Gluconeogenesis, Glycogen Metabolism, and the Pentose Phosphate Pathway

Objectives:
I. Describe gluconeogenesis and its metabolic role.
   A. Cite two reasons why gluconeogenesis is not the simple reverse of glycolysis.
   B. Compare glycolysis and gluconeogenesis.
      1. What enzymes are different?
      2. Why are additional enzymes involved / necessary?
   C. Discuss the control points of gluconeogenesis.
      1. Which enzyme(s) is(are) allosteric?
      2. Describe the allosteric effectors and how they effect the enzymes.
      3. Compare / Contrast the control points of gluconeogenesis with the control points of glycolysis.
II. Describe the process of glycogenolysis.
   A. Name the enzyme(s) that catalyzes glycogenolysis (the breakdown of glycogen).
   B. Discuss the control points of glycogenolysis.
      1. Which enzyme(s) is(are) allosteric?
      2. Describe the allosteric effectors and how they effect the enzymes.
III. Describe the process of glycogenesis.
   A. Name the enzyme(s) that catalyzes glycogenesis (the synthesis of glycogen).
   B. Discuss the control points of glycogenesis.
      1. Which enzyme(s) is(are) allosteric?
      2. Describe the allosteric effectors and how they effect the enzymes.
IV. Pentose Phosphate Pathway or Hexose Monophosphate Shunt.
   A. What are the important products of the Pentose Phosphate Pathway?
   B. Why are these products important to the cell?
   C. Discuss the importance of the oxidative phase of the pathway.
      1. Discuss the control points of the oxidative phase of the Pentose Pathway.
         a) Describe the allosteric effectors and how they effect the enzymes.
   D. Discuss the importance of the recovery (regeneration) phase of the pathway.
      1. What is the biochemical importance of the products?
V. Explain the roles of glycolysis, gluconeogenesis, glycogenolysis, and glycogenesis in controlling blood sugar levels.
VI. Integrate glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, and the pentose phosphate pathway.
   A. Allosteric control points.
   B. The effects of glucagon, insulin, and epinephrine on these pathways and on blood sugar levels.
   C. Summarize the regulation of blood glucose levels by glycolysis, gluconeogenesis, and glycogenolysis in the liver.
   D. Summarize the regulation of glucose metabolism by glycolysis, gluconeogenesis, glycogenesis in skeletal muscle.
VII. Cori Cycle
   A. What is the Cori Cycle?
   B. What is the function of the Cori Cycle?
   C. How does it accomplish its function?

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D. Can the Cori Cycle function indefinitely?

VIII. Ask yourself “What If Questions”; e.g., Blood glucose levels drop because of exercise, what hormone is released, which tissues respond, how???

Background

Glycolysis, the TCA Cycle, and the Electron Transport/Oxidative Phosphorylation Pathway when functioning together generate a large quantity of ATP by the complete oxidation of glucose to CO₂ and H₂O. There are several enzymatic reactions and/or pathways that utilize carbohydrates that need to be examined. Pathways for:

1. the synthesis new glucose from three and four carbon metabolic intermediates
2. the synthesis of glycogen from glucose
3. the release of glucose-6-phosphate and glucose from glycogen for entry into metabolism

Finally, the PENTOSE PHOSPHATE PATHWAY (HEXOSE MONOPHOSPHATE SHUNT) will be discussed. This pathway serves three functions

1. It generates NADPH for reductive biosynthesis.
2. It generates ribose for nucleotide biosynthesis.
3. It converts excess pentoses into hexoses for entry into the other pathways of carbohydrate metabolism.

Gluconeogenesis

The body strives to maintain a glucose concentration of about 1 mg/mL in the blood. It is maintained at this level in order to have a constant stable supply for the glucose dependent tissues. Four tissues are dependent upon glucose alone for energy generation. Red blood cells are absolutely glucose dependent since they have only glycolysis for energy generation. In the fed state, nervous tissue, adrenal medulla, and testis/ovaries use only glucose for their energy generation. In the starvation state these three tissues can adapt to other energy sources if the starvation comes on slowly. After a meal, Insulin stimulates all of the tissues of the body to absorb glucose from the blood and utilize it for energy generation and biosynthesis. Between meals, during a short fast, when blood glucose levels begin to fall, most tissues utilize fatty acids or amino acids to meet their energy needs sparing the glucose that remains for the four glucose dependent tissues. Glucose is released from stored glycogen and is synthesized to meet the needs of the glucose dependent tissues.

