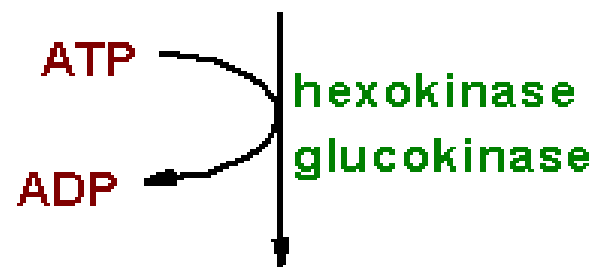


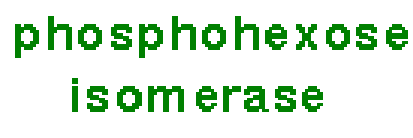
# **METABOLIC BIOCHEMISTRY**

## **REGULATION OF GLYCOLYSIS**

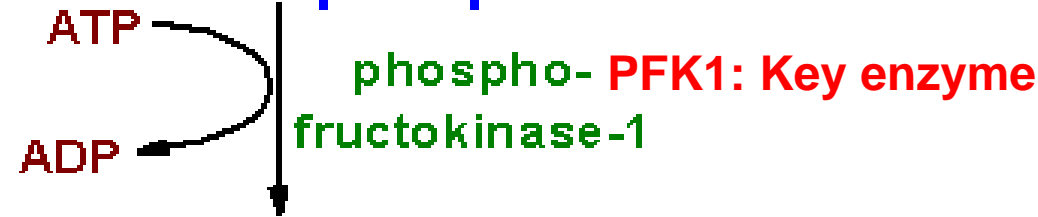
# Glucose



## Glucose-6-phosphate



## Fructose-6-phosphate



## Fructose-1,6-bisphosphate



## Glyceraldehyde-3-phosphate

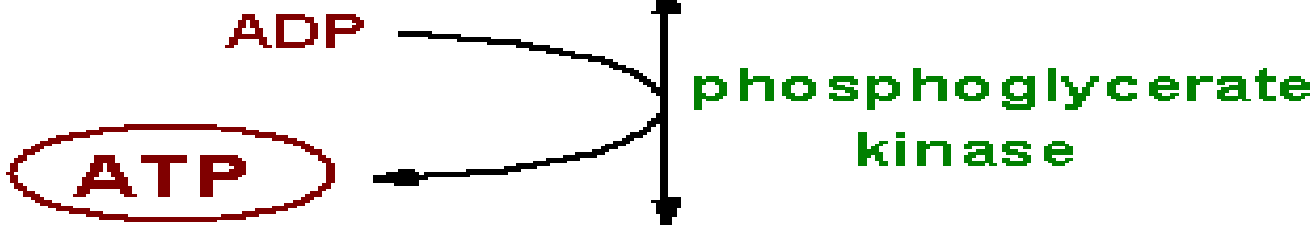
## Dihydroxyacetone phosphate



**Glyceraldehyde-3-phosphate**



**1,3-bisphosphoglycerate**



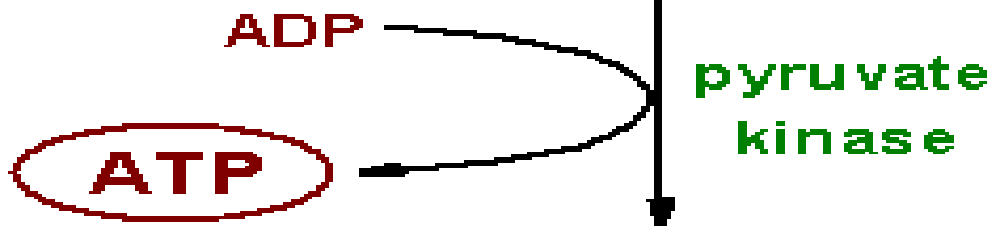
**3-phosphoglycerate**

**phosphoglycerate mutase**

**2-phosphoglycerate**

**enolase**

**Phosphoenolpyruvate**



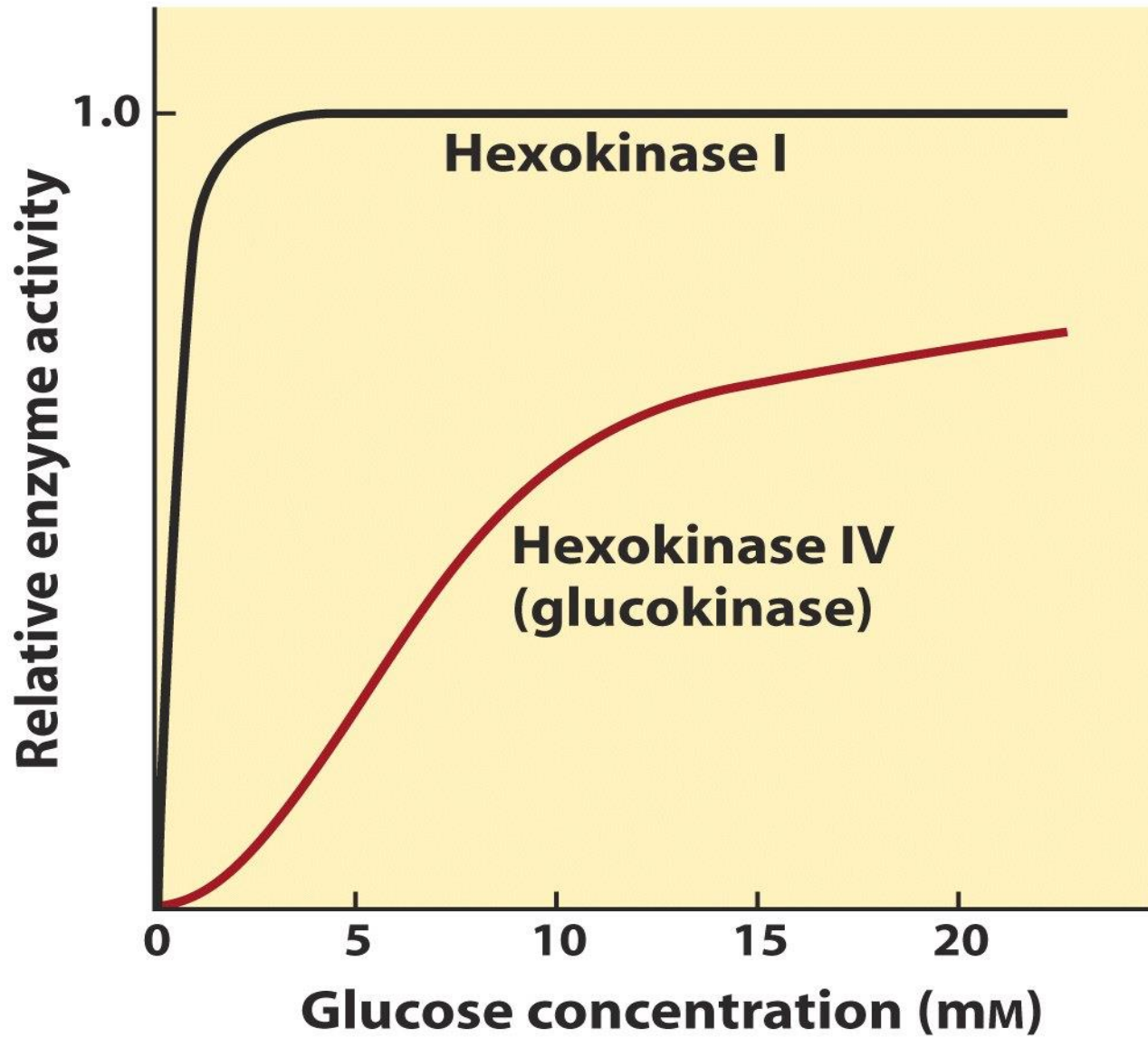
**Pyruvate**

# Glycolysis Is under Tight Regulation

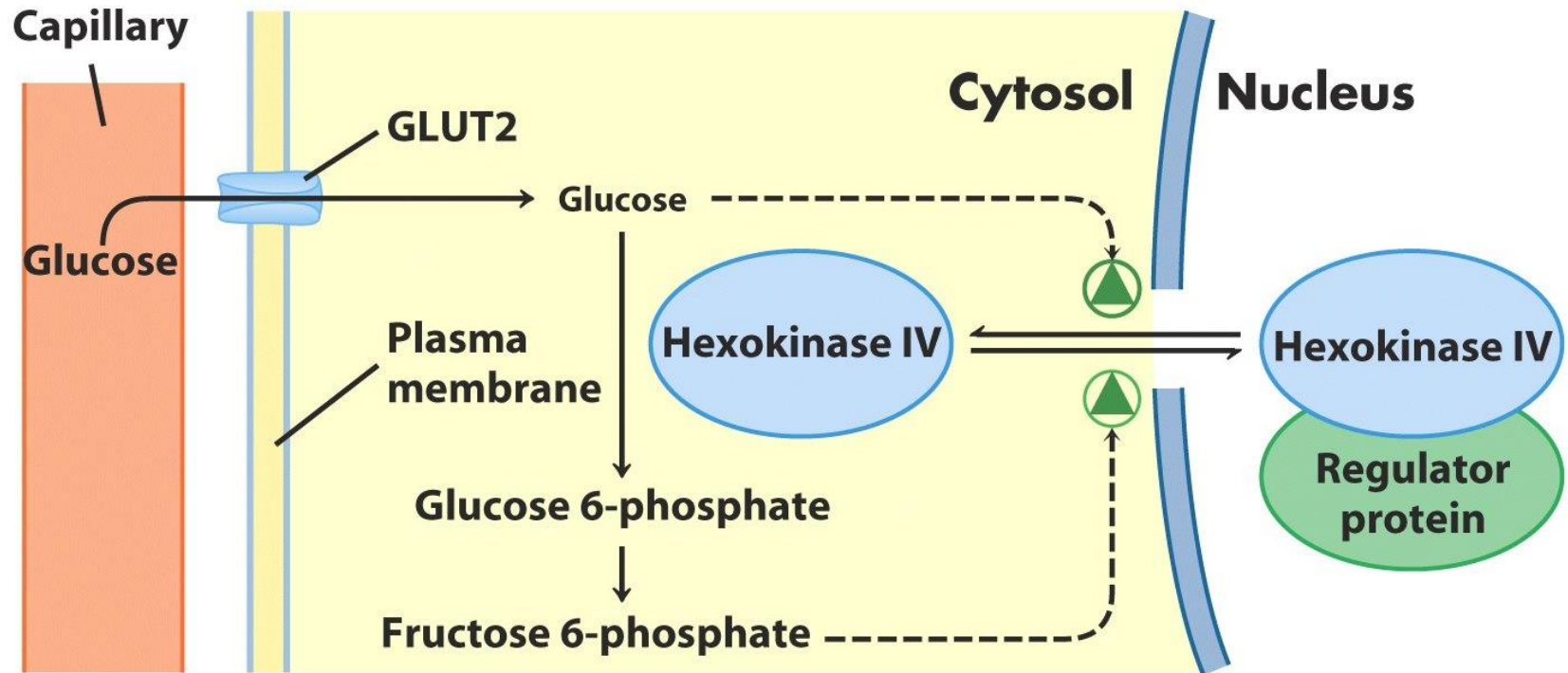
- The flux of glucose through the glycolytic pathway is regulated to maintain nearly **constant ATP levels**
- The required adjustment in the rate of glycolysis is achieved by a complex interplay among ATP consumption, NAD regeneration, and allosteric regulation of three glycolytic enzymes: **hexokinase, PFK-1, and pyruvate kinase**
- glycolysis is regulated by the hormones **glucagon, epinephrine, and insulin**
- By changes in the **expression** of the **genes** for several glycolytic enzymes.

