

METABOLISM

MID – Lecture 13

Regulation of Glycolysis

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ

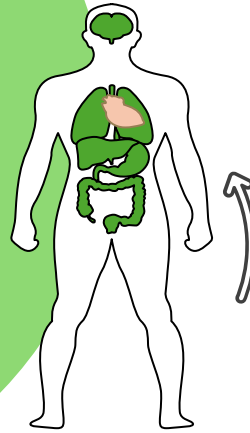
اللهم استعملنا ولا تستبدلنا

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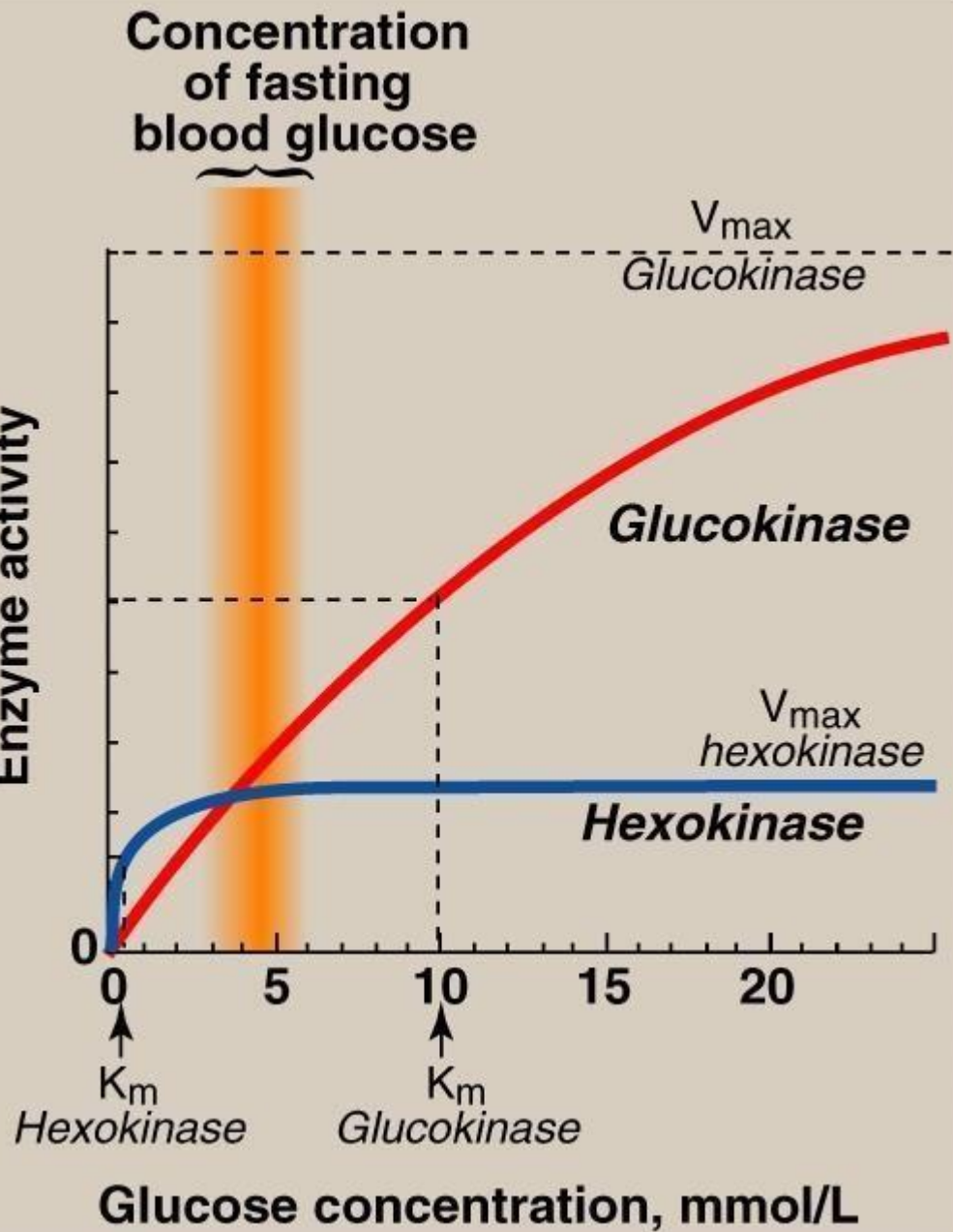
- Laith Joudeh



Quiz on the previous lecture.

Glucokinase and Hexokinase Activity

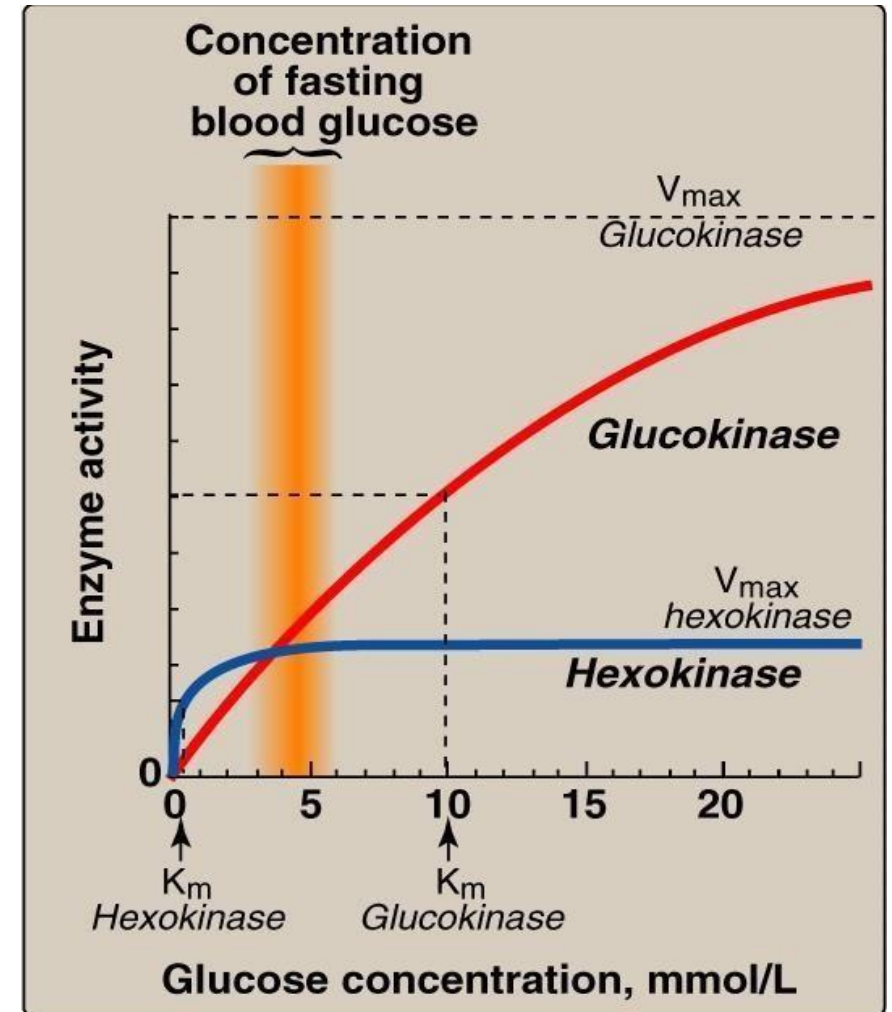
Enzyme activity



The first regulatory step in glucose metabolism is the phosphorylation of glucose to glucose-6-phosphate, catalyzed by either hexokinase or glucokinase. Hexokinase is active when glucose concentrations are low. It has a low V_{max} and a low K_m , indicating a high affinity for glucose. In contrast, glucokinase, which is activated at higher glucose concentrations, has a high V_{max} and a high K_m , reflecting its lower affinity for glucose. The activation of glucokinase is also induced by insulin.

