بسم الله الرحمـٰن الرحيم

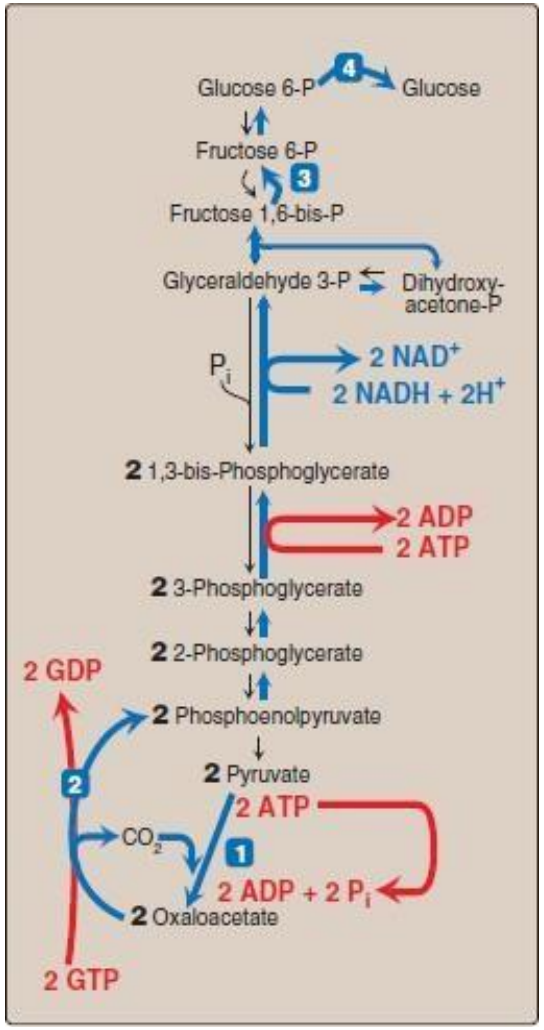
(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Lecture 14

Gluconeogenesis pt. 2 & glycogen metabolism

Written & Reviewed by : Rawan Okour

Energy requirements of gluconeogenesis



When does gluconeogenesis occur?

Gluconeogenesis takes place during **fasting**, especially long-term fasting. Its purpose is to provide a **continuous supply of glucose** when dietary glucose and glycogen stores are depleted.

- **Why is it important?**

This pathway is **urgent and essential**, not a luxury.

Your body activates it **only when it MUST produce glucose**, mainly for tissues that rely **exclusively** on glucose, such as:

- **Brain**
- **Adrenal medulla**
- **RBCs (indirectly, through lactate recycling)**

Fatty acids can generate large amounts of energy for many tissues, so the body **does not waste energy** producing glucose for cells that **do not require it**.

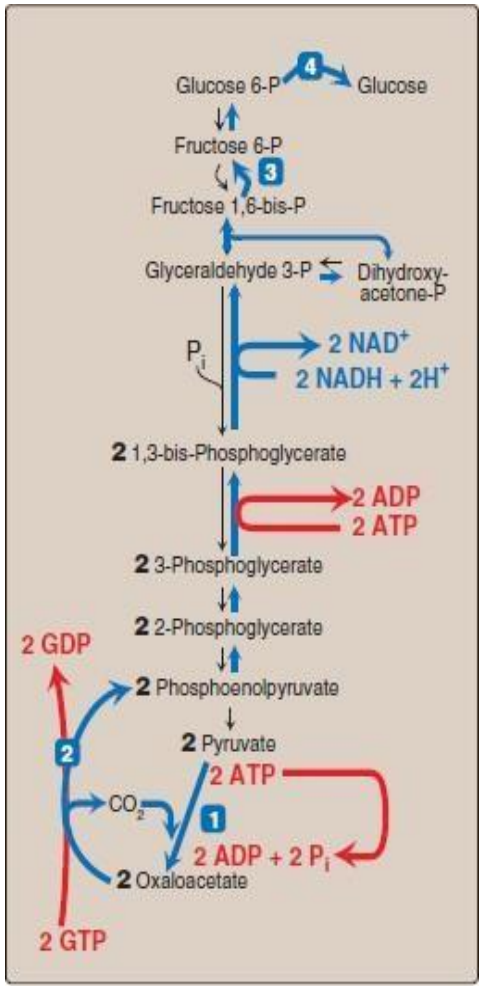
- **Energy use vs glycolysis**

Glycolysis **produces** ATP.

Gluconeogenesis **consumes** ATP, even though it occurs during fasting.

This is because the body is trying to **build up glucose**, not break it down.

Energy requirements of gluconeogenesis



How much energy is needed to make ONE glucose molecule?

To form **1 molecule of glucose**, gluconeogenesis requires:

- **2 ATP** → for pyruvate → oxaloacetate
- **2 GTP** → for oxaloacetate → PEP (repeated twice)
- **2 ATP** → later steps (3-phosphoglycerate → 1,3-bisphosphoglycerate)

➡ **Total energy consumed = 6 high-energy molecules**

(4 ATP + 2 GTP, and GTP is energetically equivalent to ATP

Step-by-step clarification:

- Converting **pyruvate → oxaloacetate** consumes **2 ATP**.
- Converting **oxaloacetate → PEP** consumes **2 GTP**.
- Several steps in the middle (PEP → 2-phosphoglycerate → 3-phosphoglycerate) do **not** require energy in either direction.
- Conversion **3-phosphoglycerate → 1,3-bisphosphoglycerate** consumes ATP (opposite of glycolysis).
- Conversion **1,3-BPG → glyceraldehyde-3-P** uses **2 NADH** (repeated twice).
- Isomerization and aldolase steps do **not** require energy.
- **Fructose 1,6-bisphosphate → fructose 6-phosphate** releases an inorganic phosphate, but **not enough energy** to form ATP.
- **Glucose-6-phosphate → glucose** also releases phosphate but **does not generate ATP**.

➡ **Final energy cost to produce one glucose:**

6 ATP equivalents (4 ATP + 2 GTP)

Energy conservation in fasting

Because this pathway runs during **fasting**, the body is selective:

It produces glucose **only for essential tissues** to preserve the limited energy reserves.

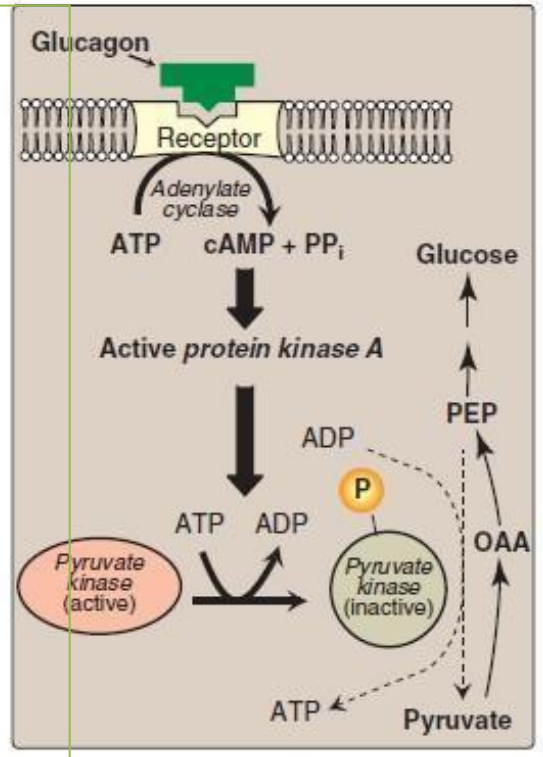
Regulation of gluconeogenesis

- Mainly by:

1. The circulating level of glucagon

- Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bisphosphatase and inhibition of PFK-1
- Inhibition of pyruvate kinase
- Glucagon increases the transcription of the gene for PEP-carboxykinase

2. The availability of gluconeogenic substrates



3.Slow adaptive changes in enzyme activity due to an alteration the rate of enzyme synthesis or degradation, or both

Gluconeogenesis is regulated at several levels:

1. Hormonal Regulation (Mainly by Glucagon)

During fasting, **glucagon is high**.

It activates:

•**Adenylate cyclase → cAMP → Protein Kinase A (PKA)**

•PKA **phosphorylates** key enzymes.

Effects:

•**Pyruvate kinase** is phosphorylated → **inhibited**

→ This stops glycolysis and allows gluconeogenesis to proceed.

•The **bifunctional enzyme (PFK-2/FBP-2)** is phosphorylated →

- **PFK-2 OFF → ↓ fructose 2,6-bisphosphate**
- ↓ F-2,6-BP **inhibits glycolysis** and **activates gluconeogenesis**.

•Glucagon increases the **gene expression** of several enzymes, especially:

- **PEP carboxykinase (PEPCK)**
  ➔ Increased expression enhances the rate of gluconeogenesis.

2. Substrate Availability

Gluconeogenesis depends heavily on substrates delivered from tissues:

•Amino acids from **muscle**

•Lactate from **RBCs and muscles**

•Glycerol from **adipose tissue**

More substrates = faster gluconeogenesis.

3. Allosteric Regulation

Enzymes in gluconeogenesis can be activated or inhibited by **allosteric molecules**, which bind and change enzyme activity.