# Hexokinase and Glucokinase

- **Hexokinase** performs step 1 of glycolysis in most tissues, including muscle and brain. It has a low  $K_m$  (high affinity) for glucose, so it permits initiation of glycolysis even when blood **glucose levels are relatively low**. However, its  **$V_{max}$  is relatively low**.
- Hexokinase is inhibited by the product of its reaction, glucose-6-phosphate. This is a very important regulatory step, since it prevents the consumption of too much cellular ATP to form G6P when glucose is not limiting.
- **Glucokinase**, found in the liver and pancreatic B- cells, requires a much higher glucose concentration for maximal activity. It is thus most active when **glucose is very high** in the portal vein, immediately after consumption of a carbohydrate-rich meal. **It has a high  $V_{max}$** , allowing the liver to effectively remove excess glucose, and minimize hyperglycemia after eating.
- Glucokinase is inhibited by F6P.



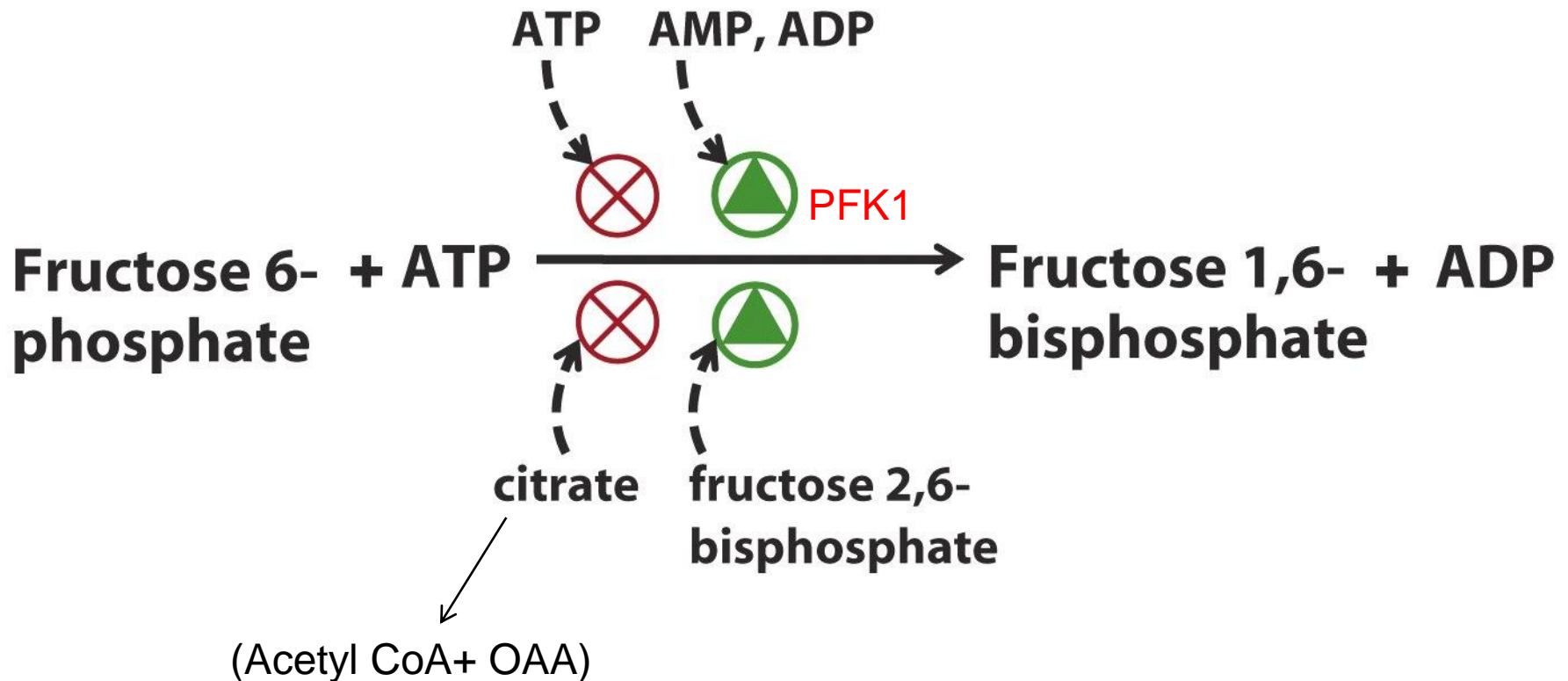
## Regulation of **hexokinase IV** (glucokinase) by sequestration in the nucleus.



The regulatory protein inhibits glucokinase by forming a complex with this enzyme in the presence of fructose 6-phosphate.

The protein inhibitor of glucokinase is a nuclear binding protein that draws glucokinase into the nucleus when the fructose 6-phosphate concentration in liver is high and releases it to the cytosol when the glucose concentration is high.

# Phosphofructokinase-1(PFK1)

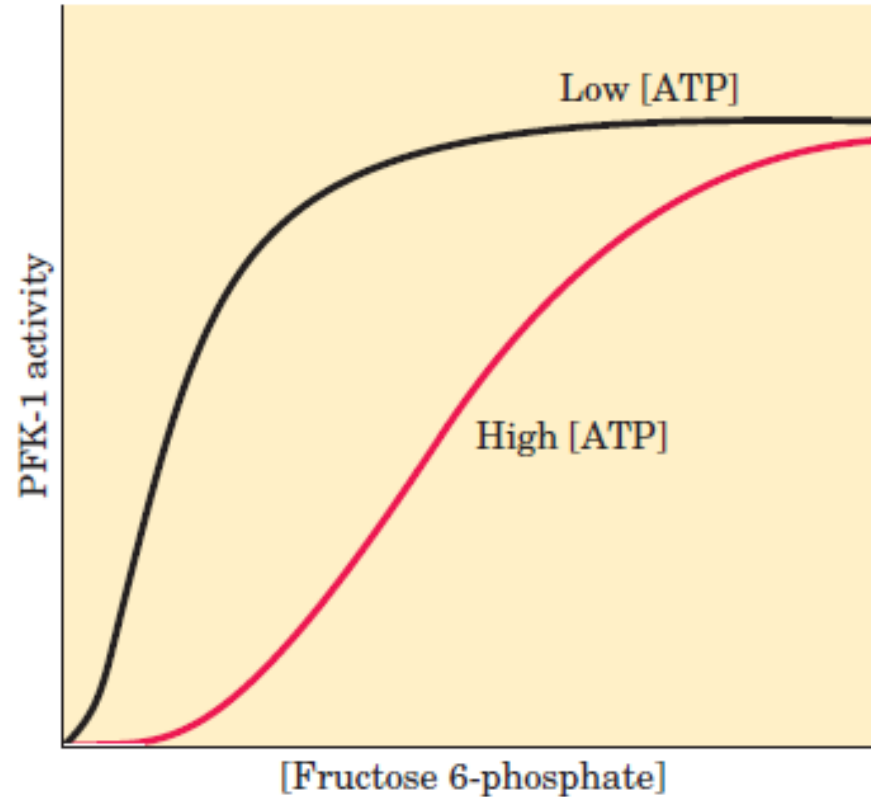


The **most important allosteric regulator** of both glycolysis and gluconeogenesis is **fructose 2,6-bisphosphate, F2,6BP**, which is not an intermediate in glycolysis or in gluconeogenesis.