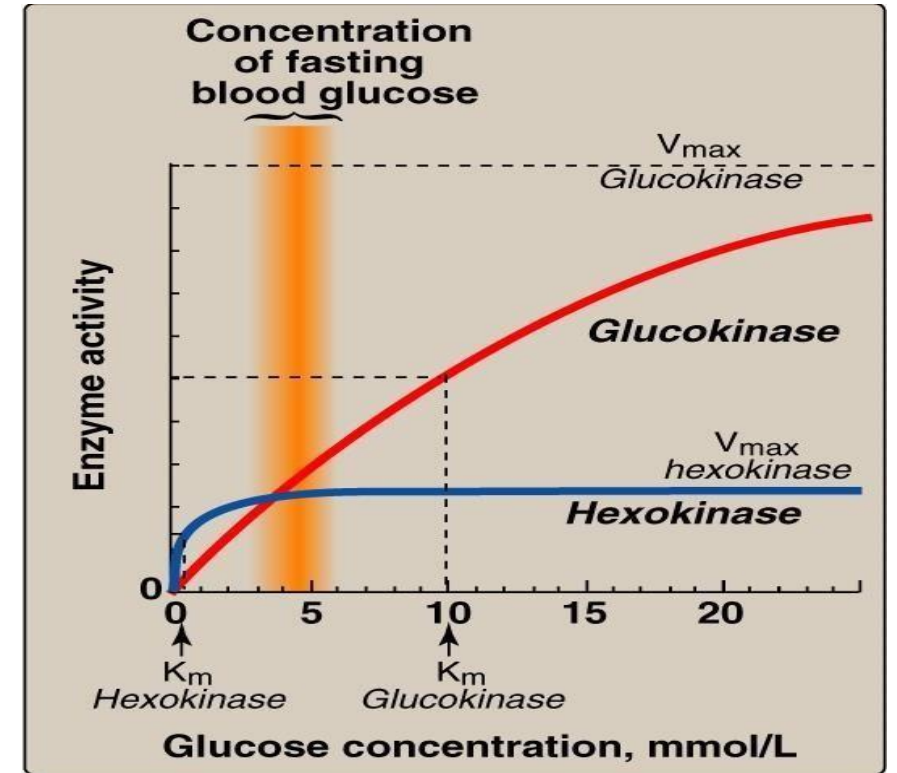
The orange region represents the concentration of glucose under fasting conditions. When you consume a meal, there is a period of 30 minutes to 1 hour during which glucose levels rise in the bloodstream as a result of digestion and absorption. The increase in blood glucose levels depends on the amount of sugar consumed. During this period, blood glucose can become significantly elevated (even in non-diabetic individuals). For this reason, blood glucose tests for diabetes are typically performed in a fasting state to avoid interference from recent food intake. Approximately two hours after eating, blood glucose levels begin to decrease as insulin acts to lower them. The minimum level reached is known as fasting blood sugar. Although it does not drop to zero, this stable fasting level is crucial for maintaining a specific solute concentration. This concentration is necessary to uphold stable osmotic pressure, regulate water movement within blood vessels, and help maintain normal blood pressure.

At fasting blood sugar levels, hexokinase is operating at its V_{max} , while glucokinase has not even reached half of its V_{max} . This indicates that glucokinase is more active in the well-fed state rather than during fasting.



Is blood sugar an inert parameter? No, it is highly dynamic. Reaching fasting blood sugar levels does not mean that glucose levels remain completely stable. Instead, glucose levels decrease and must be continually replenished to maintain a consistent concentration. This fluctuation (increase and decrease) is particularly significant in tissues that are heavily dependent on glucose as their primary energy source, such as the brain, adrenal medulla, and lens, which rely **exclusively** on glucose.

In contrast, many other tissues have alternative energy sources, such as fatty acids. These tissues prefer to use fatty acids over glycogen, especially because glycogen stores are relatively limited (we will study this in detail later).



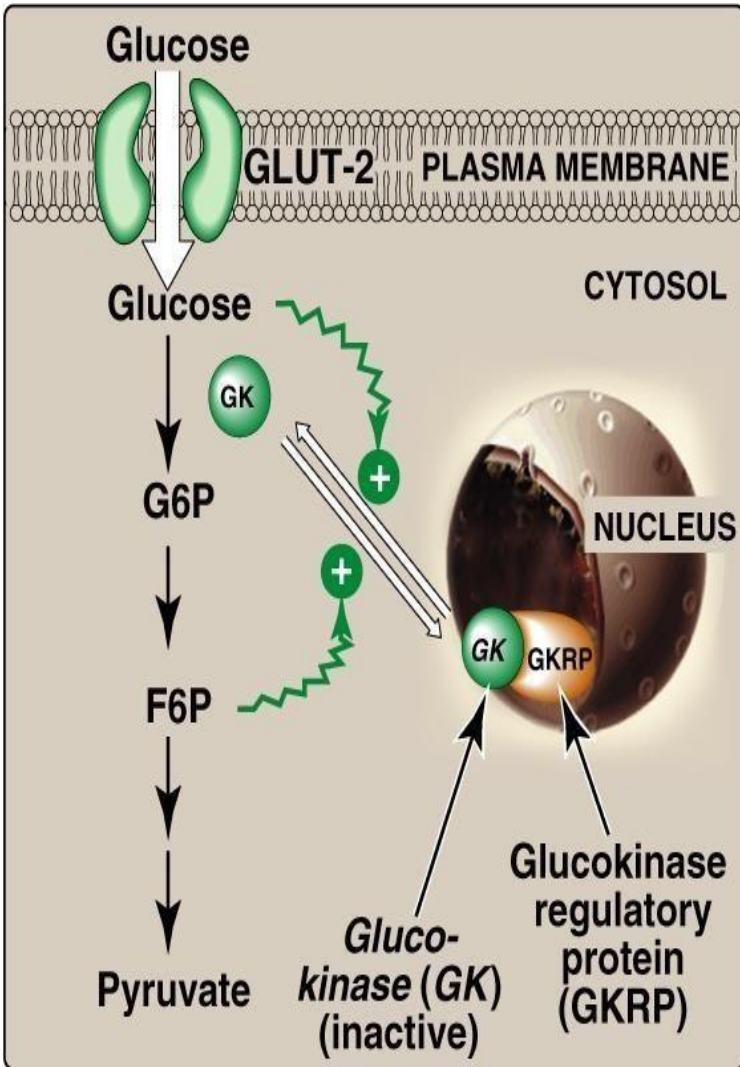
Glucokinase Regulation

Glucokinase regulation is linked to specific conditions, especially high insulin concentrations and well-fed states. When glucose levels rise, cells take it up through glucose transporters, especially GLUT-4, induced by insulin. Inside the cell, glucose undergoes metabolism, starting with phosphorylation by glucokinase or hexokinase.

