

Gluconeogenesis

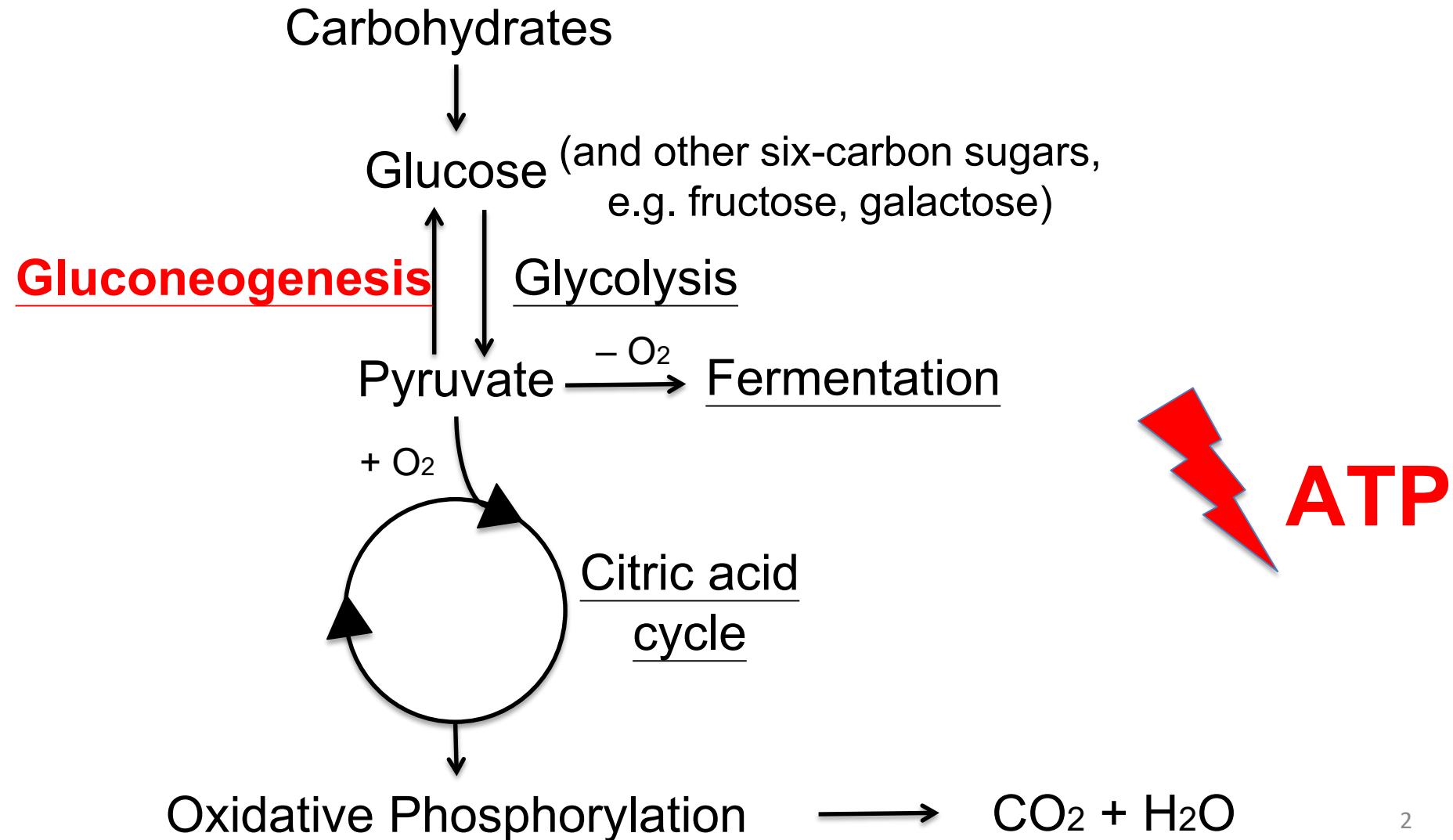
Liang Zhang

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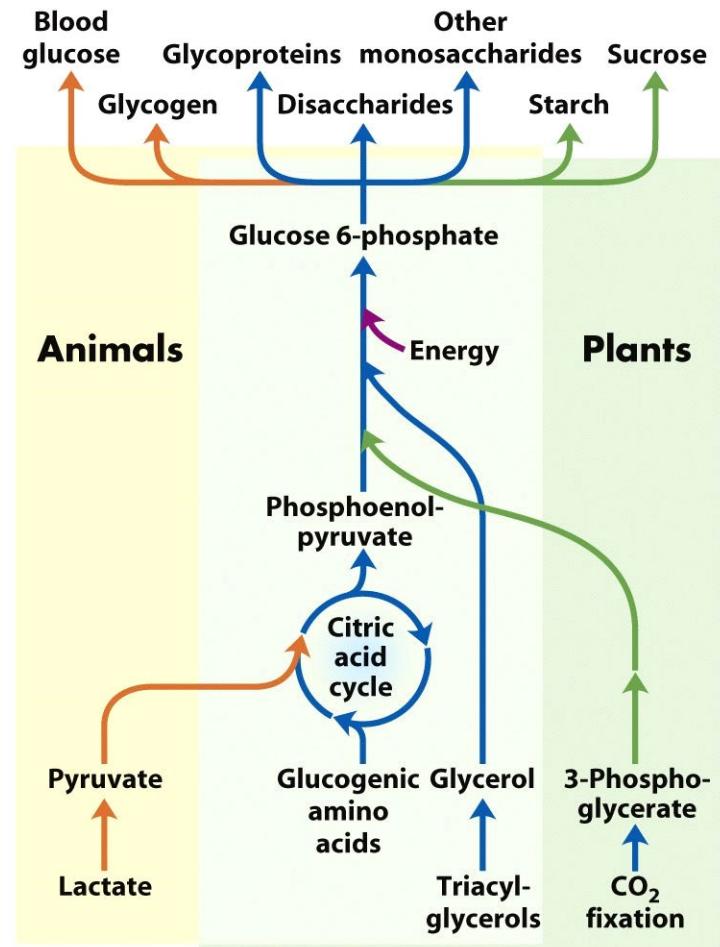
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Overview



Gluconeogenesis

- Gluconeogenesis = Glucose + new formation
- Gluconeogenesis is the process where glucose is made from pyruvate or related three- and four-carbon compounds.
- It is necessary when the supply of glucose from the glycogen stores in muscles and the liver is low. It happens between meals, fasting and after vigorous exercise.
- This process occurs in all animals, plants, fungi, and microorganisms.



Gluconeogenesis

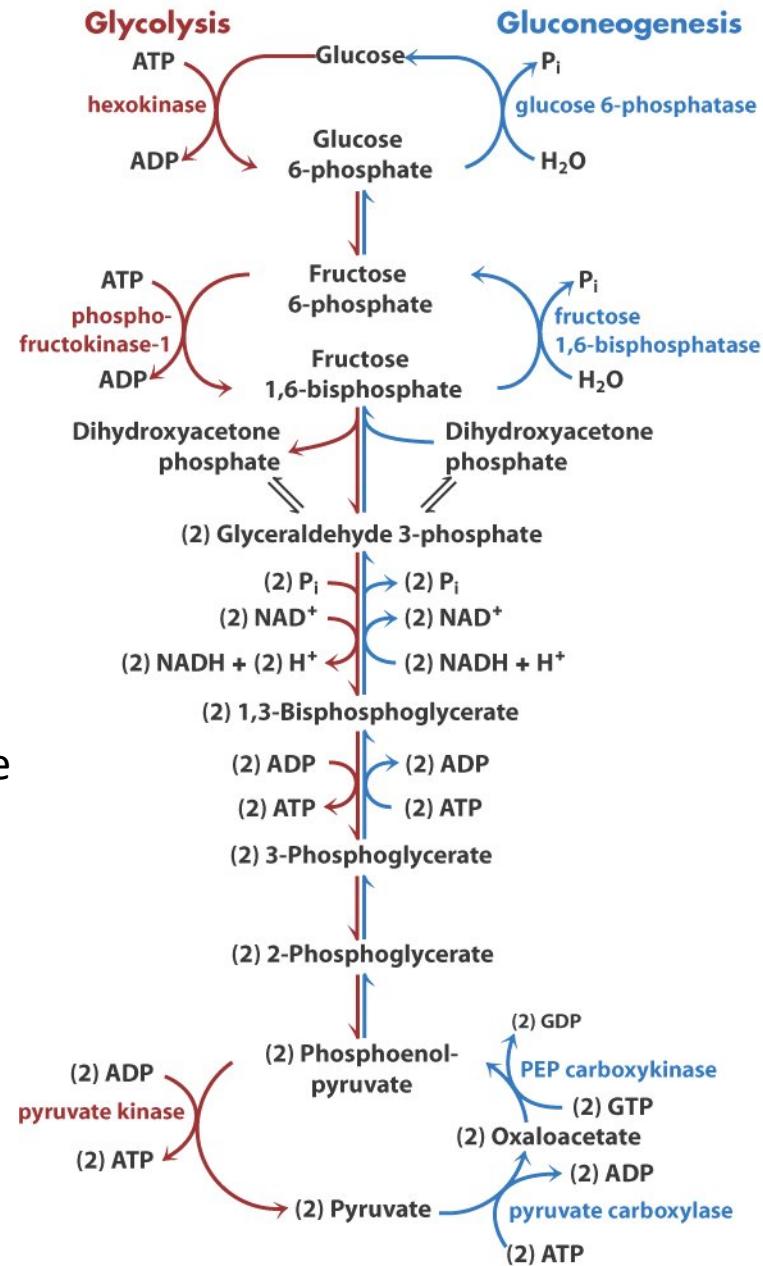
- The precursor molecules are mainly three-carbon compounds, such as lactate, pyruvate, glycerol, and some amino acids.
- It occurs in the liver, kidney and epithelial cells of small intestine.
- ***The Cori cycle*** -- lactate produced during vigorous exercise in muscle by anaerobic glycolysis returns to the liver, where it is converted back to glucose and further into glycogen.
- In plant **seedlings**, fats and proteins stored are converted to sucrose for transport throughout the developing plants.
- In microbes, simple organic compounds, such as acetate, lactate, and propionate in the growth medium are used to convert back to glucose.



Gluconeogenesis and glycolysis are similar but not identical

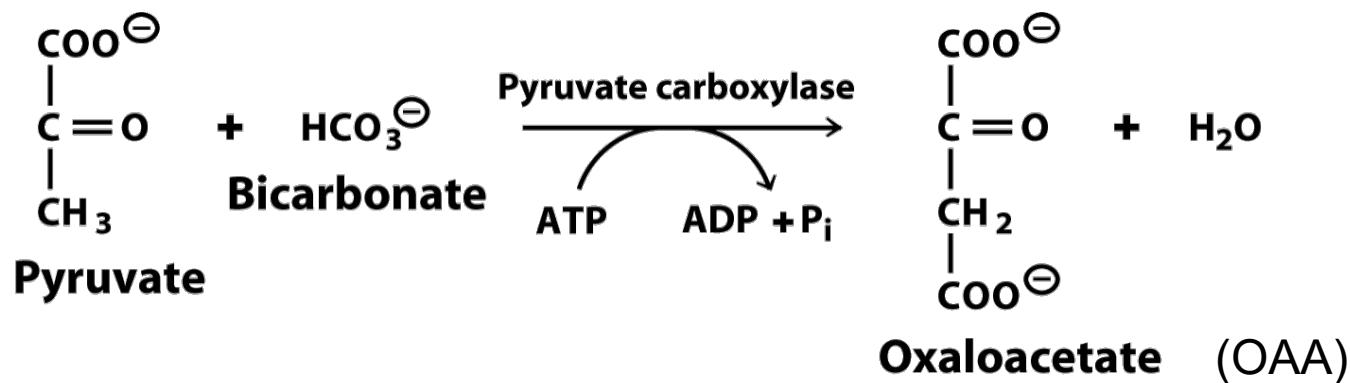
- The two processes are not identical pathways running in opposite directions.
- 7 out of 10 enzymatic reactions in gluconeogenesis are the reverse of glycolytic reactions.
- There are three reactions in glycolysis that are practically irreversible in vivo and thus cannot be used in gluconeogenesis.

Step	Reaction	Enzyme
1	$\text{Glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP} + \text{H}^+$	Hexokinase
3	$\text{Fructose 6-phosphate} + \text{ATP} \rightarrow \text{fructose 1,6-bisphosphate} + \text{ADP} + \text{H}^+$	Phosphofructokinase
10	$\text{Phosphoenolpyruvate} + \text{ADP} + \text{H}^+ \rightarrow \text{pyruvate} + \text{ATP}$	Pyruvate kinase



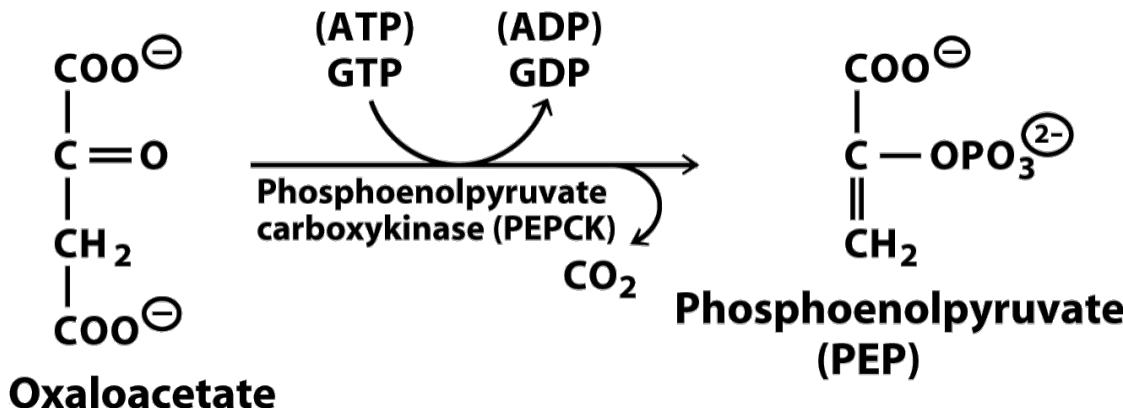
Carboxylation of pyruvate to form oxaloacetate

The first step in gluconeogenesis is the carboxylation of pyruvate to form oxaloacetate at the expense of a molecule of ATP. **This reaction take place inside the mitochondria.**



- Pyruvate carboxylase can be allosterically activated by acetyl CoA.
- Bicarbonate is formed when CO_2 dissolves in water.