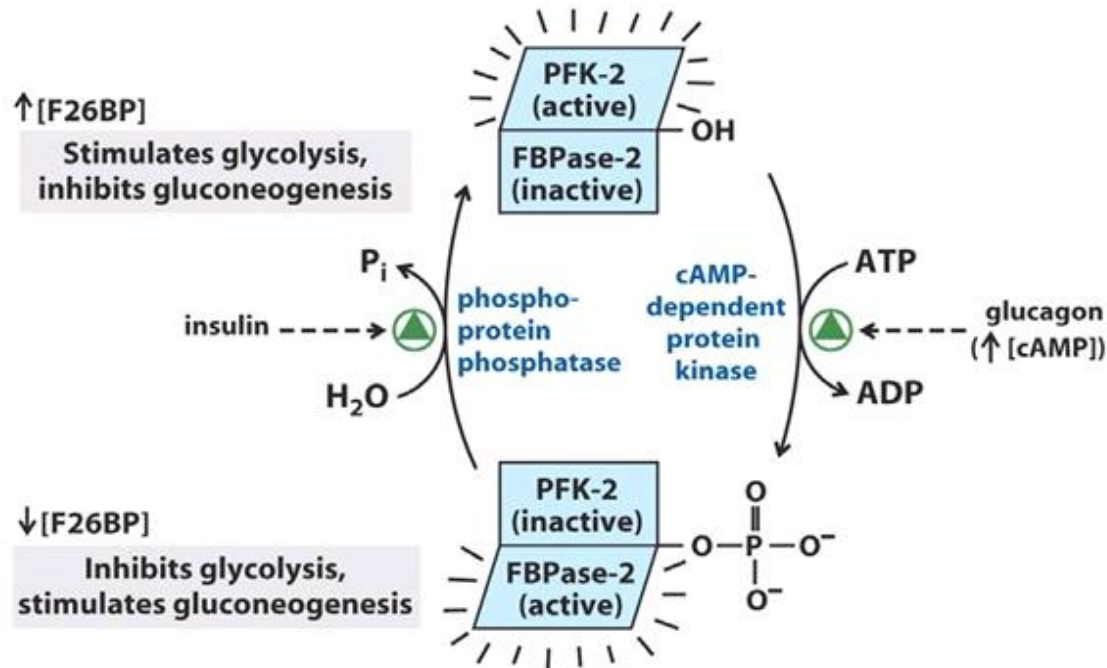
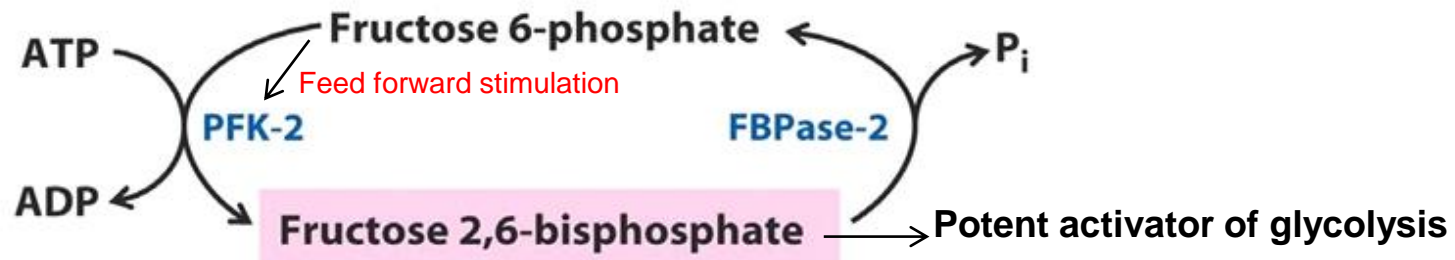
# Phosphofructokinase-1 (PFK1)

ATP allosteric inhibitor



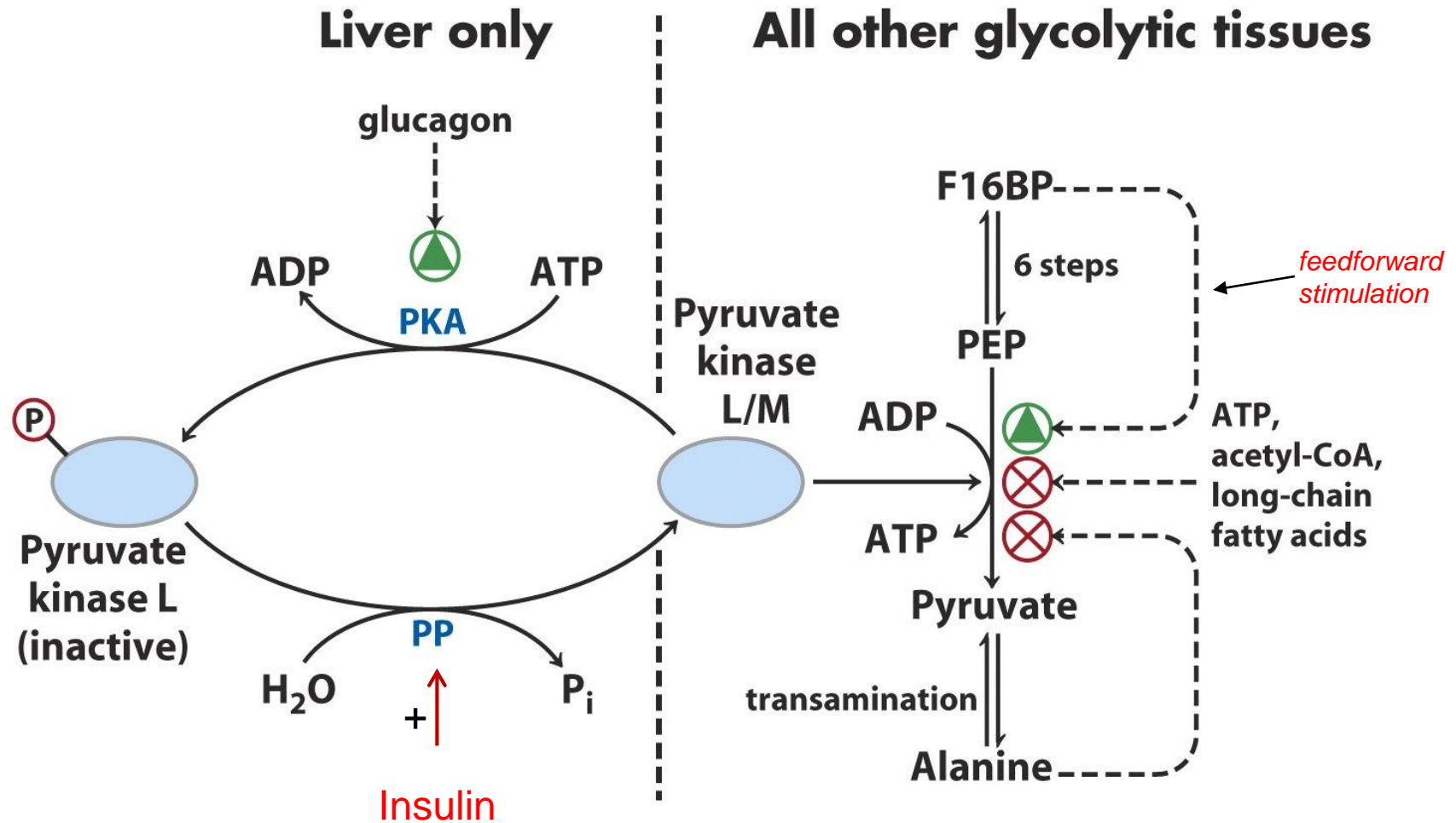
(b)

# Regulation of fructose 2,6-bisphosphate level



Insulin/Glucagon ratio (fed/starve state): **Glucagon**: high in starvation, as blood glucose levels are low, therefore it favors gluconeogenesis in Liver. **Insulin**: on the contrary favors glycolysis.

# Regulation of pyruvate kinase



# Pyruvate kinase

- Pyruvate kinase is the third regulated enzyme of glycolysis. Like PFK, pyruvate kinase is regulated both by allosteric effectors and by covalent modification (phosphorylation). Pyruvate kinase is activated by **F-1,6-BP** in the liver, a second example of **feedforward stimulation**. ATP and alanine act as allosteric inhibitors of pyruvate kinase.
- Phosphorylation of pyruvate kinase is regulated by blood glucose level, just like PFK. High glucagon (low blood sugar) causes phosphorylation, which in this case renders the enzyme inactive.
- Hormonal regulation of glycolysis ensures coordination among different tissues and organs. As we will see later, the same hormones that regulate the rate of glycolysis also regulate gluconeogenesis and the metabolism of glycogen, a stored form of glucose.

# Pyruvate kinase

- The liver isozyme (L form), but not the muscle isozyme (M form), is subject to further regulation by phosphorylation.
- When low blood glucose causes glucagon release, cAMP-dependent protein kinase phosphorylates the L isozyme of pyruvate kinase, inactivating it. This slows the use of glucose as a fuel in liver, sparing it for export to the brain and other organs.



# Pyruvate Kinase Deficiency

- Genetic defects of this enzyme cause the disease known as pyruvate kinase deficiency, a lack of pyruvate kinase slows down the process of glycolysis.
- One example is red blood cells, which in a state of pyruvate kinase deficiency rapidly become deficient in ATP and can undergo hemolysis. Therefore, pyruvate kinase deficiency **can cause hemolytic anemia and an increase in plasma bilirubin.**
- A discrepancy between red blood cell energy requirements and ATP generating capacity produces irreversible membrane injury resulting in cellular distortion, rigidity, and dehydration. This leads to premature erythrocyte destruction by the spleen and liver.

# GLUCONEOGENESIS



**Gluconeogenesis** occurs mainly in **liver**.

Synthesis of glucose from pyruvate utilizes many of the same enzymes as **Glycolysis**.

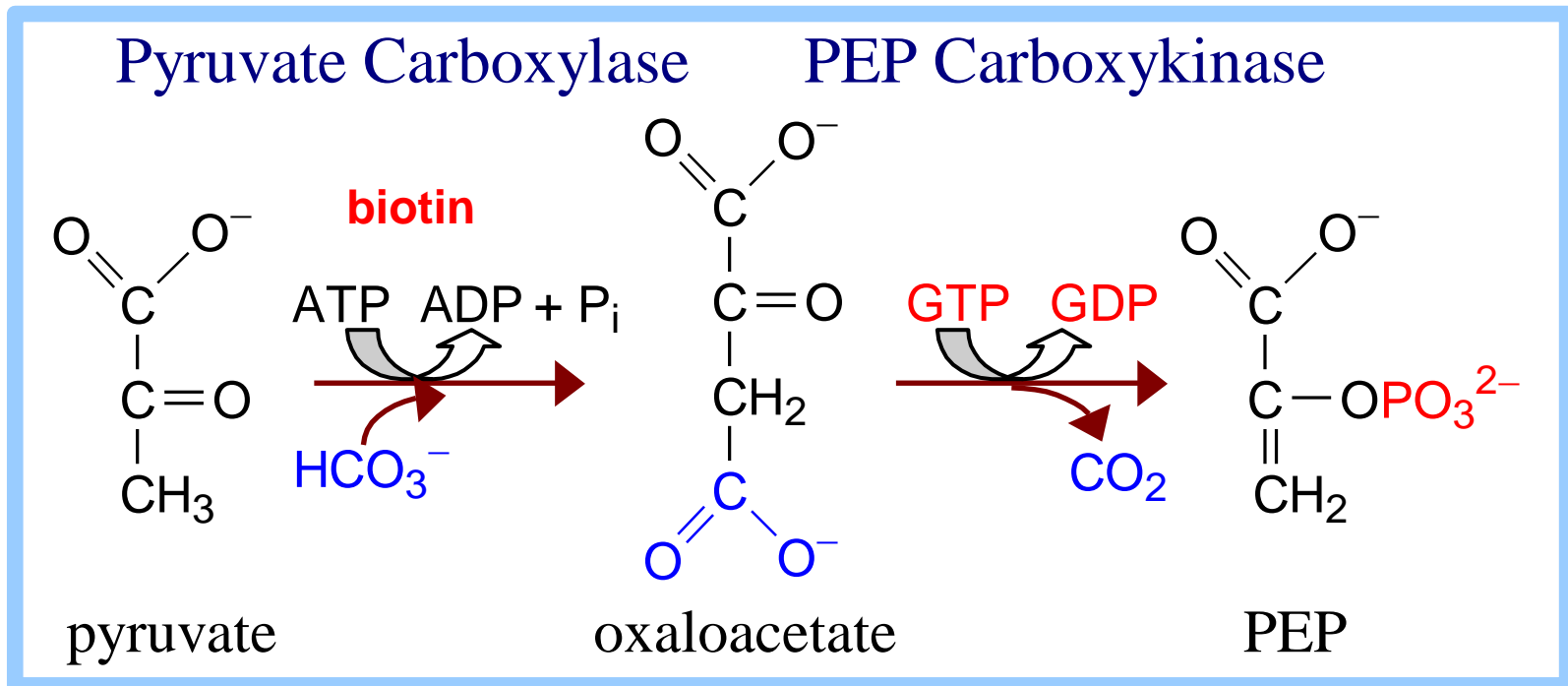
**Three** Glycolysis reactions have such a large negative  $\Delta G$  that they are essentially **irreversible**.

