Gluconeogenesis

Ahmed Salem, MD, MSc, PhD, FRCR

Carbohydrates metabolism II Aerobic metabolism	1.Gluconeogenesis Synthesis of glucose from lactate, amino acids and glycerol 2. Krebs cycle 3. Electron transport and oxidative phosphorylation 4.Inhibitors of electron transport and oxidative phosphorylation

Gluconeogenesis: Definition

- Metabolic process by which glucose in synthesised from non-carbohydrate precursors:
    - Lactate
    - Glucogenic amino acids (major source of glucose after glycogen is depleted)
    - Glycerol (part of TAG)
    - Odd chain fatty acids (rare); Propionyl coA (minor source)

Physiological importance

- Maintains blood glucose level especially in starvation
    - Brain has a **minimum obligatory requirement of 120g glucose/ day** → provided in case of starvation via gluconeogenesis
        - Appx 60% of total CHO intake by body is metabolized by brain
    - Glucose main source of energy for anaerobic tissues (RBCs, muscles during exercise)

- Control of acid-base balance
    - Production of lactate in excess to clearance → metabolic acidosis
    - Re-synthesis of glucose from lactate is a major route for lactate disposal

- Glucose required in adipose tissue as a precursor of glycerol

- Glucose is precursor of milk sugar lactose in mammary gland

- Glucose is needed to maintain the intermediates of the TCA

Important facts about gluconeogenesis

- **Sites of occurrence:** partially in mitochondria and partially in cytosol of liver (85-90%) & kidney cortex

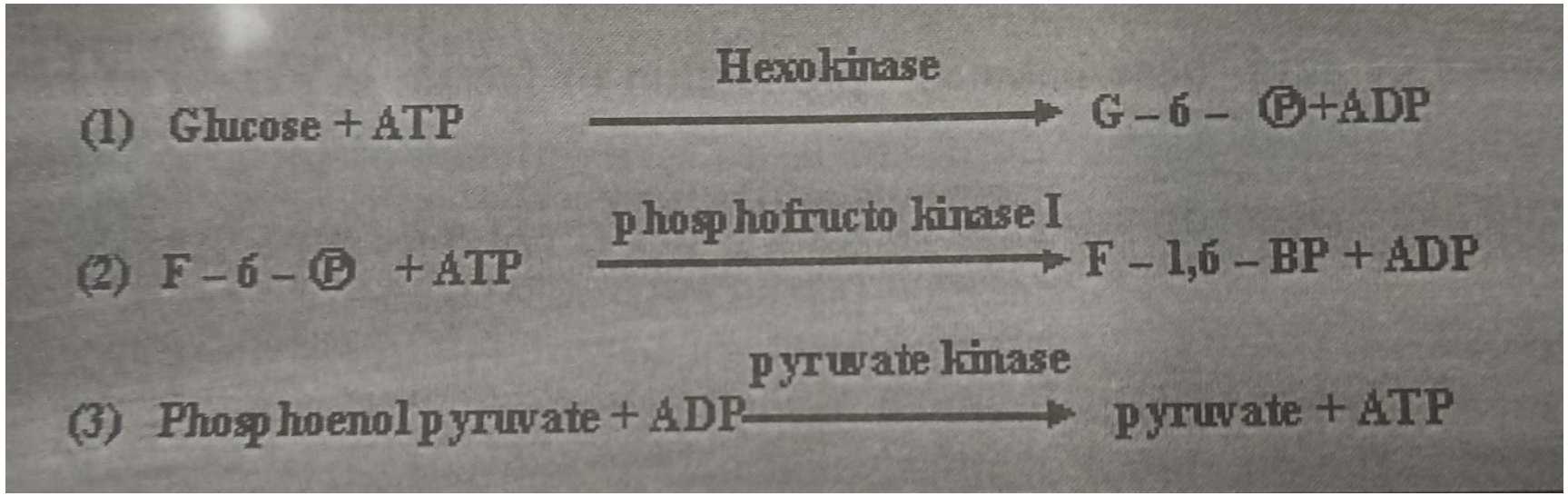
- Conditions characterised by active gluconeogenesis:
  - Prolonged fasting/ starvation → starts 6-8 h after last meal and fully active 12-18 (after depletion of liver glycogen)
  - Cushing's syndrome (high cortisol level)/ DM
  - Cortisone and ACTH therapy
  - To get rid of increased lactate (severe muscular exercise, lactate from RBCs)
  - Unbalanced diet (low CHO, high fat)

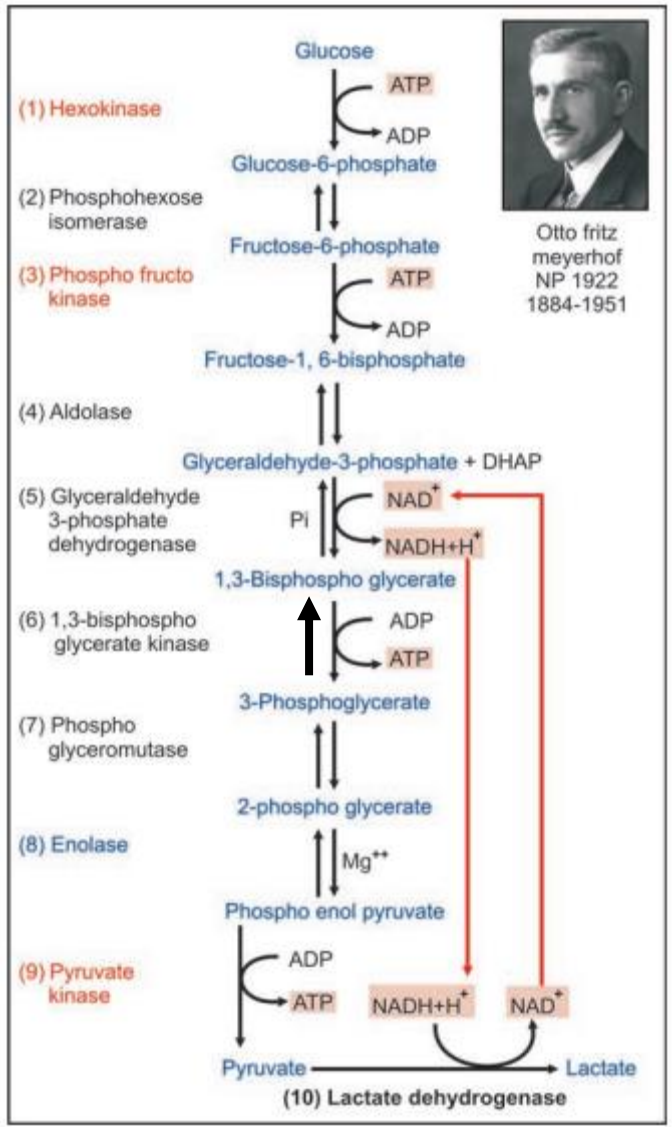
After an overnight fast, glycogenolysis and gluconeogenesis make approximately equal contributions to blood glucose. As glycogen reserves become increasingly depleted, gluconeogenesis becomes progressively more important

