Gluconeogenesis

- Color Index:

- Important.
- Extra Information.
- **Doctors slides.**

Objectives:

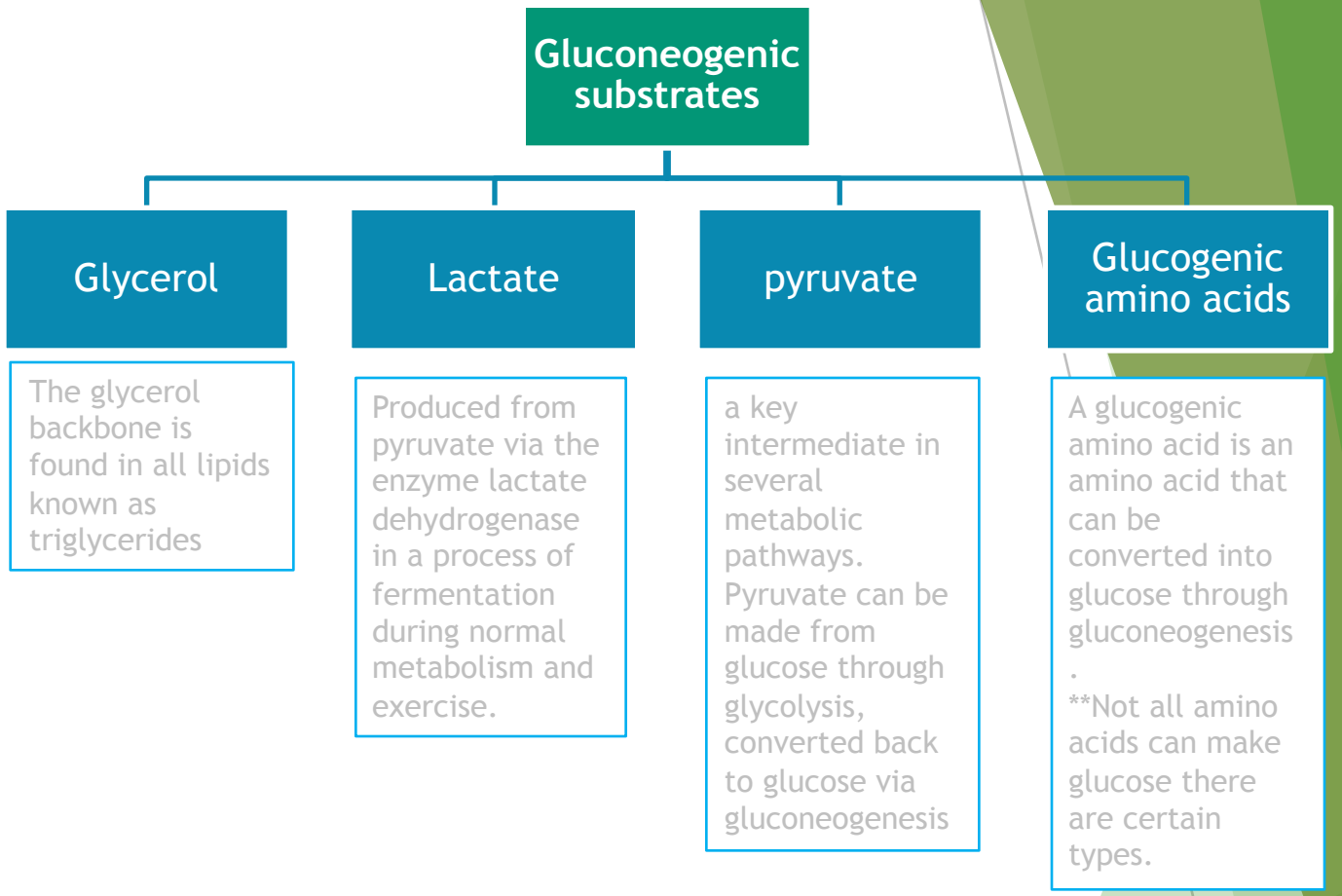
- The importance of gluconeogenesis as an important pathway for glucose production
- The main reactions of gluconeogenesis
- The rate-limiting enzymes of gluconeogenesis
- Gluconeogensis is an energy-consuming, anabolic pathway

Gluconeogenesis

- Gluconeogenesis is an energy consuming, anabolic pathway.
- It is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates .
- Gluconeogenesis is important to provide the body with glucose when there is no external source of glucose (during prolonged fasting or starvation) .
- Occurs in Liver mainly, and in Kidney
- **During Overnight fast:**
  - 90% of gluconeogenesis occurs in liver
  - 10% of gluconeogenesis occurs in Kidneys .
- Gluconeogenesis occurs in both mitochondria and cytosol .

EXCEPTION! if gluconeogenesis starts by Glycerol, it will need only the cytosol

Our bodies will metabolize all glycogen stores within the first 18 hours of fasting, then will start looking for another source to make glucose



Gluconeogenesis

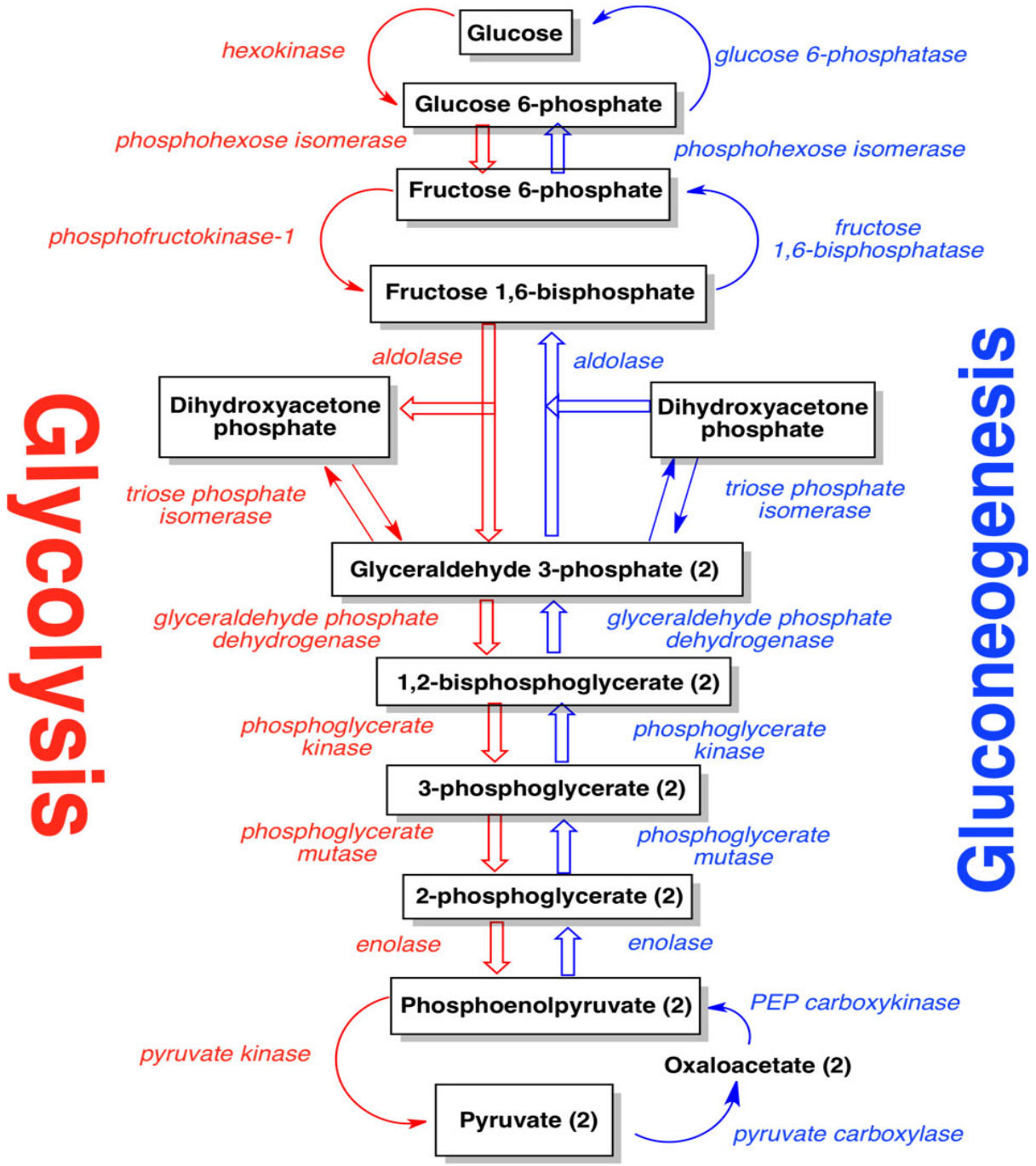
- Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate.
- Three of the reactions are irreversible and must be reversed by four alternate reactions that energetically favor the synthesis of glucose .