GLUCONEOGENESIS is the synthesis of “new” glucose from three or four carbon precursors. The three carbon precursors for gluconeogenesis are lactate, pyruvate, and glycerol. Lactate is obtained from the constant anaerobic glycolysis in the Red Blood Cell and the occasional anaerobic glycolysis in Skeletal Muscle. Pyruvate is obtained primarily from amino acid catabolism, and glycerol is from triacylglycerol catabolism. Oxaloacetate is the four carbon precursor. It is obtained from excess TCA cycle intermediates and from amino acid catabolism. Gluconeogenesis is a cytosolic process occurring primarily in the liver and kidney. Under normal conditions the liver performs about 90% of the gluconeogenesis in the human animal, kidney about 10%, and the small intestine less than 1%. The liver employs gluconeogenesis to maintain blood glucose levels using lactate, amino acid carbon skeletons, and glycerol as the starting materials. Gluconeogenesis in the kidney primarily employs the carbon skeletons of amino acids as
precursors. The amino group is removed from the amino acid as $\text{NH}_4^+$ and used by the kidney to buffer excreted metabolic acids. During starvation kidney can perform up to 50% of the gluconeogenesis necessary to sustain the organism. Kidney takes over this process during starvation in order to produce sufficient $\text{NH}_4^+$ to buffer the metabolic acids and to free the liver for ketone body synthesis. The small intestine performs gluconeogenesis on the glycolytic intermediates released from the ingested food and absorbed by the cells of the small intestine. The resulting glucose is released into the blood.

Skeletal muscle, while containing the enzymes required for gluconeogenesis, has a very limited gluconeogenic capacity (<1% of the glucose produced). The lactate produced by anaerobic glycolysis in skeletal muscle is transported to the liver and converted to glucose by the liver. The subunit composition and $K_m$ of skeletal muscle lactate dehydrogenase would require extremely high concentrations of lactate in order for the lactate to pyruvate reaction to occur. The necessary concentration is difficult to attain in skeletal muscle due to the efficiency of the transporter that moves lactate from the cell into the blood. Gluconeogenesis in skeletal muscle is primarily used to reduce the concentration of glycolytic intermediates after a bout of prolonged or extreme contraction. Skeletal muscle converts the intermediates prior to pyruvate back to glucose-6-phosphate and stores this glucose as glycogen. Any pyruvate formed by prolonged contraction is either converted to lactate and the lactate is released to the liver for gluconeogenesis or when the muscle is again well oxygenated it is converted to acetyl-CoA for the TCA cycle.

**Gluconeogenesis - The Pathway**

Entry of glycerol into gluconeogenesis will be discussed with triacylglycerol metabolism. This discussion centers around the utilization of lactate, pyruvate, and oxaloacetate for gluconeogenesis. Since seven of the ten reactions of glycolysis are freely reversible, these same “glycolytic” enzymes are used during gluconeogenesis, but in the “reverse” direction. There are three irreversible steps in glycolysis, the reactions catalyzed by Pyruvate Kinase, Phosphofructokinase-1, and Hexokinase. Different enzymes must be used to bypass these irreversible steps during gluconeogenesis.

With lactate, pyruvate, or oxaloacetate as the precursors, the first steps of gluconeogenesis is the conversion of these starting materials into phosphoenolpyruvate. Since the reaction catalyzed by Pyruvate Kinase is irreversible, a different set of enzymes need to be employed for this conversion. The conversion of each of these three precursors into phosphoenolpyruvate follow slightly different paths. Remember these reactions are occurring primarily in the liver (≈90%) and to a lesser extent in the kidney (≈10%), small intestine (≈1%), and skeletal muscle (≪1%).