Gluconeogenic pathway

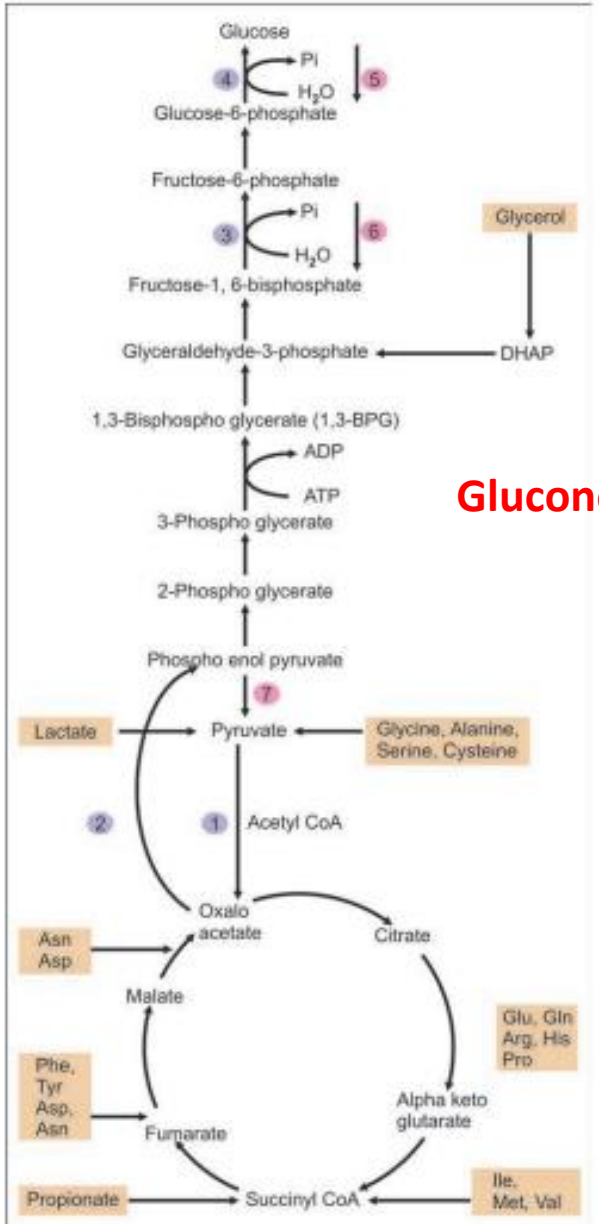
- **Pretty much** the reversal of glycolysis (but not just reversal of glycolysis)
    - Gluconeogenesis & glycolysis need to be reciprocally regulated (when glycolysis is active, gluconeogenesis is shut down)

- 3 irreversible reactions of glycolysis need to be overcome:





Glycolysis



Gluconeogenesis

Key gluconeogenic enzymes:
1=Pyruvate carboxylase; 2=Phospho enol pyruvate carboxykinase; 3=Fructose-1, 6-bisphosphatase; 4=Glucose-6-phosphatase

Key glycolytic enzymes:
5=Hexokinase; 6=Phosphofructokinase; 7=Pyruvate kinase.
Substrates for gluconeogenesis are shown inside brown squares.