- ◆ **Hexokinase** (or Glucokinase)
- ◆ **Phosphofructokinase1(PFK1)**
- ◆ **Pyruvate Kinase.**

These steps must be **bypassed** in Gluconeogenesis.

**Two** of the bypass reactions involve simple **hydrolysis** reactions.

# First bypass



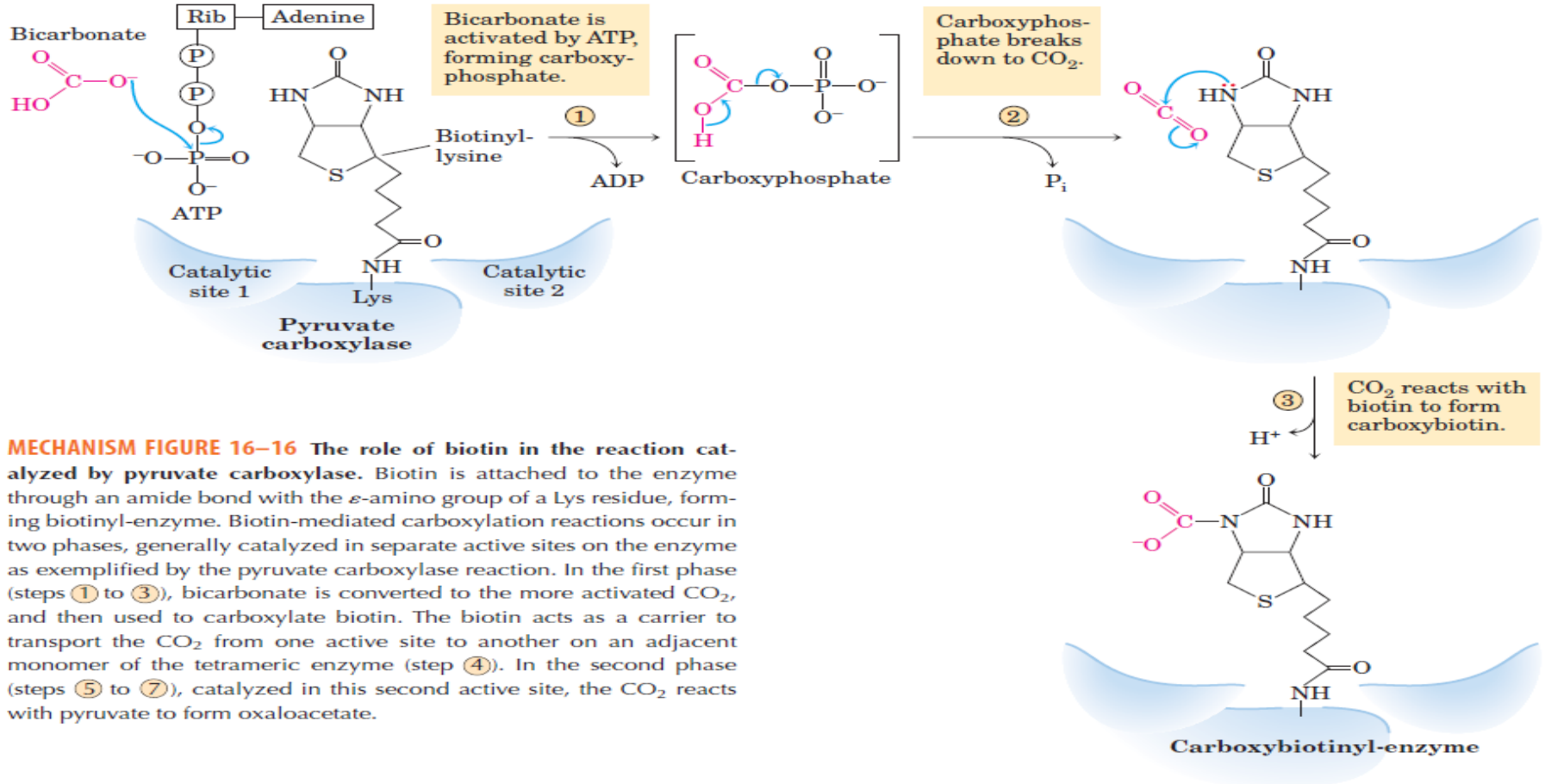
Bypass of Pyruvate Kinase (2 enzymes):

**Pyruvate Carboxylase** (**Gluconeogenesis**) catalyzes:

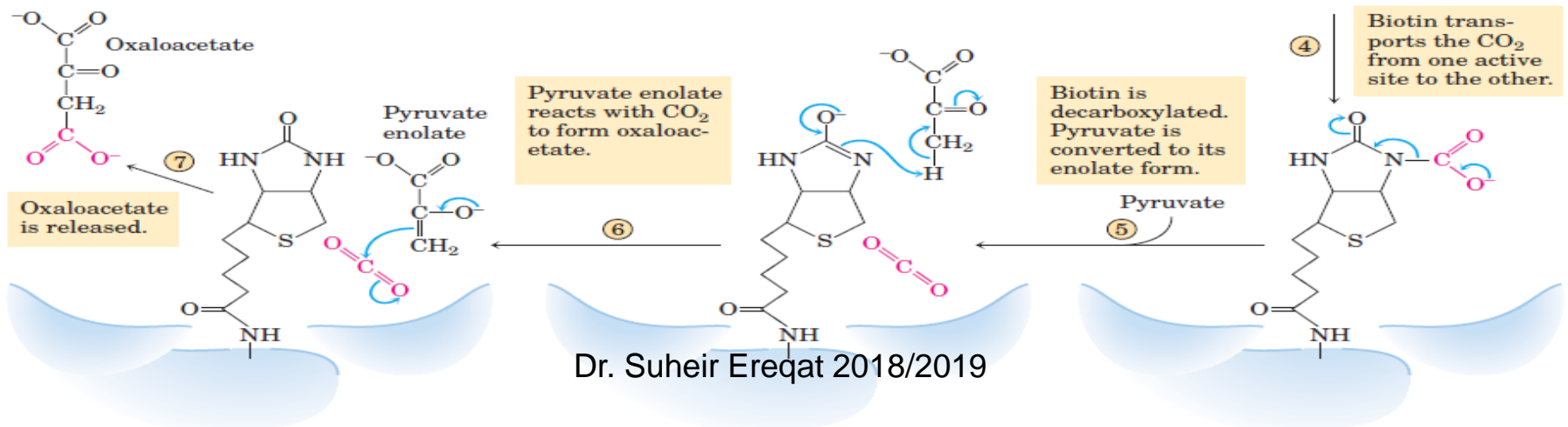


**PEP Carboxykinase** (**Gluconeogenesis**) catalyzes:

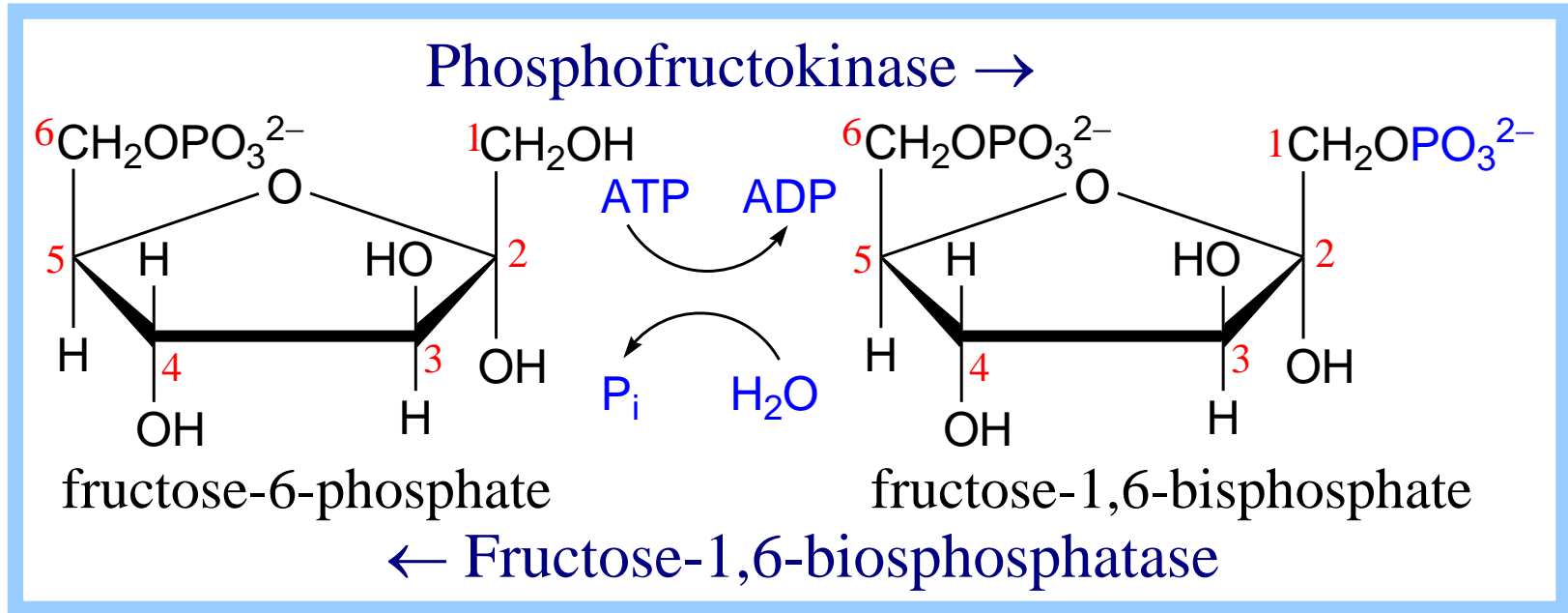




**MECHANISM FIGURE 16-16** The role of biotin in the reaction catalyzed by pyruvate carboxylase. Biotin is attached to the enzyme through an amide bond with the  $\epsilon$ -amino group of a Lys residue, forming biotinyl-enzyme. Biotin-mediated carboxylation reactions occur in two phases, generally catalyzed in separate active sites on the enzyme as exemplified by the pyruvate carboxylase reaction. In the first phase (steps ① to ③), bicarbonate is converted to the more activated  $\text{CO}_2$ , and then used to carboxylate biotin. The biotin acts as a carrier to transport the  $\text{CO}_2$  from one active site to another on an adjacent monomer of the tetrameric enzyme (step ④). In the second phase (steps ⑤ to ⑦), catalyzed in this second active site, the  $\text{CO}_2$  reacts with pyruvate to form oxaloacetate.