The lactate from anaerobic glycolysis (RBC always; skeletal muscle under “stressed” conditions) is converted to pyruvate by Lactate Dehydrogenase in the cytosol. The pyruvate is transported from the cytosol into the mitochondria and once in the mitochondria the pyruvate is converted to oxaloacetate by the action of Pyruvate Carboxylase. The enzyme requires Biotin as a prosthetic group. This reaction is one of the anapleurotic reactions of the TCA cycle previously discussed.

This “new” oxaloacetate is converted to phosphoenolpyruvate by the action of the mitochondrial isoenzyme of Phosphoenolpyruvate Carboxykinase (PEP Carboxykinase). The resulting phosphoenolpyruvate is transported out of the mitochondria for gluconeogenesis.
When pyruvate from amino acid catabolism is the starting material, the pyruvate is either produced in the...
mitochondria or transported from the cytosol to the mitochondria. Once in the mitochondria the pyruvate is converted to oxaloacetate by the action of Pyruvate Carboxylase. The oxaloacetate generated from pyruvate is then converted to malate by the action of the mitochondrial isoenzyme of Malate Dehydrogenase, the TCA cycle enzyme running in the reverse direction. Malate is transported from the mitochondria to the cytoplasm by a transport protein, and once in the cytoplasm it is converted back to oxaloacetate by the action of the cytoplasmic isoenzyme of Malate Dehydrogenase. The net result of these three reactions is the conversion of pyruvate in the cytoplasm to oxaloacetate and a NADH in the cytoplasm. Oxaloacetate in the cytoplasm is converted to phosphoenolpyruvate by the action of the cytoplasmic isoenzyme of Phosphoenolpyruvate (PEP) Carboxykinase. The resulting phosphoenolpyruvate enters gluconeogenesis.

The starting material oxaloacetate is generated in the mitochondria from amino acid catabolism or it is drawn out of the TCA cycle to slow the pathway. The oxaloacetate is converted to malate by the action of the mitochondrial isoenzyme of Malate Dehydrogenase, the TCA cycle enzyme running in the reverse direction. Malate is transported from the mitochondria to the cytoplasm by a transport protein, and once in the cytoplasm it is converted back to oxaloacetate by the action of the cytoplasmic isoenzyme of Malate Dehydrogenase. The oxaloacetate formed in the cytoplasm is converted to phosphoenolpyruvate by the action of the cytoplasmic isoenzyme of Phosphoenolpyruvate (PEP) Carboxykinase and the resulting phosphoenolpyruvate enters gluconeogenesis.

Although, it may not be immediately apparent, there is logic behind these different pathways. During gluconeogenesis, NADH is required in the cytoplasm for the Glyceraldehyde-3-phosphate Dehydrogenase step. In the cytoplasm the ratio of \([\text{NADH}] / [\text{NAD}]\) is normally very low, about \(8 \times 10^{-4}\). These different pathways generate NADH in the cytoplasm, assuring that there is sufficient NADH for gluconeogenesis.

Two phosphoenolpyruvate molecules are converted to fructose-1,6-bisphosphate by the glycolytic enzymes catalyzing the “reverse” reactions. Once fructose-1,6-bisphosphate is synthesized for gluconeogenesis a new enzyme is employed to bypass the irreversible phosphofructokinase-1 step of glycolysis. The conversion of fructose-1,6-bisphosphate to fructose-6-phosphate is catalyzed by the enzyme Fructose-1,6-bisphosphatase.

Gluconeogenesis in the liver, and to a lesser extent the kidney, is stimulated by the hormone glucagon and in this case the new glucose is used to maintain blood glucose levels. In these two tissues the glucose-6-phosphate is converted to glucose by the action of the hydrolase Glucose-6-phosphatase. Glucose-6-phosphatase is found in liver, kidney, and the small intestine. The liver (primarily) and kidney (secondarily & during starvation) uses this reaction to maintain blood glucose concentrations. Small intestine uses this enzyme as a digestive enzyme. Glucose-6-phosphatase is part of a multimeric intrinsic protein embedded in the membrane of the smooth endoplasmic reticulum (ER) of these tissues.