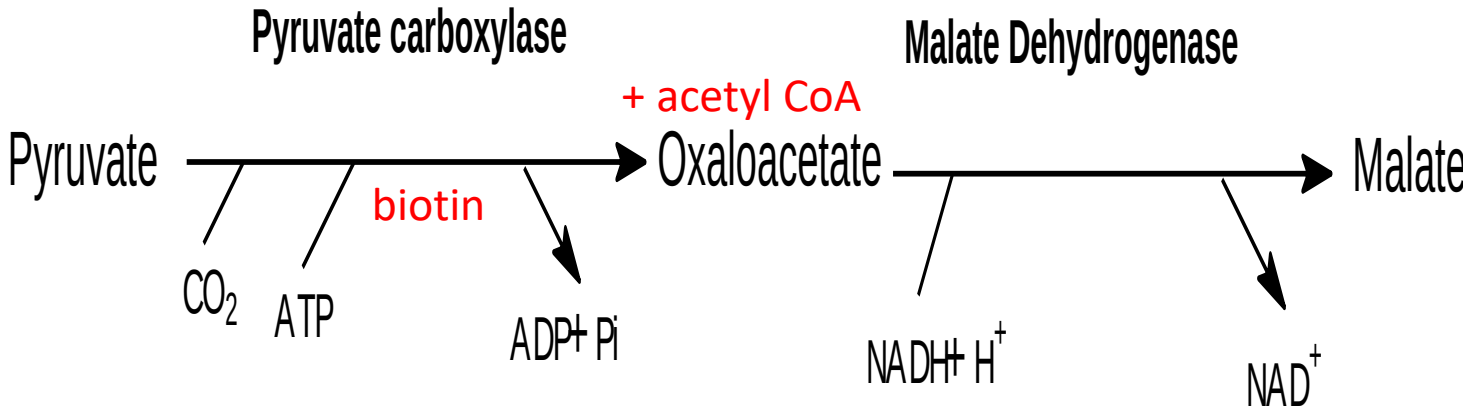
Table 9.7. Key enzymes

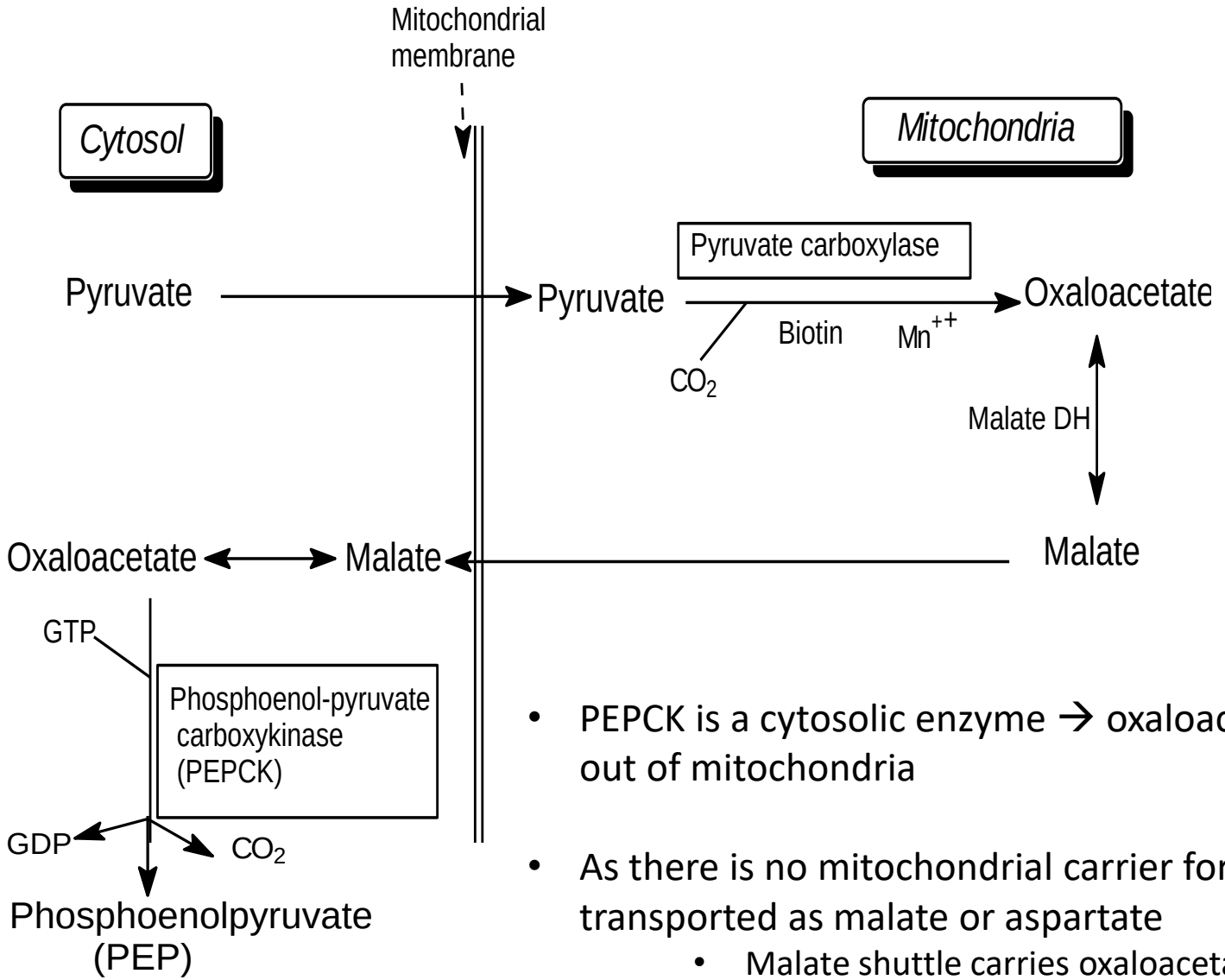
Irreversible steps in glycolysis	Corresponding key gluconeogenic enzymes
Pyruvate kinase (Step 9)	Pyruvate carboxylase; Phosphoenol pyruvate-carboxy kinase
Phosphofructokinase (Step 3)	Fructose-1,6-bisphosphatase
Hexokinase (Step 1)	Glucose-6-phosphatase

1) FROM LACTATE & PYRUVATE: **This requires:**

(A) Reversal of Pyruvate Kinase Reaction: (Dicarboxylic acid shuttle).

- Pyruvate generated in cytosol is transported to mitochondria and converted to oxaloacetate
- Pyruvate carboxylase like many CO2 fixing enzymes needs **biotin**

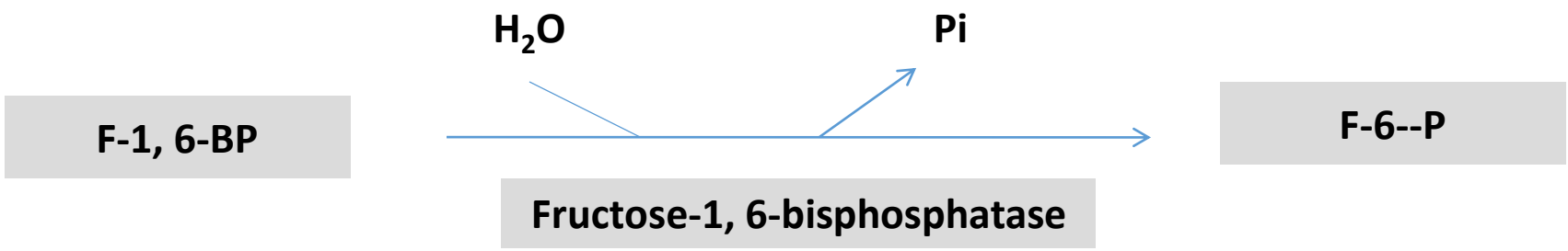




- PEPCK is a cytosolic enzyme → oxaloacetate must be transported out of mitochondria
- As there is no mitochondrial carrier for oxaloacetate, it is transported as malate or aspartate
  - Malate shuttle carries oxaloacetate and reducing equivalents
  - Aspartate shuttle does not require preliminary reduction step, depends of availability of glutamate and α ketoglutartae

(B) **Reversal of the phosphofructokinase reaction:**

Fructose-1, 6-bisphosphatase is the ***KEY ENZYME*** of gluconeogenesis.

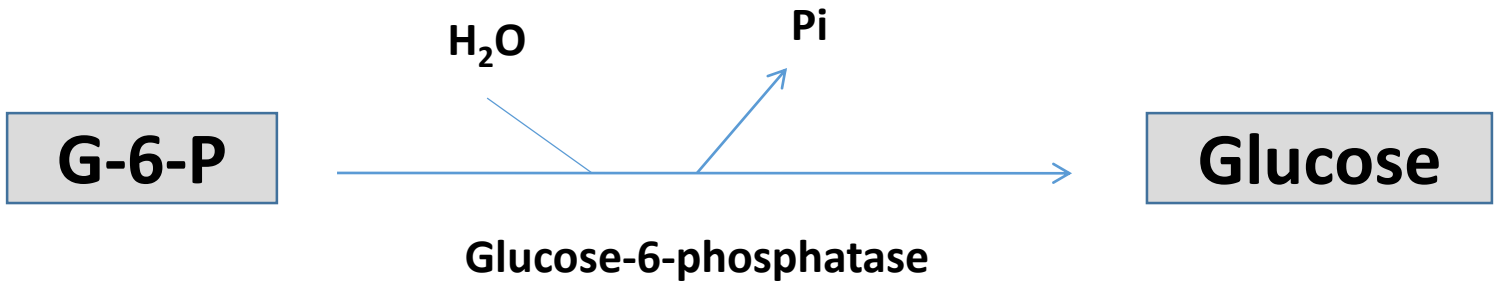


Fructose-1, 6-bisphosphatase enzyme is not found in heart, smooth muscle or adipose tissue , so gluconeogenesis does not occur in these sites.

