Carbohydrate Metabolism

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TCA Cycle
(Krebs Cycle, Citric acid cycle)

- It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2.
- This oxidation provides energy for the production of the majority of ATP in most animals, including humans.

TCA Cycle

- The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle……Oxidative phosphorylation

TCA Cycle

- The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor.
- Most of the body's catabolic pathways converge on the TCA cycle.
- Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic reactions.

TCA Cycle

- The citric acid cycle also supplies intermediates for a number of important synthetic reactions.
- For example;

- The cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it provides building blocks for the synthesis of some amino acids
- Therefore, this cycle should not be viewed as a closed circle, but instead as a traffic circle with compounds entering and leaving as required.

Reactions of TCA Cycle

- In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA), and then is regenerated as the cycle is completed.
- Thus, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consump- tion of intermediates.

!!! Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 existing.

A. Oxidative decarboxylation of pyruvate

- Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle.
- This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane.
- Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex.
- Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.

Component enzymes of PDH complex

- The pyruvate dehydrogenase complex (PDH complex) is a multimolecular aggregate of three enzymes:
- ✓ pyruvate dehydrogenase (PDH or E1, also called a decarboxylase),
- ✓ dihydrolipoyl transacetylase (E2)
- ✓ dihydrolipoyl dehydrogenase (E3).
- Each catalyzes a part of the overall reaction.
- Their physical association links the reactions in proper sequence without the release of intermediates.
- In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the complex also contains two tightly bound regula- tory enzymes, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase.

Coenzymes of PDH complex

- The PDH complex contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions shown in previos slide.
- E1 requires thiamine pyrophosphate (TPP),
- E2 requires lipoic acid and CoA, and
- E3 requires FAD and NAD^+.

B. Synthesis of citrate from acetyl CoA and oxaloacetate

- The condensation of acetyl CoA and oxaloacetate to form citrate (a tricarboxylic acid) is catalyzed by citrate synthase.
- In humans, citrate synthase is not an allosteric enzyme. It is inhibited by its product, citrate.
- Substrate availability is another means of regulation for citrate synthase.
- The binding of oxaloacetate causes a conformational change in the enzyme that generates a binding site for acetyl CoA.

C. Isomerization of citrate

- Citrate is isomerized to isocitrate by aconitase, an Fe-S protein.
- Aconitase is inhibited by fluoroacetate, a compound that is used as a rat poison.

D. Oxidation and decarboxylation of isocitrate

- Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate, yielding the first of three NADH molecules produced by the cycle, and the first release of CO_2.
- This is one of the rate-limiting steps of the TCA cycle.
- The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+}, and is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

E. Oxidative decarboxylation of α-ketoglutarate

- The conversion of α-ketoglutarate to succinyl CoA is catalyzed by the α-ketoglutarate dehydrogenase complex, a multimolecular aggregate of three enzymes.
- The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDH complex.

E. Oxidative decarboxylation of α-ketoglutarate

- The reaction releases the second CO_2 and produces the second NADH of the cycle.
- The coenzymes required are thiamine pyrophosphate, lipoic acid, FAD, NAD+, and CoA.
- α-Ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}.
- However, it is not regulated by phosphorylation/ dephosphorylation reactions as described for PDH complex.

F. Cleavage of succinyl CoA

- Succinate thiokinase (also called succinyl CoA synthetase—named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA.
- This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP).
- GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:
- GTP+ADP →← GDP+ATP
- The generation of GTP by succinate thiokinase is another example of substrate-level phosphorylation !!!

G. Oxidation of succinate

- Succinate is oxidized to fumarate by succinate dehydrogenase, as FAD (its coenzyme) is reduced to $FADH_2$.
- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.

H. Hydration of fumarate

- Fumarate is hydrated to malate in a freely reversible reaction catalyzed by fumarase (also called fumarate hydratase).

I. Oxidation of malate

- Malate is oxidized to oxaloacetate by malate dehydrogenase.
- This reaction produces the third and final NADH of the cycle.

HOW MUCH ENERGY IS PRODUCED BY THE TCA CYCLE ???

- Two carbon atoms enter the cycle as acetyl CoA and leave as CO_2.
- The cycle does not involve net consumption or production of oxaloacetate or of any other intermediate.
- Four pairs of electrons are transferred during one turn of the cycle:

✓ Three pairs of electrons reducing 3 NAD^+ to NADH and

✓ 1 pair reducing FAD to $FADH_2$.