Formation of phosphoenolpyruvate



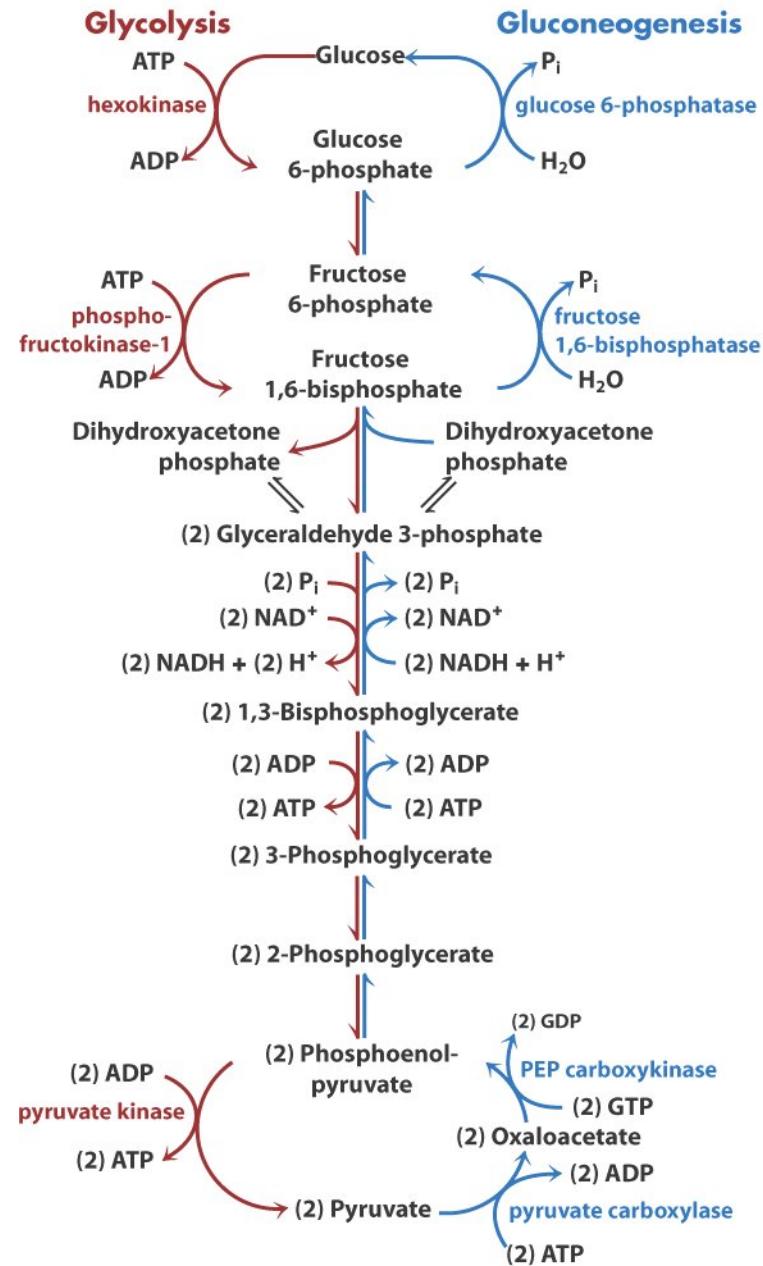
There are two different versions of PEPCK: the enzyme found in bacteria, protists, fungi and plants uses **ATP** as the phosphoryl group donor; the animal version uses **GTP**. This reaction also take place inside the mitochondria.

Regulation: a *decrease in blood glucose level* leads to an increased secretion of **glucagon** from pancreas. This leads to *increased transcription of the PEPCK gene* in the liver and *increased synthesis of PEPCK*. Insulin (abundant in the fed state) acts in opposition to glucagon at the level of the gene, reducing the rate of synthesis of PEPCK.

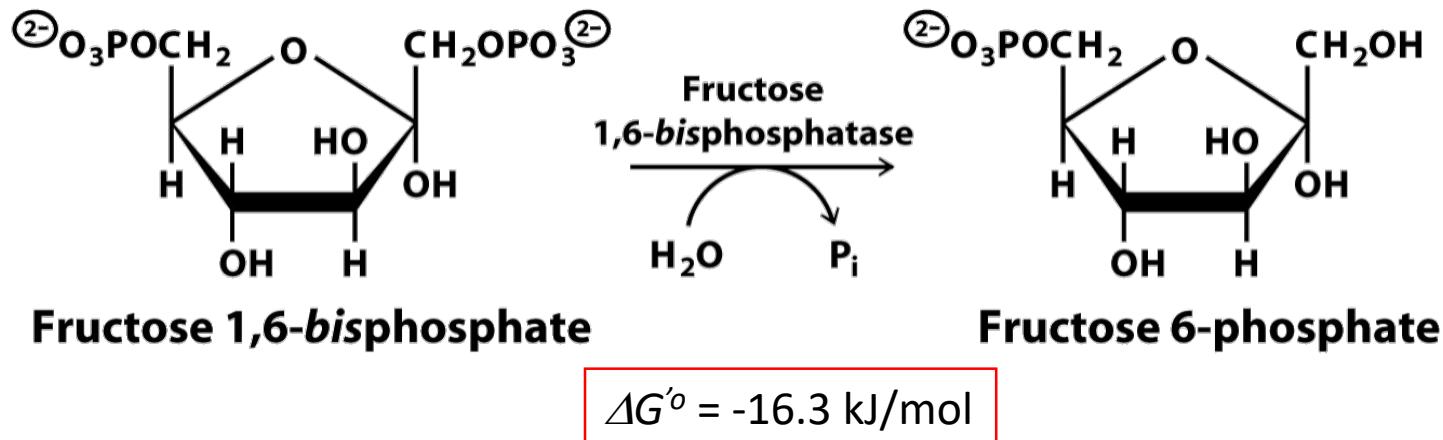
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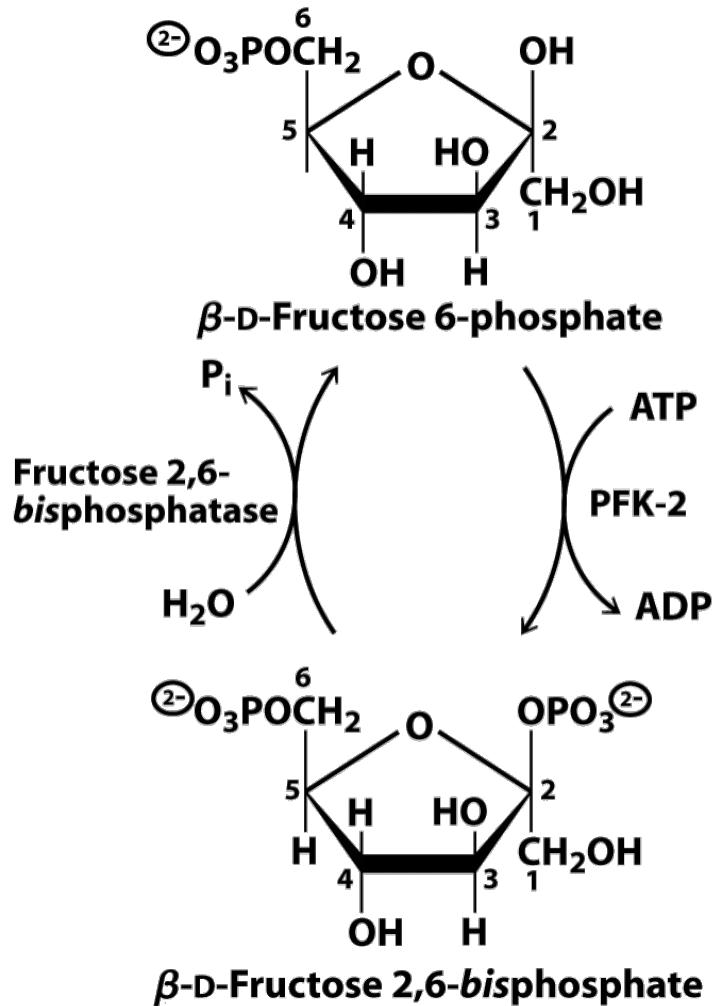
Fructose 1,6-bisphosphatase



Hydrolysis of the phosphate ester is associated with a large negative free energy, making this reaction **metabolically irreversible**.

The enzyme fructose 1,6-biphosphatase, is allosterically inhibited by AMP and fructose 2,6-biphosphate. Thus the interconversion of fructose 6-phosphate and fructose 1,6-biphosphate is reciprocally controlled by the [fructose 2,6-biphosphate].

Major regulator: fructose 2,6-bisphosphate



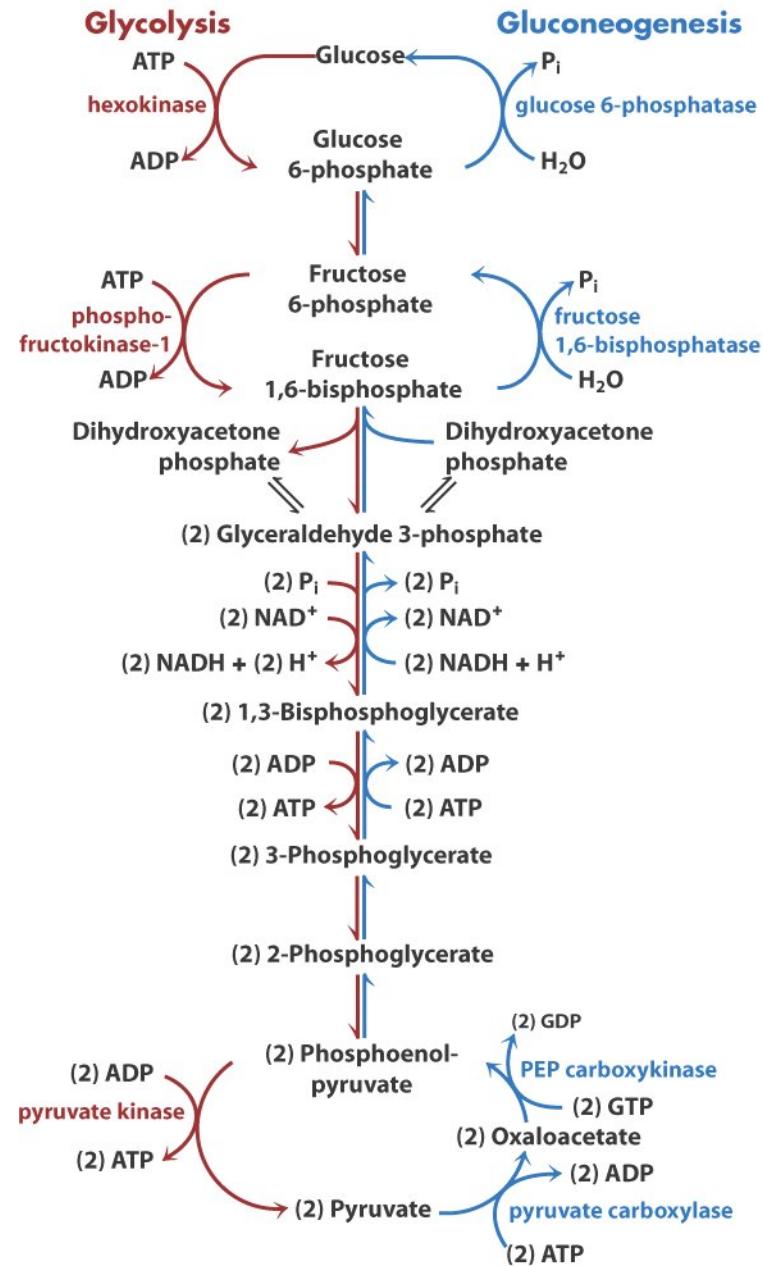
In the **liver**, the concentration of fructose 6-phosphate rises when blood-glucose concentration is high, and the abundance of fructose 6-phosphate accelerates the synthesis of F-2,6-BP (produced via hormone-induced [insulin] covalent modification of PFK-2).

Hence, *an abundance of fructose 6-phosphate leads to a higher concentration of F-2,6-BP, which in turn stimulates phosphofructokinase-1*. Such a process is called *feed-forward stimulation*.

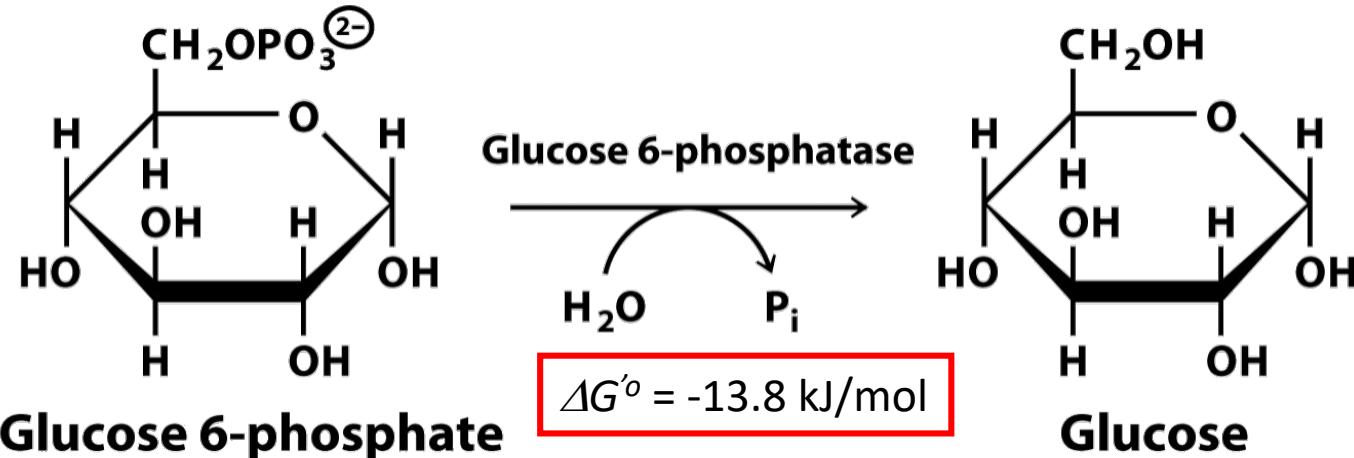
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Glucose 6-phosphatase



Again, the hydrolysis of the phosphate ester is associated with a large negative free energy, making this reaction metabolically irreversible.