The 4 alternate reactions in gluconeogenesis to the 3 irreversible glycolytic steps:

Glycolysis enzymes	Gluconeogenesis enzymes
Pyruvate kinase	1) Pyruvate carboxylase 2) PEP-CK
PFK-1	3) Fructose 1,6 bisphosphatase
Glucokinase/Hexokinase	4) Glucose 6-phosphatase

موضحة في الصورة باللون الأحمر

موضحة في الصورة باللون الأزرق



1. Glycerol:

Glycerol is released during the hydrolysis of triacylglycerol in adipose tissue and is delivered by the blood to the liver.

Gluconeogenic precursors are (non-carbohydrates) molecules that can be used to produce a net synthesis of glucose.

#Recall

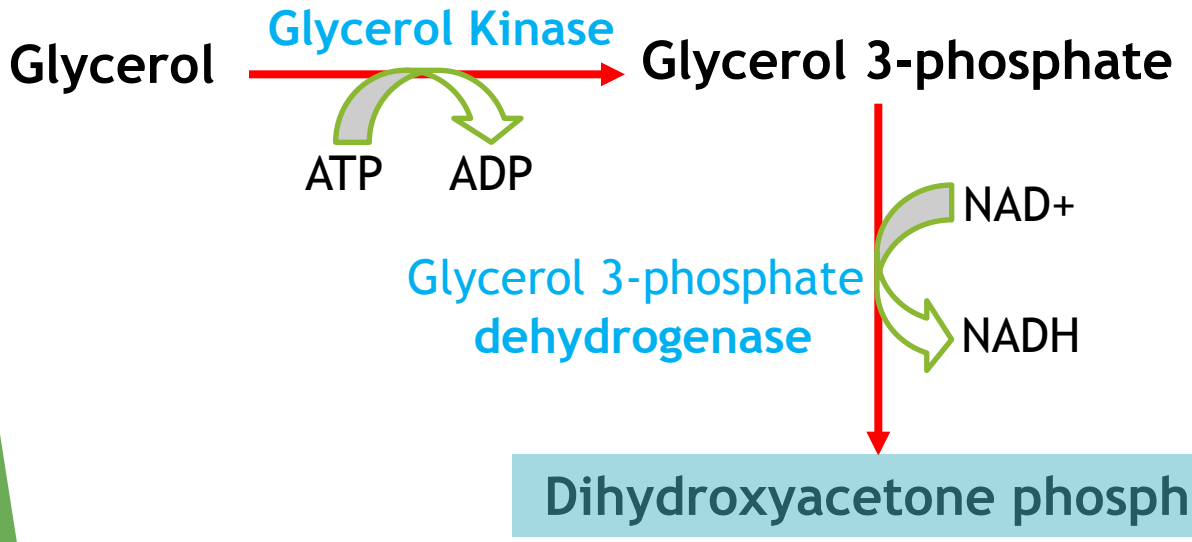
Gluconeogenic Substrates:

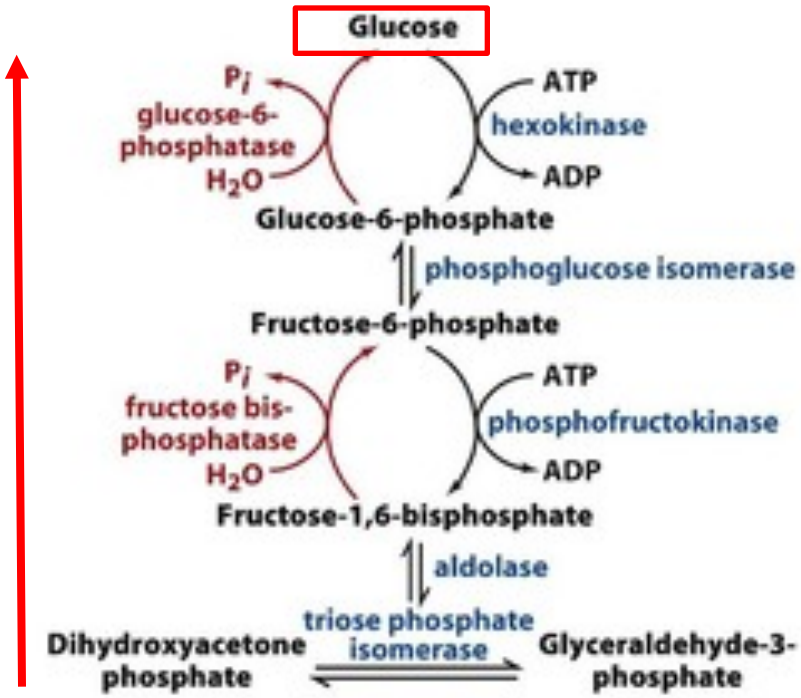
- Glycerol
- Lactate
- Glucogenic amino acids
- Pyruvate

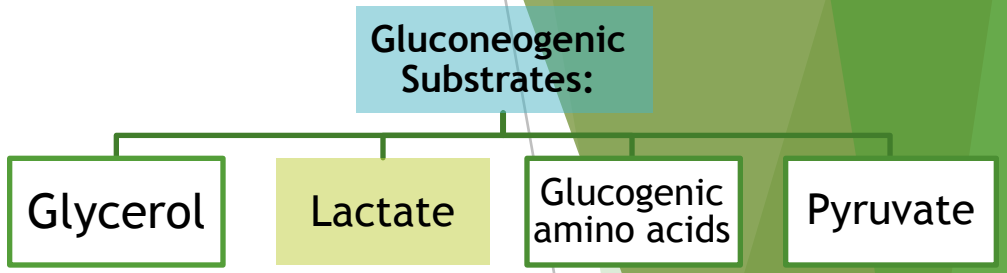
Important notes:

- Glycerol kinase is only present in liver & kidneys
- Gluconeogenesis of glycerol occurs only in the cytosol

Why can't adipocytes phosphorylate glycerol? Because they essentially **lack glycerol kinase.**



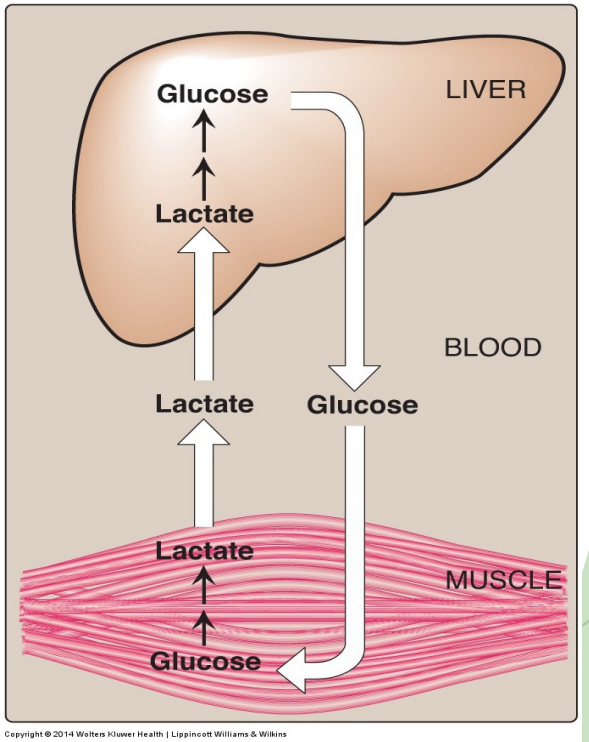


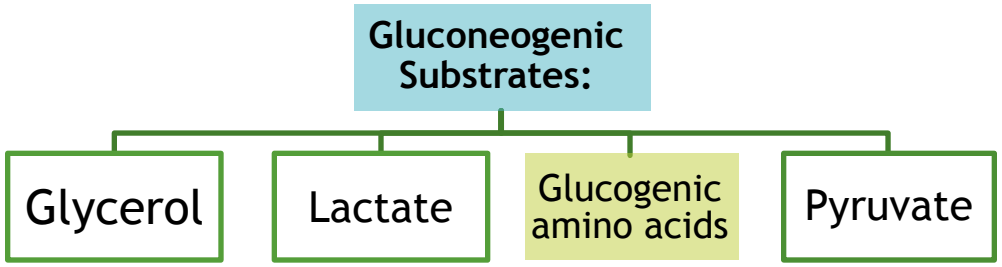


2. Lactate:

Lactate is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBCs. In the Cori cycle, bloodborne glucose is converted by exercising muscle to lactate, which diffuses into the blood. The lactate is taken up by the liver and reconverted to glucose, which is released back into circulation.

*Lactate is converted to pyruvate and then to glucose.





*The amino acids in the red boxes must be MEMORIZED along with the intermediates they produce

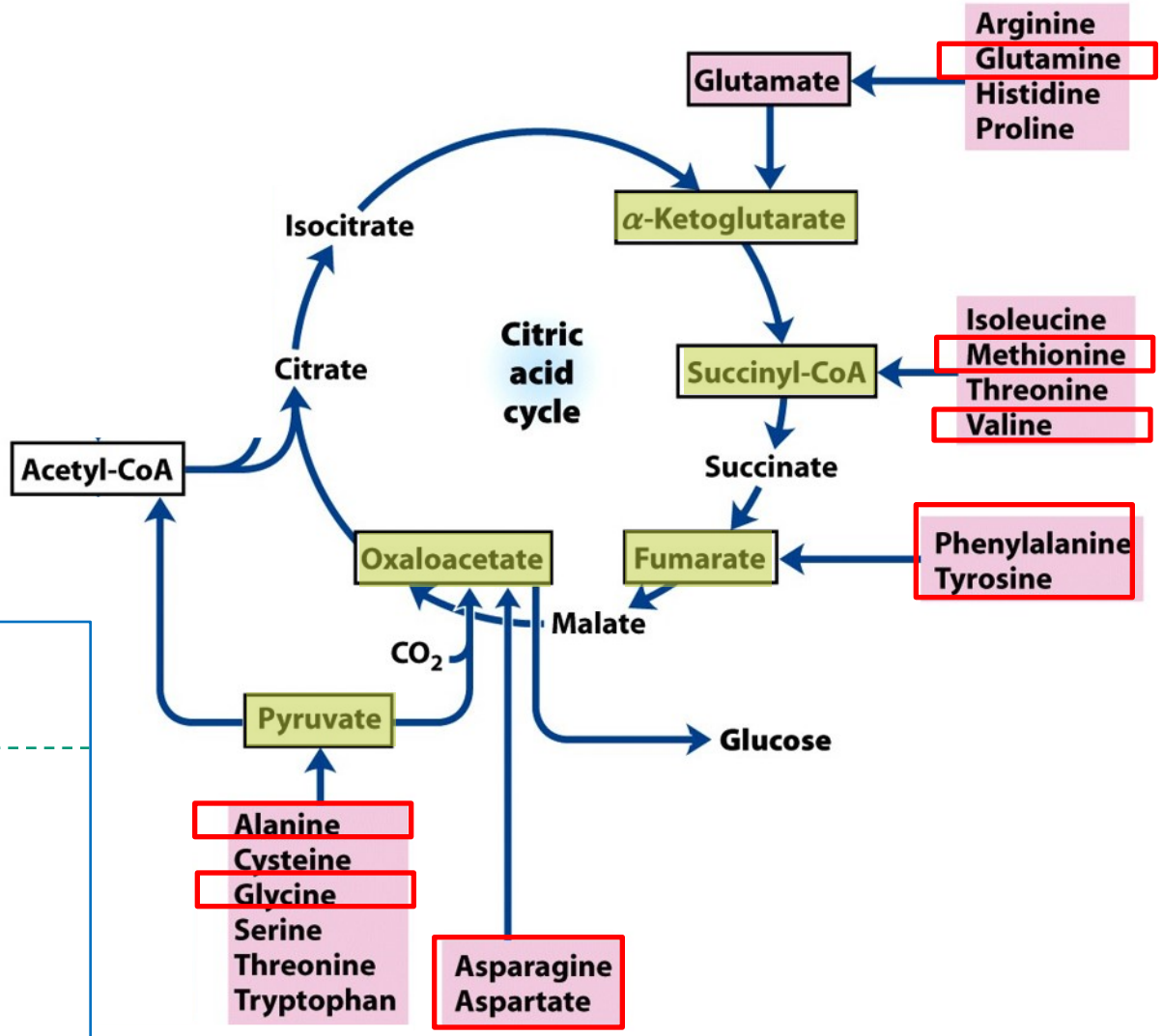
3. Glucogenic Amino Acids:

The catabolism of glucogenic amino acids produces either:

- **pyruvate**
- Or one of the **intermediates in the Krebs Cycle.**

For example; catabolizing asparagine produces oxaloacetate (an intermediate) which can be converted later to glucose.