This system consists of six different proteins:

1. Glucose-6-phosphate transport protein
2. Glucose-6-phosphatase catalytic subunit
3. Glucose-6-phosphatase regulating protein
4. Microsomal phosphate transport protein
5. Microsomal phosphate/pyrophosphate transport protein
6. Microsomal glucose transport protein (GluT7).
Glucose-6-phosphate transport protein brings glucose-6-phosphate into the lumen of the ER where the Glucose-6-phosphatase catalytic subunit / Glucose-6-phosphatase regulating protein complex catalyzes the hydrolytic removal of phosphate. Microsomal phosphate transport protein and/or Microsomal phosphate/pyrophosphate transport protein moves the phosphate into the cytoplasm and Microsomal glucose transport protein (GluT7) moves the glucose into the cytoplasm. As the cytoplasmic concentration of glucose increases above that in plasma the GluT2 transport protein moves the glucose from the liver/kidney cell to the blood.
Energetics of Gluconeogenesis

When the cell uses lactate or pyruvate as the precursor for gluconeogenesis, six high energy phosphate bonds are required for the synthesis of one glucose molecule. Two high energy phosphate bonds are used for the conversion of lactate or pyruvate to phosphoenolpyruvate; one from ATP the other from GTP. A third is used to convert 3-phosphoglycerate to 1,3-bisphosphoglycerate. Since two lactate or pyruvate are required for one glucose a total of six high energy phosphate bonds are required. Oxaloacetate as a precursor spares two high energy phosphate bonds; only four high energy phosphate bonds are required per glucose synthesized.

Control of Gluconeogenesis

Pyruvate carboxylase is an allosteric enzyme activated by Acetyl-CoA and ATP. In the absence of acetyl-CoA this enzyme has little activity. Fructose-1,6-bisphosphatase is also an allosteric enzyme. It is allosterically inhibited by AMP and Fructose-2,6-bisphosphate.

Insulin also plays a role in controlling gluconeogenesis. Insulin, when present after a carbohydrate rich meal, inhibits the synthesis of Phosphoenolpyruvate carboxykinase, Fructose-1,6-bisphosphatase, and Glucose-6-phosphatase. Glucagon has the opposite effect, it stimulates the synthesis of these three enzymes.
Glycogen Metabolism - An Overview

Glycogen is the storage form of glucose in mammalian tissues. All tissues are able to store glycogen to a greater or lesser extent. Liver and skeletal muscle are the major sites of glycogen storage. The kidney ranks third. Heart muscle, platelets, and adipose tissue store a small but measurable amount. Liver and kidney store glycogen in times of high blood glucose concentrations, in times of plenty. When blood glucose begins to drop below normal levels the glycogen is broken down and the resulting glucose is released into the blood. Muscle stores glycogen as a quick source of glucose for anaerobic muscle contraction.

Glycogenolysis - Glycogen Breakdown

GLYCOGENOLYSIS is the metabolic release of glucose-6-phosphate and glucose from glycogen by the action of three enzymes. The enzyme Glycogen Phosphorylase A (or more simply Phosphorylase A {“A” indicates the “active” form}) catalyzes the PHOSPHOROLYSIS of glycogen at the numerous nonreducing ends of the molecule. Phosphorylase A belongs to the hydrolysis class of enzymes. Hydrolysis is the cleavage of a chemical bond by adding HO-H across the bond; phosphorolysis is similar, except HO-PO$_3$$^{2-}$ is added across the chemical bond. The reaction catalyzed by Glycogen Phosphorylase A is depicted above. Each
Phosphorylase A catalyzes its reaction a glucose-1-phosphate and glycogen molecule one residue shorter is produced.

