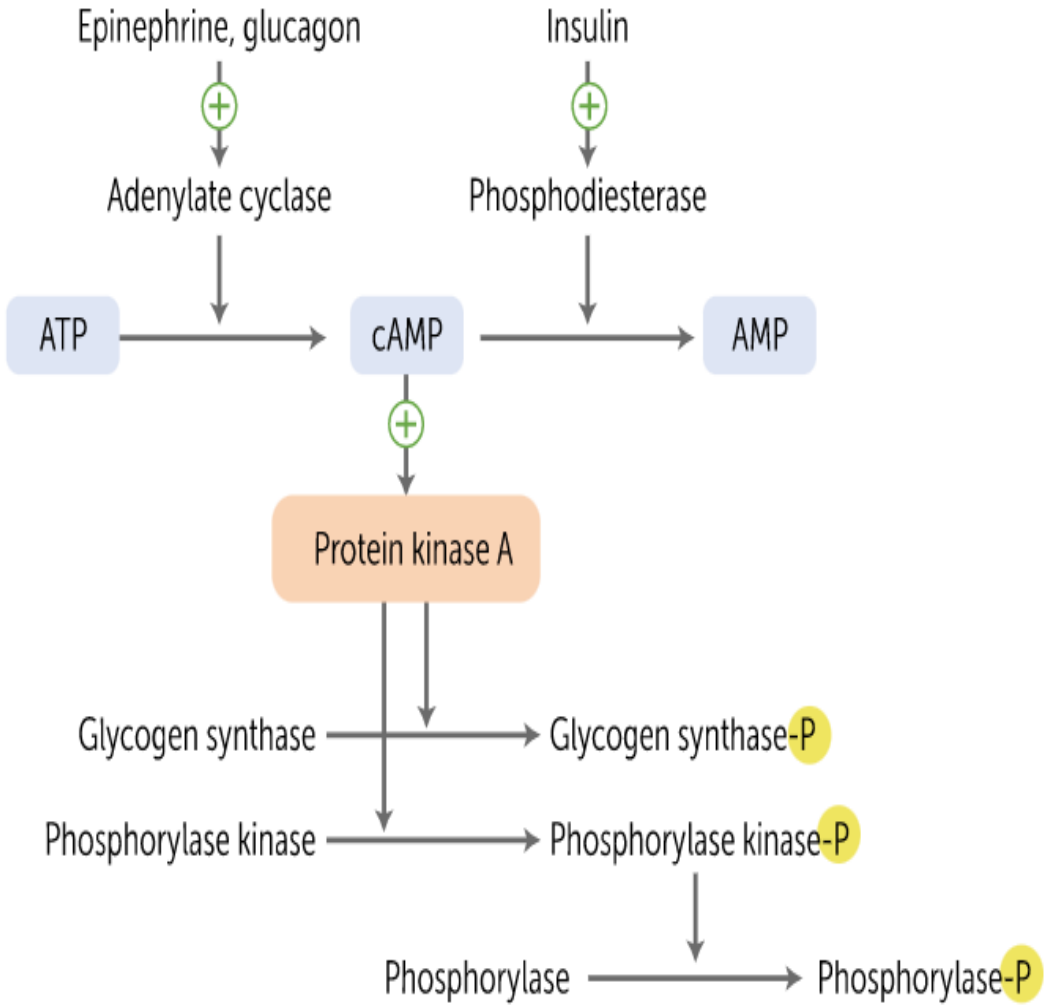
- **Activates cAMP formation in liver**

Epinephrine:

- **Activates cAMP formation in muscle**

Cyclic AMP (cAMP):