(C) **Reversal of the Hexokinase Reaction:**

- G-6-phosphatase is present in the liver & (kidney, & intestines) lesser extent
  - Liver provides >85% of glucose produced in body
    - This proportion ↓ in prolonged starvation → kidney production ↑
  - Totally absent in brain, muscles and adipose tissues

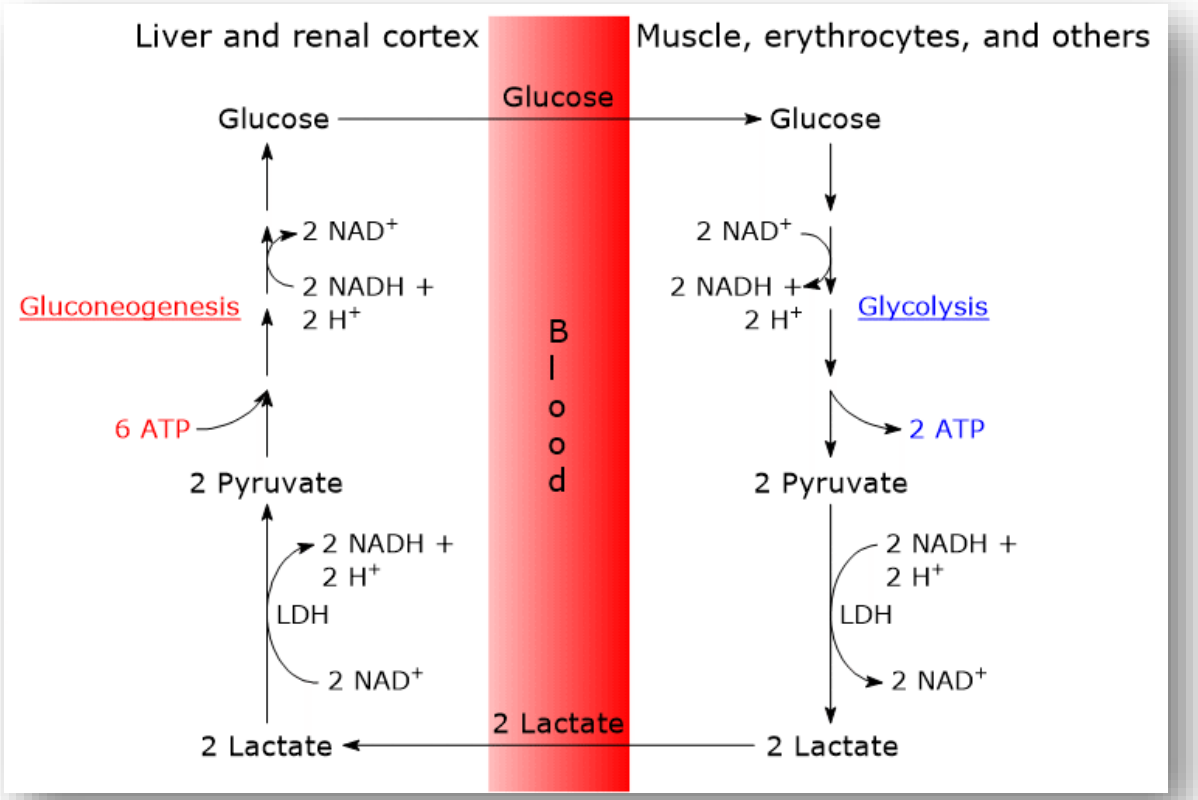
- In skeletal muscles gluconeogenesis ends in G-6-P which cannot leave the cell , but G-6-P can form glycogen



Gluconeogenic precursors

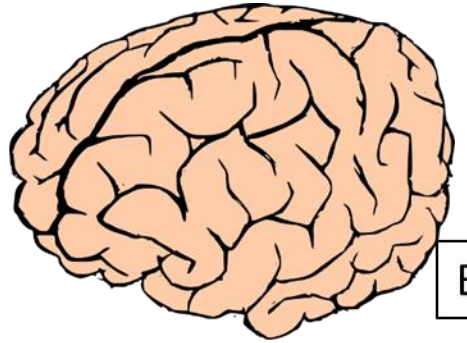
- Lactate
  - End product of anaerobic metabolism of glucose in muscle, RBCs
  - It is transported from muscle, RBCs to liver where it is reoxidised by LDH to pyruvate which is converted to glucose

- Cori cycle
  - Occurs between [RBCs and muscles in vigorous exercise] and liver
  - Clears blood and tissues from lactate **to give glucose in the liver**
  - **→ prevents lactic acidosis**



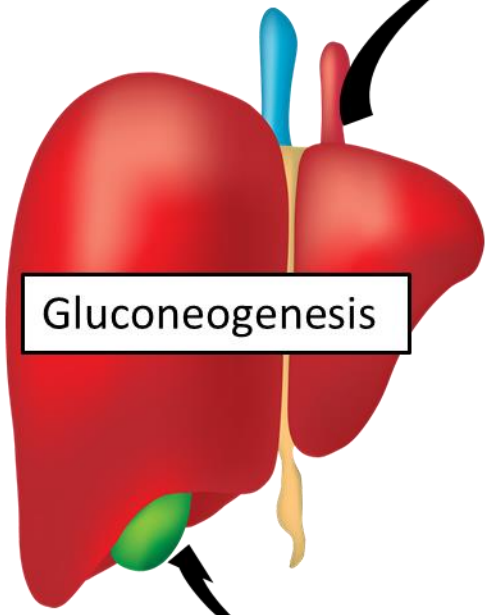
Cori Cycle (Lactic Acid Cycle)

Blood Glucose
70-110 mg/dl
(3.5-6 mmol/l)



Brain

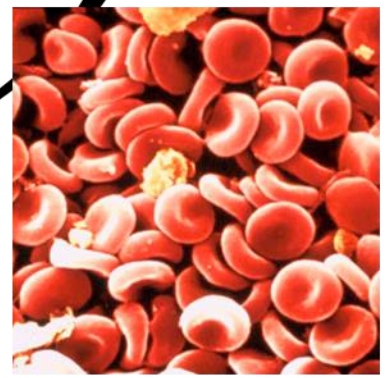
Liver and kidney



Gluconeogenesis

Cori Cycle: in fasting, insulin level is decreased while glucagon, adrenaline and cortisol levels increase. This stimulates gluconeogenesis in the liver and kidney tubules, which converts lactate into glucose to maintain blood level. Most of the glucose is directed to the brain and red blood cells. Less amounts go to skeletal muscles, which rely on ketone bodies and fatty acids for energy. Red cells convert glucose all the time to lactate but brain and skeletal muscles also produce lactate under anaerobic conditions which in the brain could be contributed to by morphine respiratory depressants. Lactate is released to the blood and taken by the liver to be reconverted back to glucose.