Phosphorylase A is forced to stop the phosphorolysis process four glucose residues from an $\alpha_1 \rightarrow 6$ branch point. Glycogen phosphorylase A is non reactive toward the last four glucose residues of a glycogen branch; this structure does not fit into its substrate/active site. This shortened “branch” of the glycogen “tree” is called the Limit Dextrin. Before Phosphorylase can continue its action, the branch point of the limit dextrin must be removed. This is accomplished by the action of Debranching Enzyme or Oligo (1,6 $\rightarrow$ 1,4) Glucantransferase. This enzyme catalyzes a two step reaction. First, it moves 3 of the 4 remaining glucose residues from the limit dextrin to the non reducing end of a nearby glycogen branch. Second, it hydrolytically cleaves the $\alpha_1 \rightarrow 6$ branch point releasing a molecule of glucose.
The glucose-1-phosphate that is formed by the action of glycogen phosphorylase is converted to glucose-6-phosphate by the reversible action of Phosphoglucomutase. In liver and kidney, the stored glycogen is used to maintain blood glucose levels. The glucose-6-phosphate coming from glycogen is converted to glucose by Glucose-6-Phosphatase. Once in the blood stream, the glucose is used by other tissues, especially nervous tissue, red blood cells, and the adrenal medulla. In all other tissues, especially skeletal muscle cells the glucose-6-phosphate enters glycolysis for energy generation.

Control of Glycogenolysis

Glycogen Phosphorylase is controlled by reversible covalent modification. When blood glucose concentrations drop Glucagon is released from the pancreas and Protein Kinase A is activated in the liver. In skeletal muscle Epinephrine, via the Gs effector system activates Protein Kinase A Glycogen Phosphorylase Kinase (or more simply Phosphorylase Kinase), a very specific protein kinase, is activated when phosphorylated by Protein Kinase A. The phosphorylated active form of Glycogen Phosphorylase Kinase then adds phosphate to Glycogen Phosphorylase converting the enzyme from the totally inactive form Glycogen Phosphorylase B to the active form Glycogen Phosphorylase A. Glycogen Phosphorylase A is an allosteric enzyme. In the liver it is allosterically inhibited by Glucose. In skeletal muscle it is allosterically activated by Ca²⁺ and AMP and allosterically inhibited by ATP. Phosphoprotein Phosphatase 1 catalyzes the hydrolytic removal of phosphate from Phosphorylase A converting it into the inactive Phosphorylase B.

Glycogenesis - Glycogen Synthesis

In times of high blood glucose concentrations the body stores some of the glucose in the form of glycogen. Liver and skeletal muscle are the major sites of glycogen storage. Kidney, heart, platelets, and adipose tissue store glycogen to a lesser extent. GLYCOGENESIS is the pathway that stores glucose as glycogen.

Before glucose can be polymerized into glycogen it must be activated. Glucose is phosphorylated by Hexokinase forming glucose-6-phosphate. This reaction traps the glucose in the cell and forms a pool of glucose-6-phosphate that is drawn upon, based on cellular needs, by the various metabolic pathways. To activate the glucose-6-phosphate for GLYCOGENESIS and other anabolic polymerization reactions it is converted to glucose-1-phosphate by the reversible action of Phosphoglucomutase and the Glucose-1-phosphate is then coupled to UTP by the action of UDP-Glucose pyrophosphorylase forming UDP-Glucose and P₂O₇(pyrophosphate). This reaction is energy expensive since the release of pyrophosphate from UTP and its subsequent hydrolysis into phosphate ions by Inorganic pyrophosphatase liberates the same amount of energy as the hydrolysis of two ATP to ADP. UDP-glucose is the activated form of glucose used for biosynthesis and...
galactose metabolism.

*De novo* glycogen synthesis (GLYCOGENESIS) starts on a protein called **Glycogenin**. Glycogenin forms a tight complex with **Glycogen Synthase**, and once this complex is formed a glucose from UDP-glucose is transferred to a specific **Tyrosine** residue on **Glycogenin**. **Glycogenin** is the catalyst for this first step in the synthesis process. The anomeric carbon of glucose, the reducing end of glucose, is attached to the tyrosine of glycogenin. **Glycogenin** now catalyzes the formation of a short polysaccharide by transferring seven (7) additional glucose residues from UDP-glucose to the glucose residues previously attached to **glycogenin**. All of the glycosidic bonds in this short polymer are \( \alpha 1 \rightarrow 4 \). This short oligosaccharide (8 glucose residues) synthesized on glycogenin serves as a substrate for the two enzymes that synthesize the remainder of the glycogen “tree”.