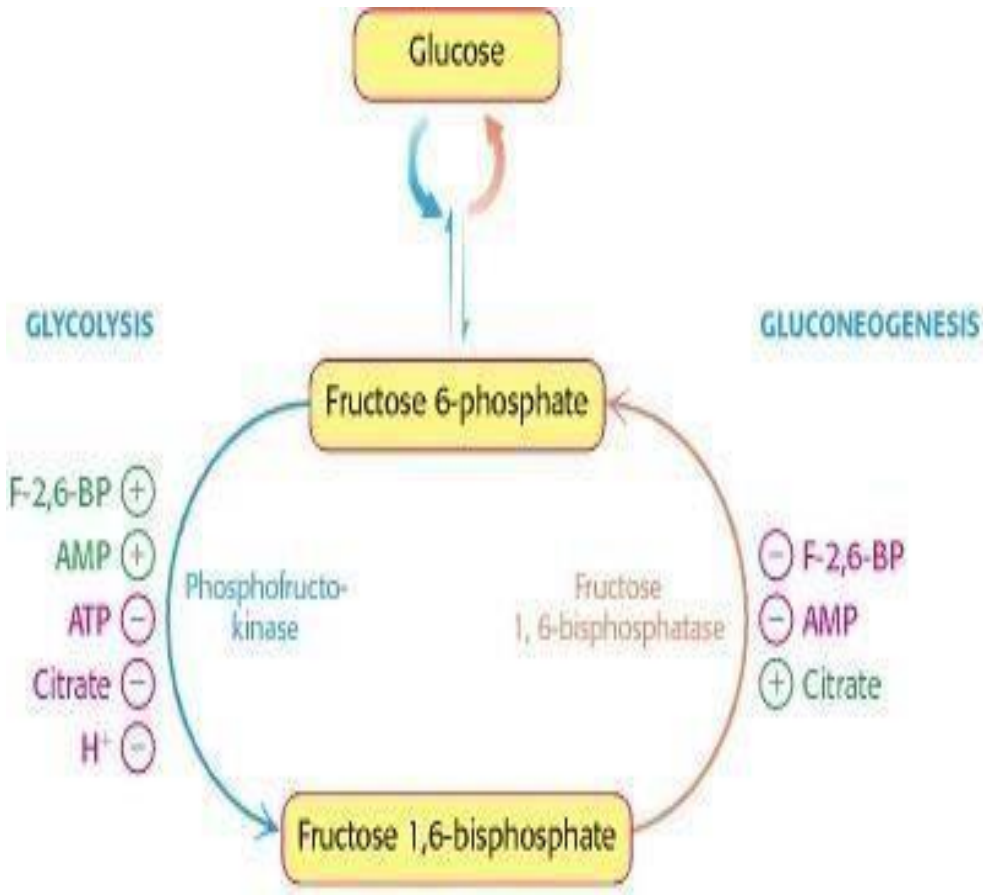
When glucokinase is inactive, it is sequestered in the nucleus, bound to the glucokinase regulatory protein (GKRP). Since glycolysis occurs in the cytosol, this sequestration effectively separates glucokinase from the glycolytic pathway. The regulatory protein keeps glucokinase in the nucleus under low-glucose conditions.

When glucose concentrations are high, glucose promotes the dissociation of glucokinase from its regulatory protein, allowing glucokinase to translocate from the nucleus to the cytosol, where it becomes active and catalyzes the phosphorylation of glucose. As a result, glucose is converted to glucose-6-phosphate, which is then isomerized to fructose-6-phosphate.

When the concentration of fructose-6-phosphate (F6P) rises, it signals the need to downregulate glycolysis by promoting the sequestration of glucokinase into the nucleus. This regulatory mechanism ensures that when glycolysis is already highly active and F6P levels are elevated, further activation is unnecessary, leading to the inhibition of glucokinase.



Allosteric Regulators of PFK1



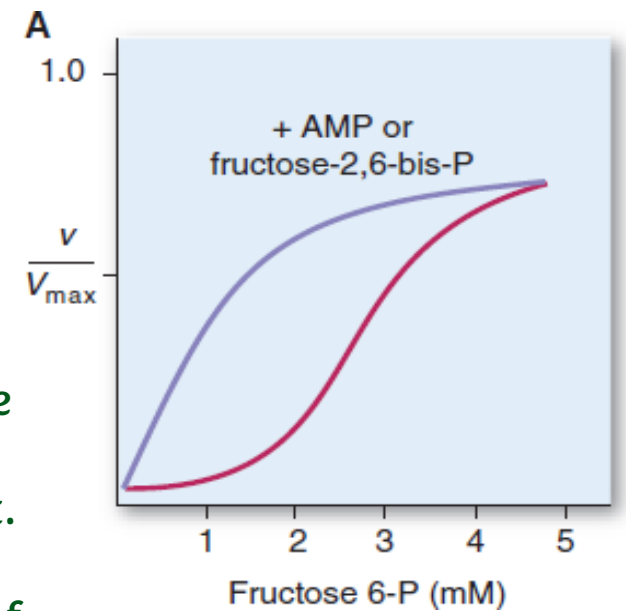
- The second regulated step in glycolysis is **step 3**, catalyzed by phosphofructokinase-1 (PFK-1), which is controlled by multiple allosteric regulators, both positive and negative. Positive regulators include fructose-2,6-bisphosphate (F-2,6-BP) and AMP.
- Fructose-2,6-bisphosphate is a regulatory molecule that differs from the glycolytic intermediate fructose-1,6-bisphosphate. It acts as a switch by activating glycolysis and inhibiting gluconeogenesis.
- AMP, which signals a low energy state in the cell, also activates PFK-1 to promote glycolysis and simultaneously inhibits gluconeogenesis, ensuring that energy production is prioritized.
- Negative regulators of PFK-1 include ATP, citrate, and protons (H⁺). ATP indicates a high energy state, signaling that there is no need to break down more glucose, and thus inhibits PFK-1 to prevent unnecessary glycolysis.
- Citrate, a product of the Krebs cycle, reflects sufficient energy availability and inhibits PFK-1, preventing further glucose breakdown.
- High proton levels suggest active oxidative phosphorylation and high energy production. Elevated proton concentration can lead to cellular acidosis, so PFK-1 is inhibited to prevent excessive glycolytic activity that could worsen the acidic environment. Together, these regulators ensure glycolysis is finely tuned to the cell's energy demands.

Regulation of PFK1 by Fructose 2,6- bisphosphate

In the figure, we are plotting the concentration of fructose-6-phosphate against the reaction velocity, which is expressed as a ratio. Although velocity is presented as a ratio, it still effectively represents the overall reaction rate, regardless of the format.