Examples:

- **Acetyl-CoA activates pyruvate carboxylase**
- **AMP inhibits fructose-1,6-bisphosphatase**

4. Enzyme Concentration

The level of an enzyme depends on:

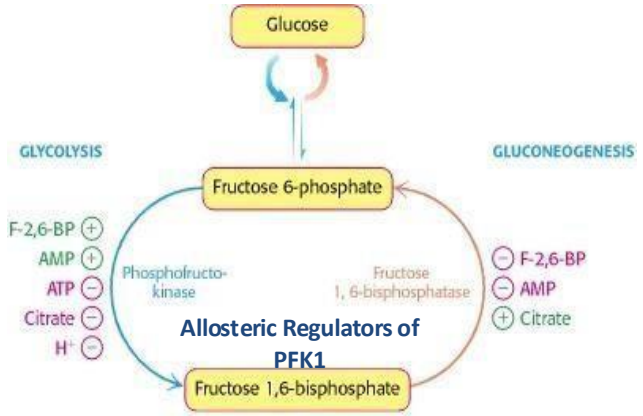
- **Gene expression**
- **mRNA stability**
- **Protein degradation mechanisms**
- **Enzyme half-life**

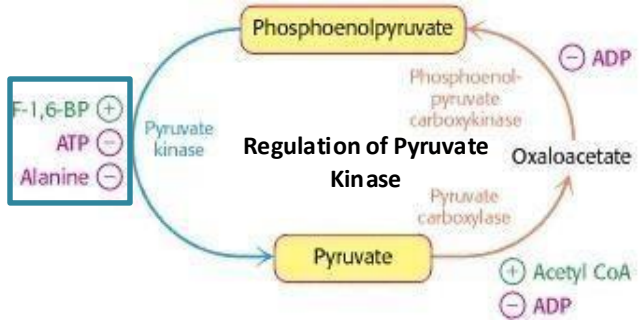
A higher concentration of enzymes → higher gluconeogenesis rate.

Regulation includes:

- mRNA degradation by **microRNAs**
- Increased **proteasomal degradation**
- Mutations that prolong protein half-life (dangerous → can lead to cancer)
- Drugs may be needed to inhibit **overactive or long-lived proteins**

Regulation of gluconeogenesis





Key Regulators of Glycolysis

A. Phosphofructokinase-1 (PFK-1)

•Stimulated by:

- **Fructose-2,6-bisphosphate (F-2,6-BP)**
- **AMP**

•Inhibited by:

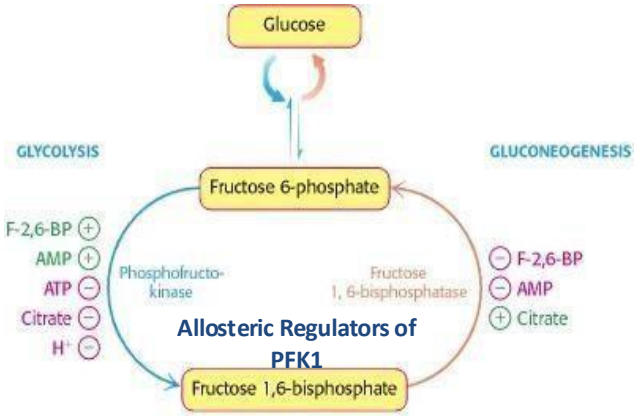
- **ATP** (high energy state)
- **Citrate** (signals high energy and abundant biosynthetic intermediates)

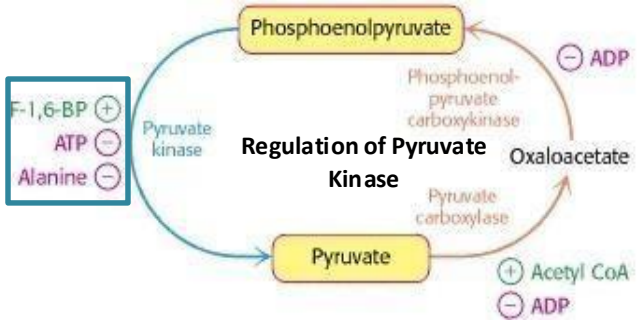
Concept:

•High **F-2,6-BP** = fed state → **activate glycolysis**

•High **ATP or citrate** = high energy → **inhibit glycolysis**

Regulation of gluconeogenesis





2. Key Regulators of Gluconeogenesis

A. Fructose-1,6-bisphosphatase (FBPase-1)

•Inhibited by:

- **Fructose-2,6-bisphosphate**
- **AMP**

•Activated by:

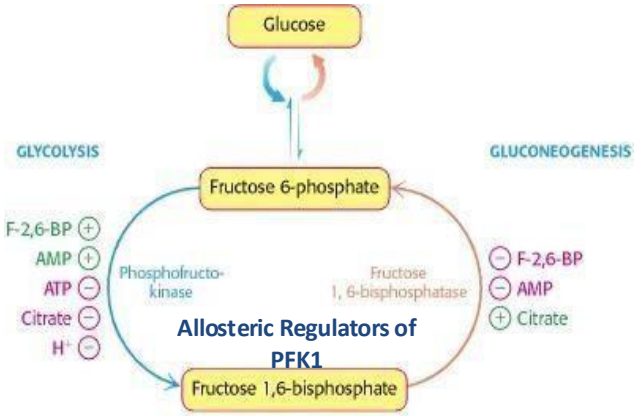
- **High ATP**
- **High citrate**

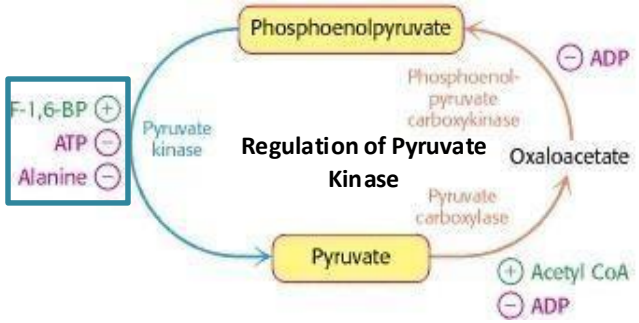
Concept:

•High **F-2,6-BP** = we are *not* fasting → **gluconeogenesis OFF**

•High **citrate/ATP** = high energy → **gluconeogenesis ON**

Regulation of gluconeogenesis





3. Regulation of Pyruvate ↔ PEP

This step is **irreversible** in glycolysis but occurs in **two steps** in gluconeogenesis:

A. Glycolysis (one-step)

•**Pyruvate → Acetyl-CoA** (via pyruvate dehydrogenase) – not reversible.

B. Gluconeogenesis (two-step)

1.Pyruvate → Oxaloacetate (OAA)

1. Enzyme: **Pyruvate carboxylase**
2. **Activated by acetyl-CoA**
    1. (Comes from **fatty acid oxidation** → indicates **fasting** and **high energy**)

2.Oxaloacetate → Phosphoenolpyruvate (PEP)

1. Enzyme: **PEP carboxykinase**

Why acetyl-CoA activates pyruvate carboxylase

•During **fasting**, fatty acids are oxidized → produce **acetyl-CoA**.

•High acetyl-CoA = plenty of energy available → **turn on gluconeogenesis.**

ATP effect

•**High ATP** = high energy → support gluconeogenesis

•**Low ATP (high ADP/AMP)** = low energy →

- **inhibit pyruvate carboxylase**
- **inhibit PEP carboxykinase**
- Because gluconeogenesis is energy-consuming, it cannot proceed in low energy.

Regulator	High Level Means	Effect on Glycolysis	Effect on Gluconeogenesis
Fructose-2,6-bisphosphate	Not fasting, enough energy	**Activates**	**Inhibits**
Citrate	High energy	**Inhibits**	**Activates**
AMP (low ATP)	No energy	**Activates**	**Inhibits**
ATP (high)	High energy	**Inhibits**	**Activates**
Acetyl-CoA	Fasting → fatty acid oxidation	—	**Activates pyruvate carboxylase**

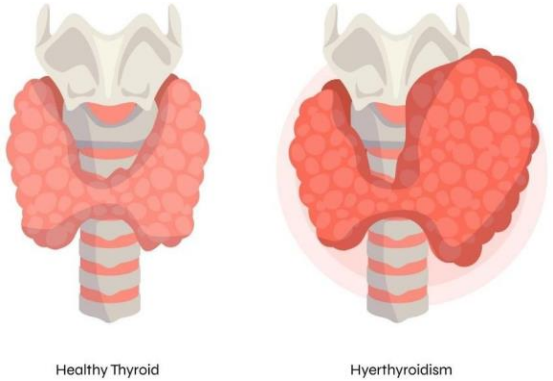
Summary

F-2,6-BP → activates **glycolysis**, inhibits **gluconeogenesis**.

- **ATP & citrate high** → inhibit **glycolysis**, activate **gluconeogenesis**.
- **Acetyl-CoA** activates **pyruvate carboxylase** (fasting signal).
- **Low ATP** stops gluconeogenesis because it needs energy.

Application: Gluconeogenesis and diseases, hyperthyroidism as an example

- ✓Hyperthyroidism is a hypermetabolic state
- ✓Associated with increased rates of gluconeogenesis and glycogenolysis.
- ✓Thyroid hormone, specifically the biologically active triiodothyronine (T_3), binds to its nuclear receptor and enhances *PEPCK* and *G6PC* expression



- ✓Excess thyroid hormone stimulates proteolysis and lipolysis to promote substrate delivery to the liver

Hyperthyroidism as an Application of Gluconeogenesis

Hyperthyroidism is considered one of the most common endocrine disorders, affecting around 13% of the population. It represents a *hyper-metabolic* state in which all metabolic pathways become accelerated. Individuals with hyperthyroidism typically present with **low body weight**, **increased appetite**, **heat intolerance**, **sweating**, **high energy consumption**, and **reduced sleep**, because the thyroid gland becomes overactivated and secretes excessive amounts of thyroid hormones.