# Second bypass



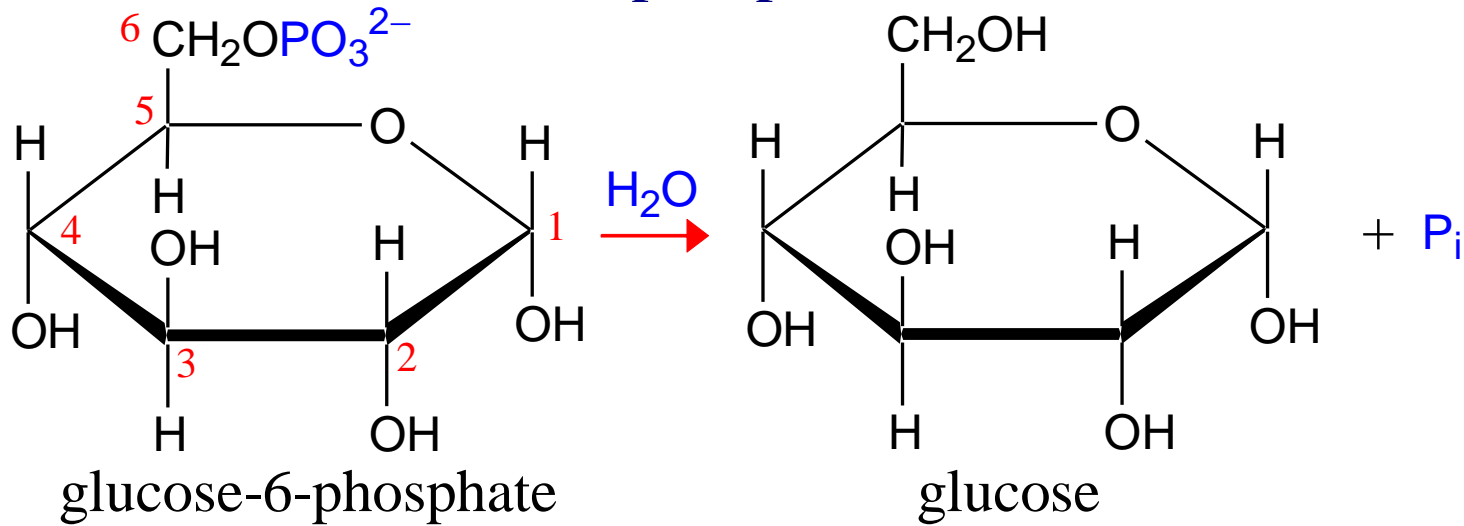
**Phosphofruktokinase(PFK1)** (**Glycolysis**) catalyzes:  
**fructose-6-P + ATP → fructose-1,6-bisP + ADP**

**Fructose-1,6-bisphosphatase** (**Gluconeogenesis**)  
catalyzes:



# Third bypass

## Glucose-6-phosphatase



**Hexokinase** or **Glucokinase** (**Glycolysis**) catalyzes:



**Glucose-6-Phosphatase** (**Gluconeogenesis**) catalyzes:



The **source of pyruvate and oxaloacetate** for gluconeogenesis during fasting or carbohydrate starvation is mainly **amino acid catabolism**.

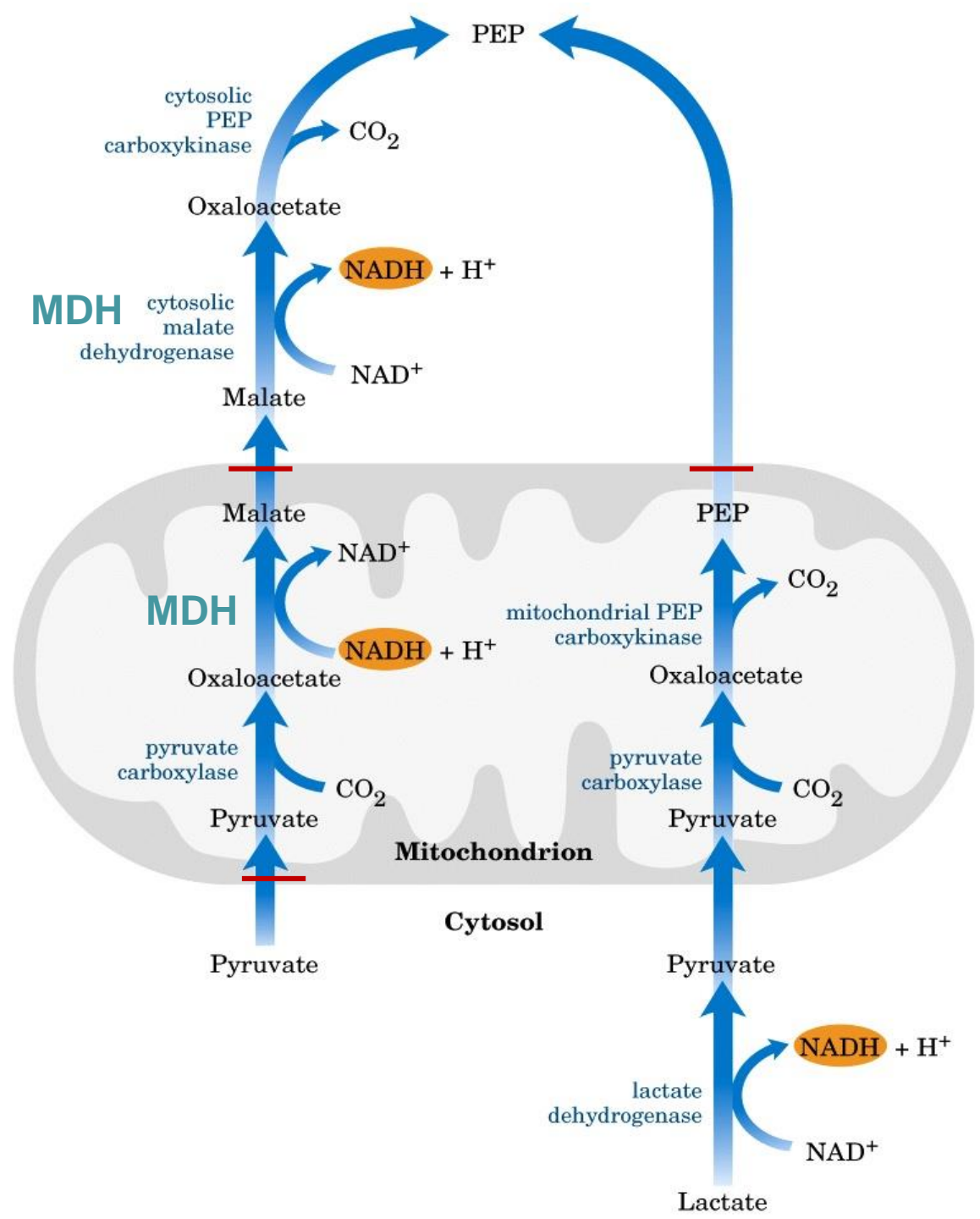
Some amino acids are catabolized to pyruvate, oxaloacetate.

**Muscle proteins** may break down to supply amino acids. These are transported to liver where they are deaminated and converted to gluconeogenesis inputs.

