"Gluconeogenesis"

I. OVERVIEW

Tissues Requiring Continuous Glucose Supply

- Some tissues require a continuous supply of glucose as a metabolic fuel:
    - Brain
    - Erythrocytes
    - Kidney medulla
    - Lens and cornea of the eye
    - Testes
    - Exercising skeletal muscle

Role of Liver Glycogen

- Liver glycogen is an essential postprandial source of glucose.
- In the absence of dietary intake of carbohydrate:
    - Liver glycogen can meet glucose needs for <24 hours.

Glucose Production During Prolonged Fast

- During a prolonged fast, hepatic glycogen stores are depleted.
- Glucose is then made from noncarbohydrate precursors.

Nature of Gluconeogenesis

- Formation of glucose does not occur by simple reversal of glycolysis.
- Reason:
    - The overall equilibrium of glycolysis strongly favors pyruvate formation.
- Instead:
    - Glucose is synthesized de novo by a special pathway: gluconeogenesis.
    - This pathway requires:
        - Mitochondrial enzymes
        - Cytosolic enzymes

Clinical Note

- Deficiencies of gluconeogenic enzymes cause hypoglycemia.

Site of Gluconeogenesis

- After an overnight fast:
    - Approximately 90% of gluconeogenesis occurs in the liver.
    - The remaining ~10% occurs in the kidneys.
- During prolonged fasting (48 hours or longer):
    - Kidneys become major glucose-producing organs.
    - They contribute approximately 40% of total glucose production.
- The small intestine can also make glucose.

II. SUBSTRATES

General Overview

- Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose.
- Most important gluconeogenic precursors:
    - Glycerol
    - Lactate
    - α-keto acids obtained from the metabolism of glucogenic amino acids
- All amino acids are glucogenic except:
    - Leucine
    - Lysine

A. Glycerol

- Source:
    - Glycerol is released during the hydrolysis of triacylglycerols (TAGs) in adipose tissue.
    - It is delivered by the blood to the liver.

- Metabolism:
    - Glycerol is phosphorylated by glycerol kinase to form glycerol 3-phosphate.
    - Glycerol 3-phosphate is then oxidized by glycerol 3-phosphate dehydrogenase to dihydroxyacetone phosphate.
    - Dihydroxyacetone phosphate is an intermediate of glycolysis and gluconeogenesis.

B. Lactate

- Source:
    - Lactate from anaerobic glycolysis is released into the blood by:
        - Exercising skeletal muscle
        - Erythrocytes (cells that lack mitochondria)

- Cori Cycle:
    - Lactate is taken up by the liver.
    - It is oxidized to pyruvate, which is then:
        - Converted to glucose
        - Released back into the circulation

C. Amino Acids

- Source:
    - Amino acids produced by hydrolysis of tissue proteins are the major sources of glucose during a fast.
- Metabolism:
    - Their metabolism generates α-keto acids, such as:
        - Pyruvate, which is converted to glucose
        - α-ketoglutarate, which can:
            - Enter the tricarboxylic acid (TCA) cycle
            - Form oxaloacetate (OAA), a direct precursor of phosphoenolpyruvate (PEP)

Note:

- Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA cannot give rise to a net synthesis of glucose

- Examples:
    - Acetoacetate
    - Lysine
    - Leucine
- Reason:
    - Due to the irreversible nature of the pyruvate dehydrogenase complex (PDHC), which:
        - Converts pyruvate to acetyl CoA
- These compounds instead give rise to ketone bodies and are termed ketogenic.

III. REACTIONS

Overview

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

- These irreversible reactions are unique to gluconeogenesis.

A. Pyruvate Carboxylation

1. General Mechanism

- The first roadblock in glucose synthesis from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase (PK).

- In gluconeogenesis:

    - Pyruvate is carboxylated by pyruvate carboxylase (PC) to oxaloacetate (OAA).
    - OAA is converted to PEP by PEP-carboxykinase (PEPCK).

2. Biotin

- PC requires the coenzyme biotin, which is:

    - Covalently bound to the ε-amino group of a lysine residue in the enzyme.

- ATP hydrolysis drives the formation of an enzyme-biotin-carbon dioxide (CO_2) intermediate.
    - This intermediate carboxylates pyruvate to form OAA.
    - (Note: HCO_3^- provides the CO_2.)
- The PC reaction occurs in the mitochondria of liver and kidney cells.
- The PC reaction has two purposes:
    - To allow production of PEP, an important substrate for gluconeogenesis.
    - To provide OAA that can replenish TCA cycle intermediates that may become depleted.
- Muscle cells also contain PC but:
    - Use the OAA product only for the replenishment (anaplerotic) purpose.
    - Do not synthesize glucose.
- (Note: Pyruvate carrier protein moves pyruvate from the cytosol into mitochondria.)