Pyruvate: Alanine, Glycine	
Intermediates:	**Glucogenic Amino Acid:**
Oxaloacetate	Asparagine, Aspartate.
Fumarate	Phenylalanine, Tryosine.
Succinyl-CoA	Methionine, Valine.
a-ketoglutarate	Glutamine (after it's converted to **glutamate**.)



Pyruvate Carboxylation

- The carboxylation occurs in the **liver** and **kidney**, exactly in Mitochondria .
- So Pyruvate has to travel from cytoplasm to mitochondria why ?
- Because Pyruvate carboxylase is only found in matrix of mitochondria .

Pyruvate carboxylase: is an enzyme of the ligase class that catalyzes (depending on the species) the physiologically irreversible carboxylation of pyruvate to form oxaloacetate (OAA).

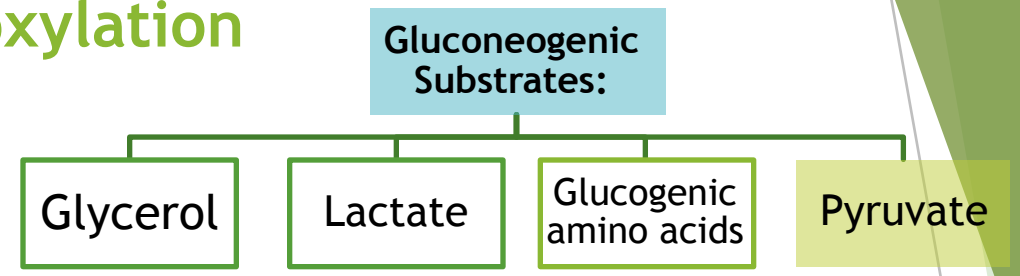
Biton: coenzyme that makes CO2 more active to bind.

To compare ☺

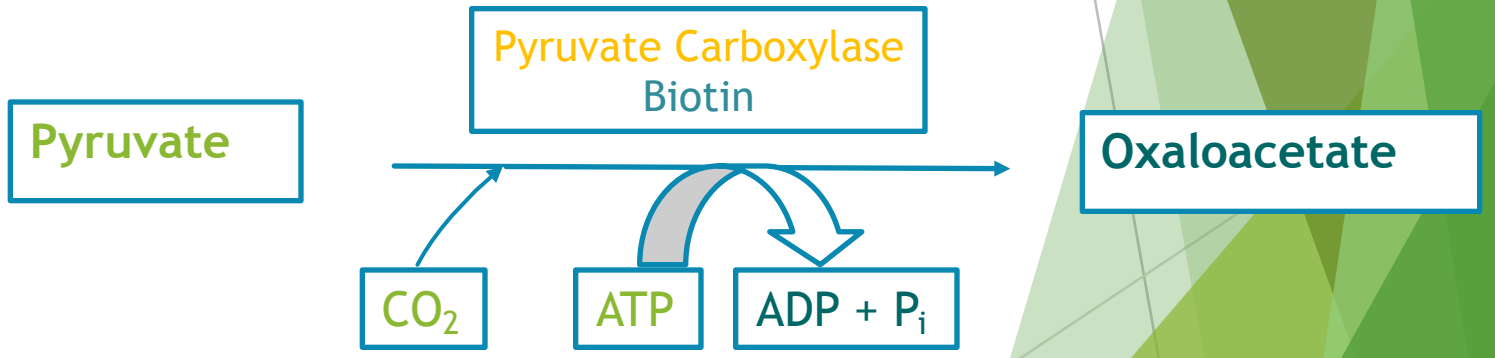
In glycolysis:
to convert from PEP into Pyruvate we need just one enzyme which is (Pyruvate Kinase) .

In gluconeogenesis:
to convert Pyruvate into PEP we need two enzymes in two steps these enzymes are (Pyruvate carboxylase + PEP-CK)

Explained in next slide



General Idea ☺
Substrate: Pyruvate
Chemical Reaction: Carboxylation
Enzyme: Pyruvate Carboxylase
Co-Enzyme: Biotin
Product: Oxaloacetate *Intermediate*



Explanation for the two steps

الحين زي ما قلنا Pyruvate لازم يدخل الميتوكندريا ليش؟ عشان هناك راح يلقى الإنزيم Pyruvate Carboxylase اللي راح يحوله إلى Oxaloacetate ، **طيب المشكلة إن هذا Oxaloacetate ما يقدر يطلع من الميتوكندريا بصورته هذي** والحل طيب ؟ فيه إنزيم ثاني اسمه

Malate dehydrogenase

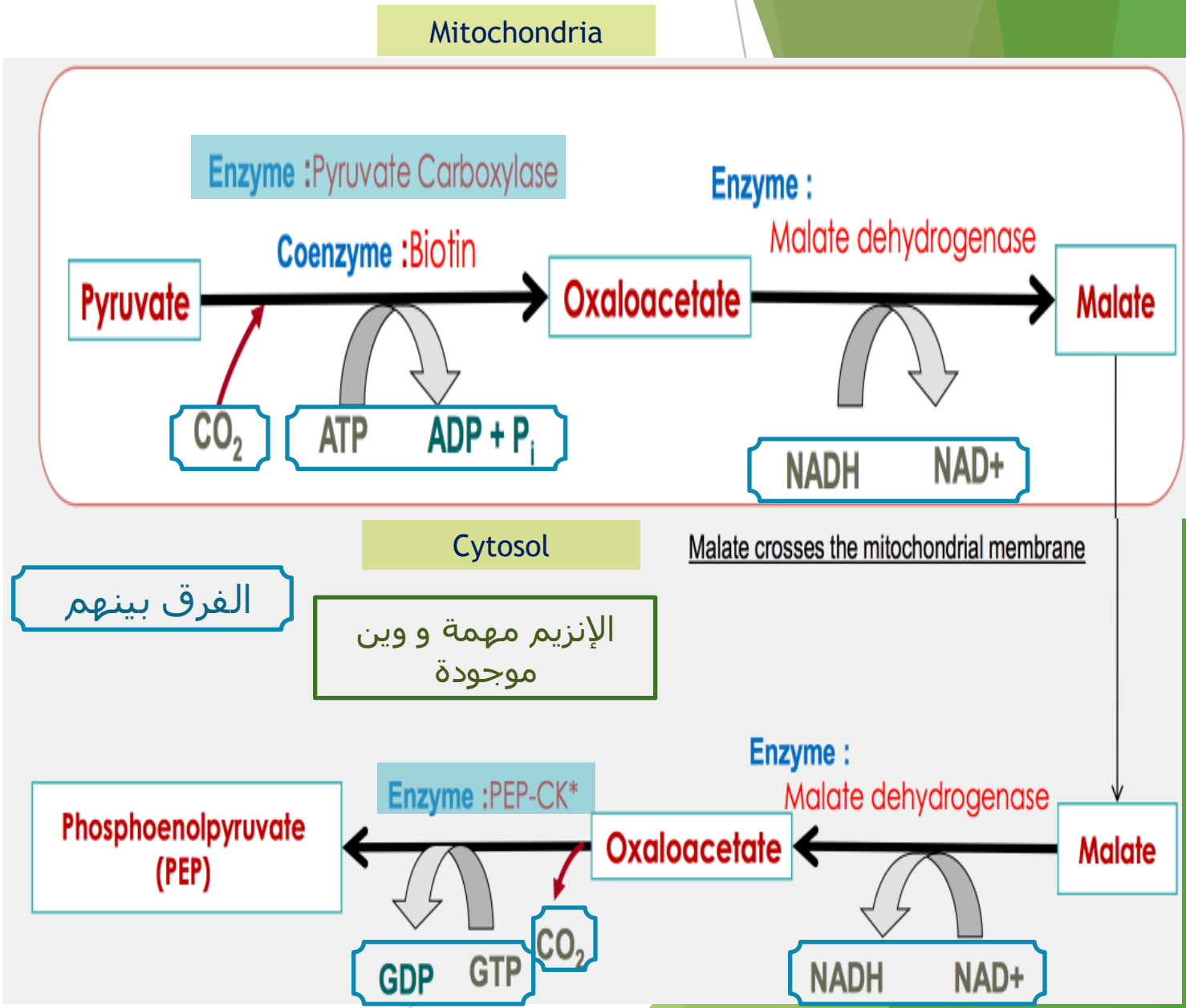
راح يحوله بشكل مؤقت إلى Malate بالاختزال نفس الإنزيم اللي كان موجود بكربس سايكل ← هذي كلها الخطوة الأولى