Remark!!! In most cases, the biosynthesis pathway ends with glucose 6-phosphate, the activated form of glucose. Glucose 6-phosphatase is only found in cells from liver, kidney, and small intestine, so only these tissues can synthesize free glucose.

Comparing glycolysis and gluconeogenesis

- In gluconeogenesis, the three irreversible steps are catalyzed by a separate set of enzymes. These reactions are also sufficiently exergonic to be effectively irreversible to make glucose.
- Thus both glycolysis and gluconeogenesis are irreversible processes in the cells. In animals, both pathways occur largely in the cytosol, and allowing coordinated regulation of the two pathways.

Net equation of gluconeogenesis:

Consume 6 ATP equivalent!!!



Comparing glycolysis and gluconeogenesis

Gluconeogenesis



under intracellular condition

$$\Delta G = -16 \text{ kJ/mol}$$

Glycolysis



$$\Delta G = -63 \text{ kJ/mol}$$

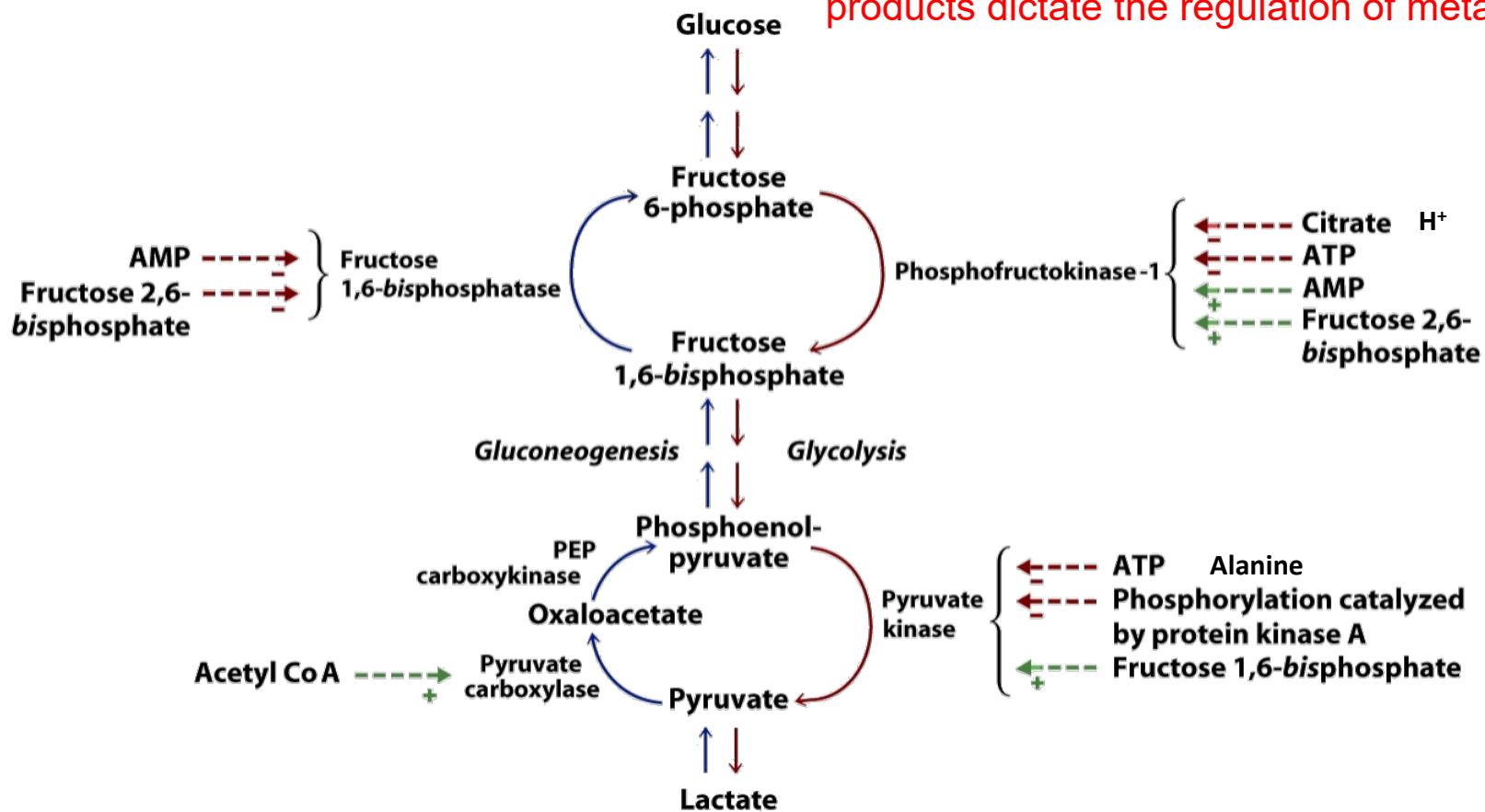
By comparing the two equations, gluconeogenesis is not just a reversal of glycolysis. Gluconeogenesis is a relatively expensive process. Much of the energy invested is to make gluconeogenesis a practically irreversible process.

Summary of objectives

- Gluconeogenesis is employed to synthesize glucose when necessary.
- Gluconeogenesis is not simply a reverse of glycolysis. **THREE** steps are different.
- Gluconeogenesis is energetically expensive.

Regulation of glycolysis/gluconeogenesis

Availability/abundance of reactants and products dictate the regulation of metabolism.



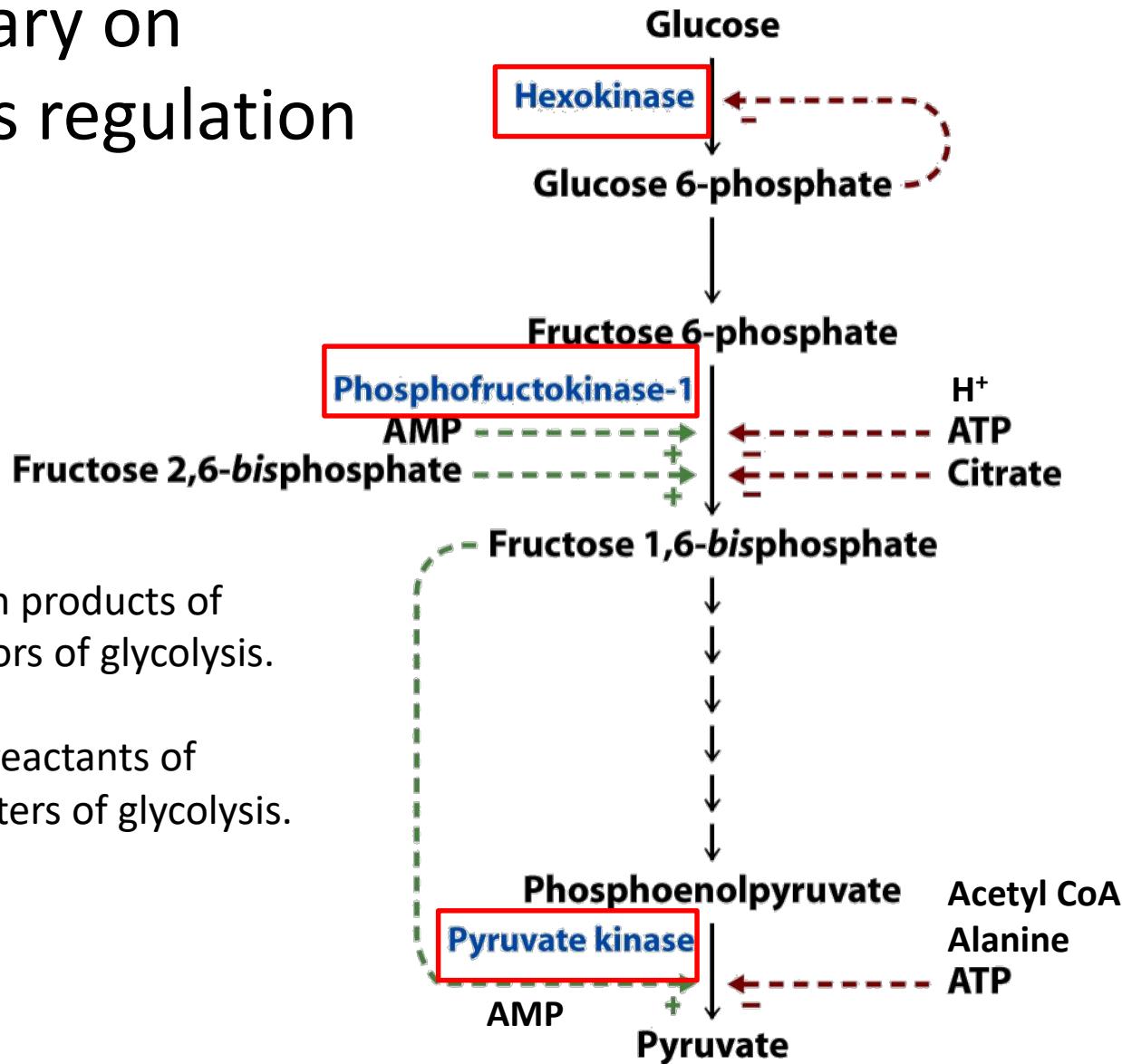
Regulation of glycolysis

In metabolic pathways, enzymes catalyzing **practically irreversible reactions** are potential sites of control!

Step	Reaction	Enzyme
1	Glucose + ATP → glucose 6-phosphate + ADP + H ⁺	Hexokinase
3	Fructose 6-phosphate + ATP → fructose 1,6-bisphosphate + ADP + H ⁺	Phosphofructokinase
10	Phosphoenolpyruvate + ADP + H ⁺ → pyruvate + ATP	Pyruvate kinase

In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are virtually irreversible (highly energetically favorable); hence, these enzymes would be expected to serve as regulatory points.

A summary on glycolysis regulation



Rule of thumb:

ATP and downstream products of glycolysis are inhibitors of glycolysis.

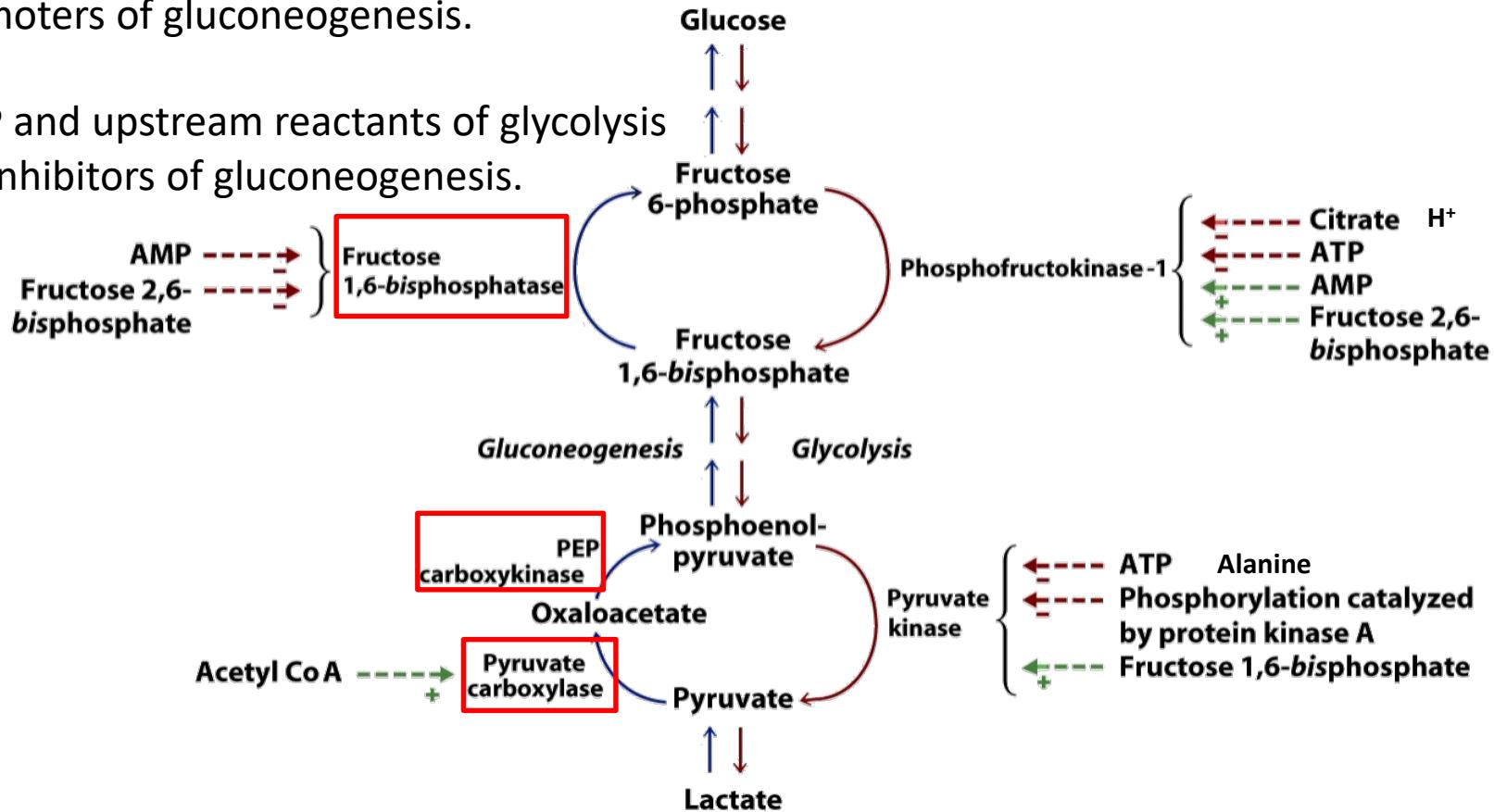
AMP and upstream reactants of glycolysis are promoters of glycolysis.