1. Thyroid Hormones (T_3 and T_4)

The thyroid gland produces two hormones:

- **T_4 (thyroxine)** – the major form secreted
- **T_3 (triiodothyronine)** – the biologically active form

T_4 can be converted into T_3 in peripheral tissues.

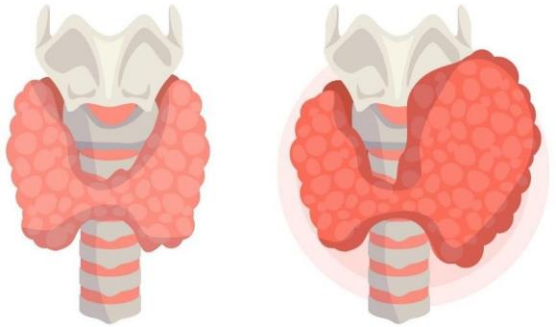
T_3 is the hormone that binds the **nuclear receptor** inside the cell and exerts metabolic effects.

2. Effect of Hyperthyroidism on Metabolism

When thyroid hormone levels are high:

- Overall metabolism increases (hypermetabolic state).
- Energy consumption rises significantly.
- The body shifts toward using stored fuels.
- Glycogen stores become depleted quickly.
- The body increases **gluconeogenesis** and **glycogenolysis**.

This explains why, despite increased appetite, patients often continue losing weight.



3. Mechanism: How T3 Stimulates Gluconeogenesis

T3 enters the cell → enters the nucleus → binds its nuclear receptor.
This receptor-hormone complex activates transcription of key gluconeogenic enzymes:

- **PEPCK** (phosphoenolpyruvate carboxykinase)
- **G6PC** (glucose-6-phosphatase)

Upregulating these enzymes increases hepatic glucose output through gluconeogenesis.

4. Effect on Fuel Mobilization

Because the body is in a hypermetabolic state, thyroid hormone promotes:

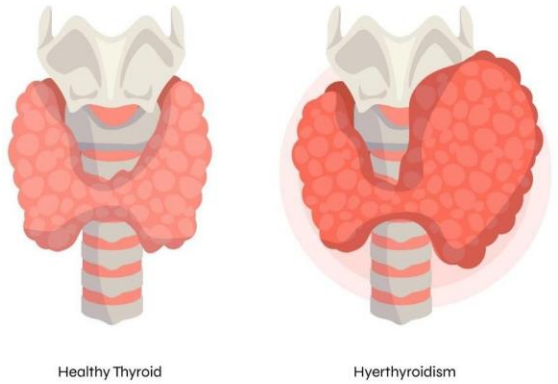
- **Proteolysis** → providing amino acids for gluconeogenesis
- **Lipolysis** → providing glycerol and fatty acids
- **Glycogen breakdown** → releasing glucose rapidly

This ensures a continuous supply of substrates to the liver.

5. Clinical Picture

People with hyperthyroidism commonly show:

- Low weight despite increased food intake
- Nervousness and irritability
- Heat intolerance
- Excess sweating
- High metabolic rate
- Increased glucose production
- Muscle wasting (due to proteolysis)
- Fat loss (due to lipolysis)

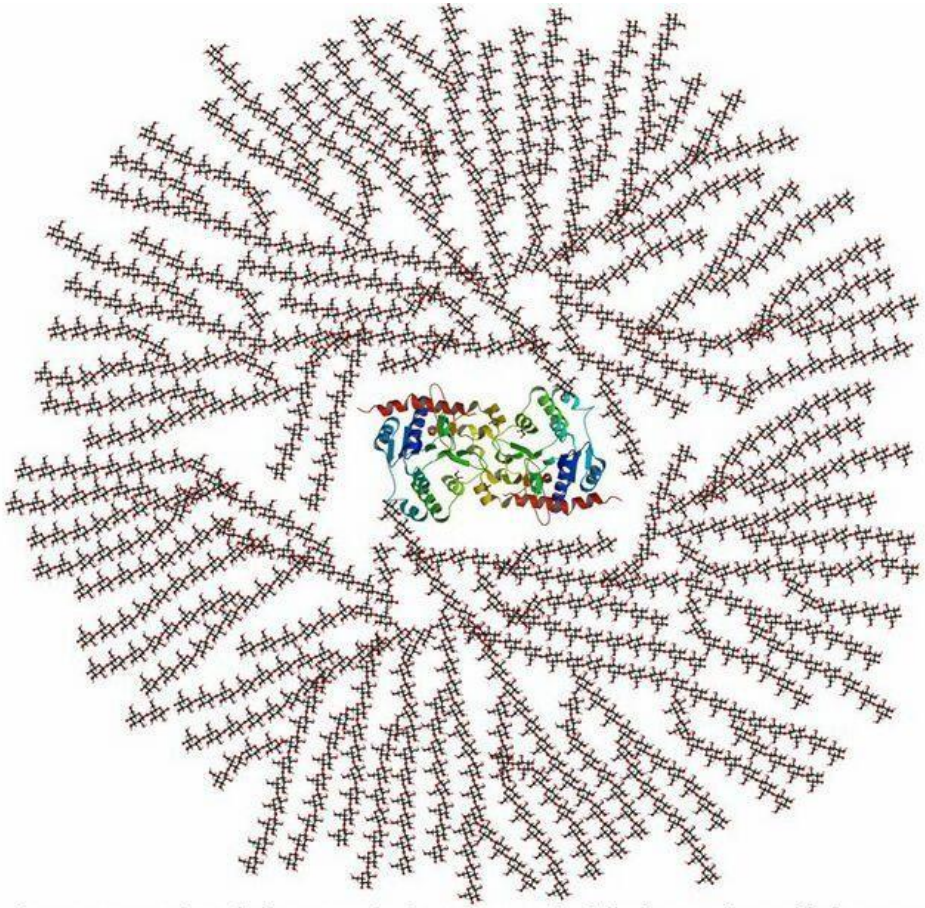


You Don’t Always Get What You Want

“Life is what happens while you’re busy making other plans.” – John Lennon

Glycogen Metabolism

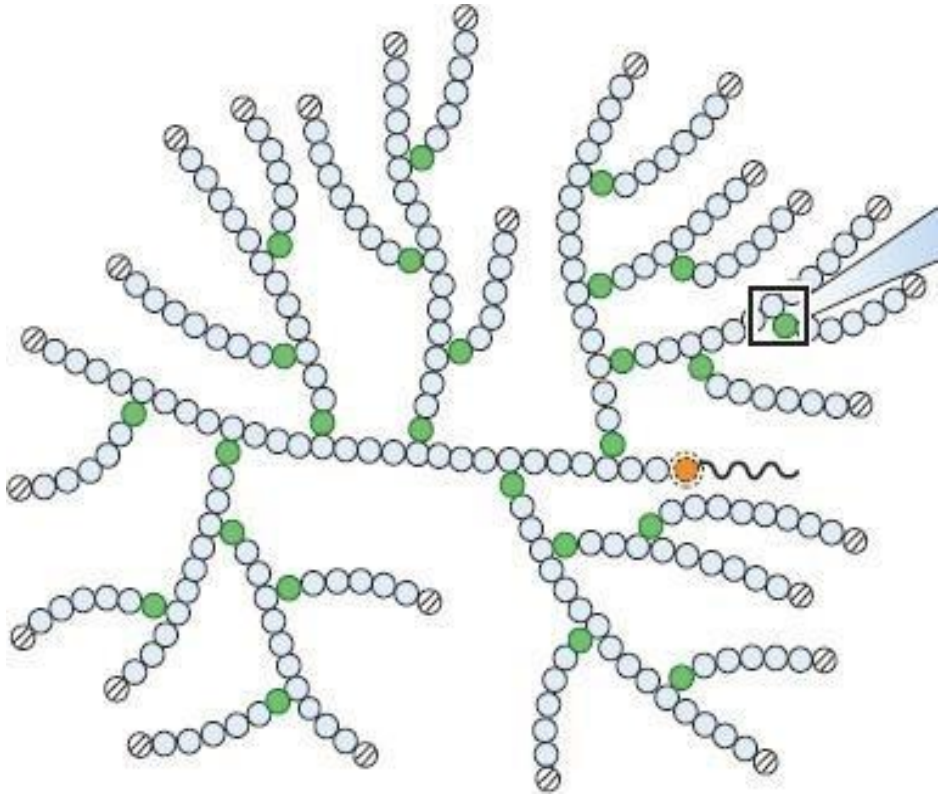
Glycogen Metabolism
Glycogen represents the **second source of glucose** after dietary intake.
Its amount in the body is **highly variable between individuals**.
When blood glucose from food reaches fasting levels (between meals), the body **shifts to glycogen degradation (glycogenolysis)** to maintain glucose supply.



A core protein of glycogenin is surrounded by branches of glucose units.
The entire globular complex may contain approximately 30,000 glucose units.

Dr. Diala Abu-Hassan

Glycogen Structure



*Extensively branched homopolysaccharide

*One molecule consists of hundreds of thousands of glucose units

1. Structure of Glycogen

Glycogen is a **homopolysaccharide** composed **only of glucose residues**.

Main Chain

- Glucose units in the main chain are connected by **α-1,4 glycosidic linkages**.
- The **first residue** in the chain has a **free anomeric carbon**, so it is the **reducing end**.
- The rest of the chain ends have carbon 4 exposed → these are **non-reducing ends**.

Why is the main chain "non-reducing" except for the first residue?

Because:

- The first glucose (anomeric carbon) is free → reducing.
- All other terminal carbons are carbon-4 → **non-reducing ends**.

2. Branching in Glycogen

Glycogen is **highly branched**.