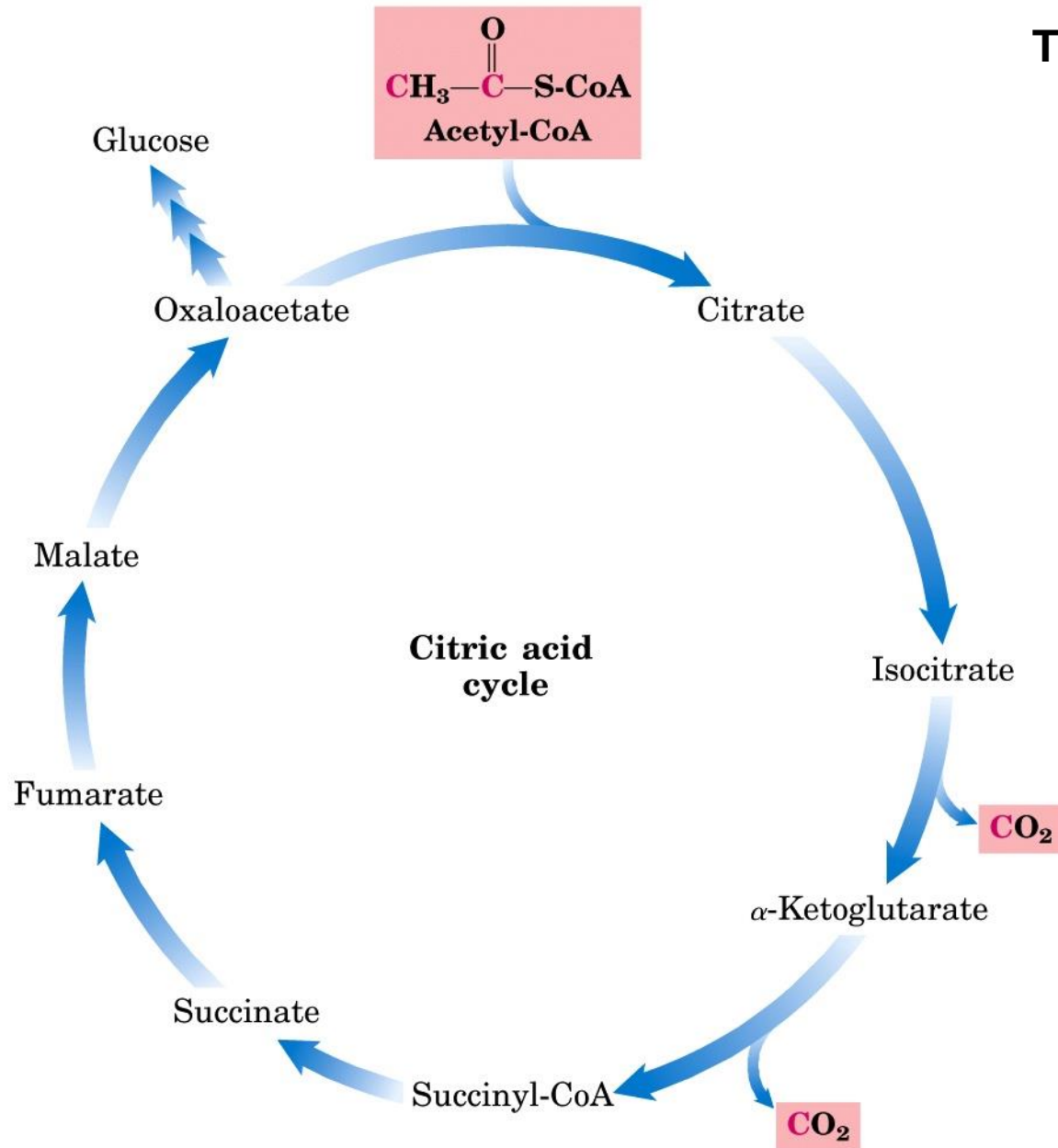
**Glycerol**, derived from hydrolysis of triacylglycerols in fat cells, is also a significant input to gluconeogenesis.

## Malate shuttle

The transport of malate from the mitochondrion to the cytosol and its reconversion there to OAA effectively moves reducing equivalents to the cytosol, where they are scarce. This path from pyruvate to PEP therefore provides an important balance between NADH produced and consumed in the cytosol during gluconeogenesis



# TCA cycle





## table 20–3

### Glucogenic Amino Acids, Grouped by Site of Entry\*

#### **Pyruvate**

Alanine

Cysteine

Glycine

Serine

Tryptophan<sup>†</sup>

#### **$\alpha$ -Ketoglutarate**

Arginine

Glutamate

Glutamine

Histidine

Proline

#### **Succinyl-CoA**

Isoleucine<sup>†</sup>

Methionine

Threonine

Valine

#### **Fumarate**

Phenylalanine<sup>†</sup>

Tyrosine<sup>†</sup>

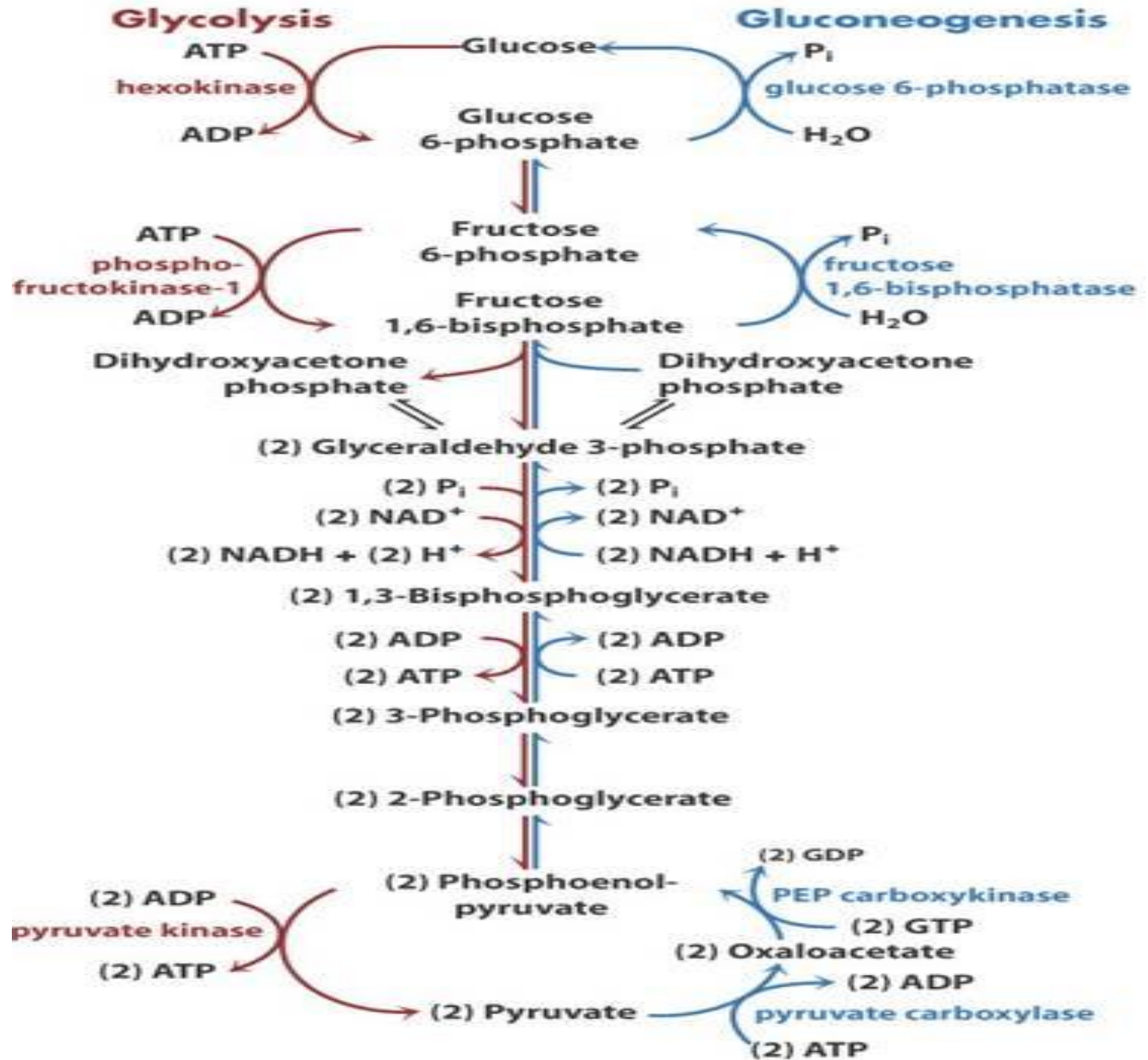
#### **Oxaloacetate**

Asparagine

Aspartate

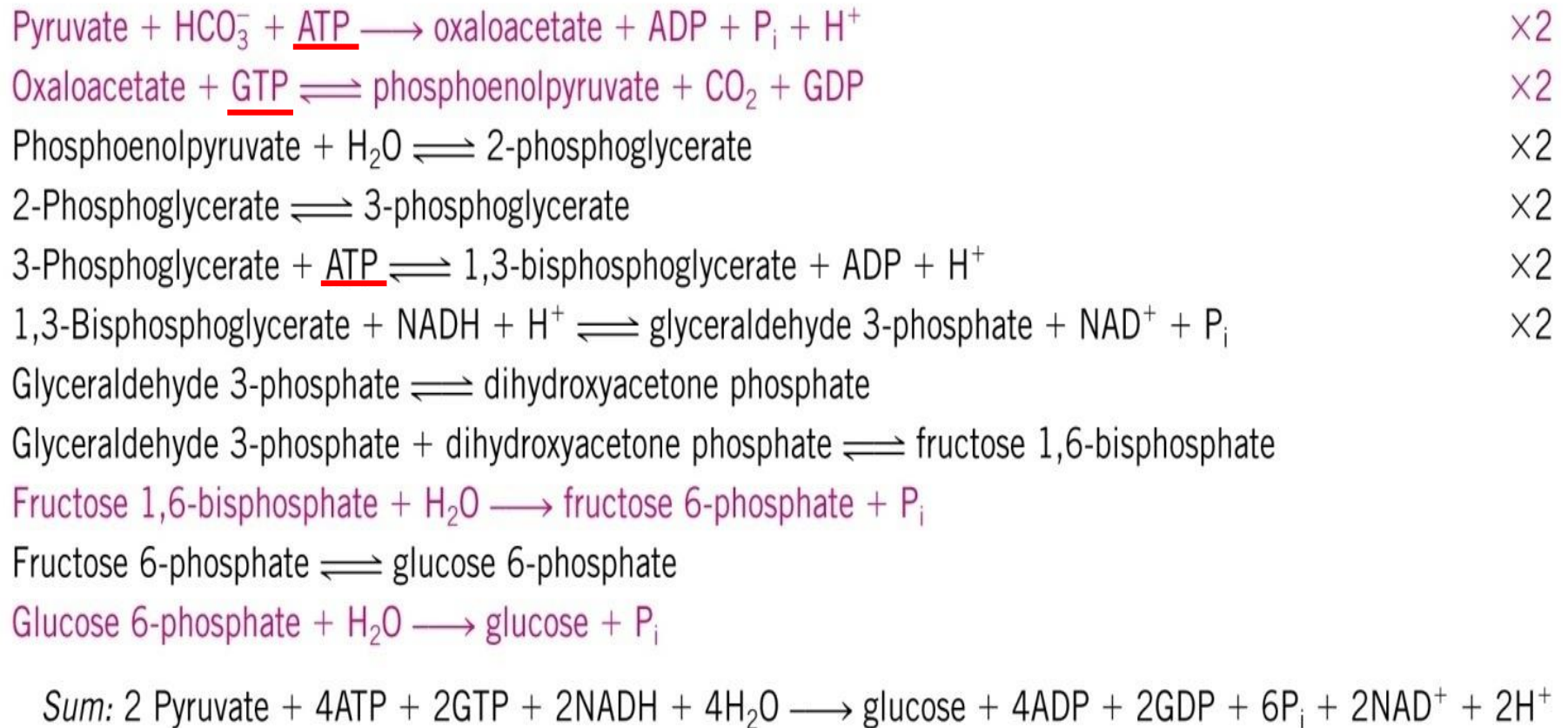
\*These amino acids are precursors of blood glucose or liver glycogen because they can be converted to pyruvate or citric acid cycle intermediates. Only leucine and lysine are unable to furnish carbon for net glucose synthesis.