Regulation of gluconeogenesis

Rule of thumb:

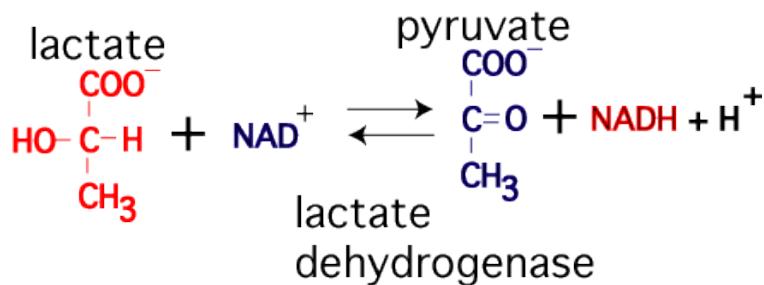
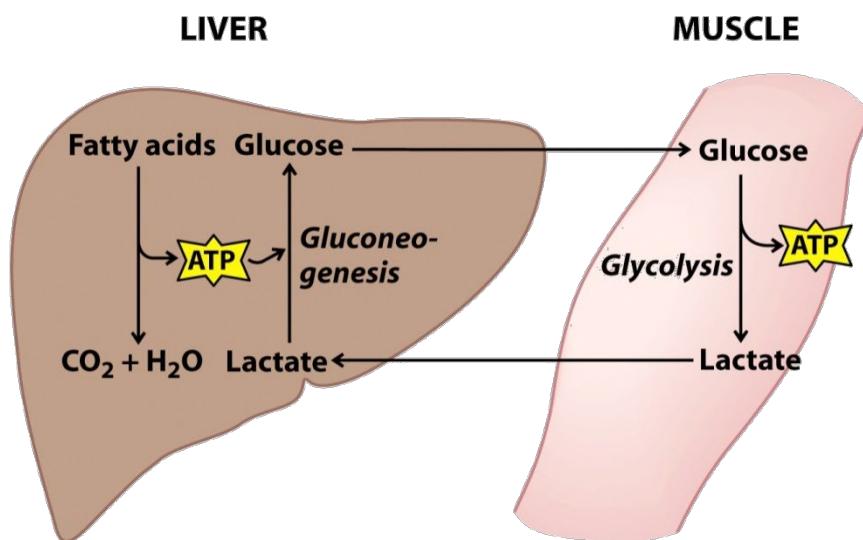
Downstream products of glycolysis are promoters of gluconeogenesis.

AMP and upstream reactants of glycolysis are inhibitors of gluconeogenesis.



Lactate as substrate

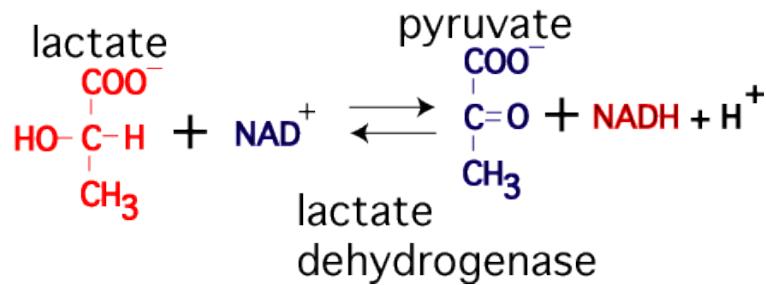
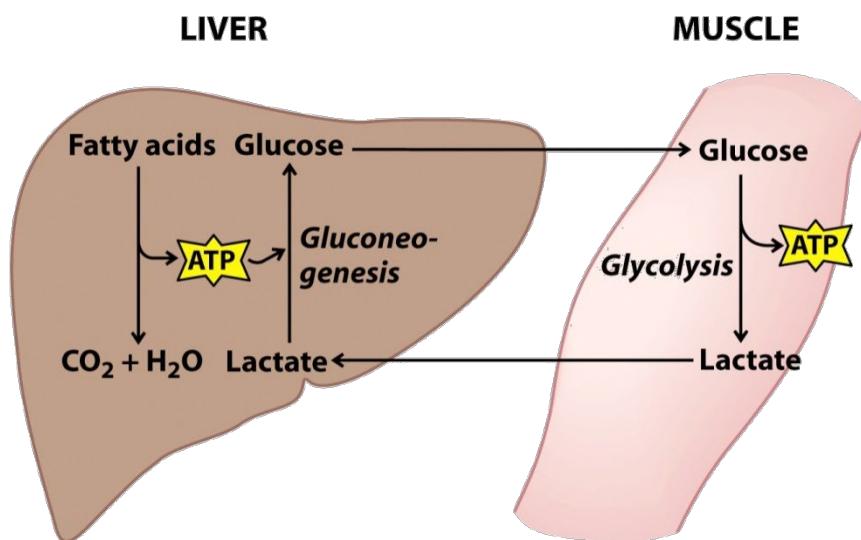
The Cori Cycle



- This cycle helps prevent lactic acidosis in muscle under anaerobic condition.
- In addition, this cycle is essential in red blood cells as the cells do not contain mitochondria.
- The significance is to preserve the oxygen molecules transported by red blood cells.

Lactate as substrate

The Cori Cycle



Carl Cori and Gerty Cori.

Summary of objectives

- The **THREE** different steps are key regulatory points of glycolysis/gluconeogenesis.
- AMP/ATP, reactants and products regulate the enzymes to control glycolysis/gluconeogenesis.
- Lactate acid is utilized in gluconeogenesis through Cori cycle, which is physiologically important.

The Citric Acid Cycle

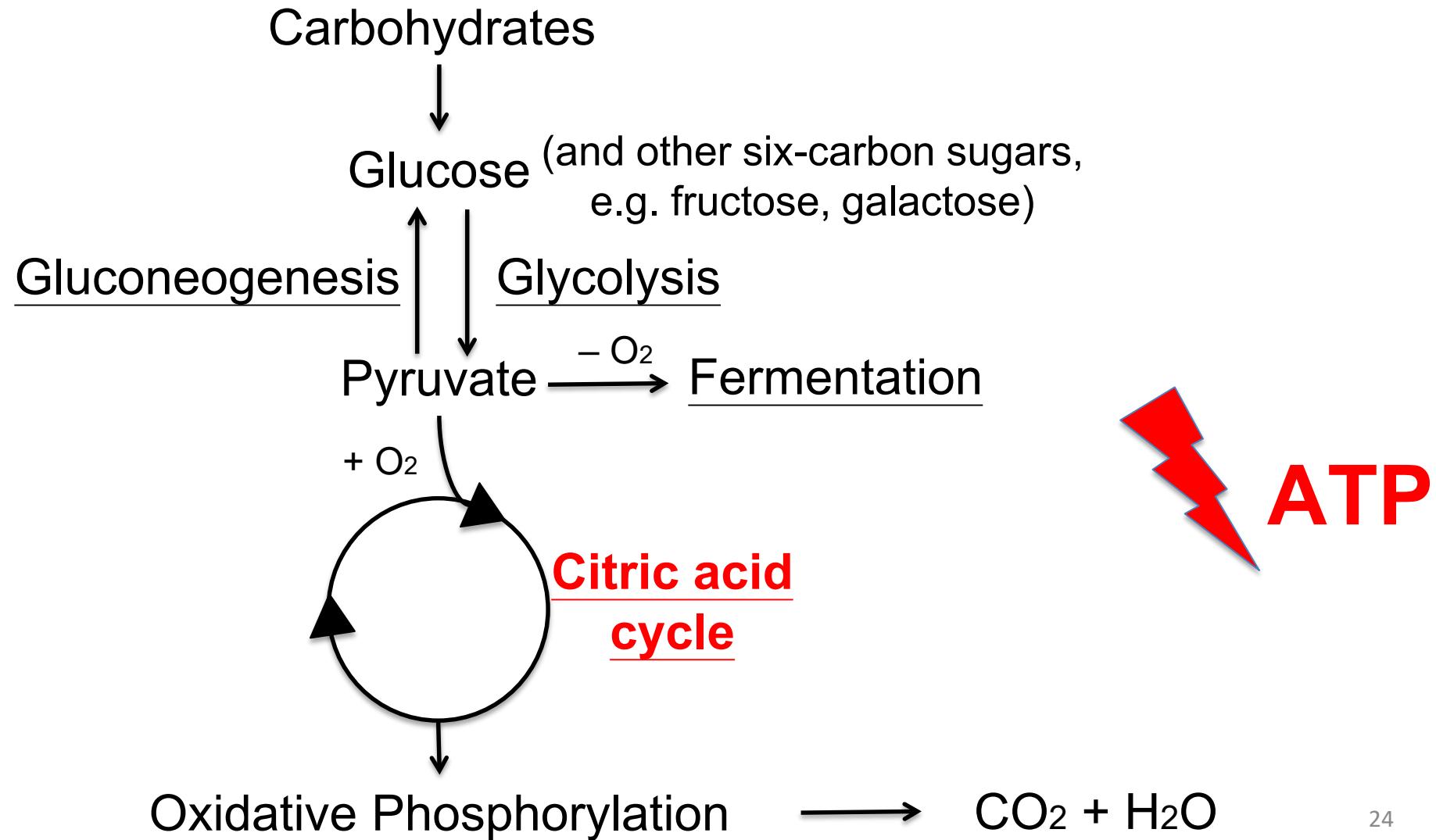
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Overview





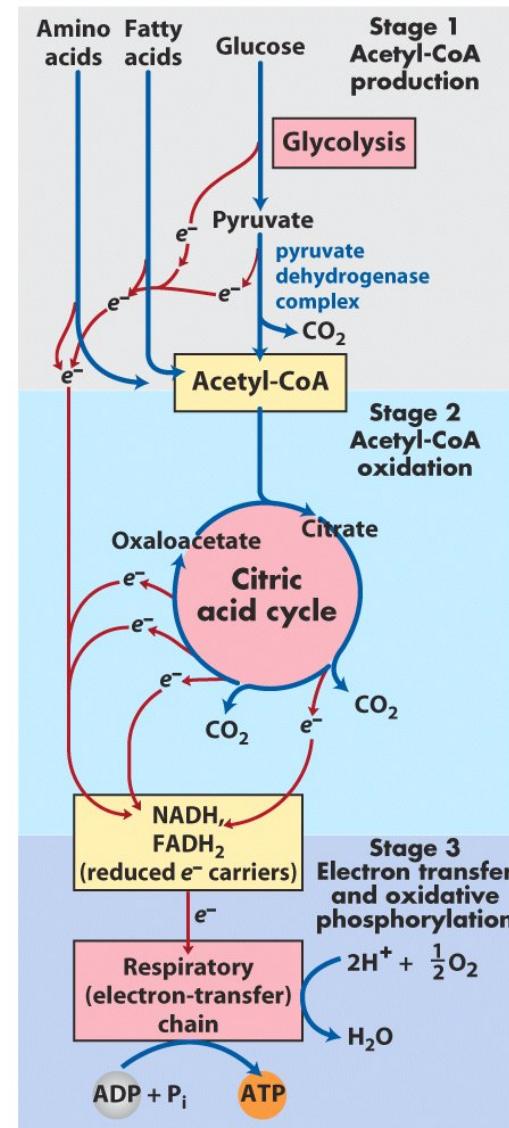
Cellular respiration

In the presence of oxygen, glucose is fully oxidized into carbon dioxide and water and the release of energy in the form of ATP.

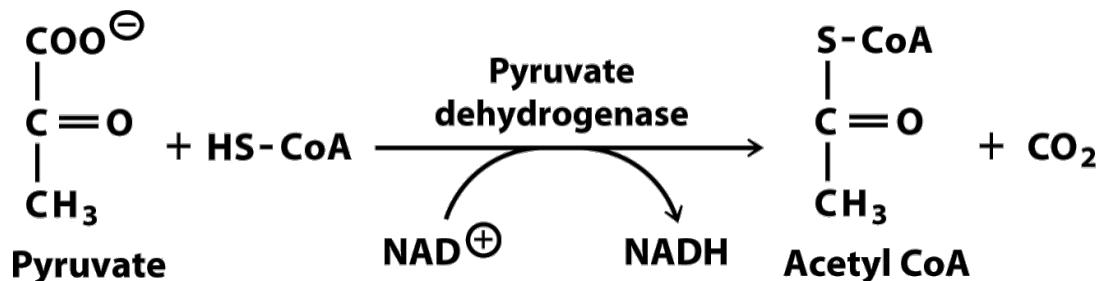
Cellular respiration refers to the molecular processes by which cells consume oxygen and produce carbon dioxide.

Three stages in the process that takes place in **mitochondria**:

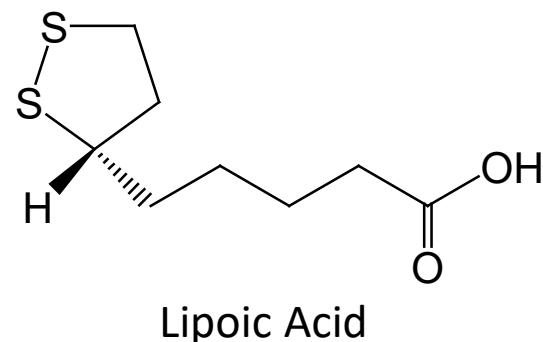
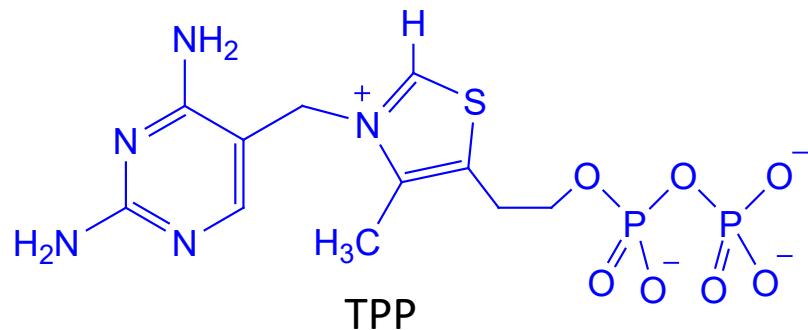
1. Making of acetyl CoA
2. Oxidation of the acetyl group by the citric acid cycle and generation of NADH or FADH₂.
3. Oxidation of NADH and FADH₂ by oxygen via the electron transport chain (oxidative phosphorylation).