The red curve, which has a sigmoidal shape, illustrates the reaction in the absence of activators. In contrast, the blue curve shows the effect of activators like AMP or fructose-2,6-bisphosphate, shifting the curve to the left. This shift indicates enhanced enzyme activity and a higher reaction velocity at lower concentrations of fructose-6-phosphate. Both curves reach the same V_{max} because V_{max} is only altered by changing the enzyme concentration, not by the presence of allosteric activators.

The shape of the blue curve approaches a near-hyperbolic form, indicating that even at very low concentrations of fructose-6-phosphate, the activators can significantly accelerate the reaction, leading to a rapid increase in velocity.

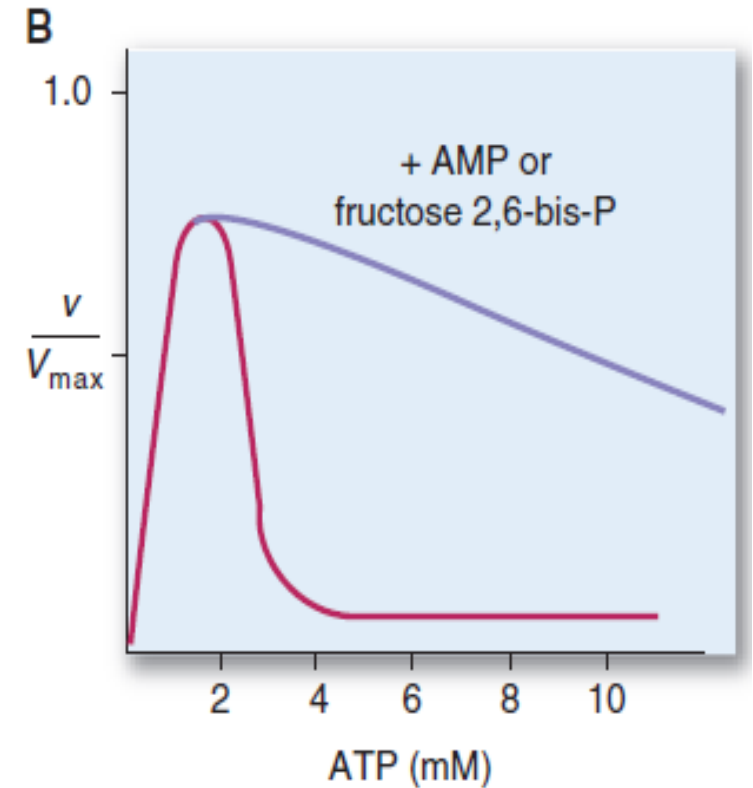


How about the other substrate?

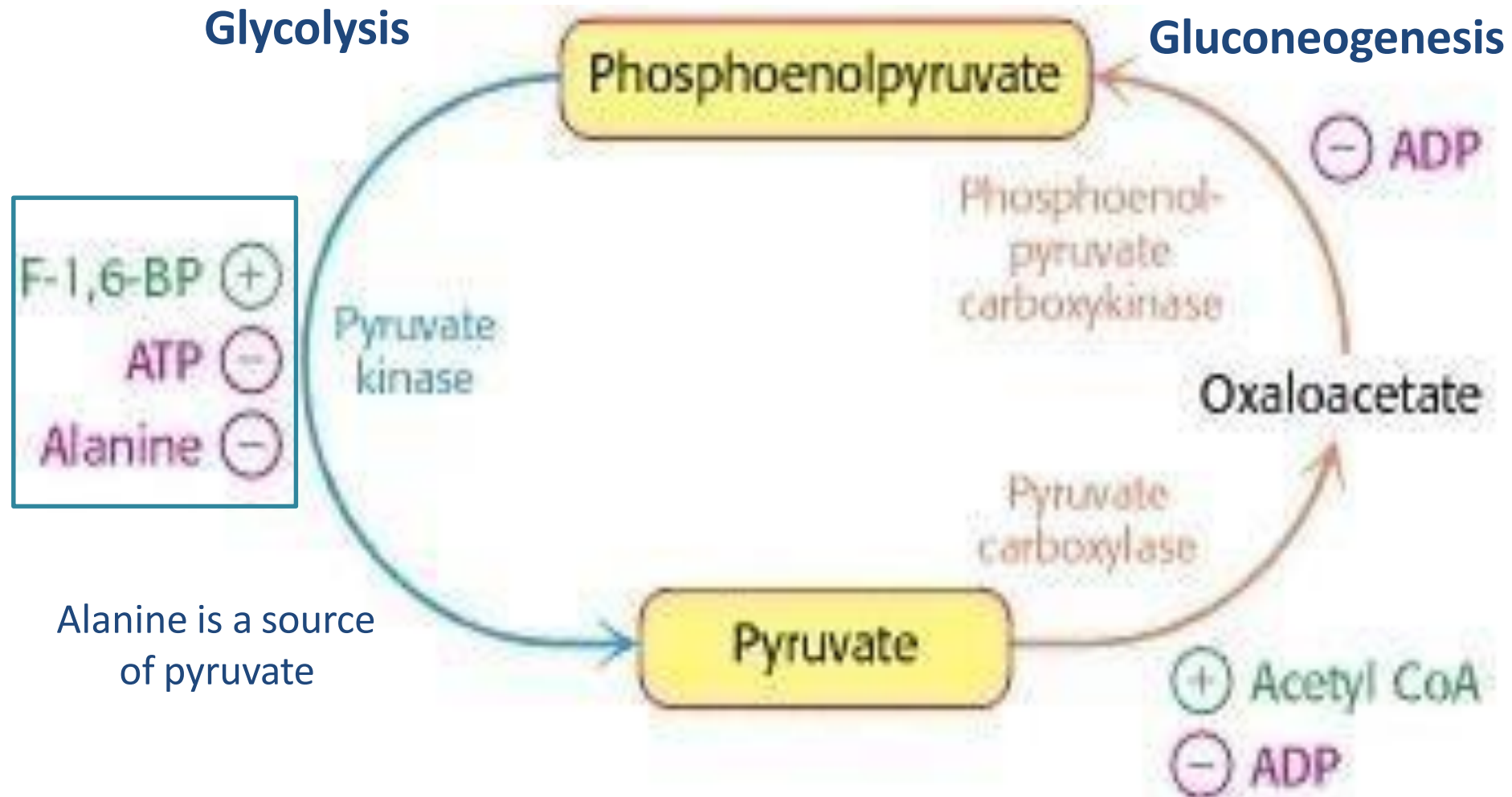
Now, we are plotting the concentration of ATP against reaction velocity. Notice how the curve initially rises sharply, then declines rapidly, and eventually reaches a plateau. In the presence of activators like AMP or fructose-2,6-bisphosphate, the decline becomes more gradual, indicating a slower decrease in velocity.

The shape of the curve reflects the dual role of ATP in this reaction: it serves as both a substrate and an allosteric regulator (specifically, a negative regulator). At low ATP concentrations, ATP primarily functions as a substrate, and it does not inhibit the enzyme. As ATP concentration increases, it signals a high energy state in the cell and begins to act as an allosteric inhibitor. ATP binds to phosphofructokinase-1 (PFK-1), reducing its activity, which explains the decline in the reaction velocity observed in the curve.