<sup>†</sup>These amino acids are also ketogenic (see Fig. 18–19).



## table 20-2

### Sequential Reactions in Gluconeogenesis Starting from Pyruvate\*



\*The bypass reactions are in red; all other reactions are reversible steps of glycolysis. The figures at the right indicate that the reaction is to be counted twice, because two three-carbon precursors are required to make a molecule of glucose. Note that the reactions required to replace the cytosolic

NADH consumed in the glyceraldehyde 3-phosphate dehydrogenase reaction (the conversion of lactate to pyruvate in the cytosol or the transport of reducing equivalents from mitochondria to the cytosol in the form of malate) are not considered in this summary.

# **METABOLIC BIOCHEMISTRY**

## **REGULATION** (Gluconeogenesis)

# Gluconeogenesis

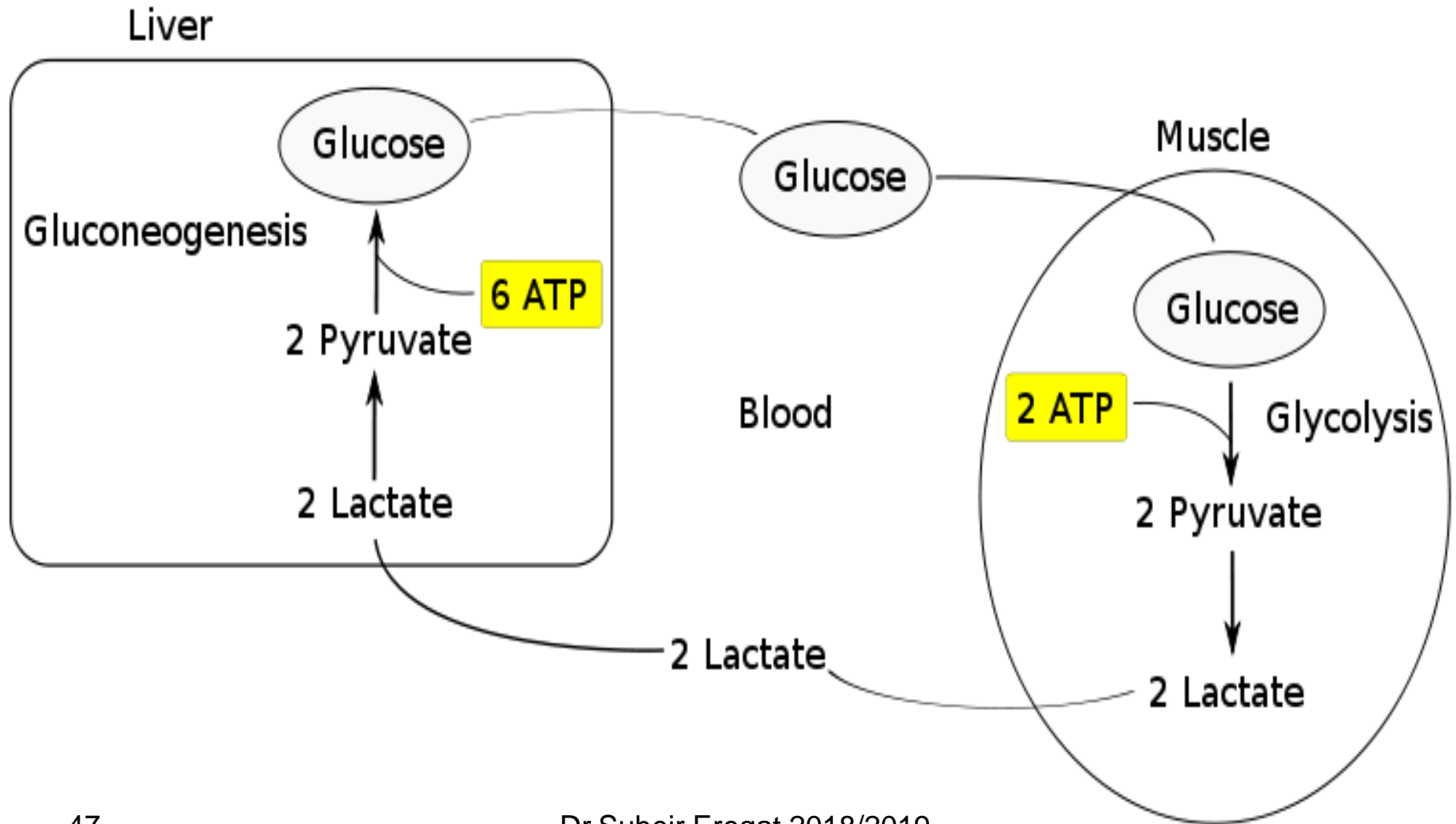
- *Gluconeogenesis involved in 2 cycles to maintain blood glucose level:*

*Cori cycle and alanine cycle*

*What is cori cycle?*

- Glucose formed circulates to tissues in blood
- Required to recycle lactate in large mammals on a recovery basis after anaerobias
- Lactate (muscles;RBC) → Liver (glycogen) is the Cori Cycle

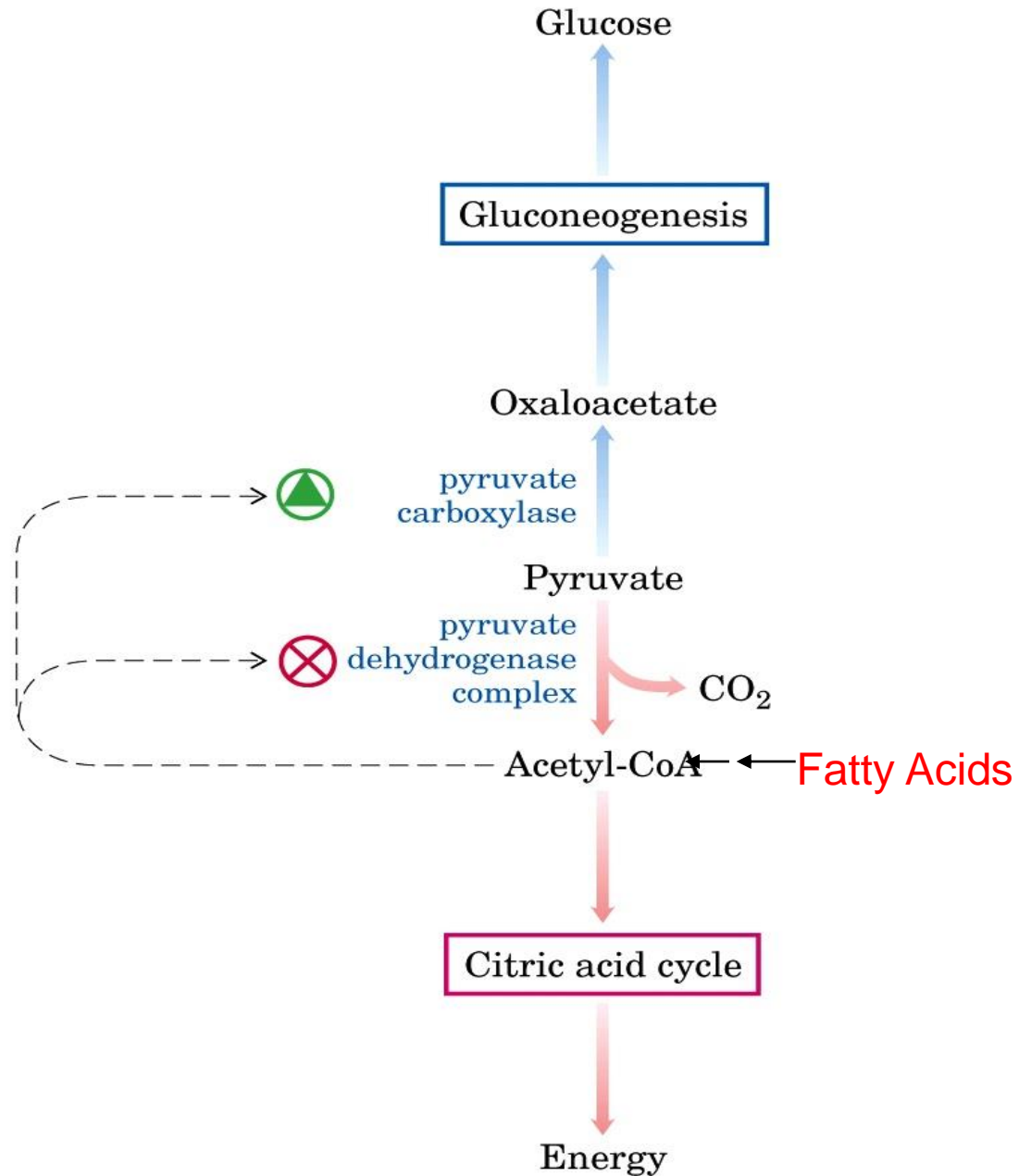
# Cori Cycle



# Two alternative fates for pyruvate

When fatty acids are readily available as fuels, their breakdown in liver mitochondria yields acetyl-CoA, a signal that further oxidation of glucose for fuel is not necessary.