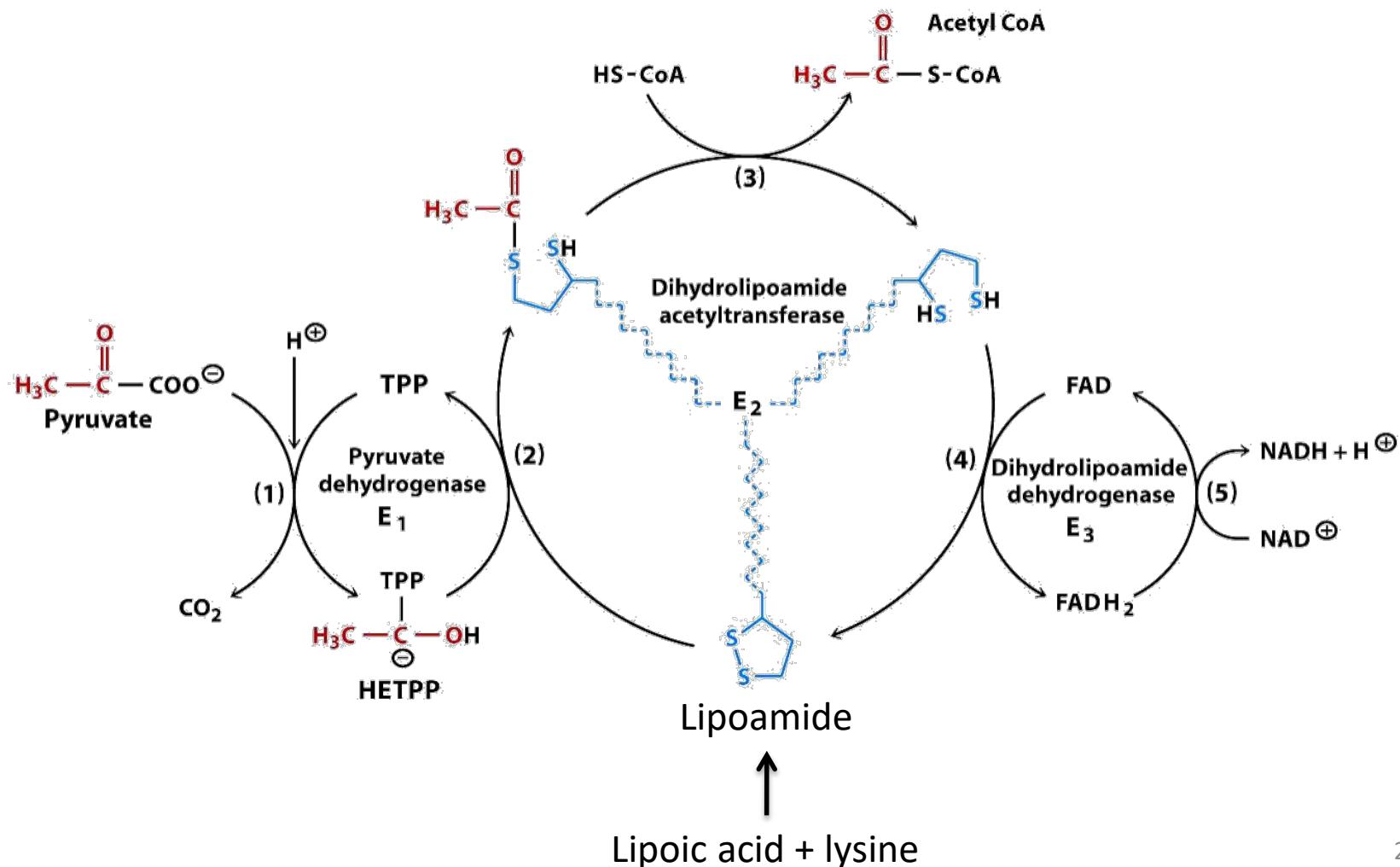
Formation of acetyl CoA from pyruvate



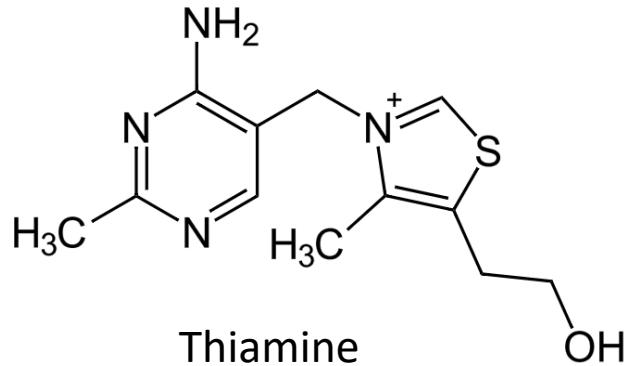
The reaction requires the participation of the three enzymes of the **pyruvate dehydrogenase complex** (abbreviated as E_1 , E_2 and E_3), and five coenzymes: **thiamine pyrophosphate (TPP)**, **lipoic acid**, **FAD**, **CoA** and **NAD^+** .



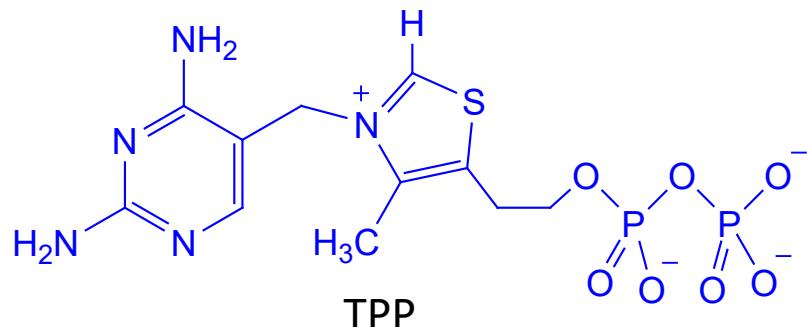
Formation of acetyl-CoA from pyruvate: An overview



Vitamin B1

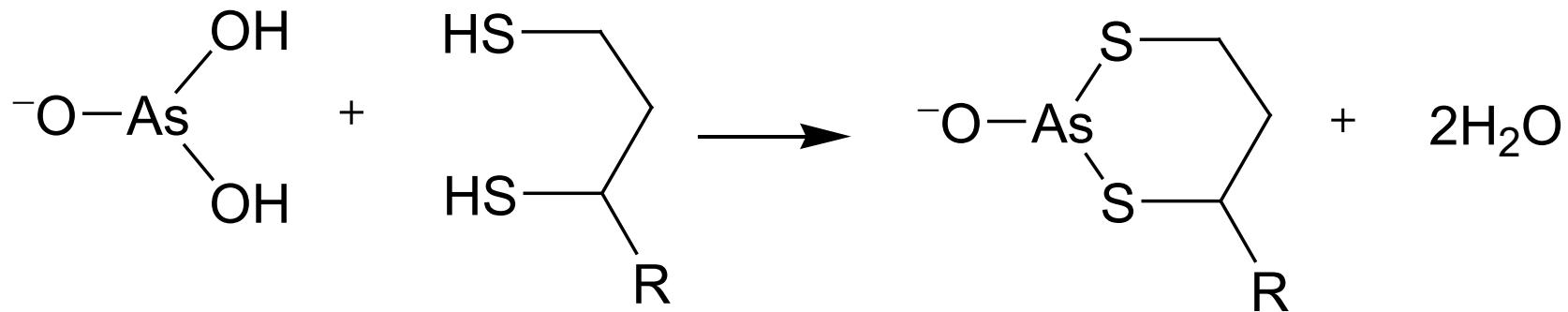


- Precursor of TPP.
- Animals can not synthesize Thiamine.
- Grains are a good source.
- But polished rice has very low content of it.
Brown rice is much better.
- Deficiency leads to Beriberi, a neurologic and cardiovascular disease.

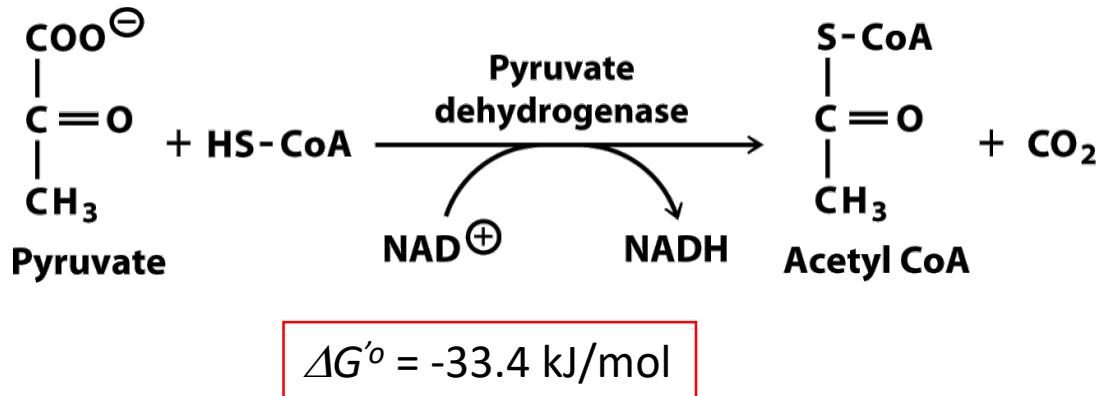


Arsenic and mercury compounds are poisonous

Arsenic has been known to be a poison since ancient times. As(III) compounds like arsenite (AsO_3^{3-}) are **toxic** because of their ability to covalently bind sulfhydryl compounds. **As(III)** compounds can inactivate lipoamide-containing enzymes, e.g. pyruvate dehydrogenase, and thus halt the respiration process. Mercury has similar mechanisms.



Formation of acetyl CoA from pyruvate



Pyruvate decarboxylation (occurs in **the mitochondrial matrix**) links glycolysis and the citric acid cycle:

It is an oxidative decarboxylation reaction, which is technically irreversible. (Which means acetyl CoA in our body can not be converted back in to pyruvate.

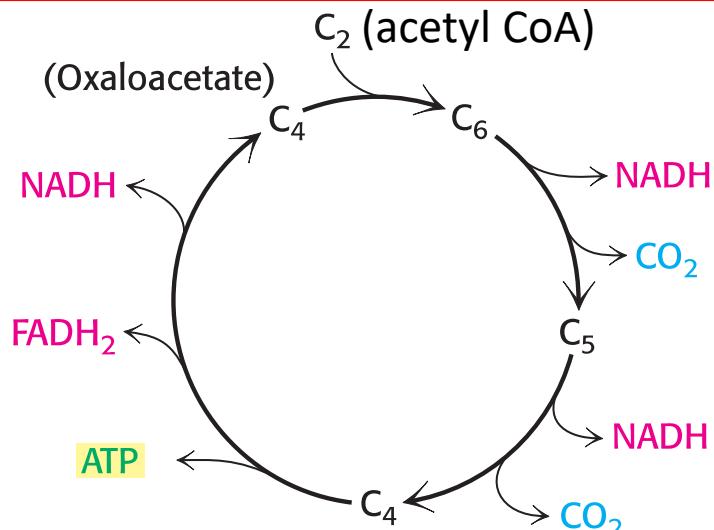
It is a preparation step, but not part of the cycle!

Summary of objectives

- In the presence of oxygen, pyruvate is converted into acetyl-CoA by the pyruvate dehydrogenase complex .
- Acetyl-CoA enters the citric acid cycle, which is connected to oxidative phosphorylation to generate more ATP.
- These steps take place in mitochondria.

Entering the Citric Acid Cycle

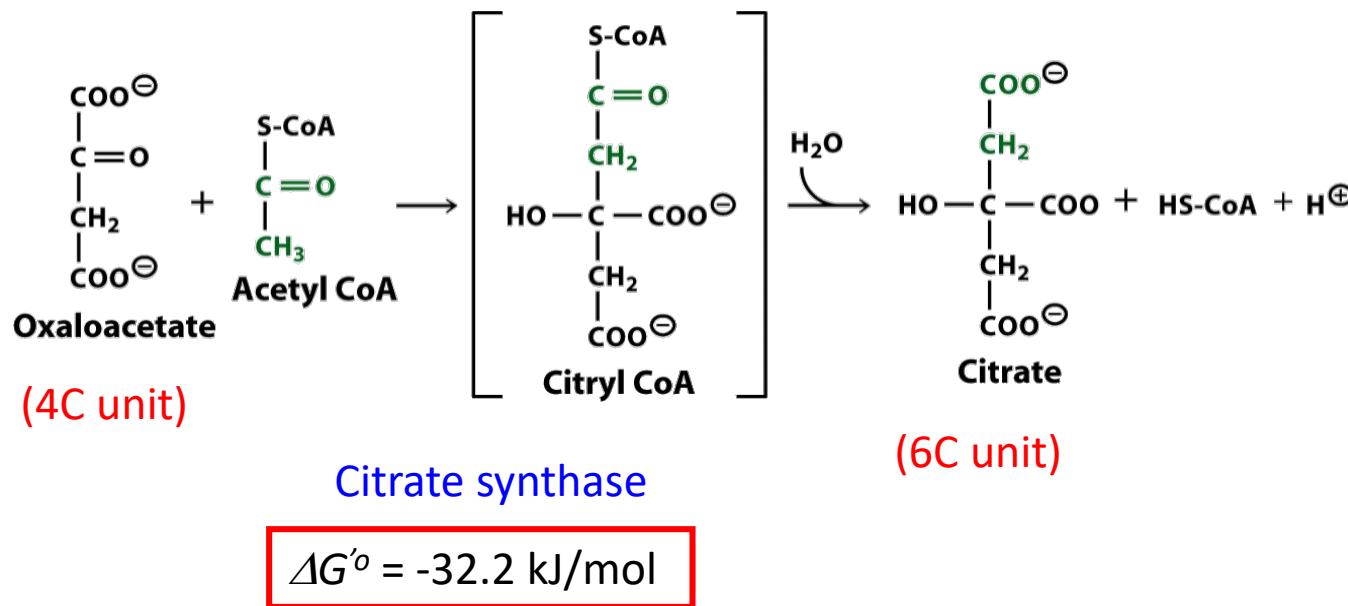
The net reaction of the citric acid cycle is:



It starts with 4C Oxaloacetate, adding 2C, sequentially getting rid off 2C, then rearranging back into Oxaloacetate.