A cell would not normally exhibit high levels of ATP and AMP simultaneously. However, when AMP or fructose-2,6-bisphosphate is added separately in an experimental setup, these molecules reduce ATP's inhibitory impact on glycolysis. Consequently, the decrease in reaction velocity caused by ATP inhibition occurs more gradually in the presence of these activators. This explains why the curve representing reaction velocity declines more slowly when activators are present.



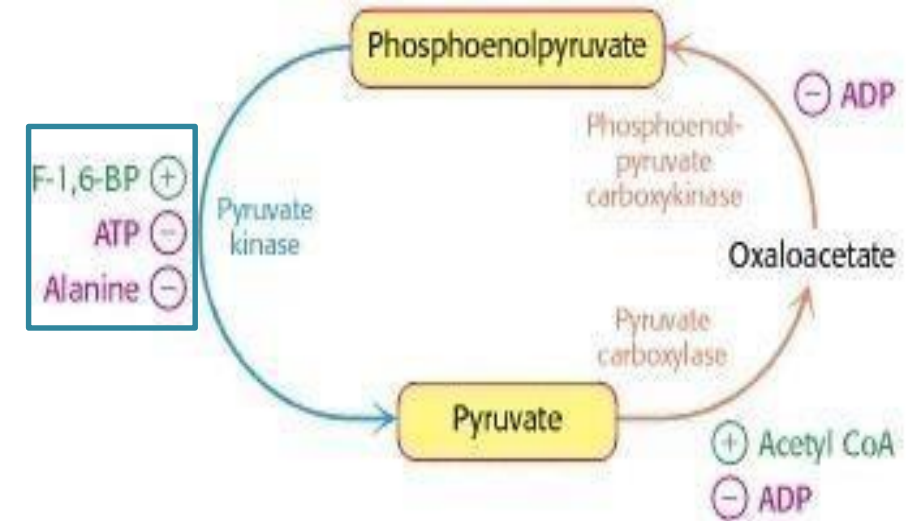
Regulation of Pyruvate Kinase



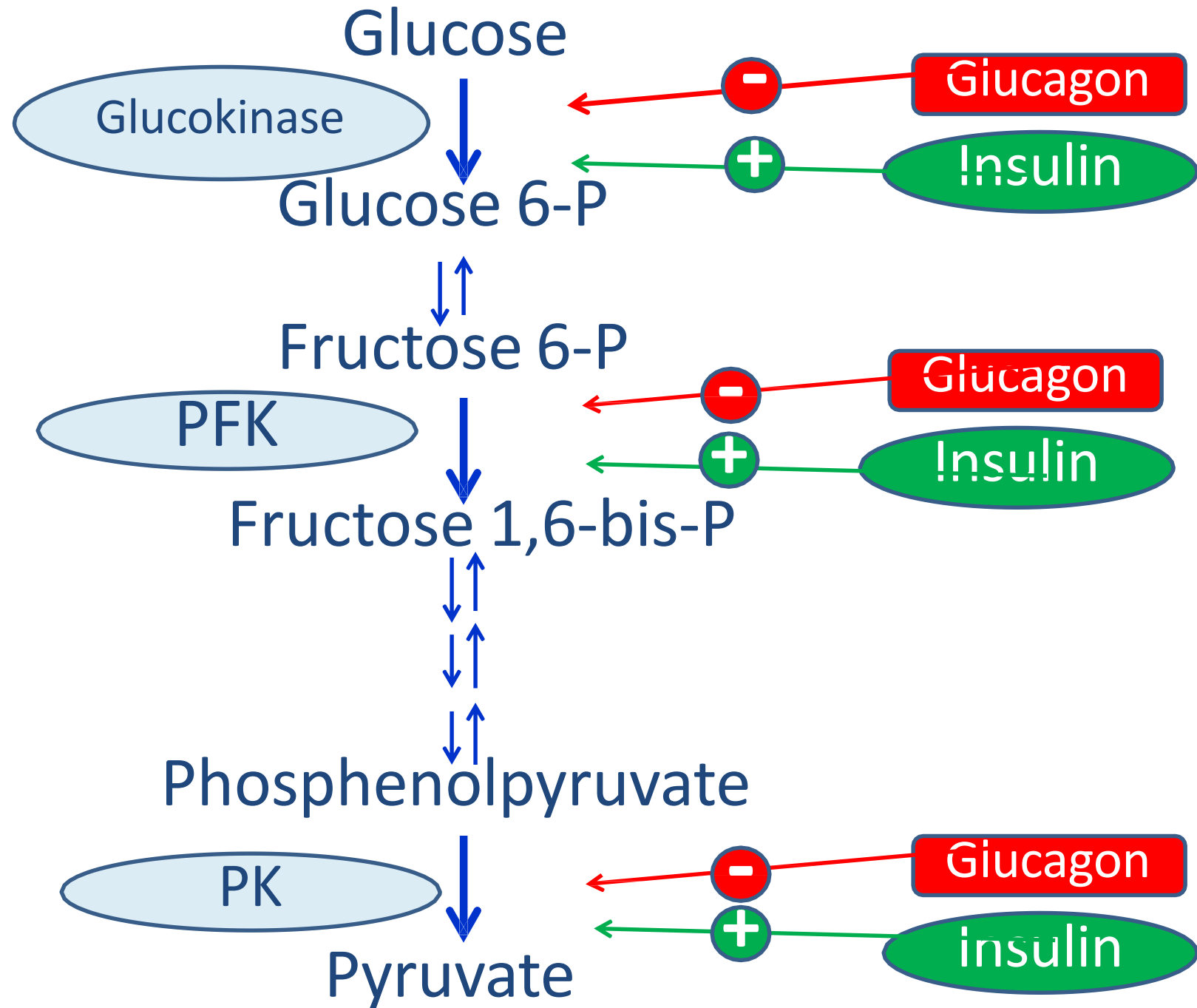
Regulation of Pyruvate Kinase

Now we will discuss the regulation of the third key enzyme in glycolysis: pyruvate kinase. This enzyme is influenced by several allosteric regulators:

1. The first regulator is fructose-1,6-bisphosphate, a glycolytic intermediate. This is an example of “feed-forward regulation”, where a product or intermediate from an earlier step in the pathway activates a later step. Specifically, fructose-1,6-bisphosphate, which is produced during step 3, acts as a positive allosteric regulator on step 10, enhancing pyruvate kinase activity.
2. In contrast, ATP acts as a negative regulator, signaling a high energy state in the cell and thereby inhibiting pyruvate kinase to prevent excessive glucose breakdown.
3. Finally, alanine serves as another negative regulator. Alanine is closely related to pyruvate because it can be converted into pyruvate through transamination, as pyruvate is the alpha-keto acid form of the amino acid alanine. Thus, high levels of alanine indicate sufficient pyruvate availability and inhibit pyruvate kinase activity.