✓ Oxidation of one NADH by the electron transport chain leads to formation of approximately 2.5 ATP, whereas oxidation of $FADH_2$ yields approximately 1.5 ATP.

REGULATION OF THE TCA CYCLE

- In contrast to glycolysis, which is regulated primarily by phosphofructokinase, the TCA cycle is controlled by the regulation of several enzyme activities.
- The most important of these regulated enzymes are those that catalyze reactions with highly negative $\Delta G0$: citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complex.
- Reducing equivalents needed for oxidative phosphorylation are generated by the pyruvate dehydrogenase complex and the TCA cycle, and both processes are upregulated in response to a rise in ADP.

Gluconeogenesis

Gluconeogenesis

- Some tissues, such as the brain, red blood cells, kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continu- ous supply of glucose as a metabolic fuel.
- Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate.
- During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols), and α-ketoacids (derived from the catabolism of glucogenic amino acids).

Gluconeogenesis

- The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation.
- Instead, glucose is synthesized by a special pathway, gluconeogenesis, that requires both mitochondrial and cytosolic enzymes.

Gluconeogenesis

- During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the kidneys providing 10% of the newly synthesized glucose molecules.
- However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production.

Substrates For Gluconeogenesis

- Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose.
- They include intermediates of glycolysis and the tricarboxylic acid (TCA) cycle.
- Glycerol, lactate, and the α-keto acids obtained from the transamination of glucogenic amino acids are the most important gluconeogenic precursors.

A. Glycerol

- Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue, and is delivered by the blood to the liver.
- Glycerol is phosphorylated by glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate—an intermediate of glycolysis.

B. Lactate

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

C. Amino acids

- Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during a fast.
- α-Ketoacids, such as α-keto-glutarate, are derived from the metabolism of glucogenic amino acids.
- These α-ketoacids can enter the TCA cycle and form oxaloacetate (OAA)—a direct precursor of phosphoenol- pyruvate (PEP).

!!! Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA (for example, acetoacetate and amino acids such as lysine and leucine) can not give rise to a net synthesis of glucose. This is due to the irreversible nature of the pyruvate dehydrogenase reaction, which converts pyruvate to acetyl CoA. [These compounds give rise instead to ketone bodies and are therefore termed ketogenic.]

WHICH REACTIONS ARE UNIQUE TO GLUCONEOGENESIS ???

- 7 glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate.
- However, 3 of the reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose.
- The reactions, unique to gluconeogenesis are ???

A. Carboxylation of pyruvate

- The first "roadblock" to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase.
- In gluconeogenesis, pyruvate is first carboxylated by pyruvate carboxylase to OAA, which is then converted to PEP by the action of PEP-carboxykinase.

PEP ➔ Pyruvate ➔ OAA

! Biotin is a coenzyme

Allosteric regulation

- Pyruvate carboxylase is allosterically activated by acetyl CoA.
- Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which the increased synthesis of OAA is required.
- For example, this occurs during fasting, when OAA is used for the synthesis of glucose by gluconeogenesis in the liver and kidney.
- Conversely, at low levels of acetyl CoA, pyruvate carboxylase is largely inactive, and pyruvate is primarily oxidized by the pyruvate dehydrogenase complex to produce acetyl CoA that can be further oxidized by the TCA cycle.

B. Transport of oxaloacetate to the cytosol

- OAA must be converted to PEP for gluconeogenesis to continue.
- The enzyme that catalyzes this conversion is found in both the mitochondria and the cytosol in humans.
- The PEP that is generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to directly cross the inner mitochondrial membrane; it must first be reduced to malate by mitochondrial malate dehydrogenase.
- Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to oxaloacetate by cytosolic malate dehydrogenase as NAD+ is reduced.
- The NADH produced is used in the reduction of 1,3-BPG to glyceraldehyde 3-phosphate, a step common to both glycolysis and gluconeogenesis.

C. Decarboxylation of cytosolic oxaloacetate

- Oxaloacetate is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase.
- The reaction is driven by hydrolysis of guanosine triphosphate (GTP).
- The combined actions of pyruvate carboxylase and PEP- carboxykinase provide an energetically favorable pathway from pyruvate to PEP.
- Then, PEP is acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate.