Skeletal muscles



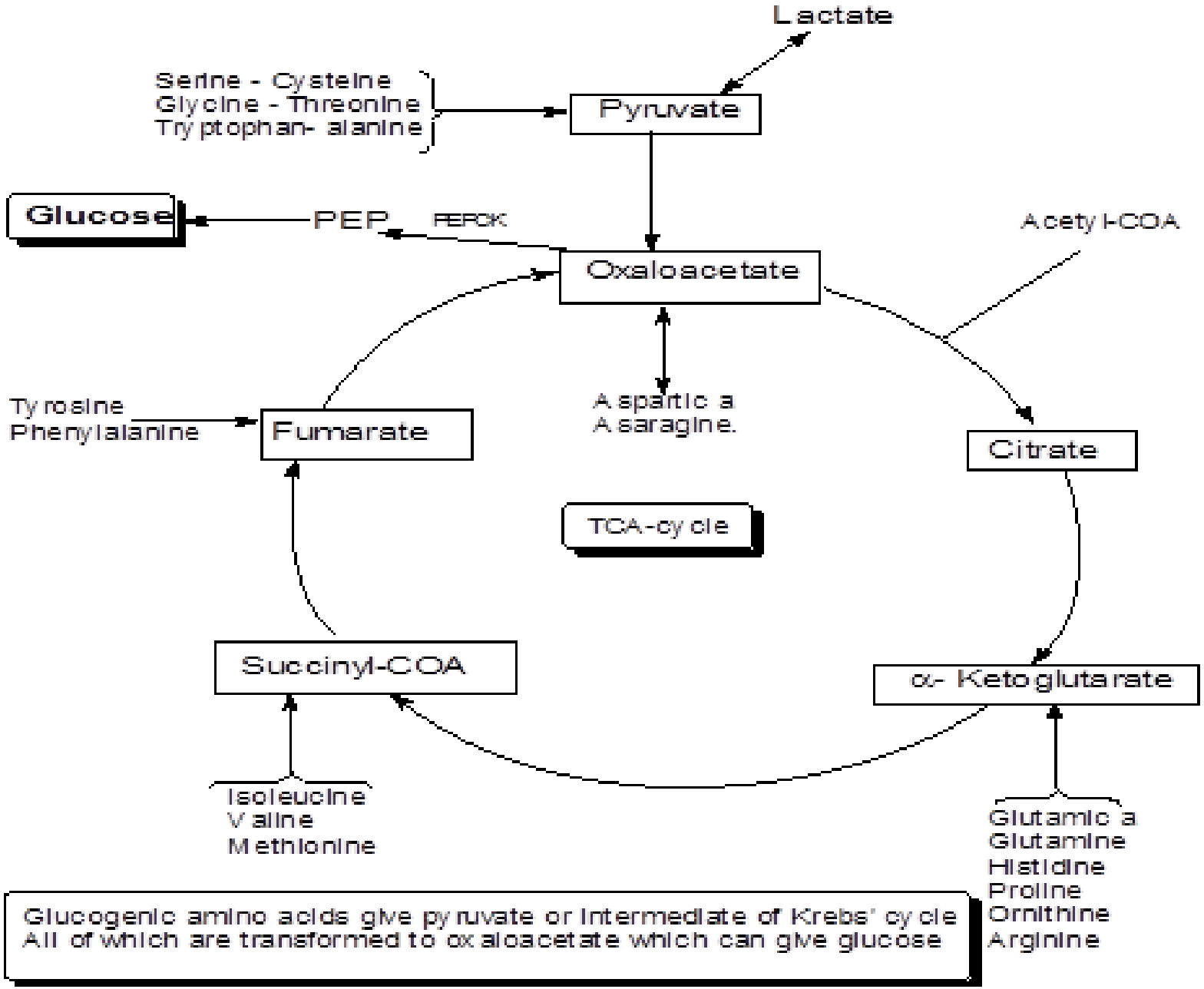
Red blood cells

Lactic acid
2-5 mmol/l
(Increased in lactic acidosis)

Glucose from amino acids:

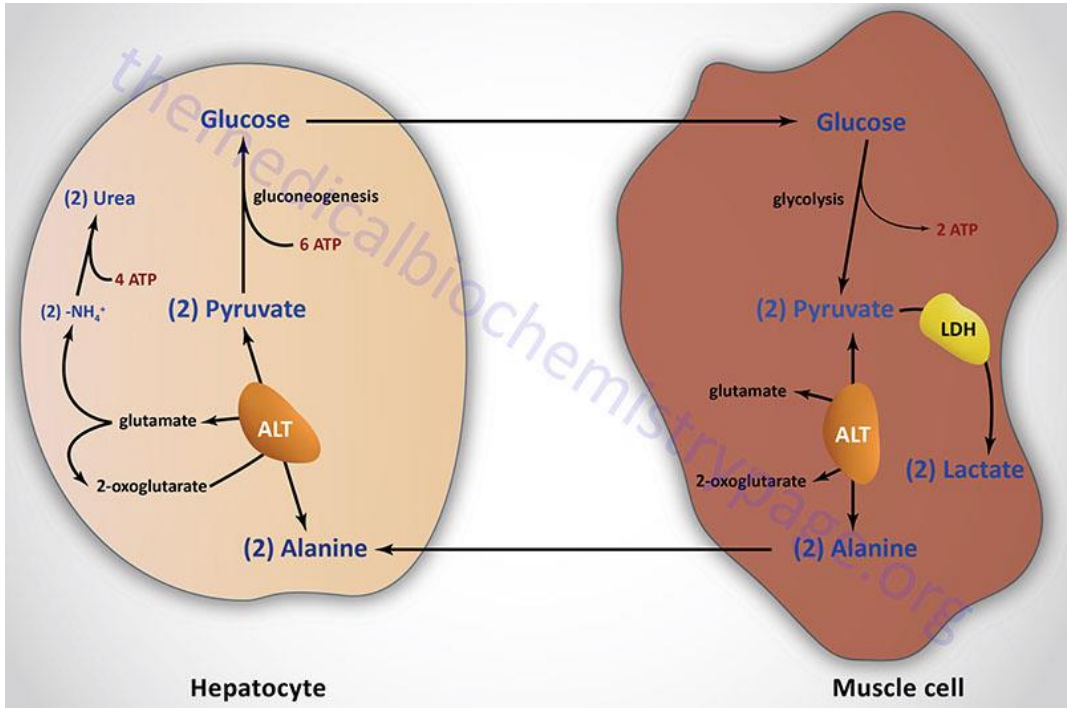
- All glucogenic and mixed amino acids can give glucose (i.e. all amino acid except leucine and lysine)