الحين هذا Malate يقدر يطلع من الميتوكندريا ويروح للسيتوبلازم، ليش ؟ لأن الهدف من هذا كله هو إني أكون جلكوز وأنزله على الدم ويروح للخلايا الثانية تستخدمه وتنتج طاقة ، طيب في السيتوبلازم يصير له أكسدة بنفس الإنزيم

Malate dehydrogenase

ويرجع يتحول إلى Oxaloacetate وهذا اللي نبغاه ليش ؟ لأن **فيه إنزيم مهم PEP–CK راح يحوله أخيراً إلى PEP** اللي بدوره بيكمل السالفة ← هذي هي الخطوة الثانية

Phosphoenolpyruvate CarboxyKinase (PEP-CK)



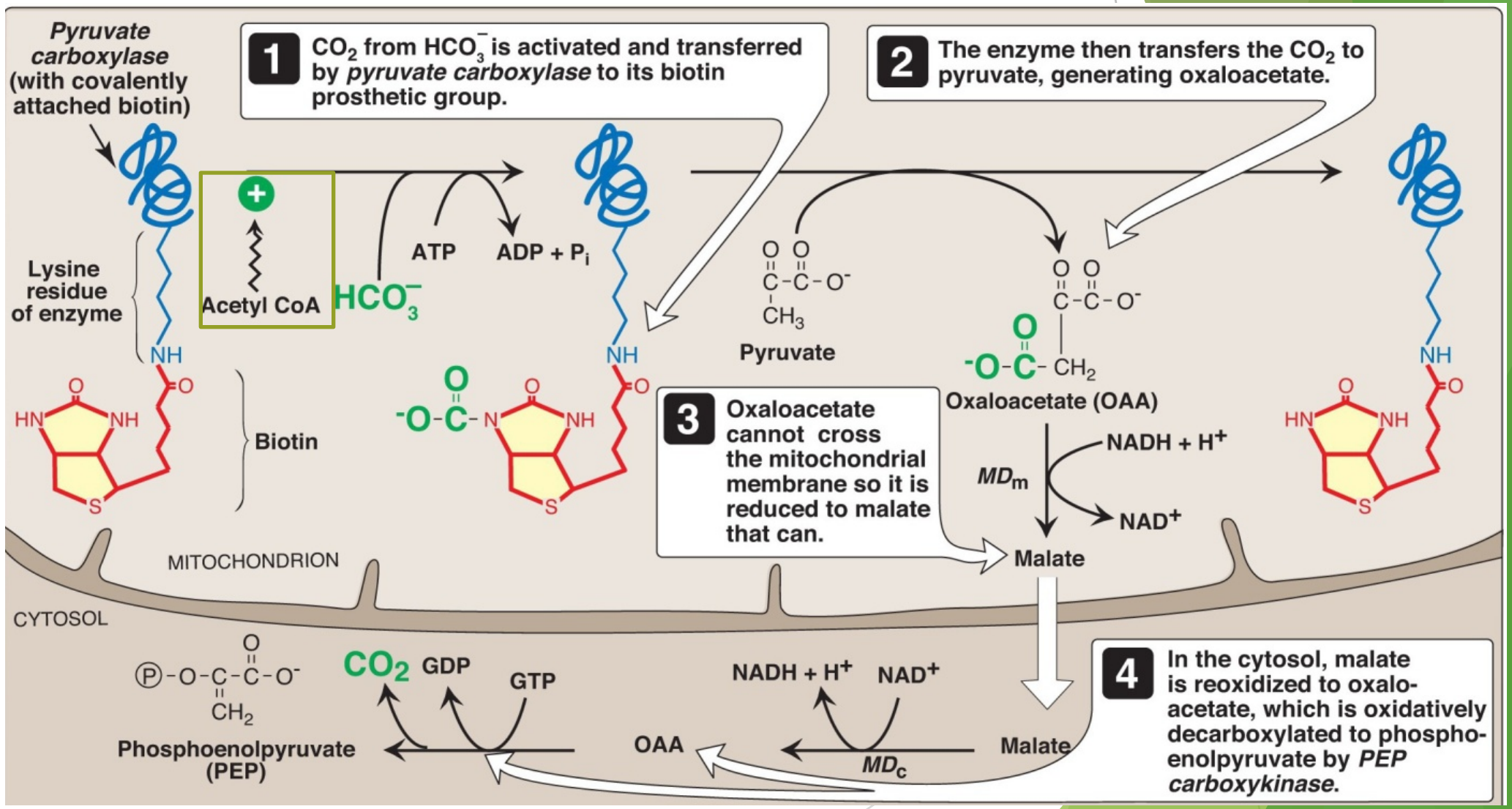
Pruvate Carboxylase and PEP-CK

Pyruvate carboxylase + PEP-CK = Pyruvate kinase

- ***Pyruvate Carboxylase is induced by Acetyl-CoA***

meaning that Acetyl-CoA is an activator for the pyruvate carboxylation reaction

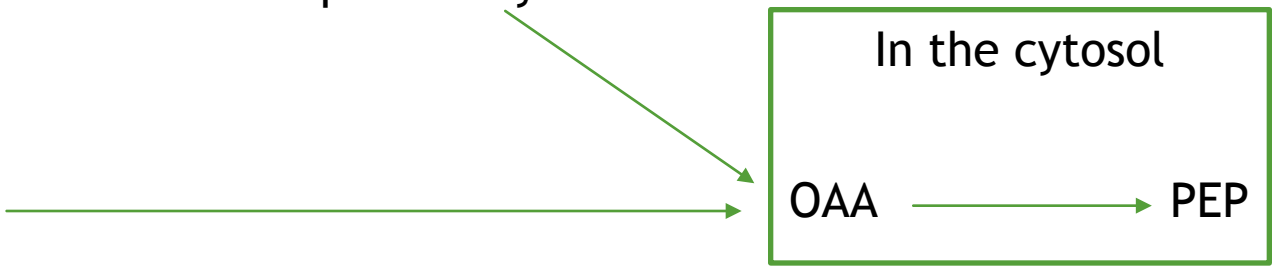
- ***What happens during fasting?***
- **Acetyl-CoA is increased via fatty acid oxidation (FAO)**



Mentioned in Boys' Lecture Only

There are 3 ways for Oxaloacetate (OAA) to transport across the mitochondrial membrane:

- OAA reduction to malate "as we mentioned previously"
- OAA conversion to aspartate
- OAA conversion to PEP (inside the mitochondria)



Gluconeogenesis Regulation:

Overview:*EXTRA EXPLANATION* While most steps in gluconeogenesis are the reverse of those found in glycolysis, the 3 regulated and endergonic reactions in glycolysis are replaced.

Glycolysis: Hexokinase/glucokinase, phosphofructokinase, and pyruvate kinase enzymes
Gluconeogenesis:

- PEP carboxykinase/pyruvate carboxylase.
- Fructose-1,6-bisphosphatase