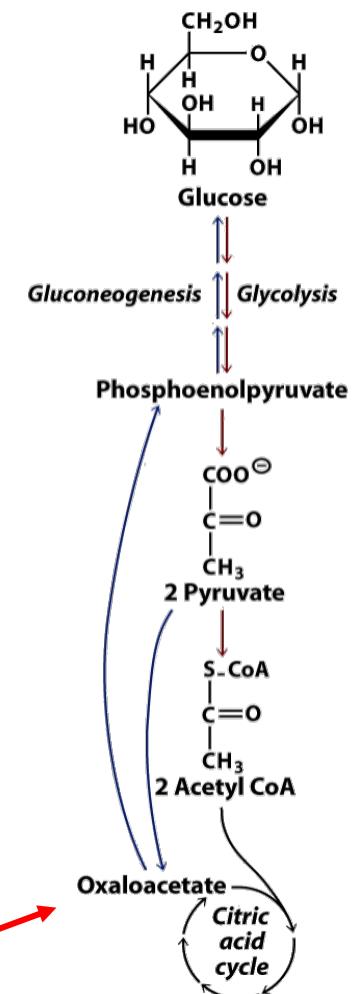
Why bother? Look at what we get out of it: 3 x NADH, 1 x FADH₂, and 1 ATP/GTP.

Oxaloacetate condenses with acetyl CoA to form citrate

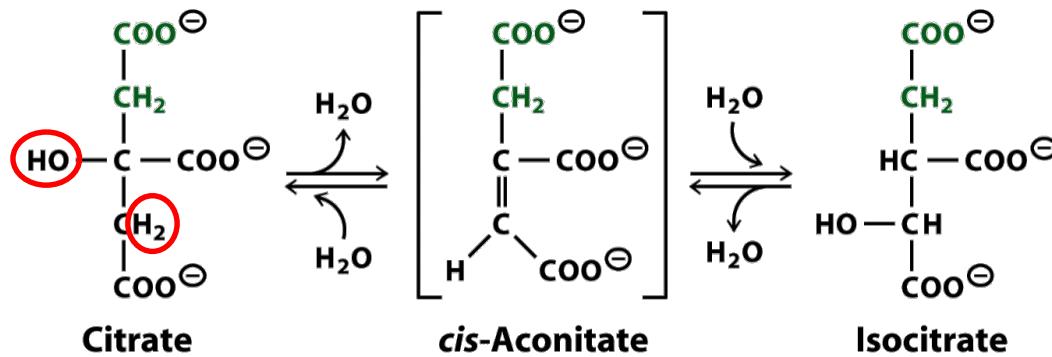


Note: the concentration of OAA is normally **very low** in the cells.

N.B. Oxaloacetate comes from here

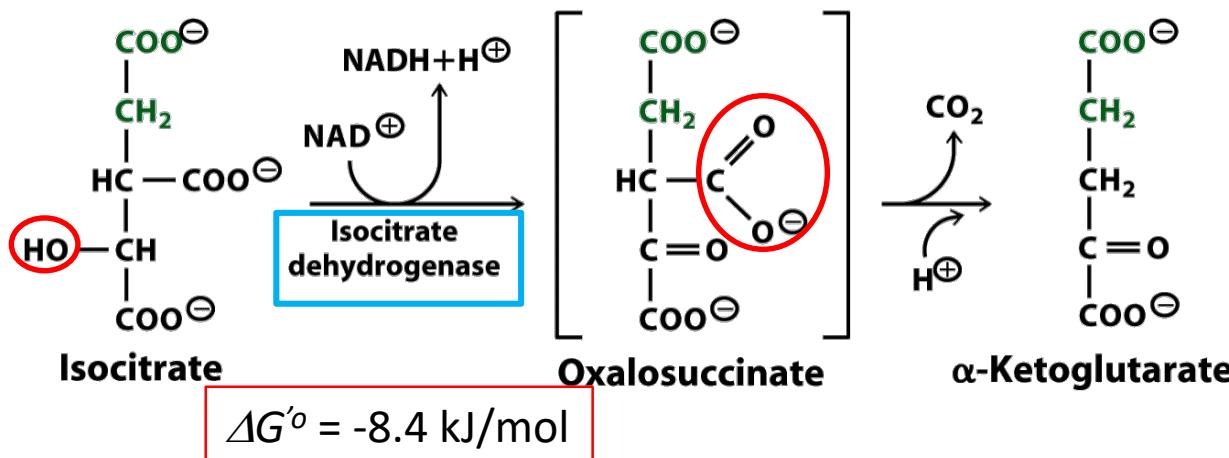


Citrate is isomerized into isocitrate

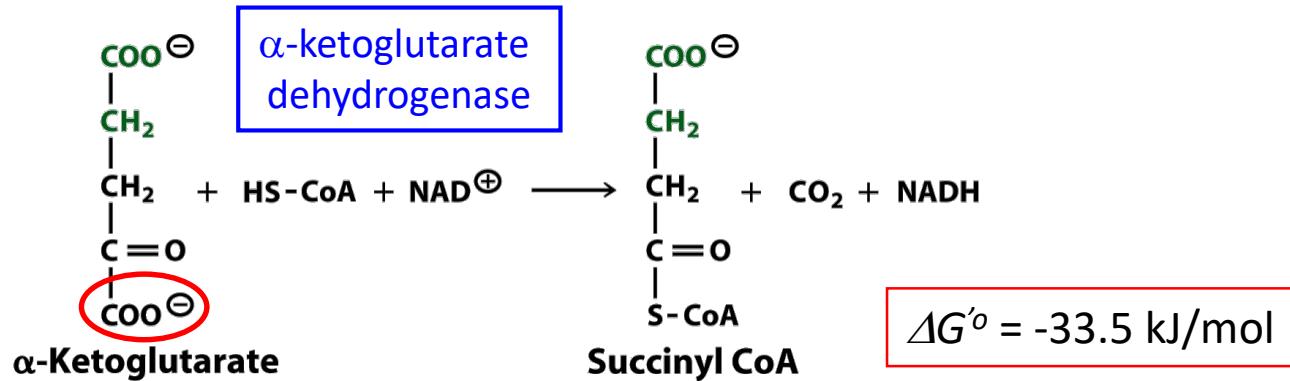


The isomerization of citrate is accomplished by a *dehydration* step followed by a *hydration* step. The result is an interchange of a hydrogen atom and a hydroxyl group.

Isocitrate is oxidized and decarboxylated to α -ketoglutarate



Succinyl CoA is formed by Oxidative Decarboxylation



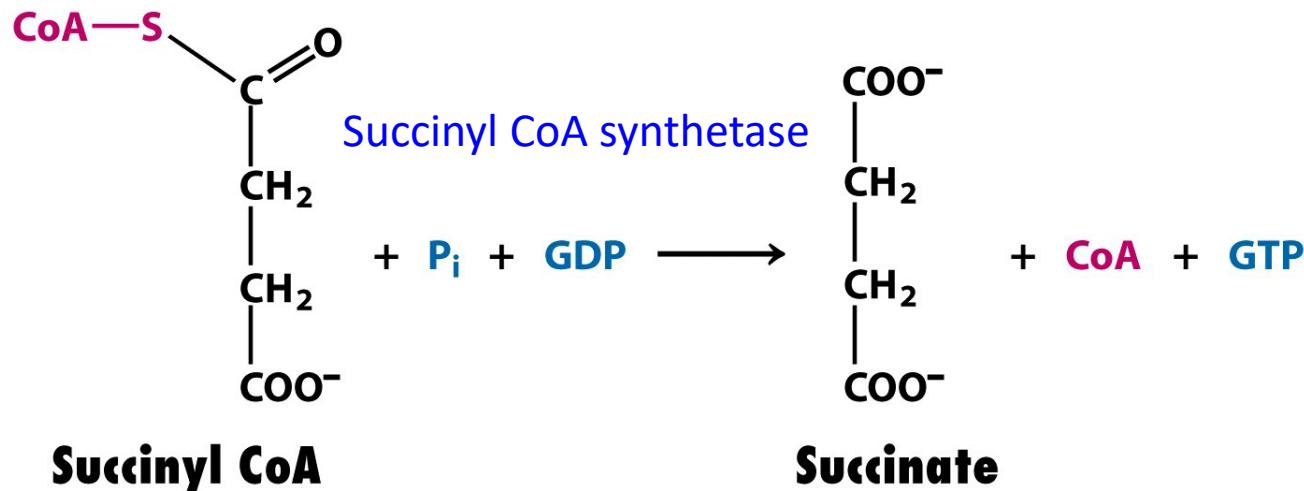
The mechanism of this reaction is **very similar** to that of the conversion of pyruvate into acetyl CoA !

Cleavage of thioester bond of succinyl-CoA

The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of guanosine diphosphate (GDP)– generation of GTP or ATP!



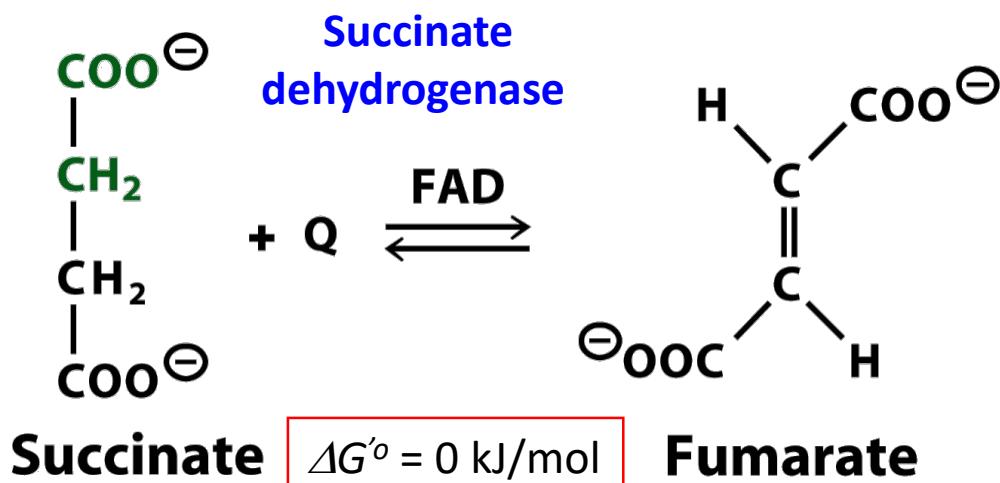
$$\Delta G'{}^o = -2.9 \text{ kJ/mol}$$



Oxaloacetate is regenerated (1)

Succinate is converted into oxaloacetate in 3 steps:

- (1) Oxidation
- (2) Hydration
- (3) Oxidation



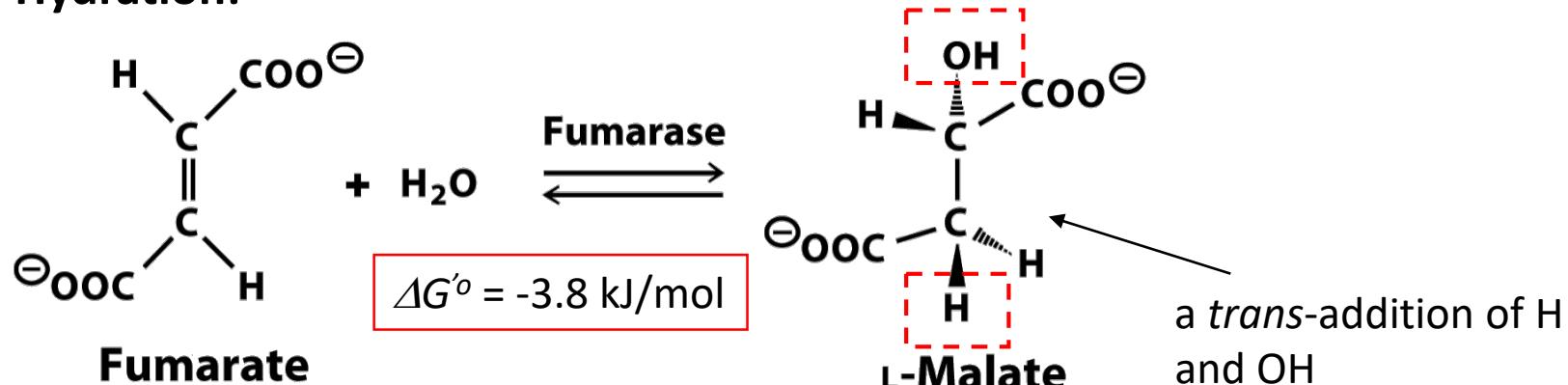
Oxidation:

the protons and electrons are passed to a quinone, which is reduced to QH_2 . More details on the next lecture.

Succinate is oxidized to fumarate by succinate dehydrogenase.