D. Dephosphorylation of fructose 1,6-bisphosphate

- Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphosphatase bypasses the irreversible phosphofructokinase-1 reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate.
- This reaction is an important regulatory site of gluconeogenesis.

Regulation by energy levels within the cell

- Fructose 1,6-bisphosphatase is inhibited by elevated levels of adenosine monophosphate (AMP), which signal an “energy-poor” state in the cell.
- Conversely, high levels of ATP and low concentrations of AMP stimulate gluconeogenesis, an energy-requiring pathway.

Regulation by fructose 2,6-bisphosphate

- Fructose 1,6-bisphosphatase, found in liver and kidney, is inhibited by fructose 2,6-bisphosphate, an allosteric effector whose concentration is influenced by the level of circulating glucagon.

E. Dephosphorylation of glucose 6-phosphate

- Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase bypasses the irreversible hexokinase reaction, and provides an energetically favorable pathway for the formation of free glucose.
- Liver and kidney are the only organs that release free glucose from glucose 6-phosphate.
- This process actually requires two proteins:

1. glucose 6-phosphate translocase, which transports glucose 6-phosphate across the endoplasmic reticulum (ER) membrane, and the ER enzyme,
2. glucose 6-phosphatase (found only in gluconeogenic cells), which removes the phosphate, producing free glucose. Specific transporters are responsible for releasing free glucose and phosphate back into the cytosol and, for glucose, into blood.

!!! Muscle lacks glucose 6- phosphatase, and therefore muscle glycogen can not be used to maintain blood glucose levels.

F. Summary of the reactions of glycolysis and gluconeogenesis

- Of the 11 reactions required to convert pyruvate to free glucose, seven are catalyzed by reversible glycolytic enzymes.
- The irreversible reactions of glycolysis catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase are circumvented by glucose 6-phosphatase, fructose 1,6-bisphosphatase, and pyruvate carboxylase/PEP-carboxykinase.

F. Summary of the reactions of glycolysis and gluconeogenesis

- In gluconeogenesis, the equilibria of the seven reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluco- neogenic enzymes.
- The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose.

REGULATION OF GLUCONEOGENESIS

- The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon, and by the availability of gluconeogenic substrates.
- In addition, slow adaptive changes in enzyme activity result from an alteration in the rate of enzyme synthesis or degradation, or both.

A. Glucagon

- This hormone from the α cells of pancreatic islets stimulates gluconeogenesis by three mechanisms.
- **1. Changes in allosteric effectors:** Glucagon lowers the level of fructose 2,6-bisphosphate, resulting in activation of fructose 1,6-bis-phosphatase and inhibition of phosphofructokinase-1, thus favoring gluconeogenesis over glycolysis
- **2. Covalent modification of enzyme activity:** Glucagon binds its G protein-coupled receptor and, via an elevation in cyclic AMP (cAMP) level and cAMP-dependent protein kinase activity, stimulates the conversion of hepatic pyruvate kinase to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose
- **3. Induction of enzyme synthesis:** Glucagon increases the transcrip-tion of the gene for PEP-carboxykinase, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Insulin causes decreased transcription of the mRNA for this enzyme.]

B. Substrate availability

- The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis.
- Decreased levels of insulin favor mobilization of amino acids from muscle protein, and provide the carbon skeletons for gluconeogenesis.
- ATP and NADH, coenzymes-cosubstrates required for gluconeogenesis, are primarily provided by the catabolism of fatty acids.

C. Allosteric activation by acetyl CoA

- Allosteric activation of hepatic pyruvate carboxylase by acetyl CoA occurs during fasting.
- As a result of increased lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β -oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O.
- As a result, acetyl CoA accumulates and leads to activation of pyruvate carboxylase.

D. Allosteric inhibition by AMP

- Fructose 1,6-bisphosphatase is inhibited by AMP—a compound that activates phosphofructokinase-1.
- This results in a reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose 2,6-bisphosphate.

THANKS FOR LISTENING, ANY QUESTIONS?

References

- Biochemistry, Lippincott's Illustrated Reviews, 5th Edition, Richard A. Harvey