3. Other Biotin-Dependent Carboxylases

- PC is one of several carboxylases that require biotin, including:
    - Acetyl CoA carboxylase
    - Propionyl CoA carboxylase
    - Methylcrotonyl CoA carboxylase

4. Allosteric Regulation

- PC is allosterically activated by acetyl CoA.
- Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which increased synthesis of OAA is required.
    - This occurs during fasting, when OAA is used for gluconeogenesis in the liver and kidneys.
- At low levels of acetyl CoA:
    - PC is largely inactive.
    - Pyruvate is primarily oxidized by the PDHC to acetyl CoA, which is then:
        - Further oxidized by the TCA cycle.

B. Oxaloacetate Transport to the Cytosol

- For gluconeogenesis to continue, oxaloacetate (OAA) must be converted to phosphoenolpyruvate (PEP) by PEP-carboxykinase (PEPCK).

- PEP production in the cytosol requires transport of OAA out of mitochondria.

- However, the inner mitochondrial membrane lacks an OAA transporter.

- Therefore:

    - OAA is reduced to malate by mitochondrial malate dehydrogenase (MD).
    - Malate is transported into the cytosol.
    - In the cytosol, malate is reoxidized to OAA by cytosolic MD, as NAD^+ is reduced to NADH.

- The cytosolic NADH is used in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate by glyceraldehyde 3-phosphate dehydrogenase.

    - This reaction is common to both glycolysis and gluconeogenesis.

Note:

- When lactate is abundant, it is oxidized to pyruvate as NAD^+ is reduced.
- The pyruvate is transported into mitochondria and carboxylated by PC to OAA.
- This OAA can be converted to PEP by the mitochondrial isozyme of PEPCK.
- The PEP is then transported to the cytosol.
- OAA can also be converted to aspartate, which is transported into the cytosol.

C. Cytosolic Oxaloacetate Decarboxylation

- In the cytosol, OAA is decarboxylated and phosphorylated to PEP by PEP-carboxykinase (PEPCK).
- The reaction is driven by hydrolysis of guanosine triphosphate (GTP).

- The combined actions of PC and PEPCK provide an energetically favorable pathway from pyruvate to PEP.

- PEP is then acted on by the reactions of glycolysis running in reverse until it becomes fructose 1,6-bisphosphate.

- The pairing of carboxylation with decarboxylation:

    - Drives reactions that would otherwise be energetically unfavorable.
    - This strategy is also used in fatty acid (FA) synthesis.

D. Fructose 1,6-Bisphosphate Dephosphorylation

- Fructose 1,6-bisphosphatase hydrolyzes fructose 1,6-bisphosphate.

- This enzyme is found in the liver and kidneys.

- The reaction:

    - Bypasses the irreversible phosphofructokinase-1 (PFK-1) reaction of glycolysis.
    - Provides an energetically favorable pathway for the formation of fructose 6-phosphate.

- This is an important regulatory site of gluconeogenesis.

1. Regulation by Intracellular Energy Levels

- Fructose 1,6-bisphosphatase is inhibited by a rise in the ratio of adenosine monophosphate (AMP) to ATP.
- This ratio signals a low-energy state in the cell.
- Conversely:
    - Low AMP and high ATP levels stimulate gluconeogenesis.
    - Gluconeogenesis is an energy-requiring pathway.

2. Regulation by Fructose 2,6-Bisphosphate

- Fructose 1,6-bisphosphatase is inhibited by fructose 2,6-bisphosphate.
    - This molecule is an allosteric effector.
    - Its concentration is influenced by the insulin/glucagon ratio.

- When glucagon is high:
    - The effector is not made by hepatic PFK-2.
    - Thus, the phosphatase is active.

Note:

- The signals that inhibit gluconeogenesis (low energy, high fructose 2,6-bisphosphate) or activate gluconeogenesis (high energy, low fructose 2,6-bisphosphate) have the opposite effect on glycolysis.

- This provides reciprocal control of the pathways that synthesize and oxidize glucose.

E. Glucose 6-Phosphate Dephosphorylation

- Glucose 6-phosphate is hydrolyzed by glucose 6-phosphatase.

- This bypasses the irreversible hexokinase/glucokinase reaction.

- Provides an energetically favorable pathway for the formation of free glucose.

Liver: Primary Organ

- The liver is the primary organ that produces free glucose from glucose 6-phosphate.

Protein Complex Required (Only in Gluconeogenic Tissue)

- Requires a complex of two proteins found only in gluconeogenic tissue:

    a. Glucose 6-phosphate translocase:
        - Transports glucose 6-phosphate across the endoplasmic reticular (ER) membrane.

    a. Glucose 6-phosphatase:
        - Removes the phosphate, producing free glucose.