Acetyl-CoA is a positive allosteric modulator of pyruvate carboxylase and a negative modulator of pyruvate dehydrogenase complex



# Glycolysis vs. Gluconeogenesis

## Gluconeogenesis



Fructose 6-phosphate

P<sub>i</sub>

ATP



← - - - - ATP



← - - - - ADP



← - - - - AMP - - - - - →



← - - - - citrate

FBPase-1

PFK-1

H<sub>2</sub>O

ADP

Fructose 1,6-bisphosphate

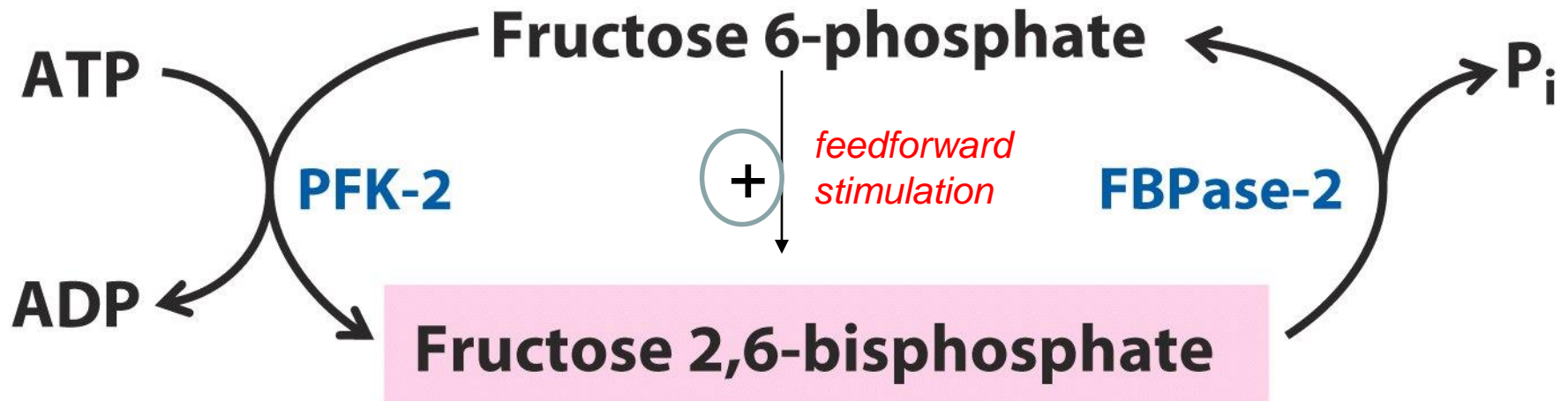


## Glycolysis

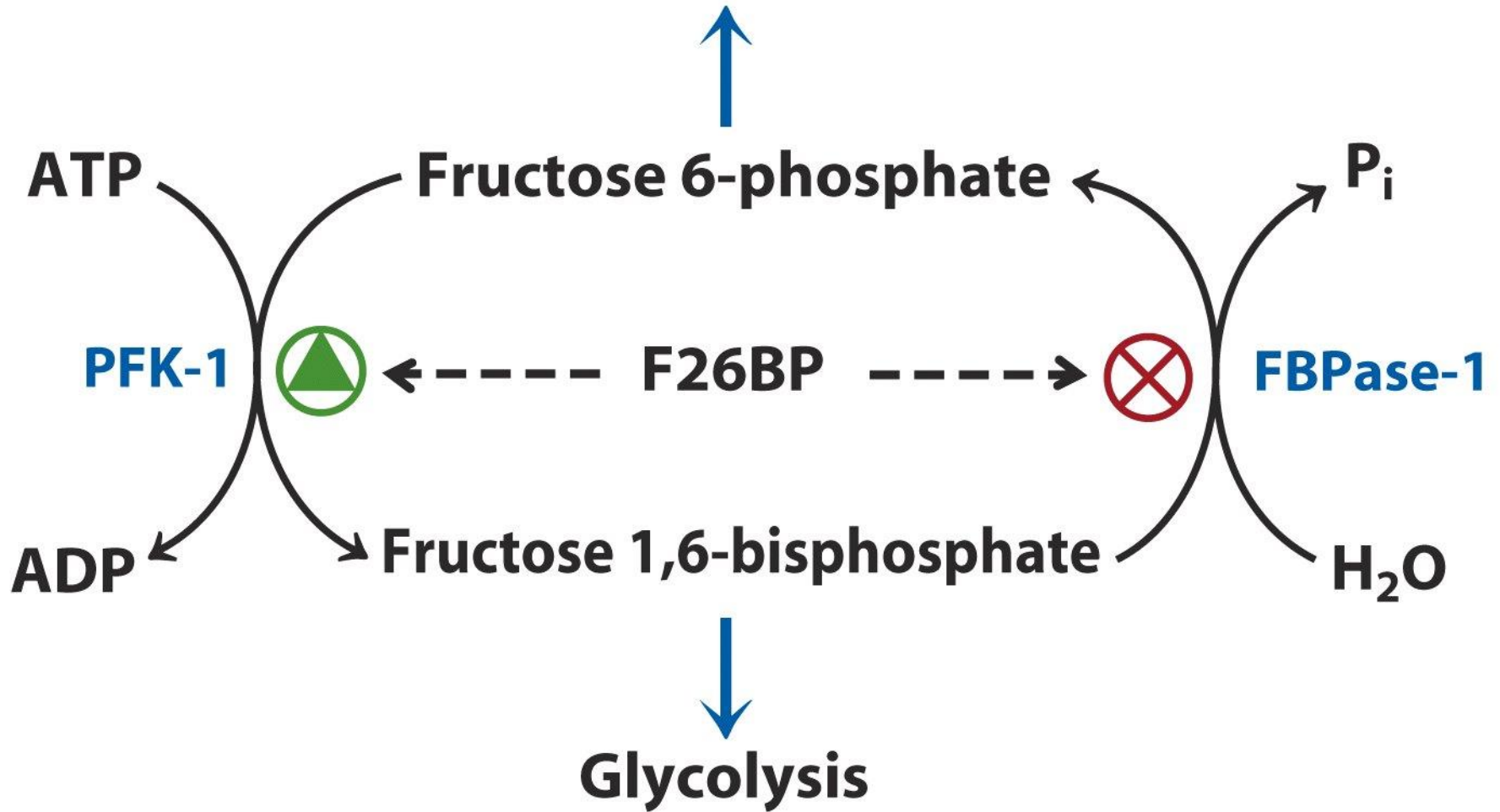
ATP/ADP,AMP ratio



# Fructose 2,6-Bisphosphate Is a Regulator of Glycolysis and Gluconeogenesis

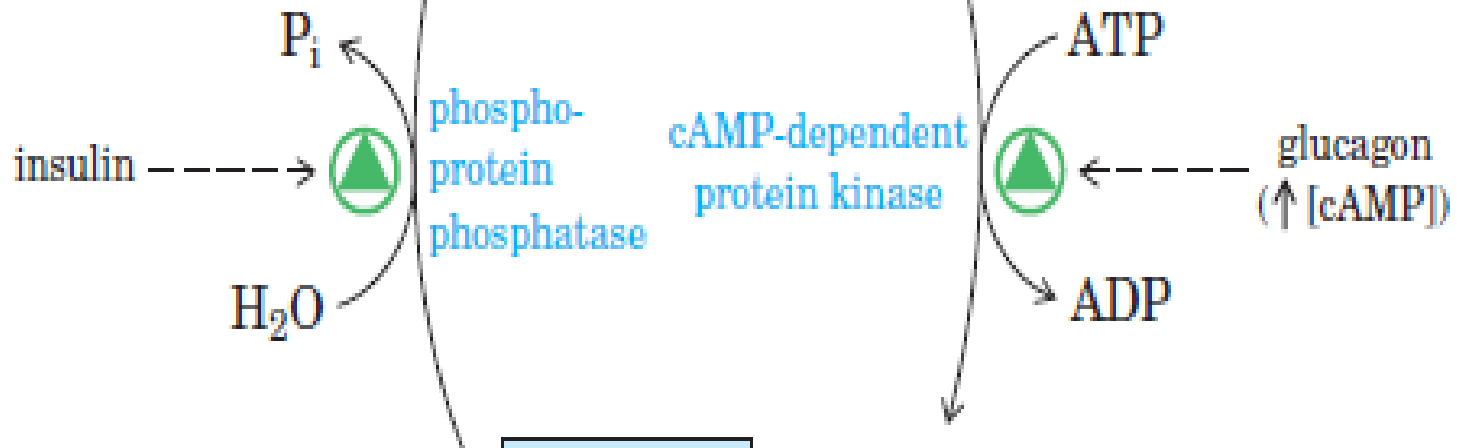
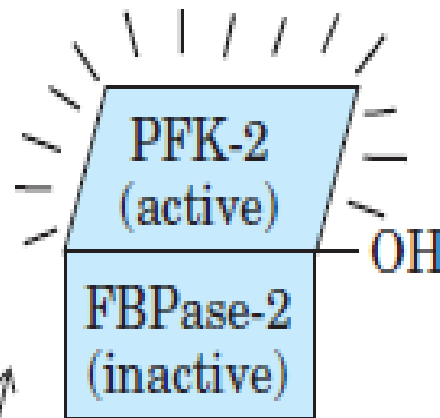


# Gluconeogenesis



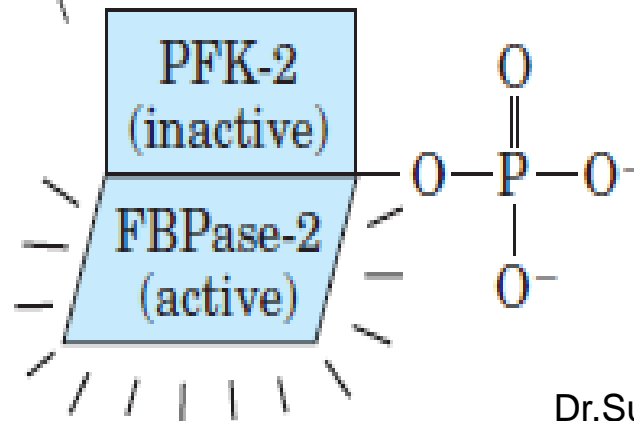
↑[F26BP]

Stimulates glycolysis,  
inhibits gluconeogenesis



↓[F26BP]

Inhibits glycolysis,  
stimulates gluconeogenesis



## To Summarize.....

- Phosphorylation of the bifunctional enzyme (PFK2/FBPase2) is regulated by blood glucose level, mediated by glucagon and insulin.
- High glucagon (low blood sugar) causes phosphorylation of the enzyme, which results in conversion of F-2,6-BP back to F6P, removing its stimulatory effect on PFK, and therefore slowing the rate of glycolysis.
- Glucagon promotes transcription of the gene for PEP carboxykinase
- Conversion of F6P to F-2,6-BP is also stimulated by high levels of F6P. This is an example of **feed forward** stimulation .
- Feedforward regulation ensures that intermediates on metabolic pathways do not accumulate uselessly.
- F-2,6-BP is also an important regulator of the process of gluconeogenesis, where glucose is synthesized from pyruvate.