- Amino acids give pyruvate or intermediates of Krebs' cycle
  - → both can be converted to oxaloacetate which by PEPCK can give phosphoenolpyruvate (PEP)
  - PEP by reversal of glycolysis can form glucose or glycogen

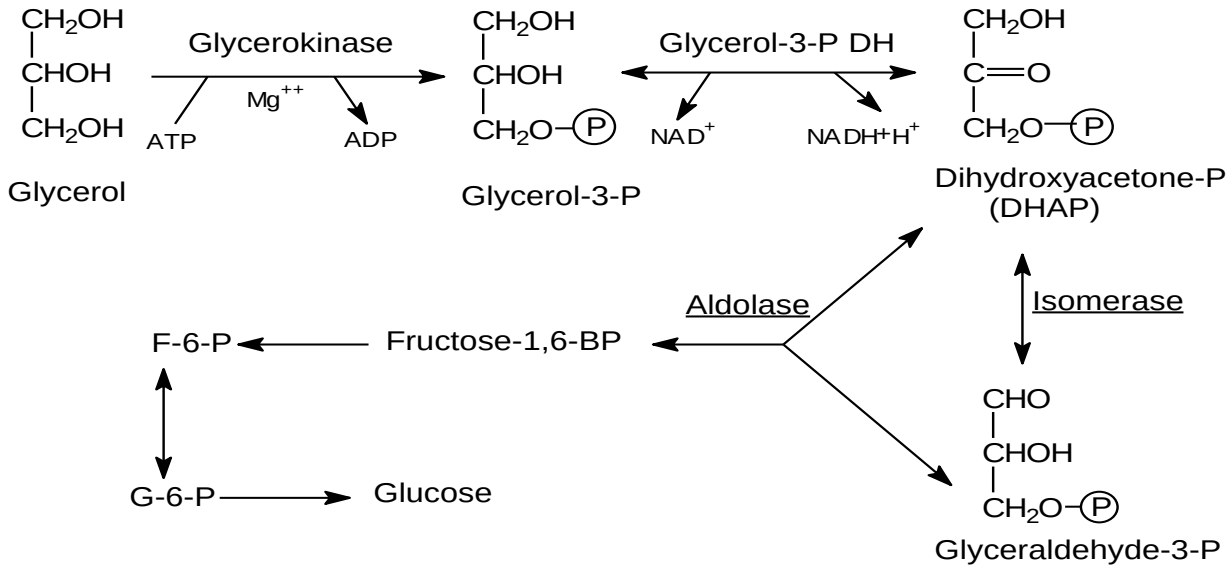

Lactate
Serine - Cysteine
Glycine - Threonine
Tryptophan- alanine
Pyruvate
Glucose
PEP
PEPCK
Acetyl-COA
Oxaloacetate
Tyrosine
Phenylalanine
Fumarate
Aspartic a
Asaragine.
Citrate
TCA-cycle
Succinyl-COA
α- Ketoglutarate
Isoleucine
Valine
Methionine
Glutamic a
Glutamine
Histidine
Proline
Ornithine
Arginine
Glucogenic amino acids give pyruvate or Intermediate of Krebs' cycle
All of which are transformed to oxaloacetate which can give glucose

Glucose-Alanine cycle

- Alanine is transported from muscle to liver, transaminated → pyruvate → glucose
- Glucose can enter glycolytic pathway to form pyruvate which is transaminated → alanine
- Glucose-alanine cycle is of primary importance in conditions of starvation
- **Importance**
  - Transfer if 3C of pyruvate to the liver to give glucose
  - Transfer of NH3 in non-toxic form from muscle to liver to be converted to urea
  - Related to Cori cycle

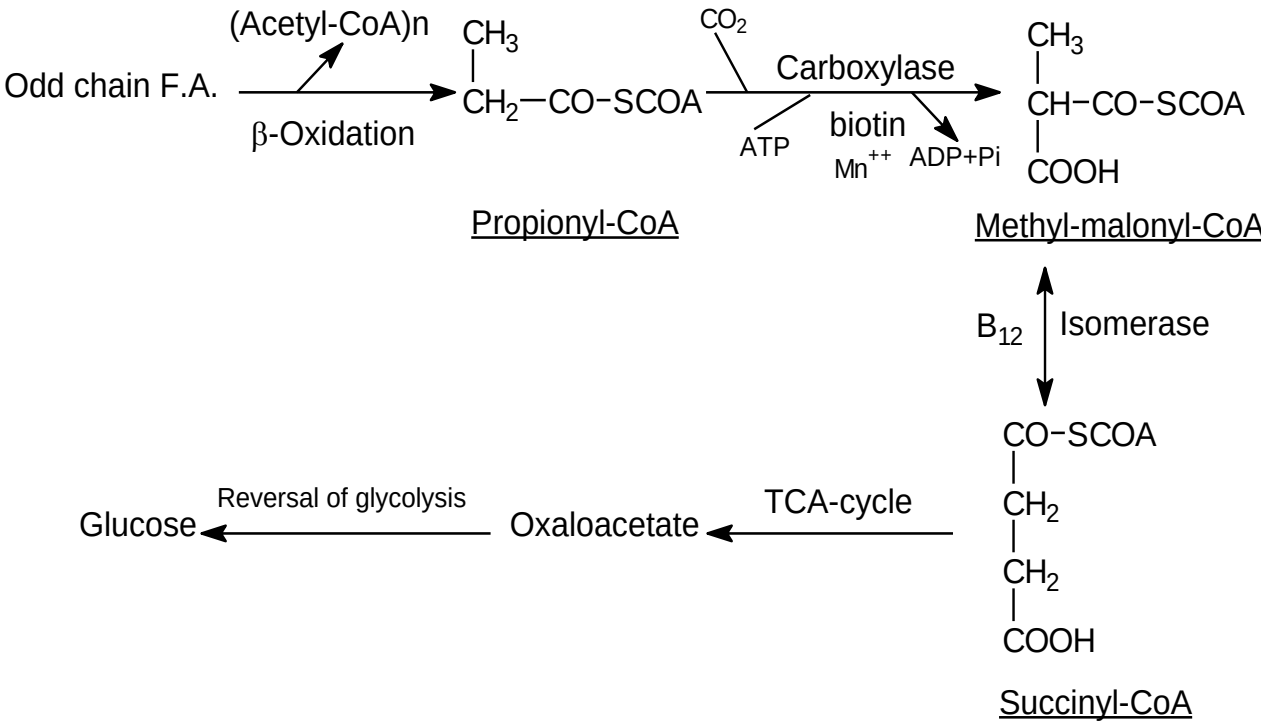


GLUCOSE FROM GLYCEROL:



- Glycerol results from hydrolysis of TAG in adipose tissue
- In **liver & kidney**, glycerol is converted to glycerol 3-P
  - Adipose tissue cannot utilise glycerol as it lacks glycerol kinase enzyme
- DHAP is point of entry into gluconeogenesis
- Glycerol release from adipose tissue is ↑ in stress

GLUCOSE FROM ODD CHAIN FATTY ACIDS: It is <u>rare conversion</u>

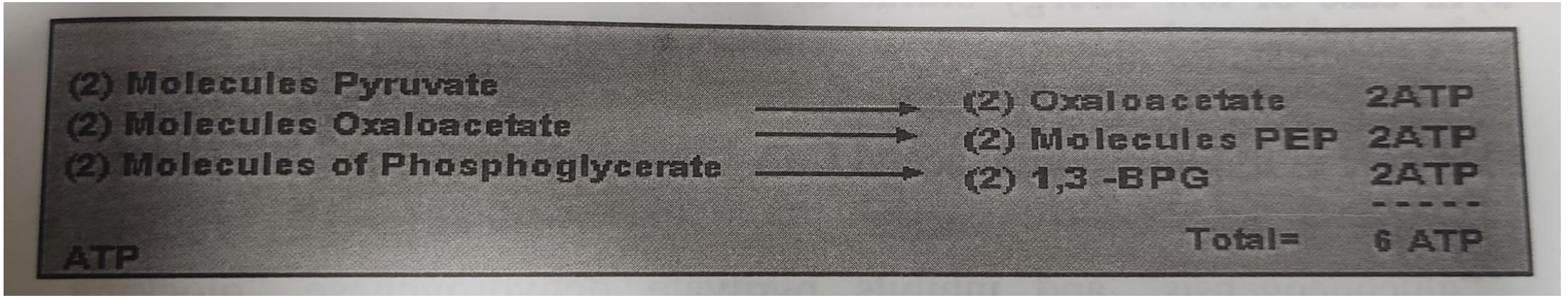


- Propionate is not a significant gluconeogenic precursor
  - Derived from catabolism of odd chain FA & isoleucine, valine, methionine, threonine
- **<u>Even chain FAs cannot be converted to glucose as the pyruvate dehydrogenase reaction is strictly irreversible</u>**
- Propionate enters gluconeogenesis through the formation of succinyl coA which is converted to oxaloacetate

Energy requirements for gluconeogenesis

- Gluconeogenesis is a costly metabolic process

- Energy requirements for gluconeogenesis depends on starting point:
  - If start with pyruvate → 6 ATP
  - If start with oxaloacetate → 4 ATP
  - If start with glycerol → 2 ATP



Regulation of gluconeogenesis

- Gluconeogenesis and glycolysis are reciprocally regulated
    - Inhibition of glycolysis → stimulation of gluconeogenesis

- 4 key enzymes of gluconeogenesis:
    - Pyruvate carboxylase (PC)
    - Phosphoenolpyruvate carboxykinase (PEPCK)
    - **Fructose 1, 6 –Bisphosphatase (F-1, 6-BPtase; the key enzyme)**
    - Glucose-6-phosphatase (G-6-Ptase)

- Types of regulation
    - Allosteric regulation
    - Hormonal regulation

Allosteric regulation: Pyruvate carboxylase

- Allosterically activated by acetyl coA according to different conditions:
  - Low energy status of cell → important to replenish oxaloacetate for directing TCA to provide ATP
  - Hypoglycaemia → acetyl coA produced from lipolysis & β oxidation of FAs:
    - Promotes oxaloacetate synthesis (gluconeogenesis)
    - Inhibits pyruvate dehydrogenase → blocks consumption of pyruvate
  - High energy status of cells (excess CHO) → ↑ acetyl coA → ↑ in oxaloacetate (via stimulation of pyruvate carboxylase) → to form citrate → enables FA synthesis