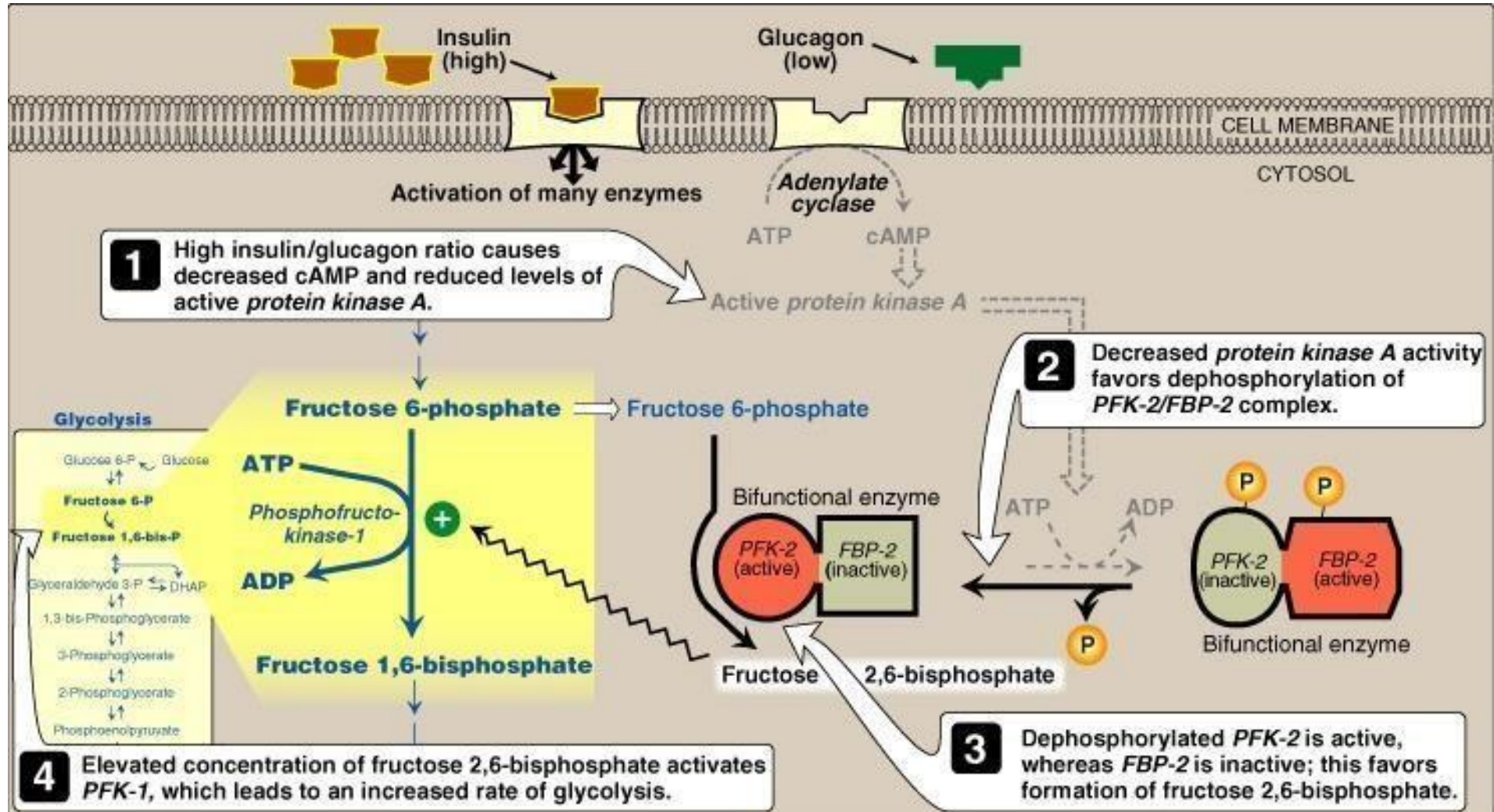
Hormonal Regulation



Explanation of the previous slide:

- The most important hormones involved in carbohydrate metabolism are insulin and glucagon, which have opposing actions. Insulin is secreted in the well-fed state to facilitate the uptake of glucose from the bloodstream into cells; where it can be used in various metabolic pathways, including glycolysis. On the other hand, glucagon is released during fasting conditions to increase blood glucose levels, ensuring a stable supply and preventing hypoglycemia and unconsciousness.
- Hormones such as cortisol, commonly known as the stress hormone, play significant roles in sugar metabolism. Stress activates the body's "fight or flight" response, prompting the release of high amounts of energy, primarily from readily available sources like sugars. This explains why individuals under chronic stress often gain weight, as stress can lead to altered eating behaviors and disrupted metabolism. Additionally, people who use cortisone (a synthetic form of cortisol) frequently experience weight gain because cortisone mimics and amplifies the effects of natural cortisol in the body, affecting metabolic processes. During stress, elevated cortisol levels boost sugar metabolism. The adrenal glands also release norepinephrine and epinephrine, further enhancing energy mobilization and the body's overall stress response.