- Branch points occur through **α-1,6 linkages**.
- A branch forms **every 8–10 glucose residues**.
- Glycogen typically contains about **13 layers of branching**.

Why does glycogen have extensive branching?

To create a **large number of non-reducing ends**, allowing:

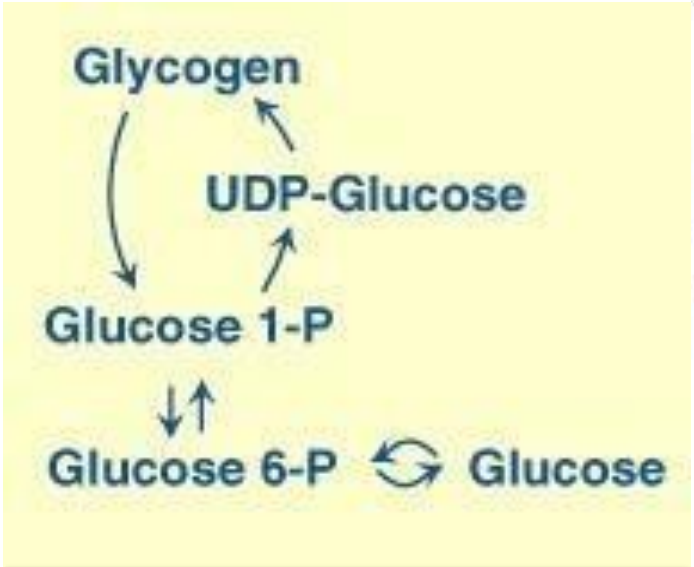
- Rapid degradation (many enzymes can work simultaneously)
- Fast release of glucose
- Efficient energy mobilization

3. Importance of Non-Reducing Ends

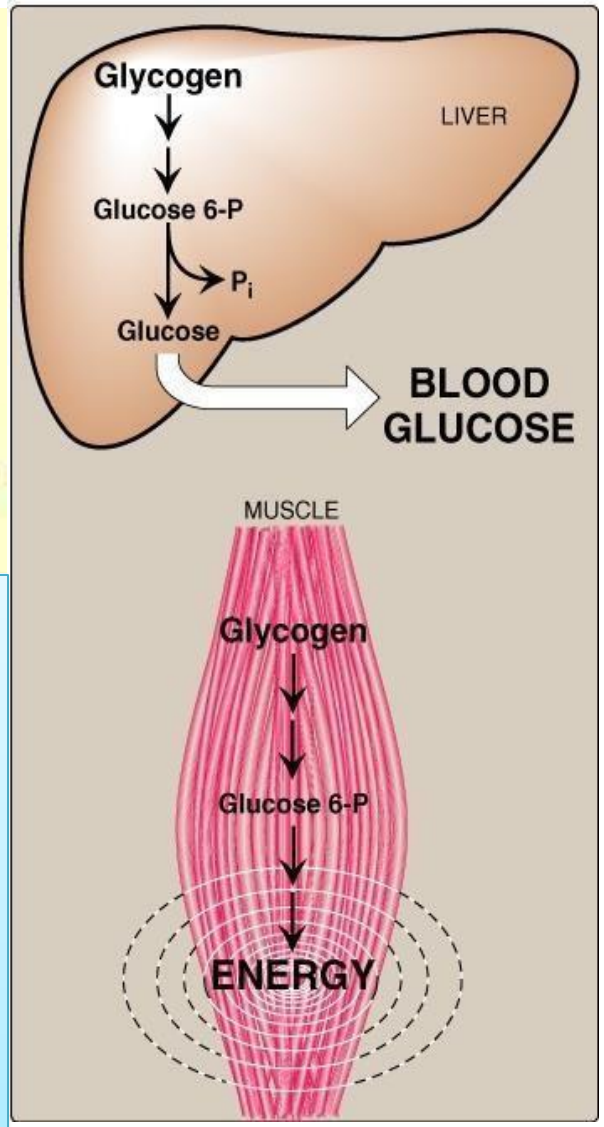
Each non-reducing end is a site where **one glucose residue** can be released at a time by glycogen-degrading enzymes.

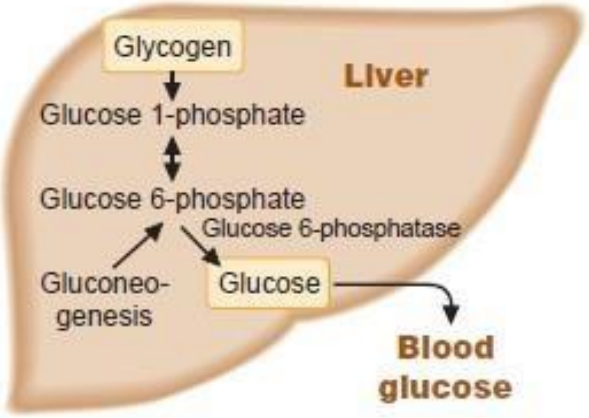
This provides a **steady and rapid source of glucose** — unlike gluconeogenesis, which is slower and hormonally regulated.

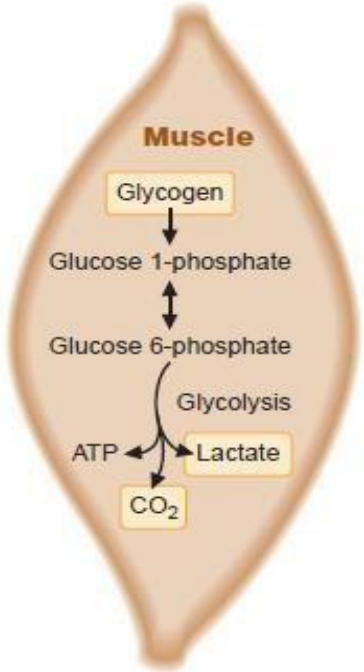
Glycogen synthesis & degradation



- Liver glycogen stores increase during the well-fed state and are depleted during fasting
- Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks).







Fates of Glucose that results from glycogen degradation

Glycogen is stored in all cells, but the **largest amounts** are found in:

- **Liver (~75 g)**
- **Skeletal muscles (~400 g)**

Liver glycogen is used to maintain *blood glucose levels* during fasting because the liver can convert glycogen into free glucose and release it into the bloodstream.

Muscle glycogen, however, is used *exclusively by the muscle cells themselves*. Muscles cannot release free glucose because they lack the enzyme **glucose-6-phosphatase**.

Glycogen Degradation (Glycogenolysis)

Liver vs Muscle

When glycogen is degraded, it does not directly give free glucose. Instead:

1.Glycogen → Glucose-1-phosphate (G1P)
(by **glycogen phosphorylase**, which adds inorganic phosphate)
2.G1P → Glucose-6-phosphate (G6P)
(by **phosphoglucomutase**)

In the liver:

- G6P → **Glucose**
(by **glucose-6-phosphatase**)
- Glucose is released into the bloodstream to maintain blood sugar.

In the muscle:

- No glucose-6-phosphatase → G6P **cannot become glucose**.
- G6P enters **glycolysis** to generate ATP for muscle contraction.
- Muscle glycogen stores (~400 g) last longer because they are large and depend on muscle activity (effort, contraction, relaxation). Each person varies.

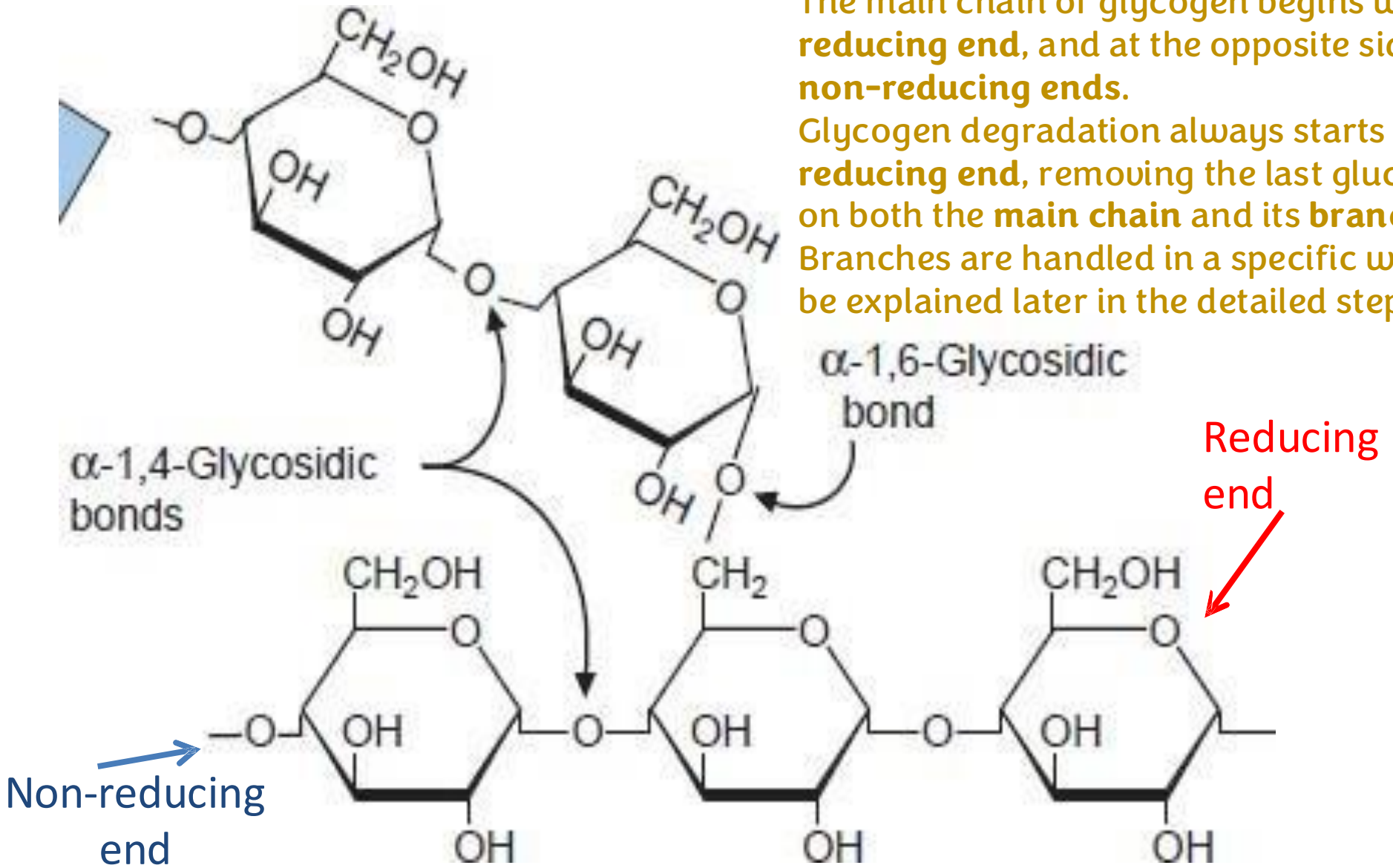
Each glycogen molecule has:

- A **reducing end** (beginning of the chain)
- Multiple **non-reducing ends** (sites of degradation)

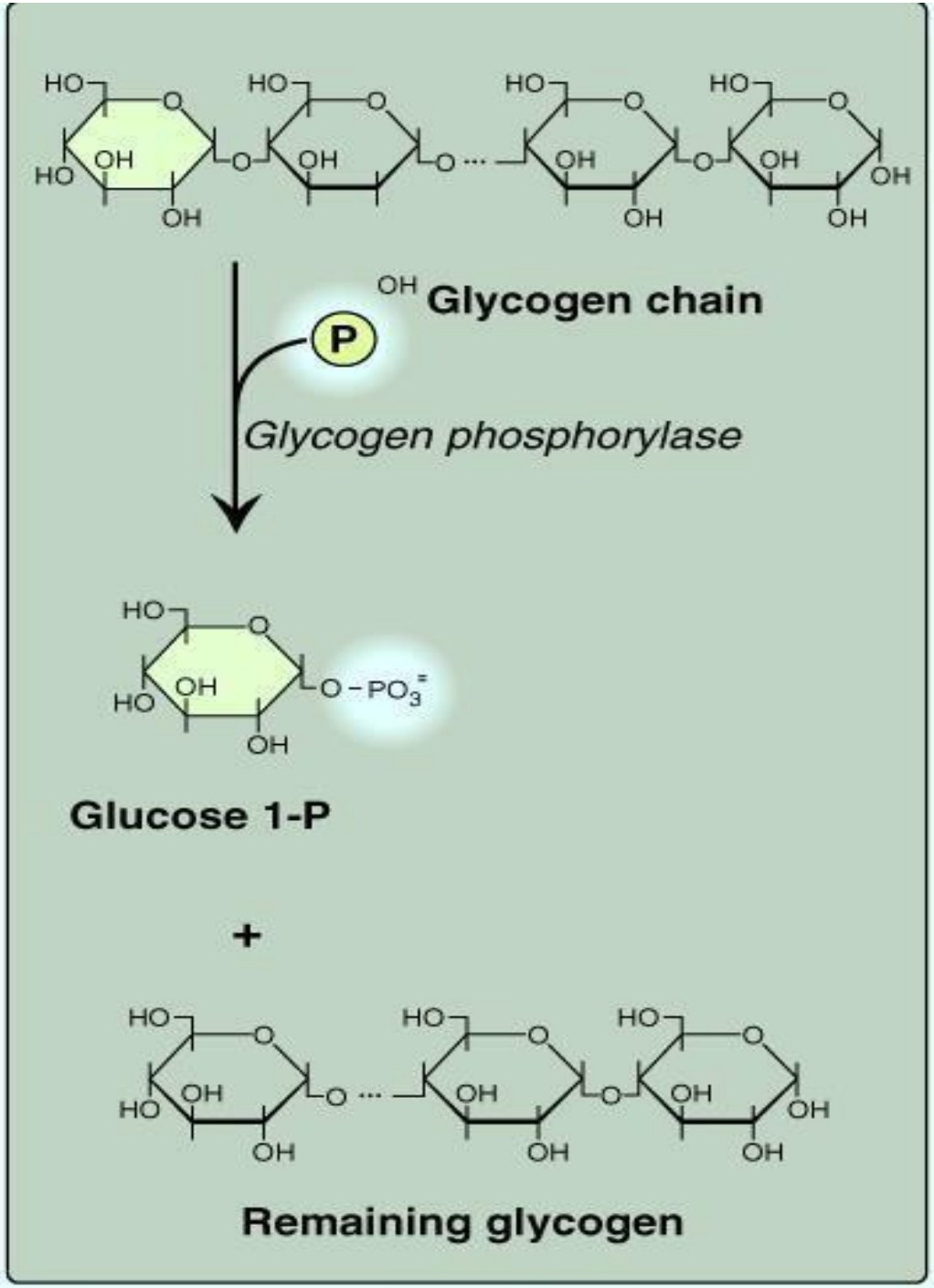
Glycogenolysis always begins at **non-reducing ends**.

Direction of Glycogen Degradation

The main chain of glycogen begins with a **reducing end**, and at the opposite side are the **non-reducing ends**.
Glycogen degradation always starts at the **non-reducing end**, removing the last glucose residues on both the **main chain** and its **branches**.
Branches are handled in a specific way, which will be explained later in the detailed steps.



Degradation of glycogen (Glycogenolysis)

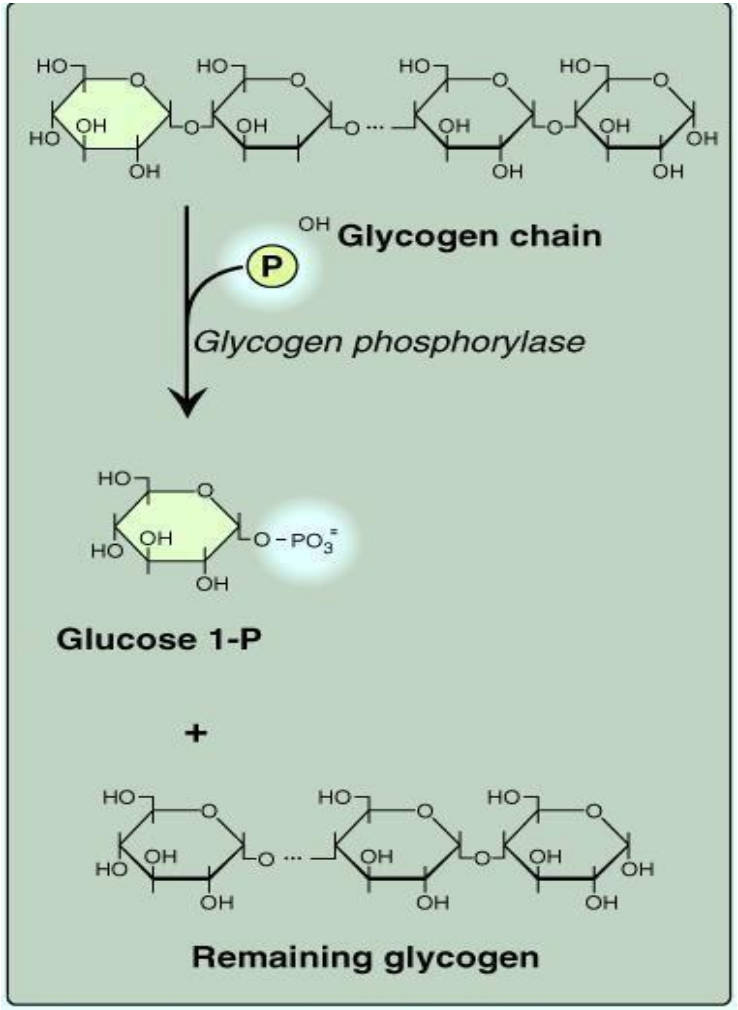


Degradation of glycogen
One glucose unit is removed at a time

Starts from the non-reducing ends

Released in the form of glucose 1-phosphate

Degradation of glycogen (Glycogenolysis)



Glycogenolysis begins on the **straight chains**, away from branch points.

1. Action of Glycogen Phosphorylase

- The **first glucose residue** of the straight chain is cleaved by **glycogen phosphorylase**.
- This enzyme adds **inorganic phosphate (Pi)** to the glucose, meaning **energy is NOT consumed**.
- The product is **glucose-1-phosphate (G1P)** and a shortened glycogen molecule.