Allosteric regulation: fructose 1,6 biphosphatase

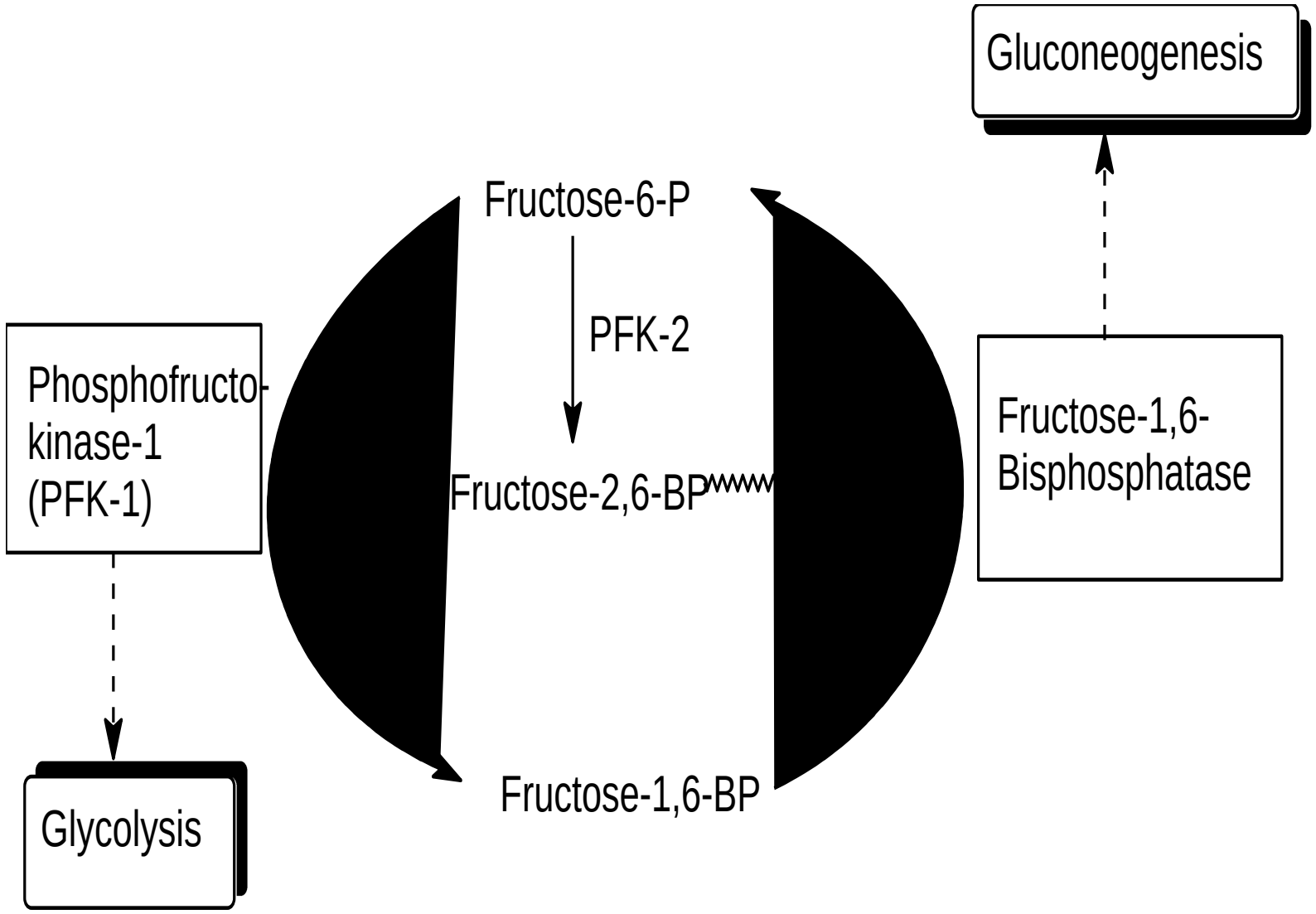
- The key enzyme of gluconeogenesis: F-1, 6-Bisphosphatase which is allosterically inhibited by F-2, 6–BP

- Fructose-2, 6-Bisphosphate: [F-2, 6–BP] is formed by phosphorylation of F-6-P by the enzyme phosphofructokinase–2 (PFK-2)

- **Fructose-2, 6-Bisphosphate** plays an important role in regulation of glycolysis and gluconeogenesis.

- CHO feeding $\rightarrow\uparrow\uparrow$ F-2, 6-BP $\rightarrow$
  - it allosterically stimulates PFK-1 and
  - inhibits F-1, 6-BPtase $\rightarrow$ stimulates Glycolysis and inhibits Gluconeogenesis

  - **So, glycolysis and Gluconeogenesis can't occur at the same time.**


Gluconeogenesis
Fructose-6-P
PFK-2
Phosphofructo-
kinase-1
(PFK-1)
Fructose-2,6-BP
Fructose-1,6-
Bisphosphatase
Glycolysis
Fructose-1,6-BP

Hormonal regulation

- Glucagon, epinephrine & glucocorticoids ↑ gluconeogenesis:
    - Induce synthesis of 4 key gluconeogenic enzymes
        - Pyruvate carboxylase, PEPC, G-6- phosphatase, F 1,6 bisphophatase
    - Repression/ inhibition of 3 key glycolytic enzymes (pyruvate kinase, PFK-1, glucokinase)
    - Promote lipolysis → ↑ fee FA → ↑ acetyl coA → activates pyruvate carboxylase
    - Release of glycerol → gives glucose in liver
    - **Glucocorticoids:**
        - promotes proteolysis of muscle protein → release of free AA → oxidation of AA → intermediates for gluconeogenesis
        - Induces transaminases
- Insulin → inhibits gluconeogenesis
    - Repressor of gluconeogenic enzymes
    - Inducer of glycolytic enzymes

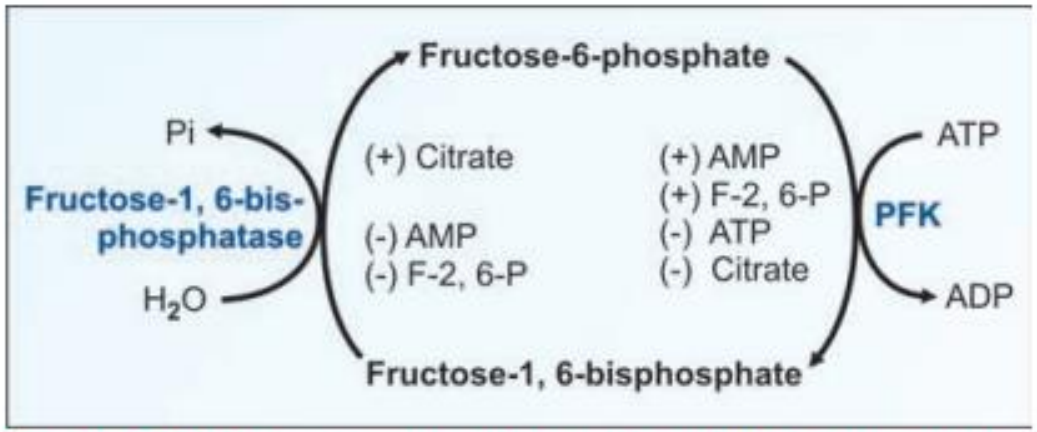


Fig. 9.32. Reciprocal regulation of PFK (glycolytic enzyme) and Fructose-1,6-bisphosphatase (gluconeogenic enzyme)

Table 9.8. Regulatory enzymes of gluconeogenesis (compare with Table 9.3)

Enzyme	*Activation*	*Inhibition*
PC	*Cortisol, Glucagon Adrenalin, Acetyl CoA*	*Insulin, ADP*
PEPCK	*do*	*Insulin*
F-1,6-bis-1 phosphatase	*do*	*F-1,6-BP, AMP F-2,6-BP*
G-6-phos-phatase	*do*	*Insulin*

Impaired gluconeogenesis

- Decreased gluconeogenesis → lactic acidosis & hypoglycaemia (could cause brain damage)
    - Blood glucose levels below 30-40mg/dL → severe hypoglycaemia

- Causes of impaired gluconeogenesis
    - Insufficiency of glucocorticoids, glucagon
    - Severe liver disease
    - Inherited deficiency of fructose 1,6 biphosphatase (hypoglycaemia, lactic acidosis, ketosis)
    - Glucose 6-phosphatase deficiency (von Gierk's disease) → severe hypoglycaemia**, lactic acidosis and hepatomegaly