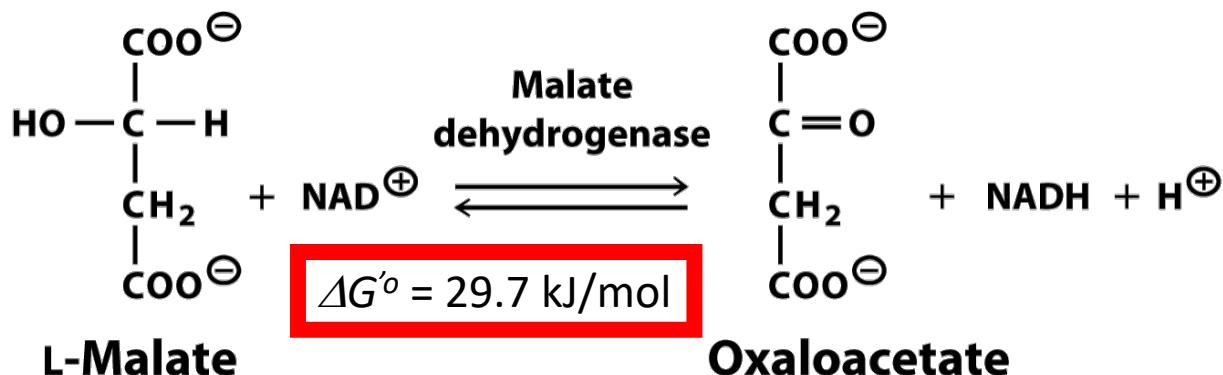
Oxaloacetate is regenerated (2)

Hydration:

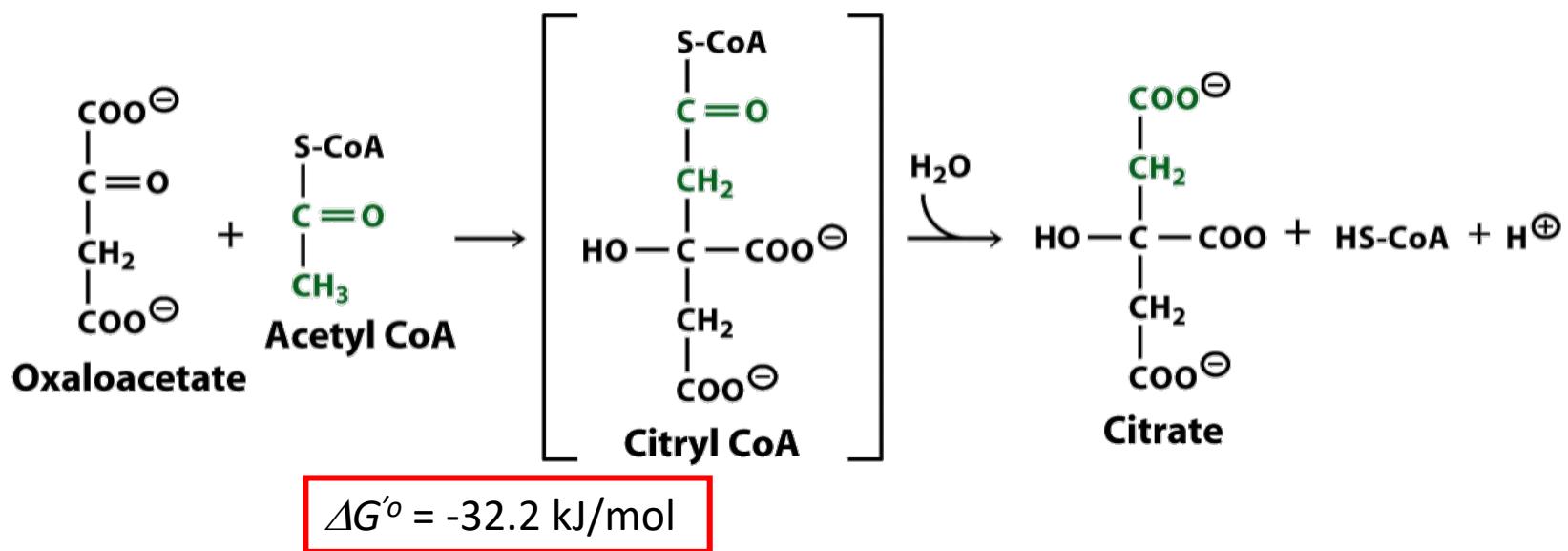
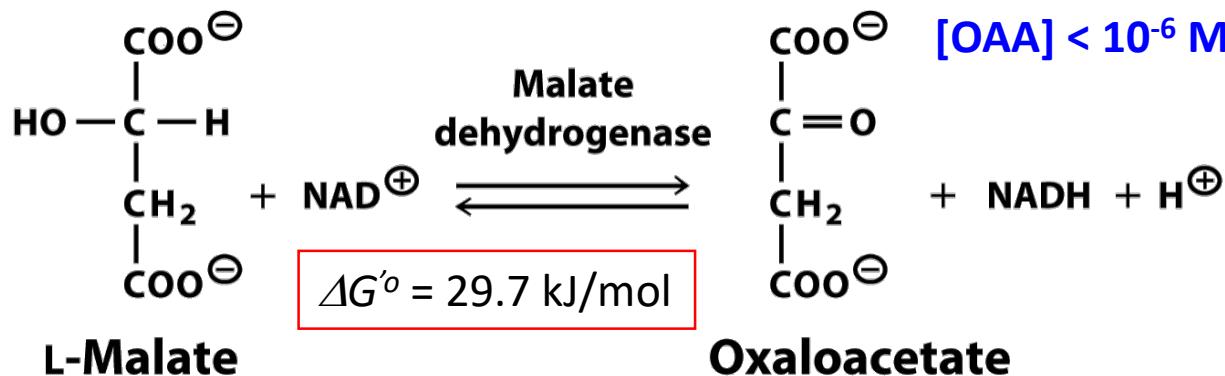


The last reaction:

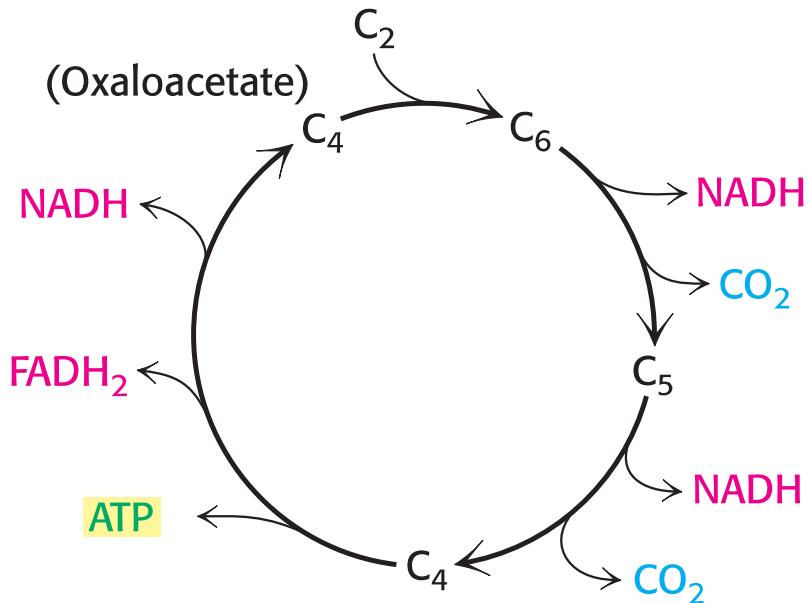
Oxidation:



Pushing and pulling



Summary



One molecule of acetyl CoA can

generate:

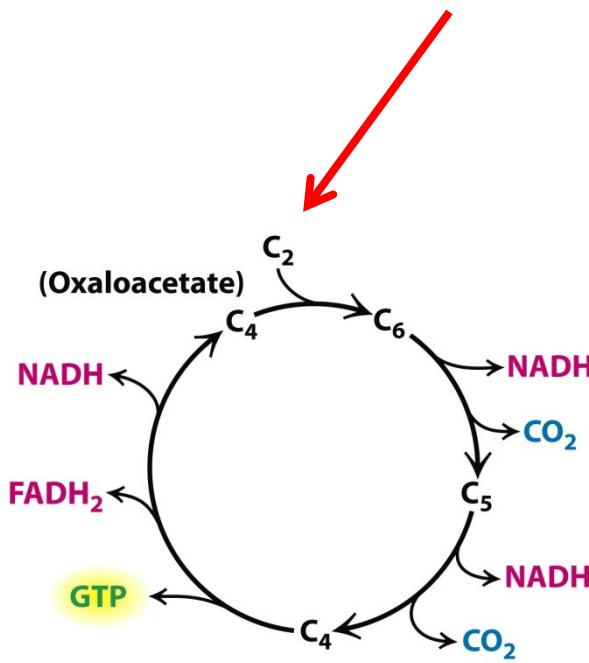
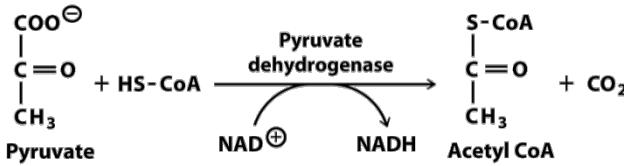
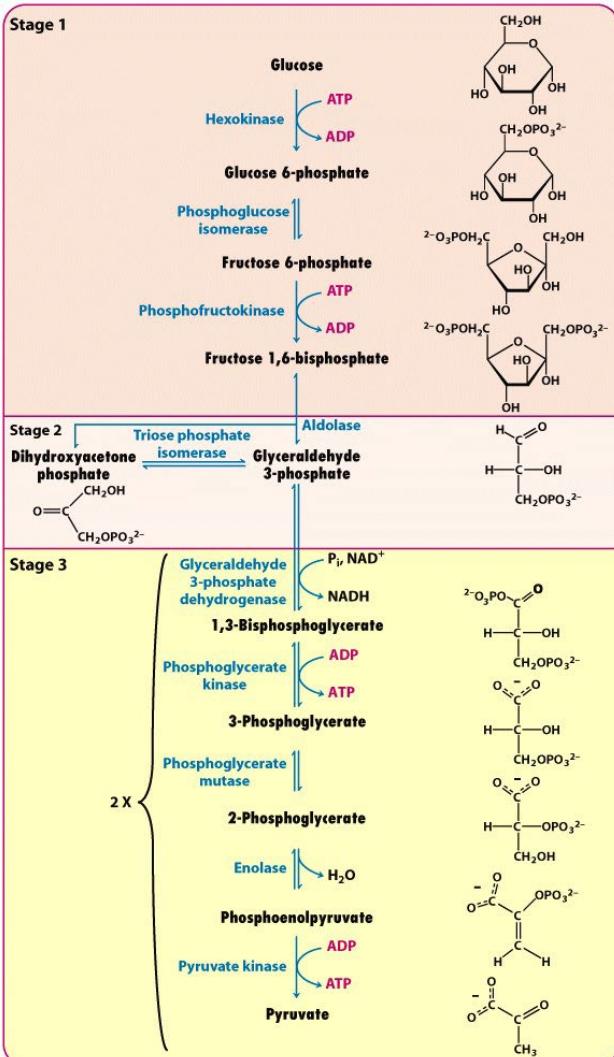
2 molecules of CO₂

3 molecules of NADH

1 molecule of FADH₂

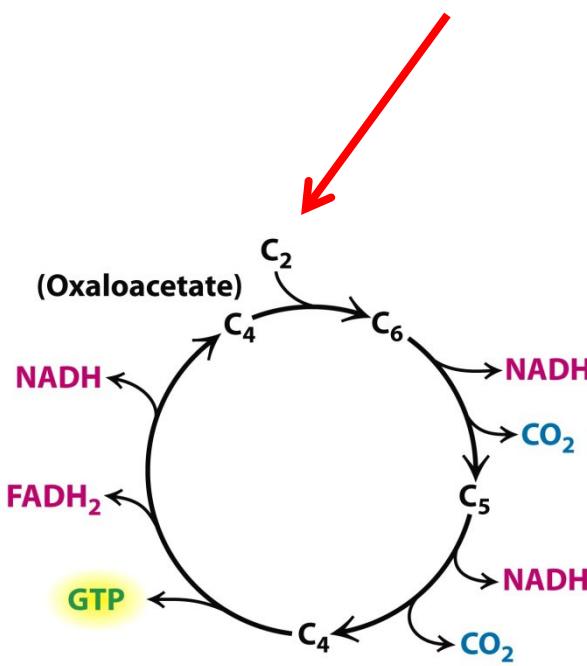
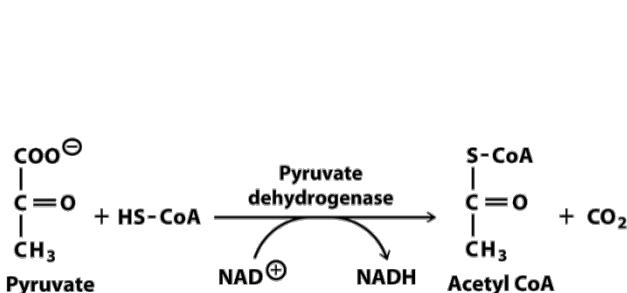
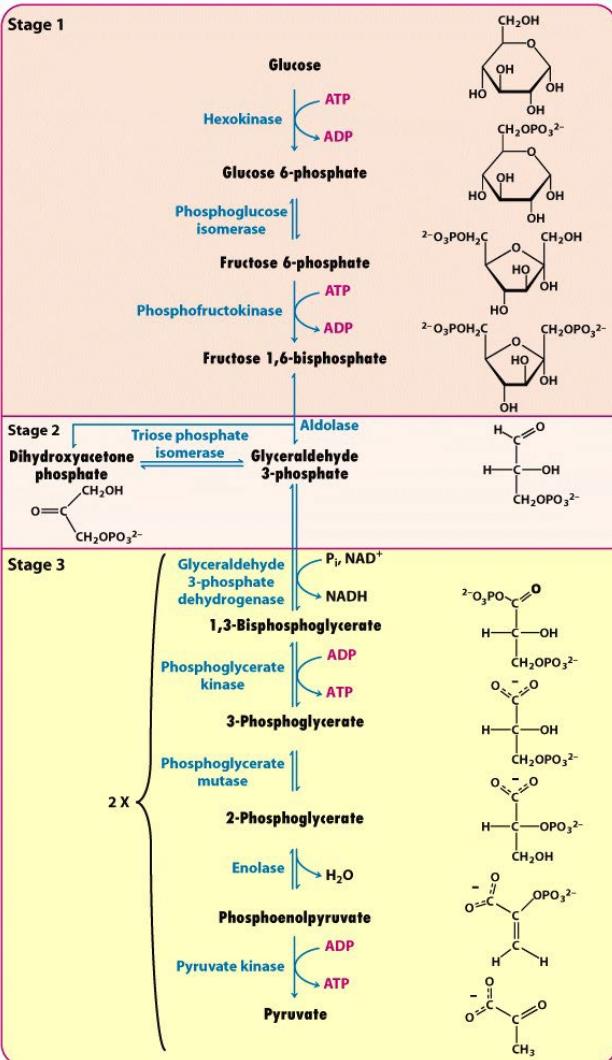
One molecule of GTP or ATP

Where does the energy come from?



- Up to here, there are only 4 ATP molecules made per glucose molecule.