Degradation of glycogen

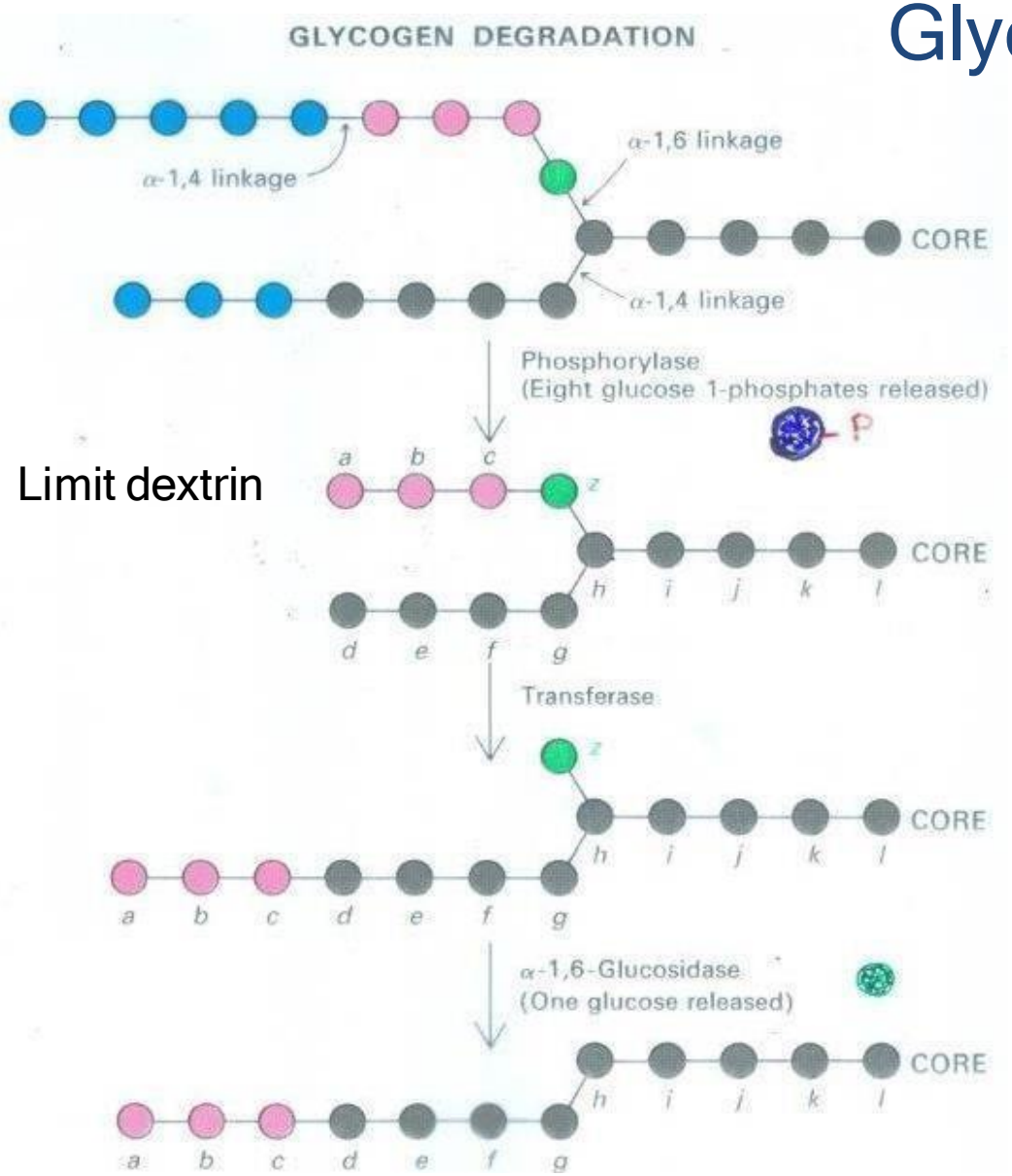
One glucose unit is

removed at a time

Starts from the non-reducing ends

Released in the form of glucose 1-phosphate

Glycogen Degradation



Debranching enzyme

G-1-P is converted in the cytosol to G-6-P by phosphoglucomutase

•The enzyme continues this process repeatedly until it approaches the branch point.

How Close Does It Go?

There is **no fixed number** of residues that define where it stops, but in practice, it stops when it is **3-4 residues away from the branch point**.

Limit Dextrin

The small segment remaining – **3 to 4 residues around the branching point** – is called **limit dextrin**.

•**"Dextrin"** means it is a sugar fragment containing a branch.

•**"Limit"** indicates that it causes a limitation – glycogen phosphorylase **cannot go past this point**, so degradation stops here.

•On the main straight chain, we do *not* use the term limit dextrin because it is not branched.

At this stage, **glycogen phosphorylase becomes inactive** until the branch is removed.

2. Debranching Enzyme (Two Functions)

A second enzyme is needed to remove the branch.

The **debranching enzyme** has **two separate activities**, and BOTH must occur to remove the branch fully

A) Transferase Activity

•The enzyme transfers **three glucose residues** (often drawn as three "pink" residues) from the branch to the main chain.

•These three residues become part of the long straight chain.

This leaves **only the branch point residue**, which is attached via an **α-1,6 bond**.

B) α-1,6-Glucosidase Activity

•The same debranching enzyme then cleaves the **α-1,6 linkage**.

•This releases the branch point residue as **free glucose** (not phosphorylated).

Now, the chain has become **straight again**, allowing glycogen phosphorylase to resume its activity

Cycle of Degradation

The overall process is **cyclic**:

1. Glycogen phosphorylase removes residues until 3–4 before a branch.
2. Debranching enzyme removes the branch (transferase + α-1,6-glucosidase).
3. Glycogen phosphorylase resumes degradation.
4. Cycle repeats at every future branch.

Important Note About the Products

•The glucose released from **α-1,6-glucosidase** is **free glucose**.

•All other glucose residues released by **glycogen phosphorylase** are **glucose-1-phosphate** (phosphorylated).

Conversion to G6P

Glucose-1-phosphate is converted to **glucose-6-phosphate (G6P)** by:

•**Phosphoglucomutase**

Lysosomal degradation of glycogen
The minor pathway

- A small amount (1-3%) of glycogen is degraded by the lysosomal enzyme, α(1–4)-glucosidase (acid maltase).
- The purpose of this pathway is unknown.
- A deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes (Type II: Pompe disease)

Glycogen degradation mainly occurs in the **cytosol** (major pathway).
But a small percentage occurs in the **lysosomes** (minor pathway).

Lysosomal Degradation (Minor Pathway)

•The lysosome is like the "digestive system" of the cell, containing many enzymes that degrade fatty acids, glycogen, and more.
•The lysosomal environment is **highly acidic** (pH ~4–5).
•Cytosol pH is **~7**.
A difference of **2 pH units = 100× difference in proton concentration.**
This means:
•Lysosomal enzymes **do not work in the cytosol**
•Cytosolic enzymes **do not work inside lysosomes**

Acid Maltase (Lysosomal α-1,4-Glucosidase)

This lysosomal enzyme:
•Degrades about **1–3% of glycogen**.
•Works only in an acidic environment.
•Despite handling a very small percentage, it is **critical**.

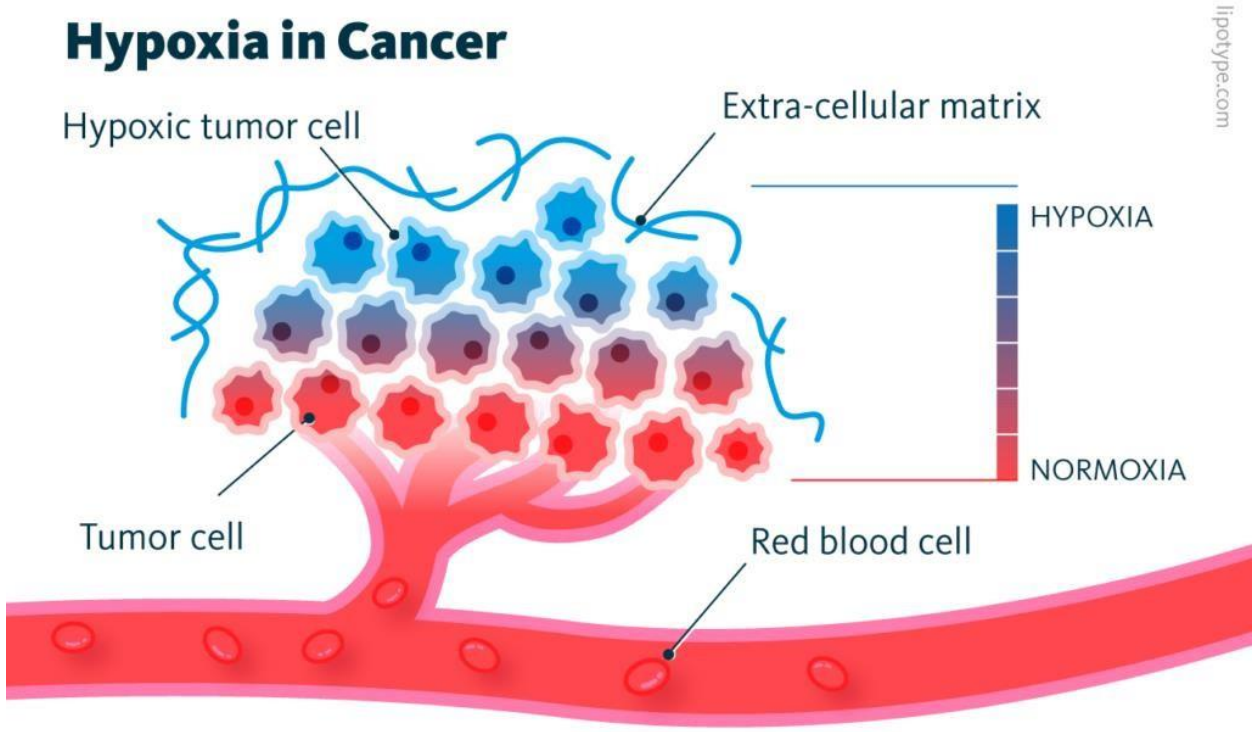
Pompe Disease

•Caused by **deficiency of acid maltase**.
•Leads to accumulation of glycogen in lysosomes.
•Lysosomes enlarge and appear like **vacuoles/bubbles** under the microscope.
•Classified as:
  - A **glycogen storage disease**
  - A **lysosomal storage disease**
•It is very rare – around **30 cases worldwide**.