Hormonal Regulation of Phosphofructokinase

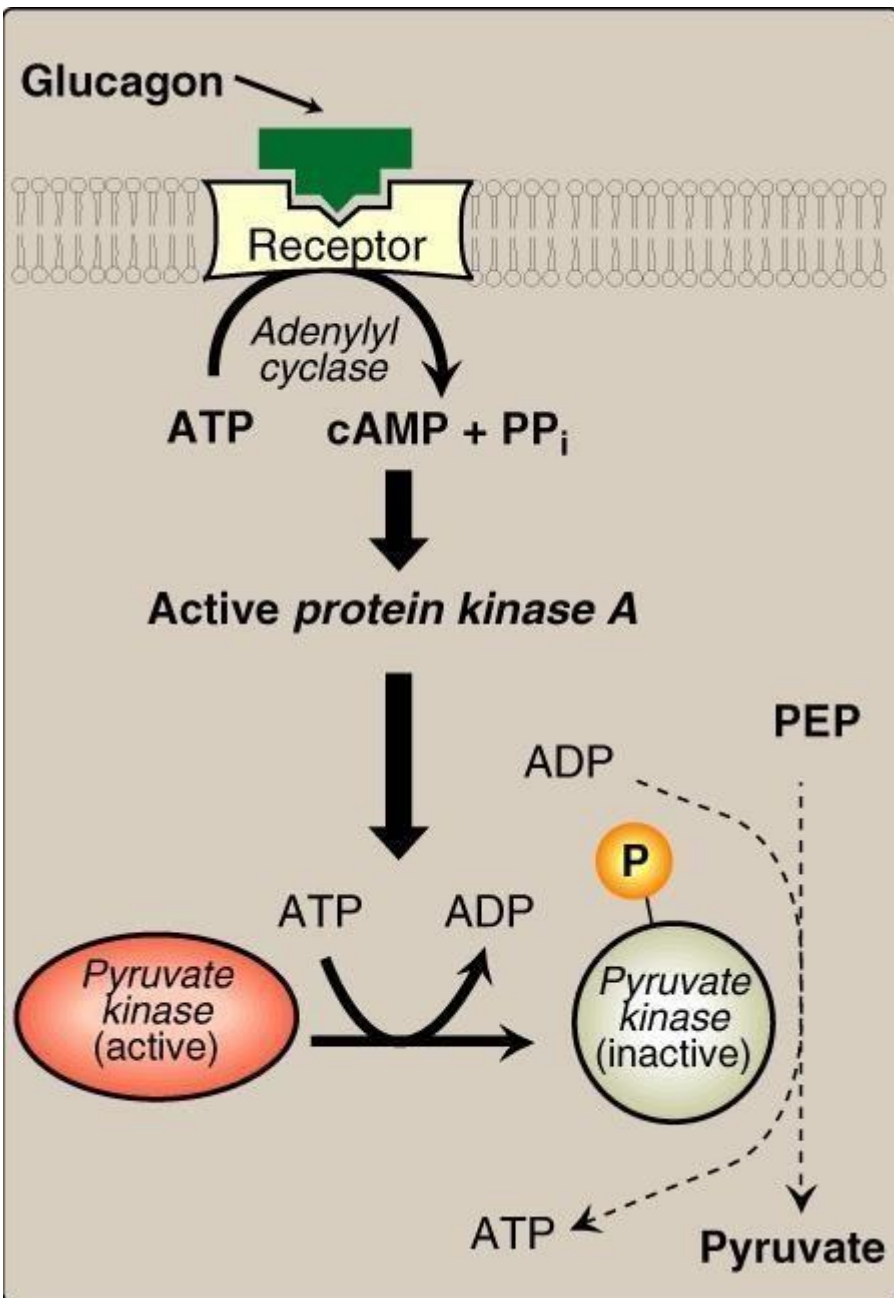


Explanation of the previous slide

- In a well-fed state, insulin levels are high, leading to the activation of insulin receptors, whereas glucagon levels are low and thus do not activate their respective receptors. Insulin binds to its receptor, a receptor tyrosine kinase, initiating phosphorylation cascades that activate various proteins and promote glycolysis. It is important to note that insulin itself does not directly inhibit or activate enzymes but instead sends signals that either inhibit or activate key metabolic pathways.
- When insulin levels are high, the kinase component of the bifunctional enzyme (PFK-2/FBPase-2) becomes active (due to low cAMP levels), while the phosphatase component is inactive. This results in an increase in the concentration of fructose 2,6-bisphosphate (F2,6BP), a potent activator of phosphofructokinase-1 (PFK-1), which enhances glycolysis. At the same time, fructose 2,6-bisphosphate inhibits gluconeogenesis, ensuring that energy production from glucose is prioritized.

Explanation of the previous slide

- Conversely, when glucagon levels are high, such as during fasting, glucagon binds to its G-protein-coupled receptor (GPCR), which activates adenylate cyclase to increase cAMP production. Elevated cAMP levels lead to the activation of protein kinase A (PKA), which then phosphorylates the bifunctional enzyme, switching off the kinase activity and activating the phosphatase activity. This reduces fructose 2,6-bisphosphate levels, thereby inhibiting glycolysis and promoting gluconeogenesis.
- The dual regulatory role of fructose 2,6-bisphosphate is crucial: at high concentrations, it activates glycolysis and inhibits gluconeogenesis, and at low concentrations, it has the opposite effect. This mechanism ensures that only one pathway—either glycolysis or gluconeogenesis—is active at a time, preventing futile cycles and efficiently managing energy resources.

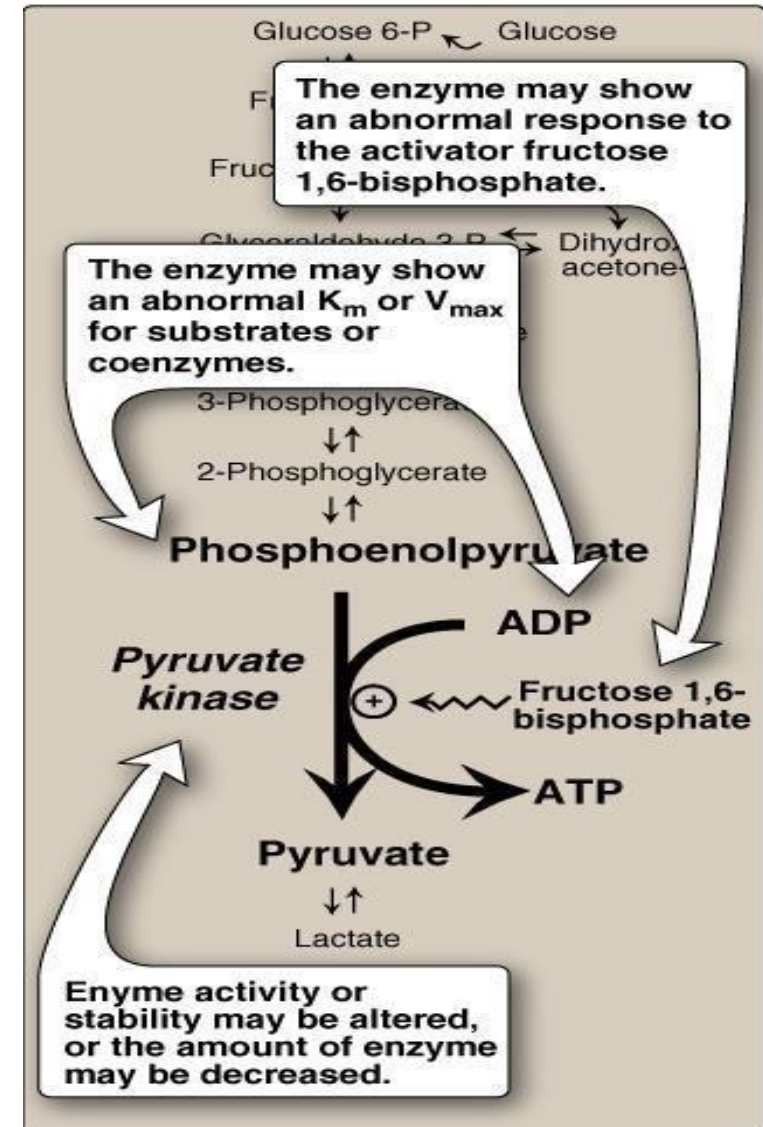


Hormonal Regulation of Pyruvate Kinase **Another target.**

Active protein kinase A inactivates pyruvate kinase by phosphorylating it.

Clinical Hint: Pyruvate Kinase Deficiency

- The most common among glycolytic enzyme deficiencies
- **RBCs** are affected
- Mild to severe chronic hemolytic anemia
- ATP is needed for Na⁺/K⁺ pump → maintain the flexible shape of the cell
- Low ATP → premature death of RBC
- Abnormal enzyme; mostly altered kinetic properties



Alterations observed with various mutant forms of pyruvate kinase

Explanation of the previous slide

- Pyruvate kinase deficiency is caused by a genetic mutation.
- Red blood cells (RBCs) have a biconcave shape, which is crucial for smooth movement through blood vessels and for bending easily as they pass through narrow capillaries.
- Since RBCs rely entirely on glycolysis for ATP production, they are significantly impacted by this condition. Without ATP, the Na^+/K^+ pump cannot function, disrupting ion gradients and compromising the cell's shape. Additionally, low ATP levels hinder the RBCs' ability to neutralize reactive oxygen species (ROS), making them more susceptible to oxidative damage.
- This condition leads to hemolytic anemia, with severity determined by the specific type of genetic mutation.