- Glucose-6-phosphatase

These enzymes are typically regulated by similar molecules, but with opposite results.
For example, acetyl CoA and citrate **activate** gluconeogenesis enzymes, while at the same time **inhibiting** the glycolytic enzyme pyruvate kinase.
This system: reciprocal control

The majority of the enzymes responsible for gluconeogenesis are found in the cytosol; the exception is mitochondrial pyruvate carboxylase.

Global control of gluconeogenesis is mediated by glucagon (released when blood glucose is low); Glucagon triggers phosphorylation of enzymes and regulatory proteins by Protein Kinase A (a cyclic AMP regulated kinase) resulting in inhibition of glycolysis and stimulation of gluconeogenesis.

Regulation of Pyruvate Carboxylase reaction

Acetyl CoA diverts pyruvate away from oxidation in Krebs cycle and toward gluconeogenesis

How? High level of Acetyl-CoA will inhibit PDH complex and stop or reduce the Glycolysi.
And stimulate Pyruvate Carboxylase to start Gluconeogenesis.

+ positive regulation

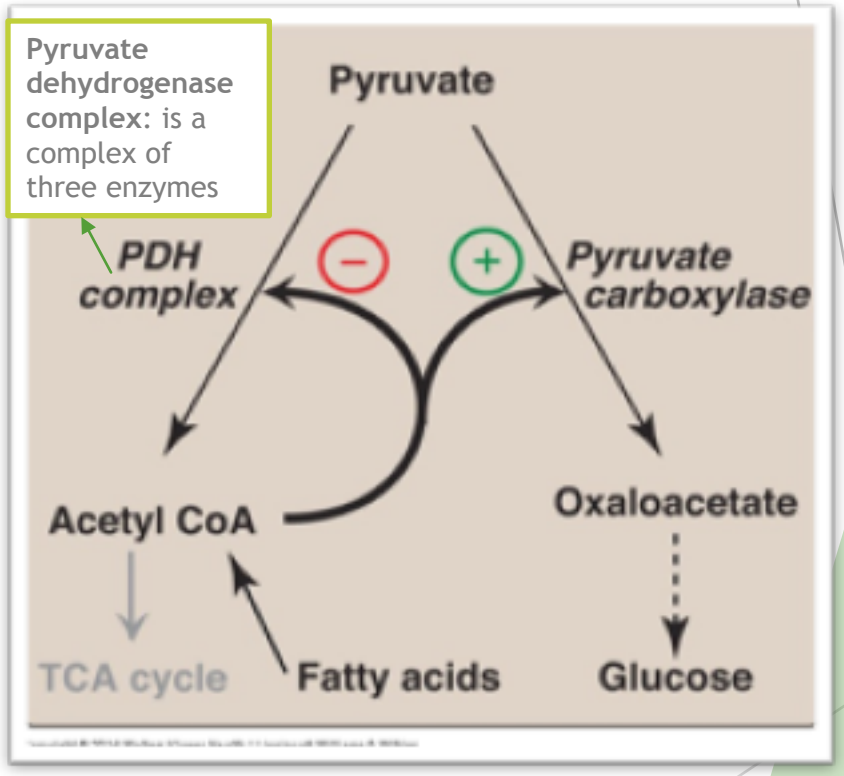
High Acetyl coA will **stimulate** the enzyme pyruvate carboxylase to make more oxaloacetate

Then, the oxaloacetate will produce more glucose

-negative regulation

High level of Avetyl-coA **inhibit** PDH complex.

PDH function: converts pyruvate carboxylase to Acetyl coA



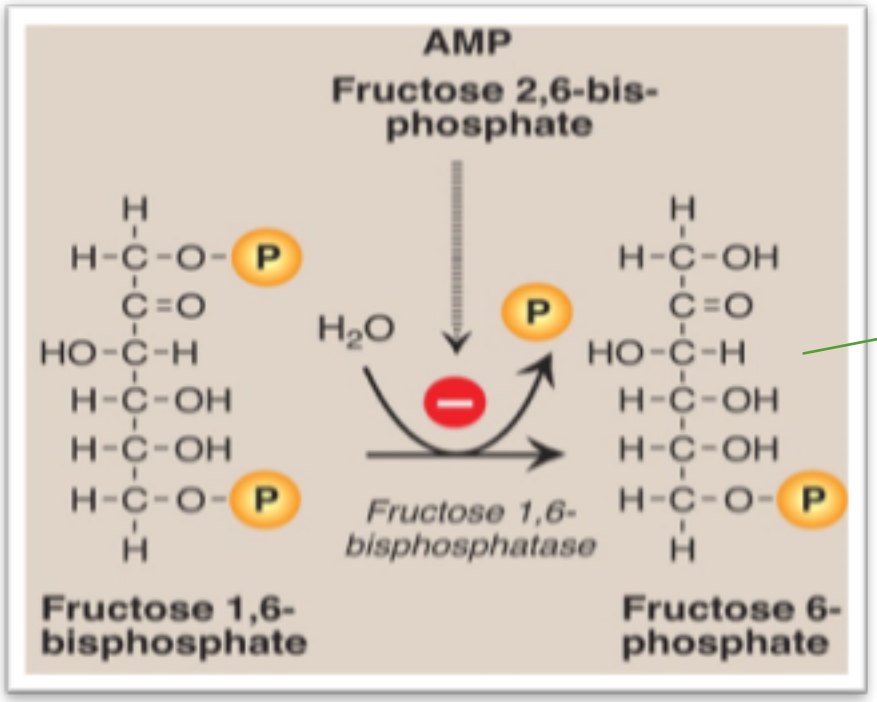
Negative regulation: inhibition
Positive regulation: activation