Application: Glycogen degradation benefits hypoxic tumor cells

In hypoxia:

- ⬆ Glycolysis
- ⬆ Glycogen breakdown



Blocking glycogen degradation can induce tumor cell senescence

Tumor cells survive **hypoxic environments** better than normal cells.
Why?
•They express many **GLUT transporters**, allowing increased glucose uptake.
•They grow rapidly, divide, synthesize proteins, and need high energy.
•Their glycolysis is already high, but under hypoxia, it increases even more.

Under Hypoxia

•Pyruvate cannot proceed normally into the Krebs cycle because oxygen is low.
•It shifts into **anaerobic glycolysis → lactate**, producing less ATP.
•Tumor cells must compensate by **breaking down even more glycogen**.

Thus, tumor cells activate **glycogen degradation** more than normal cells under hypoxic conditions.

Therapeutic Strategy

One cancer-drug strategy is to:
•**Block glycogen degradation**
•Tumor cells consume available glucose quickly
•They fail to maintain anaerobic glycolysis
•They enter energy crisis faster (حالة الهرع أسرع)

However:
•Such drugs also affect normal cells.
•Side effects occur (hair loss, diarrhea, immune suppression).
•For highly specific targeting, we would need a drug for **each cancer type**, which is difficult and requires extensive research.

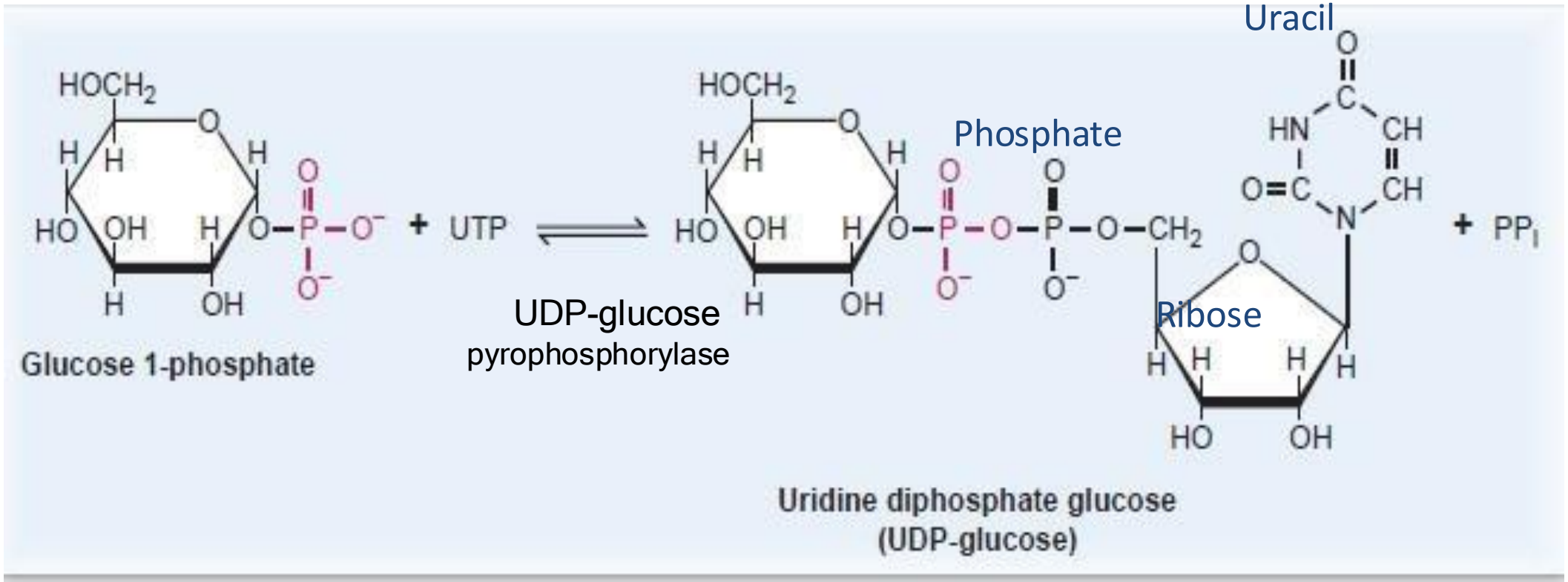
Glycogen Synthesis (glycogenesis)

Occurs in the **well-fed state**, stimulated by **insulin**.

Glycogenesis-Glycogen synthesis

Glycogen is synthesized by adding glucose one by one

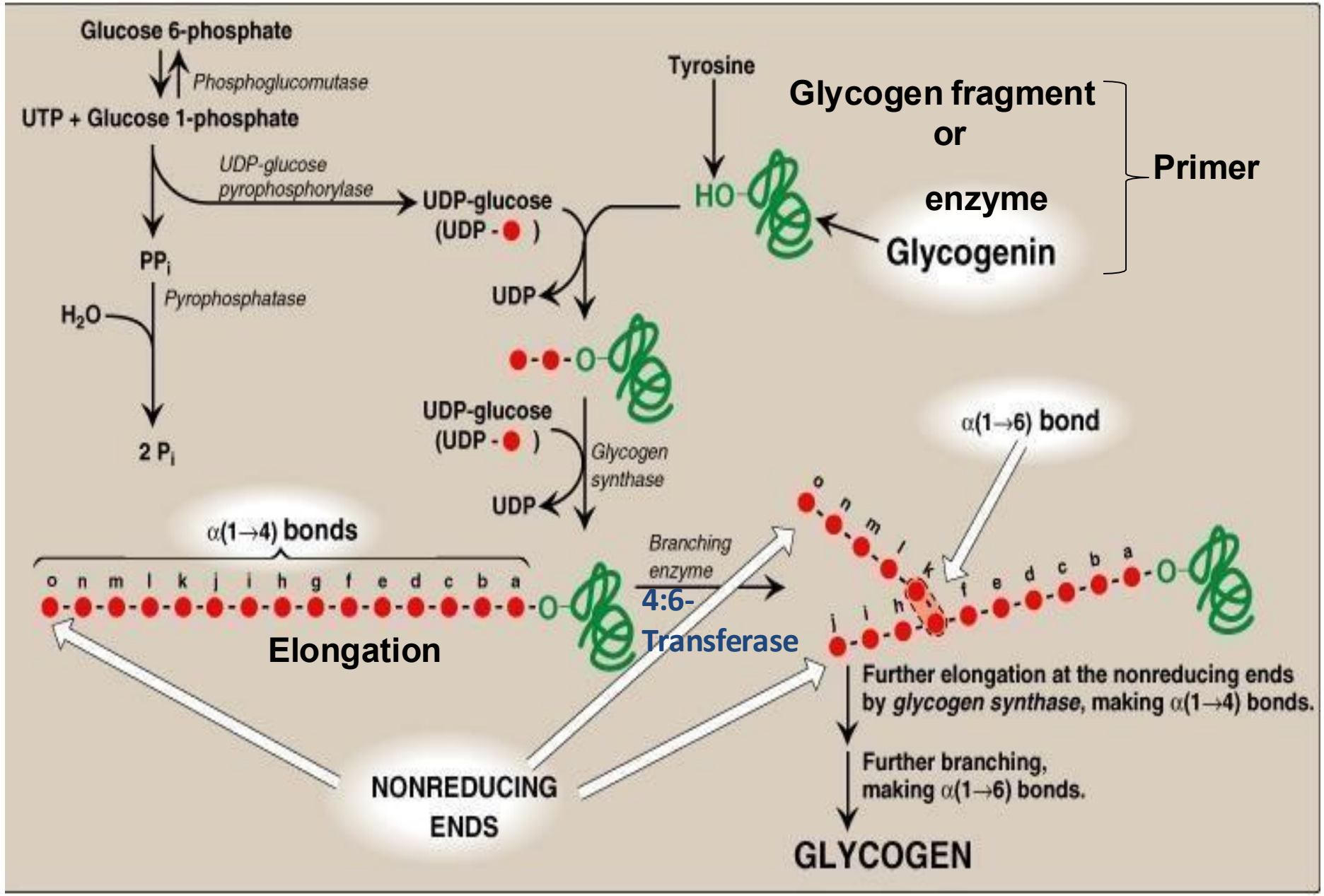
UDP-Glucose is the active donor of glucose units



Formation of UDP-Glucose

We need a specific structure of glucose so we can use it for this process, which is **UDP-glucose**.
How do we get it?
In the well-fed state, glucose is high in the blood. It will be taken up into the cells and phosphorylated into **glucose-6-phosphate** by glucokinase or hexokinase.
Then glucose-6-phosphate will be isomerized into **glucose-1-phosphate**, which reacts with **UTP**, a nucleotide that has three phosphate groups.
UTP has three phosphates, and glucose-1-phosphate has one phosphate, giving a total of four.
From the UTP we will take **UMP** and one phosphate group. The other two phosphates are released as **pyrophosphate**, which will become **inorganic phosphate**, so we used UMP.
This reaction is reversible, and it uses energy because we perform hydrolysis of UTP.
It is catalyzed by **UDP-glucose pyrophosphorylase**, which removes pyrophosphate.
This is the first step to start glycogenesis.