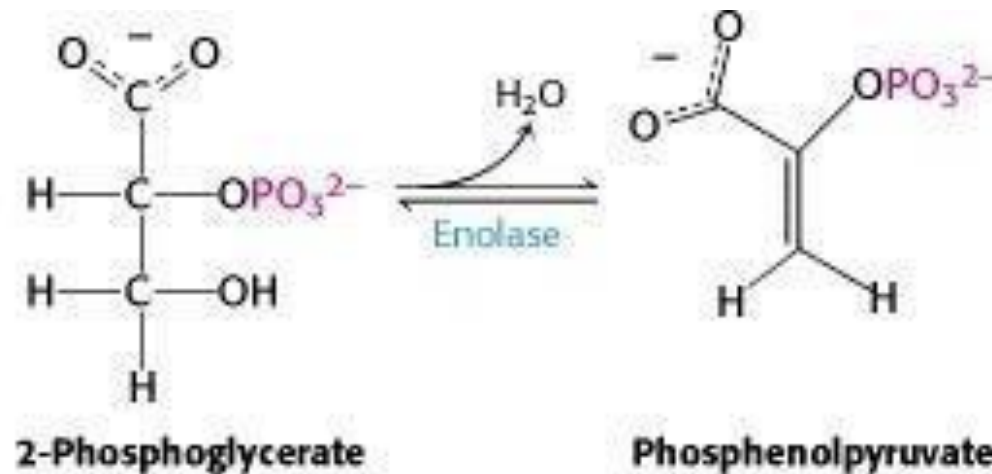
External (non-physiological) Inhibitors of Glycolysis

Inorganic Inhibitors of Glycolysis, non-physiologic

Fluoride

- Fluoride inhibits **bacterial's Enolase**

When enolase is inhibited, bacterial cells are unable to generate energy effectively through glycolysis. This energy deprivation can render the bacteria inert or even lead to cell death.



Fluoridated water → ↓ bacterial enolase →
Prevention of Dental Carries

Inorganic Inhibitors of Glycolysis, non-physiologic

Arsenic Poisoning:

- Pentavalent Arsenic (Arsenate) competes with phosphate as a substrate for GA3PDH

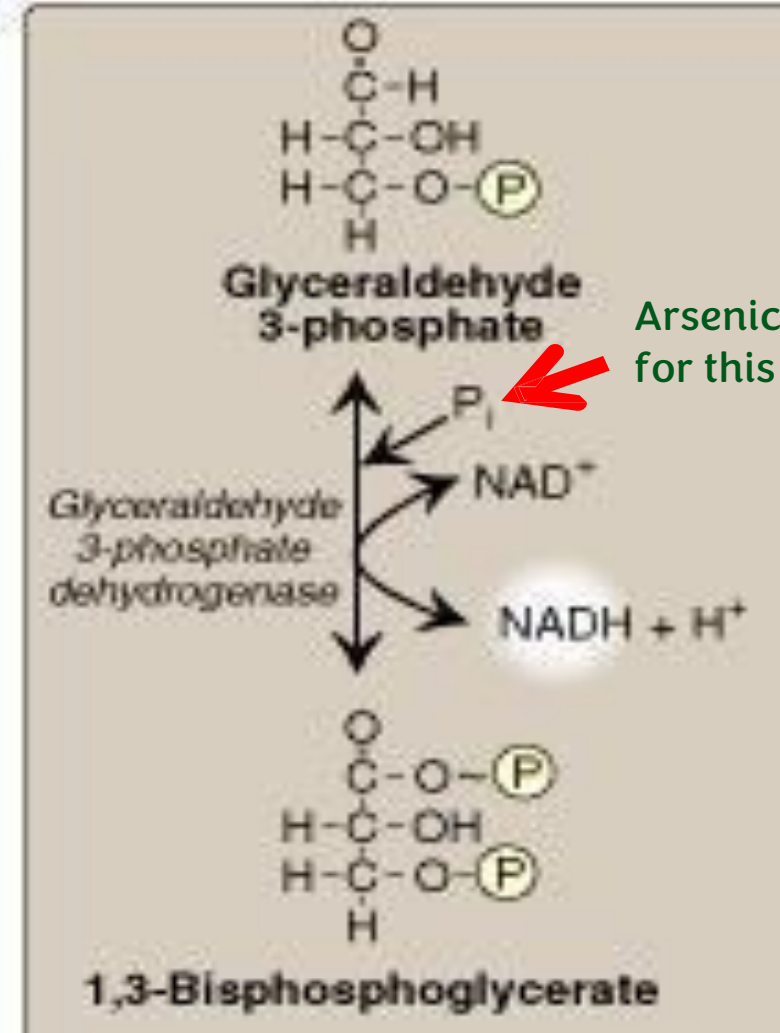
↓ ATP synthesis

- Trivalent Arsenic (Arsenite) Forms stable complex with-SH of lipoic acid

↓ Pyruvate Dehydrogenase

↓ α ketoglutarate Dehydrogenase

→ Neurological disturbances.....**DEATH**



Explanation of the previous slide

- Arsenate competes with inorganic phosphate during glycolysis, thereby reducing ATP synthesis.
- In contrast, arsenite does not directly affect glycolytic enzymes; rather, it inhibits pyruvate dehydrogenase by forming a stable complex with lipoic acid, a necessary cofactor for the enzyme. This inhibition disrupts the conversion of pyruvate to acetyl-CoA, significantly affecting energy metabolism.
- Arsenite is more harmful than arsenate because the body can obtain pyruvate through other metabolic pathways, but the disruption of pyruvate dehydrogenase has widespread effects.
- Arsenic exposure can occur through contaminated seafood and industrial processes.

Extra table to sum up:

Regulated Enzyme	Regulatory Mechanism
Glucokinase and Hexokinase	Regulation through substrate concentration (glucose) and enzyme compartmentalization Enzyme activity is increased by glucose availability
Glucokinase Regulation	Presence of glucokinase regulatory protein (GKRP) Sequestration in the nucleus when glucose levels are low <u>Both decrease glucokinase activity</u>
Phosphofructokinase-1 (PFK-1)	Allosteric regulation by ATP (inhibitor) and AMP (activator) Regulation by citrate (inhibitor)
Fructose 2,6-bisphosphate	Hormonal regulation via insulin (activator) and glucagon (inhibitor)
Pyruvate Kinase	Allosteric inhibition by ATP and alanine Hormonal regulation: Insulin activates * Glucagon inhibits
Hormonal Regulation	Insulin stimulates glycolysis Glucagon inhibits glycolysis
External Inhibitors	Fluoride: Inhibits enolase Arsenic poisoning: Inhibits enzymes by binding to active sites

For any feedback, scan the code or click on



Corrections from previous versions:

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

Additional Resources:

رسالة من الفريق العلمي:

Chapter 8: Lippincott's
illustrated reviews 7th
edition

اللهم انا نستودعك اهلنا في فلسطين والسودان، اللهم
ارحمهم وانصرهم وكن لهم معيناً ونصيراً.
واخر دعوانا أن الحمد لله رب العالمين.