NOTE ; **Acetyl CoA diverts pyruvate away from oxidation (by PDH complex to produce acetyl coA) and toward gluconeogenesis**

Fructose 1,6-Bisphosphatase

The rate of gluconeogenesis is controlled by key enzyme: fructose-1,6-bisphosphatase

- **Fructose 1,6 bisphosphatase enzyme** dephosphorylates (removes P group) Fructose 1,6 Bi-phosphate. Which turns it into Fructose 6-Phosphate.
- **(- negative regulation)** What inhibits this process?
- **AMP** and **Fructose 2,6 bisphosphate**

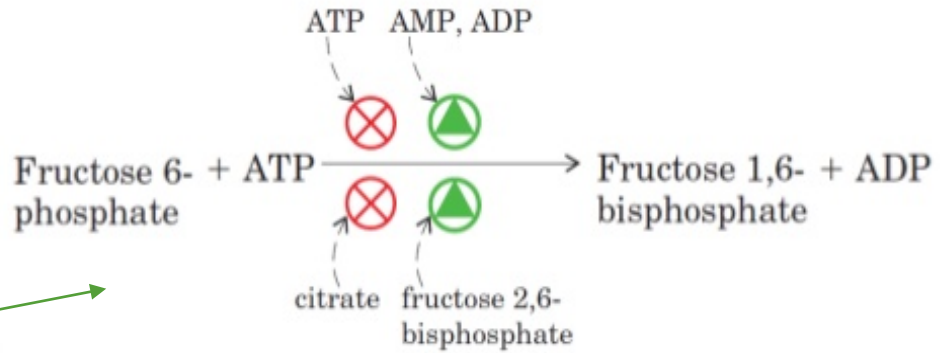


The reverse reaction

Allosteric regulation

The precise regulation of PFK1 prevents glycolysis and gluconeogenesis from occurring simultaneously.

- **Phosphofructokinase-1 (PFK-1)**
  - Enzyme has several regulatory sites at which allosteric activators or inhibitors bind
  - ATP inhibits PFK-1 by binding to an allosteric site and lowering the affinity of the enzyme for fructose 6-phosphate
  - ADP and AMP act allosterically to relieve this inhibition by ATP.
  - High citrate concentration increases the inhibitory effect of ATP.
  - Thus glycolysis is down regulated when enough ATP is present in cells.



We took this in glycolysis: Phosphofructokinase-1 (PFK-1) is one of the most important regulatory enzymes of glycolysis. It is an allosteric enzyme controlled by many activators and inhibitors. PFK-1 catalyzes the important "committed" step of glycolysis, the conversion of fructose 6-phosphate and ATP to fructose 1,6-bisphosphate and ADP.

Fructose bisphosphatase (Gluconeogensis) catalyzes the reverse of the reaction which is catalyzed by phosphofructokinase-1 (glycolysis)

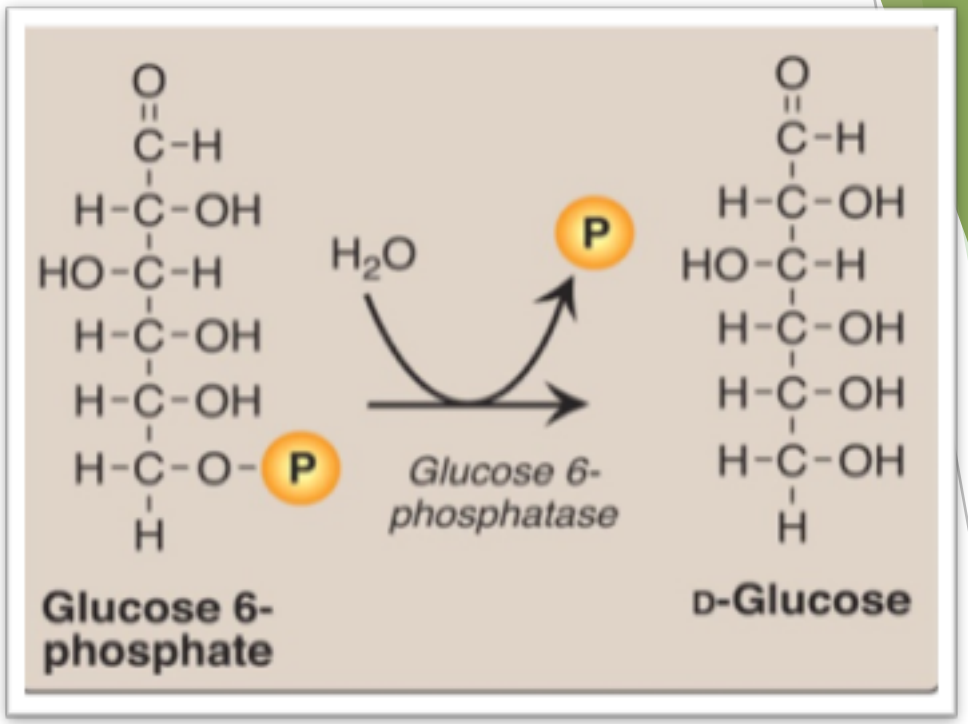
Glucose 6-Phosphatase

- Glucose 6- phosphate → D-Glucose

Done by Glucose 6-phosphatase enzyme (removal of phosphate group)

- Dephosphorylation of glucose 6-phosphate allows release of free glucose from the liver and kidney into blood.

(when glucose is phosphylated they can not go outside the cell
So. They need to Dephosphorylation to go from cell to circulation in blood to maintain blood sugar level)