- These ER membrane proteins are also required for the final step of glycogen degradation.

Glycogen Storage Diseases

- Glycogen storage disease type Ia:
    - Caused by deficiency in glucose 6-phosphatase.

- Both are characterized by severe fasting hypoglycemia:
    - Free glucose cannot be produced from gluconeogenesis or glycogenolysis.

Transport of Free Glucose

- Specific transporters move the free glucose into the cytosol and then into the blood.

F. Summary of the Reactions of Glycolysis and Gluconeogenesis

- Total of 11 reactions required to convert pyruvate to free glucose.
    - 7 reactions are catalyzed by reversible glycolytic enzymes.
    - 3 irreversible reactions:
        - Catalyzed by:
            - Hexokinase/glucokinase
            - Phosphofructokinase-1 (PFK-1)
            - Pyruvate kinase (PK)

        - These are circumvented by:
            - Glucose 6-phosphatase
            - Fructose 1,6-bisphosphatase
            - Pyruvate carboxylase (PC)
            - Phosphoenolpyruvate carboxykinase (PEPCK)

- In gluconeogenesis, the equilibria of the reversible glycolytic reactions are pushed toward glucose synthesis due to:

    - Essentially irreversible formation of:
        - PEP
        - Fructose 6-phosphate
        - Glucose
        - By the gluconeogenic enzymes

Note:

- The stoichiometry of gluconeogenesis from two pyruvate molecules:

    - Couples the cleavage of six high-energy phosphate bonds.
    - Involves the oxidation of two NADH.
    - Produces one glucose molecule.

IV. REGULATION

- Moment-to-moment regulation of gluconeogenesis is primarily determined by:
    - The circulating level of glucagon.
    - The availability of gluconeogenic substrates.
- Slow adaptive changes in enzyme amount occur due to:
    - Alteration in the rate of enzyme synthesis, degradation, or both.

A. Glucagon

- Glucagon is a peptide hormone secreted from pancreatic islet α-cells.
- It stimulates gluconeogenesis via three mechanisms:

1. Changes in Allosteric Effectors

- Glucagon lowers hepatic fructose 2,6-bisphosphate, resulting in:
    - Activation of fructose 1,6-bisphosphatase.
    - Inhibition of PFK-1.
    - This favors gluconeogenesis over glycolysis.

2. Covalent Modification of Enzyme Activity

- Glucagon binds to its G protein-coupled receptor.
- This leads to:
    - Elevation in cyclic AMP (cAMP) levels.
    - Activation of cAMP-dependent protein kinase A.
- Result: Stimulates the conversion of hepatic pyruvate kinase (PK) to its inactive (phosphorylated) form.
    - This decreases conversion of PEP to pyruvate, diverting PEP to gluconeogenesis.

3. Induction of Enzyme Synthesis

- Glucagon increases transcription of the gene for PEPCK:
    - Mediated via the transcription factor cAMP response element–binding (CREB) protein.
    - This increases availability of PEPCK enzyme as substrate levels rise during fasting.
- Cortisol (a glucocorticoid):
    - Also increases expression of the PEPCK gene.
- Insulin:
    - Decreases expression of the PEPCK gene.

B. Substrate Availability

- Availability of gluconeogenic precursors, especially glucogenic amino acids, significantly influences the rate of glucose synthesis.

- Decreased insulin levels favor:
    - Mobilization of amino acids from muscle protein.
    - These provide carbon skeletons for gluconeogenesis.

- ATP and NADH coenzymes required for gluconeogenesis are primarily provided by fatty acid (FA) oxidation.

C. Allosteric Activation by Acetyl CoA

- Allosteric activation of hepatic pyruvate carboxylase (PC) by acetyl CoA occurs during fasting.

- Due to increased TAG hydrolysis in adipose tissue, the liver is flooded with fatty acids.

- The rate of acetyl CoA formation via β-oxidation exceeds the liver's capacity to oxidize it to CO_2 and water.
    - As a result, acetyl CoA accumulates and activates PC.

Note:

- Acetyl CoA inhibits the pyruvate dehydrogenase complex (PDHC) by activating PDH kinase.

→ This diverts pyruvate toward gluconeogenesis and away from the TCA cycle

D. Allosteric Inhibition by AMP

- Fructose 1,6-bisphosphatase is inhibited by AMP, a compound that activates PFK-1.

- This leads to reciprocal regulation of:

    - Glycolysis (stimulated by AMP),
    - Gluconeogenesis (inhibited by AMP).

- Similar reciprocal regulation was previously seen with fructose 2,6-bisphosphate.

Thus, elevated AMP:

- Stimulates energy-producing pathways.

- Inhibits energy-requiring pathways.

Important Flowcharts