Where does the energy come from?



- But there are 12 NADH/FADH created that can generate more ATP molecules by another process called oxidative phosphorylation.
- NADH and FADH₂ can generate much more ATP when their electrons are transferred to the respiratory chain.

Regulation of the citric acid cycle

Purposes: ensure the **production of intermediates** in a stable steady state and without wasteful production.

Control (regulatory) points:

1. Pyruvate into acetyl CoA (by **pyruvate dehydrogenase**)
2. Acetyl CoA into the citric acid cycle (by **citrate synthase**)
3. Two other enzymes in the CAC: **isocitrate dehydrogenase** and **α -ketoglutarate dehydrogenase**.

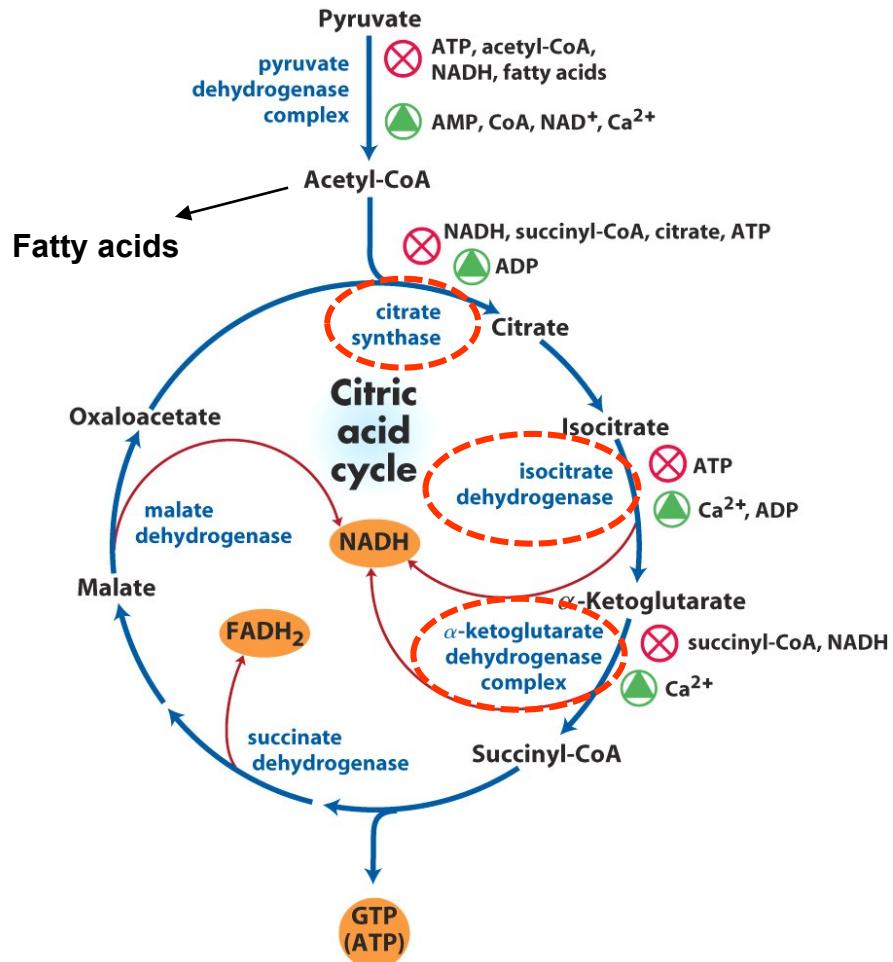
Controls of the **rate of flux** through the cycle:

- A. substrate availability
- B. inhibition by accumulating products

A summary of the control points

Three strongly exergonic steps (negative ΔG°) in the cycle are the rate-limiting steps.

1. citrate synthase
2. isocitrate dehydrogenase
3. α -ketoglutarate dehydrogenase



A summary of the control points

When food consumption is high, here are the results:

[ATP] is high or

[ATP]/[ADP] ratio is high;

[NADH] is high or

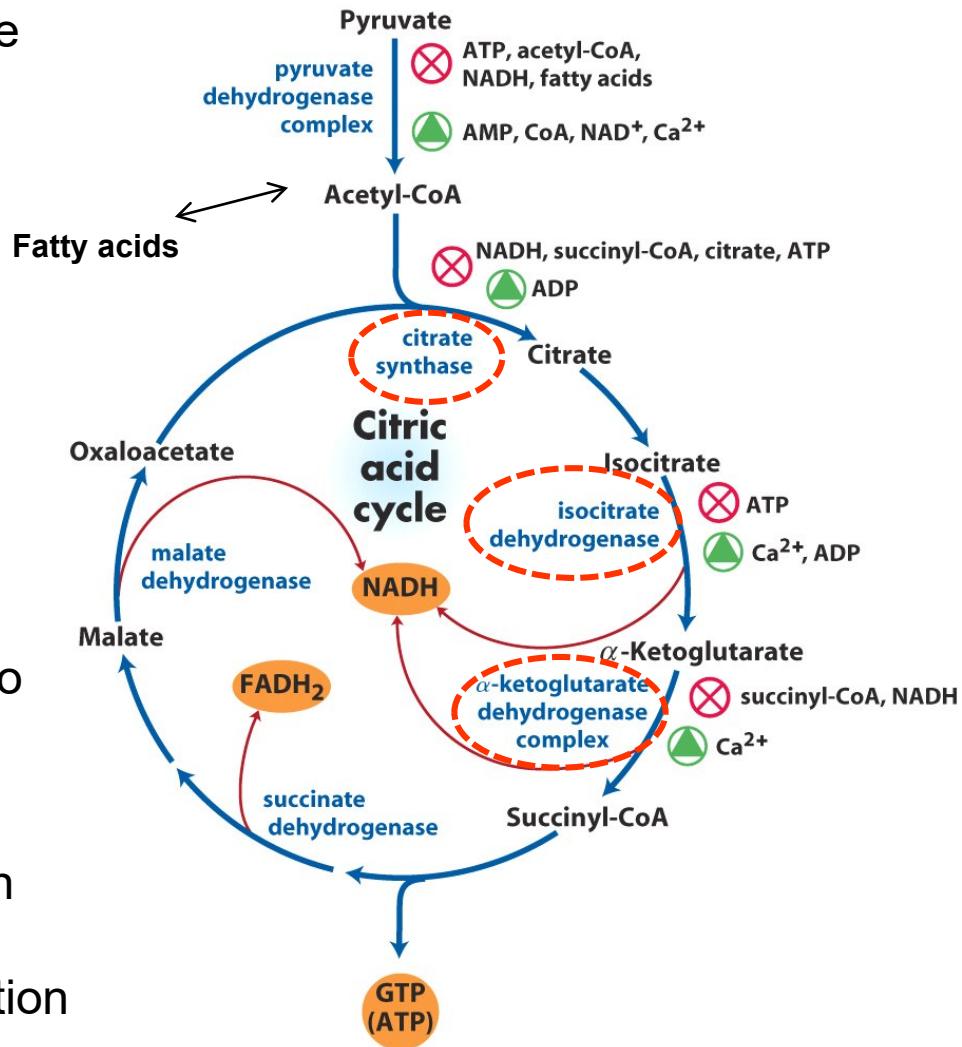
[NADH]/[NAD⁺] is high;

Citric acid cycle slows down.

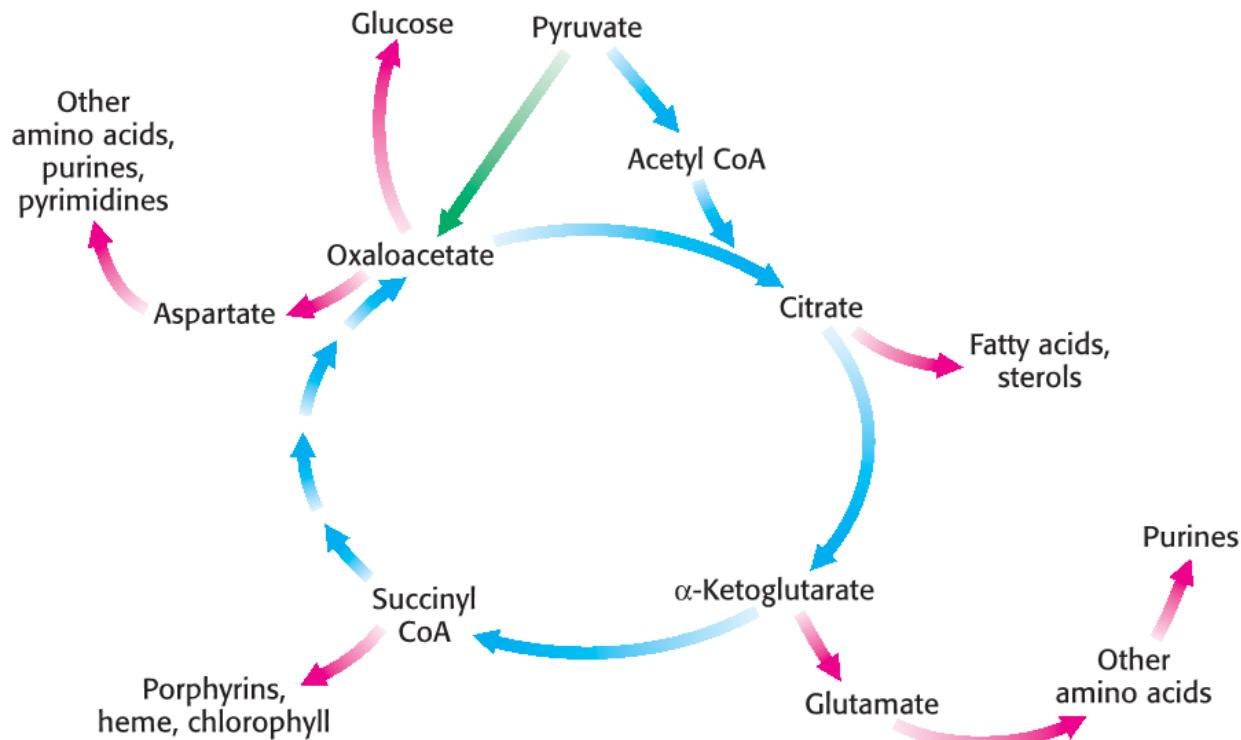
→ [Fatty acids] are high.

Therefore we need to do exercise to promote ATP → ADP + Pi

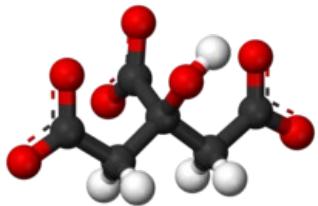
During exercise, muscle contraction requires a signal from Ca²⁺. Thus, Ca²⁺ is also important in the regulation of the citric acid cycle.



The citric acid cycle is also a source of biosynthesis precursors

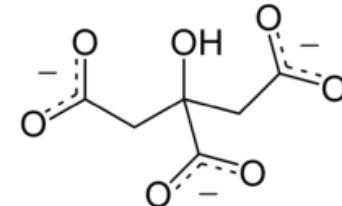


The citric acid cycle is not only a major **degradative pathway** for the generation of ATP, it also provides **intermediates for biosynthesis**.
= **Anaplerotic** (to fill up) **reactions**



Citrate in use

Food additive E331



In the food industry, citrate is used as:

1. additive to give a fruity flavor
2. buffering agent to control the pH
3. preservative/antioxidant

Produced by the fungus
Aspergillus niger in beet molasses.



CATERING TO EXHAUSTION
RECOMMENDED DOSE (ADULTS): DRINK 591 mL (1 BOTTLE) AS NEEDED.
MEDICINAL INGREDIENT: PER 591 mL: CAFFEINE 91 mg
NON-MEDICINAL INGREDIENTS: CARBONATED WATER, GLUCOSE-FRUCTOSE AND/OR SUGAR, CONCENTRATED ORANGE JUICE, CITRIC ACID, NATURAL FLAVOUR, SODIUM BENZOATE, SODIUM CITRATE, ERYTHORBIC ACID, CALCIUM DISODIUM EDTA, GUM ARABIC, COLOUR, BROMINATED VEGETABLE OIL.
CAUTION: CONTAINS CAFFEINE. NOT RECOMMENDED FOR CHILDREN, PREGNANT OR BREAST FEEDING.

INGREDIENTS

DRINKS

MILK, 1% LOWFAT: 1% Lowfat Milk, Vitamin A, Vitamin D (Federal Standard).
MINUTE MAID® ORANGE JUICE: 100% pure Orange Juice from Concentrate, Contains Pure Filtered Water, Premium Concentrated Orange Juice.
COFFEE: 100% Ground Coffee.
DECAFFEINATED COFFEE: 97% Caffeine Free, Naturally Decaffeinated Coffee.
ICE CREAM SHAKE: Milkfat and Nonfat Milk, Sugar, High Fructose Corn Syrup, Corn Syrup, Sweet Whey, Natural† and Artificial Vanilla Flavor, Guar Gum, Mono- and Diglycerides, Cellulose Gum, Sodium Phosphate, Carrageenan. †Natural flavors from plant sources
STRAWBERRY SHAKE SYRUP: Corn Syrup, Water, High Fructose Corn Syrup, Citric Acid, Artificial Flavor, Sodium Benzoate (Preservative), and Colored with Red #40.
CHOCOLATE SHAKE SYRUP: High Fructose Corn Syrup, Water, Dextrose, Cocoa (Processed with Alkali), Corn Syrup, Sweet Whey Powder, Salt, Xanthan Gum, Potassium Sorbate (Preservative), Citric Acid.
COCA COLA® CLASSIC: High Fructose Corn Syrup and/or Sucrose, Water, Caramel Color, Phosphoric Acid, Natural† Flavors, Caffeine. †Natural flavors from animal and plant sources
DIET COKE®: Water, Caramel Color, Phosphoric Acid, Sodium Saccharin, Potassium Benzene taste), Natural† Flavors, Citric Acid, Caffeine, Potassium Citrate, Aspartame, Dimethyl Sulfoxide. †Natural flavors from plant sources



Summary of objectives

- Regulatory points of the citric acid cycle (overdose of citrate is a problem).
- The citric acid cycle also provides a lot of intermediates for other metabolic reactions.