Glucose 6-phosphatase ≠ Glucokinase

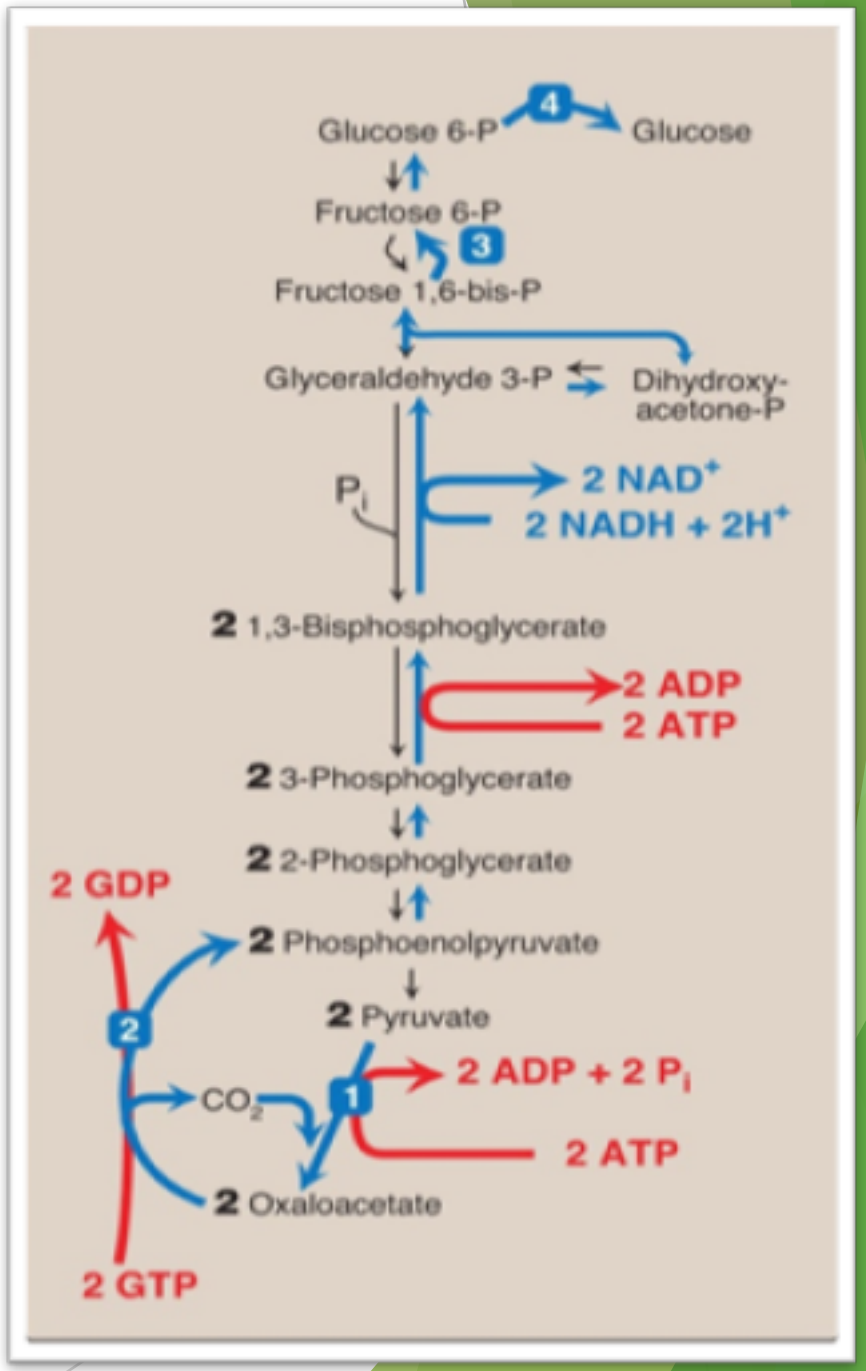
Glucokinase is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate.

Gluconeogensis:
Energy- Consumed

Six High-Energy Phosphate Bonds From Pyruvate to Glucose (From purvate to Glucose)

- 2Purvate convert to 2 Oxaloacetate -2 ATP
- 2GTP convert to 2GDP -2ATP
- 2 (3-Phosphoglycerate) convert to 2(1.3bisphosphoglycerate) -2ATP
- 2 NADH converted to 2 NAD 6ATP

NET : **0 ATP**



Gluconeogenesis: Regulation.

- **Reciprocal control**
  Gluconeogenesis & Glycolysis
- **Allosteric:**
  Acetyl CoA + (Pyruvate carboxylase)

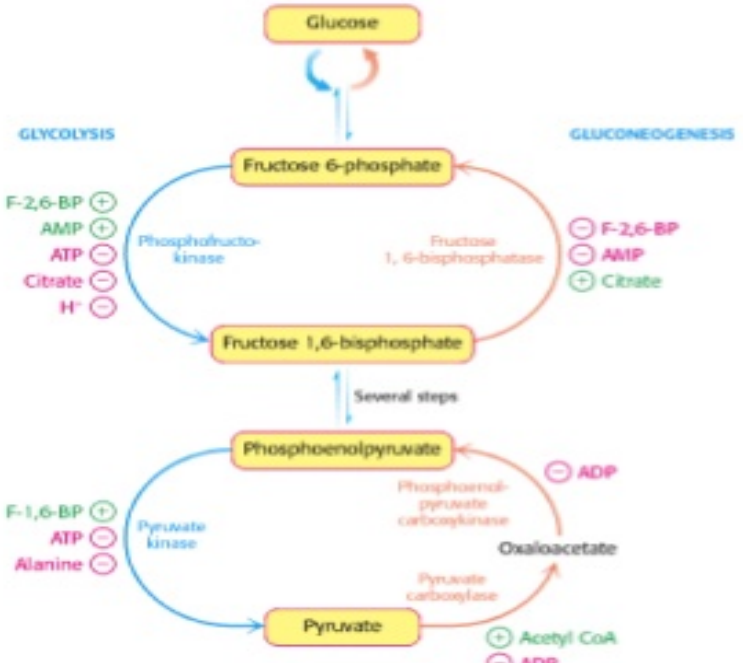
  AMP - or ATP +
  F 2,6-Bisphosphate -
  > F 1,6-bisphosphatase
- ↑**Glucagon (↓I/G ratio) stimulates gluconeogenesis**
  - Allosteric (↓ F 2,6-Bisphosphate)
  - Induction (PEP-CK)

(Insulin/Glucagon ratio) وش تعني؟
الجلوكاجون يهتم برفع مستوى الجلوكوز بالدم، بينما الأنسولين يسوي العكس..
فلذلك لتحفيز عملية (gluconeogenesis) نحتاج يكون المقام اكبر قيمة
"الجلوكاجون" من البسط "الأنسولين"..

Important!!!
Pyruvate carboxylase is only found in matrix of mitochondria

Gluconeogenesis rate-limiting enzymes:
- ❖ Pyruvate carboxylase
- ❖ PEP-CK

Reciprocal Regulation of Gluconeogenesis and Glycolysis in the Liver



- Glycolysis and Gluconeogenesis are reciprocally regulated .
- When glycolysis is on Gluconeogenesis is turned off especially in the fed state, whereas under conditions of starvation, gluconeogenesis is fully on and glycolysis is turned off.
- Both the cycles are never active at the same pace at the same time.

Reciprocal control

MCQs + videos

- The main site of gluconeogenesis is :

A- kidneys B- liver C- spleen D- lymph node

- Which one of the following is not a gluconeogenesis substrate?

A- lactate B- glycerol C- triglycerides D- glucogenic amino acid

- Gluconeogenesis and glycolysis are reciprocally uncontrolled

A- true B- false

- Anabolic pathway consumes ATP for synthesis of glucose

A- true B- false

Reactions of glycolysis : https://youtu.be/hDq1rhUkV-g

Regulation of gluconeogenesis and glycolysis : https://youtu.be/0epBvl0HTeY

1- B
2- C
3- B
4- A

- Girls team members:

1- سمية الغامدي.
2- شذا الغيهب.
3- روان سعد.
4-ربا السالم.
5- نورة الشبيب.
6- جومانا القحطاني.

- Boys team members:

1- محمد المهوس

-Team leaders:

نوره السهلي.
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