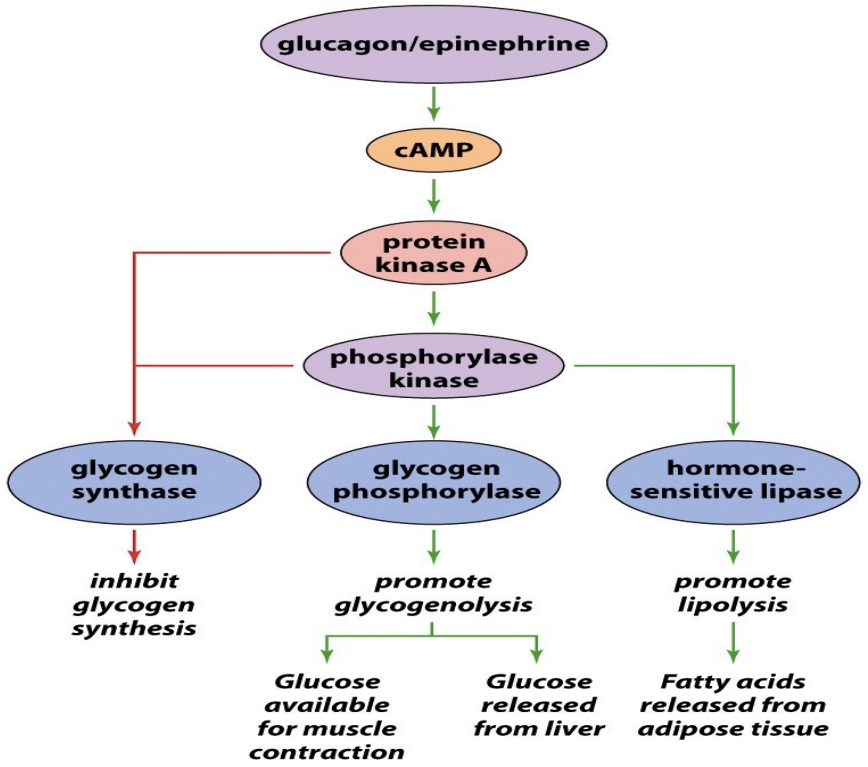
- Is formed from ATP by adenylyl cyclase at the inner surface of cell membranes
- Acts as an intracellular 2nd messenger in response to hormones; Epinephrine & glucagon
- Hydrolyzed by phosphodiesterase, so terminating hormone action; in liver insulin increases the activity of phosphodiesterase



Glucagon/Epinepherine Signaling

The cAMP cascade induced in liver by glucagon or epinephrine and acts as the opposite effect on glycogen synthesis

- Glycogen synthase is phosphorylated by:
  - Protein Kinase A
  - Phosphorylase Kinase
- Phosphorylation of Glycogen Synthase promotes
  - The “b” (less active) conformation
- **The cAMP cascade thus inhibits glycogen synthesis**
- Instead of being converted to glycogen, Glc-1-P in liver may be: Converted to Glc-6-P, & Dephosphorylated for release to the blood



Hormones involved in control of Glycogenolysis

Hormone	Source	Initiator	Effect on Glycogenolysis
Glucagon	pancreatic α-cells	Hypoglycemia	Rapid activation
Epinephrine	adrenal medulla	Acute stress, Hypoglycemia	Rapid activation
Cortisol	adrenal cortex	Chronic stress	Chronic activation
Insulin	pancreatic β-cells	Hyperglycemia	Inhibition

Regulation of Liver Glycogen Stores

State	Regulators	Response of Tissue
Fasting	Blood: Glucagon ↑ Insulin ↓ Tissue: cAMP ↑	Glycogen degradation ↑ Glycogen synthesis ↓
Carbohydrate meal	Blood: Glucagon ↓ Insulin ↑ Glucose ↑ Tissue: cAMP ↓ Glucose ↑	Glycogen degradation ↓ Glycogen synthesis ↑
Exercise and stress	Blood: Epinephrine ↑ Tissue: cAMP ↑ Ca2+ -calmodulin ↑	Glycogen degradation ↑ Glycogen synthesis ↓

Regulation of Muscle Glycogen Stores

State	Regulators	Response of Tissue
Fasting (rest)	Blood: Insulin ↓	Glycogen synthesis ↓ Glucose transport ↓
Carbohydrate meal (rest)	Blood: Insulin ↑	Glycogen synthesis ↑ Glucose transport ↑
Exercise	Blood: Epinephrine ↑ Tissue: AMP ↑ Ca^{2+} -calmodulin ↑ cAMP ↑	Glycogen synthesis ↓ Glycogen degradation ↑ Glycolysis ↑

Thankyou