Glycogen Synthesis



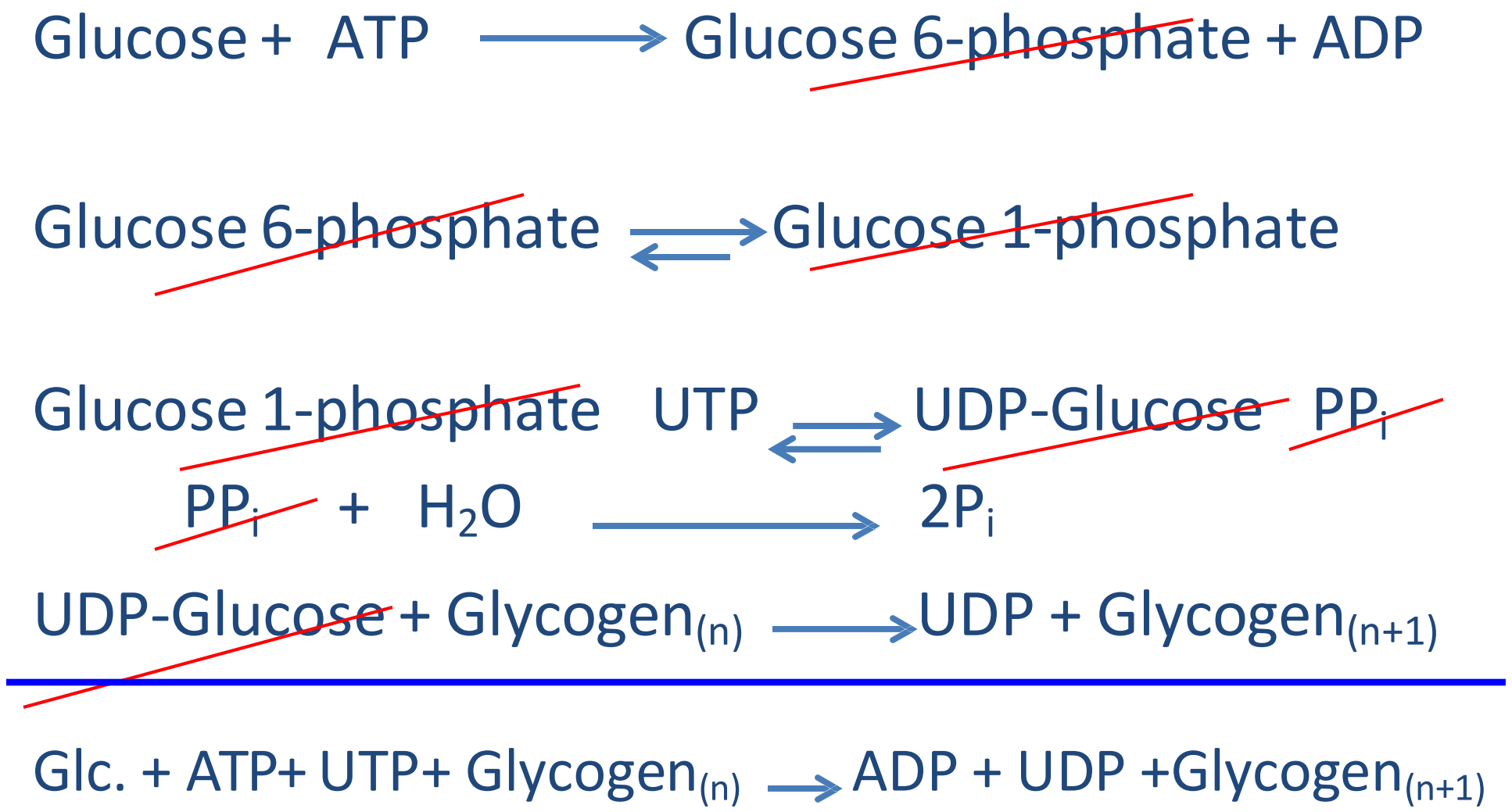
Once I have UDP-glucose, I need a base to start building up, called the **primer**.
It is either fragments of glycogen or an enzyme called **glycogenin**.
From the tyrosine residue we start connecting and building up, and at the end we release it; it will not be a part of glycogen.
Also, UDP will be released.

The enzyme that catalyzes this step is **glycogen synthase**.
Up to this step, the product is a straight chain with **α-1,4 bonds** between glucose residues, like amylose.

To introduce branches, we use the enzyme **4,6-transferase**, the **branching enzyme**.
It transfers the bond from α-1,4 to **α-1,6**.
It cuts after the j-k-l-m-n-o residues and transfers the segment to the g residue, producing an α-1,6 linkage.

Now we have a branch.
Glycogen synthase continues elongating the branch and the main chain, and the branching enzyme repeats its job.
This continues until we have **13 layers of branching**.
So it becomes an alternating cycle between glycogen synthase and the branching enzyme, resulting in a very complicated, highly and extensively branched glycogen structure with thousands, maybe millions, of residues.

Energy needed for glycogen synthesis



This is an **anabolic process**, so it requires energy.
For each glucose molecule added to glycogen, we look at the energy used:

First, glucose is phosphorylated (part of glycolysis) to glucose-6-phosphate and ADP.

Glucose-6-phosphate is isomerized to glucose-1-phosphate – no energy required here.

Glucose-1-phosphate then reacts with UTP to form UDP-glucose and pyrophosphate.

Pyrophosphate undergoes hydrolysis to inorganic phosphate.

UDP-glucose is added to glycogen to produce UDP and glycogen.

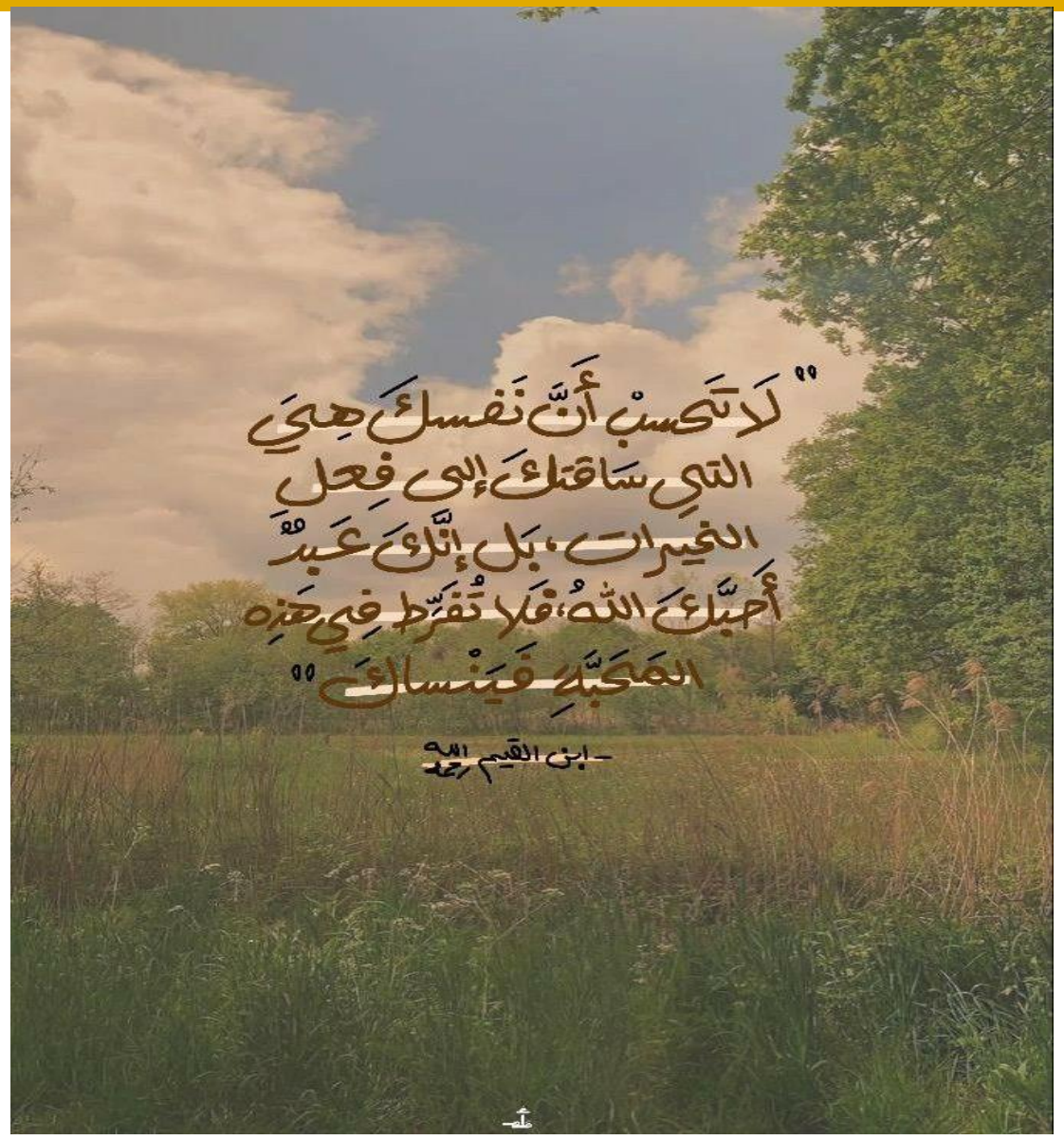
So at the end, each glucose residue requires **2 ATPs**, one as ATP and one as UTP.

Therefore, a very large amount of energy is required to produce a glycogen molecule.

For each glucose added to glycogen:
1.ATP is used to phosphorylate glucose → G6P
2.UTP is used to activate glucose → UDP-glucose
Thus, **each glucose added consumes the equivalent of 2 ATP molecules**.

Additional Resources:

رسالة من الفريق العلمي:



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			