**Glycogen Synthase** catalyzes the transfer of glucose from UDP-glucose to the C-4 hydroxyl group at the non reducing end(s) of the growing glycogen molecule.
Branching Enzyme or Amylo (1,4 → 1,6) Transglycosylase removes the last 6 or 7 glucose residues from the nonreducing end of a growing branch at least 11 residues long and transfers them to the C-6 hydroxyl of a glucose residue of the same chain or another chain forming the α1 → 6 branch point. Each branching reaction adds a new non reducing end at which growth can occur.

Control of Glycogenesis

Glycogen Synthase is controlled by reversible covalent modification and allosterically. Glycogen Synthase is a substrate for cAMP Dependent Protein Kinase (Protein Kinase A) {Gₛ effector system}, Glycogen Phosphorylase Kinase {Gₛ effector system}, Protein Kinase C {Gₛ effector system}, Calmodulin-Dependent Protein Kinase {Gₐ effector system}, and/or Glycogen Synthase Kinase 3 {Insulin system}. When Glycogen Synthase is phosphorylated, it is inactive and under very tight allosteric control. The phosphorylated form of the enzyme is called Glycogen Synthase D (Dependent). This form of the enzyme is dependent upon the concentration of glucose-6-phosphate for activity. It is strongly allosterically activated by glucose-6-phosphate. The dephosphorylated form is the active / Independent form. When dephosphorylated it is Independent of allosteric control (Glycogen Synthase I) and functioning at maximal rate regardless of the glucose-6-phosphate concentration. Phosphorylated Glycogen Synthase is dephosphorylated by Phosphoprotein Phosphatase 1.

Pentose Phosphate Pathway - Hexose Monophosphate Shunt

The PENTOSE PHOSPHATE PATHWAY or the HEXOSE MONOPHOSPHATE SHUNT meets three needs of the cell:

1. it oxidizes glucose-6-phosphate (glucose) to generate NADPH for reductive biosynthetic reactions
2. it generates RIBOSE-5-PHOSPHATE for nucleotide biosynthesis
3. it is used to convert (excess) PENTOSES into HEXOSES. These “new” hexoses can then enter the other pathways of carbohydrate metabolism.

This pathway is active

1. in tissues that synthesize fatty acids and steroids, i.e., liver, adrenal gland, and adipose tissue.
2. in tissues that are actively undergoing cell division, i.e., liver, skin, intestine, and bone marrow.
3. in tissues at a high risk of oxidative damage, i.e., the RBC.

Greater than 50% of the glucose utilized by erythrocytes is passed down this pathway. The NADPH that is generated by this pathway is used by the RBC to maintain the iron of hemoglobin in the reduced, Fe⁺², state. Oxidized hemoglobin (Fe⁺³) is non-functional.

The PENTOSE PHOSPHATE PATHWAY can be divided into an oxidative phase and a non-oxidative recovery or rearrangement phase. The oxidative phase produces NADPH from NADP⁺ as glucose-6-phosphate is
oxidized in three steps to the five carbon ketose, ribulose-5-phosphate. During the non-oxidative recovery phase pentoses from the oxidative phase or from exogenous sources are converted into hexoses that can enter “mainstream” carbohydrate metabolism.

The Pentose Phosphate Pathway - Oxidative Phase

The first step of the oxidative phase produces 6-phosphogluconolactone when glucose-6-phosphate is oxidized. The hydroxyl group on the anomeric carbon (C-1) is raised from the hemiacetal oxidation state to the oxidation state of a cyclic ester, a lactone. The ester bond is between the new carboxyl group on C-1 and the hydroxyl group on C-5. As glucose-6-phosphate is oxidized, NADP+ is reduced to NADPH. This reaction is catalyzed by Glucose-6-phosphate Dehydrogenase.

In the second step of the pathway, the cyclic ester 6-phosphogluconolactone is hydrolyzed forming 6-phosphogluconate. Lactones will spontaneously breakdown. However, the spontaneous break down is not fast enough for the cell to survive so to speed the process is catalyzed by Gluconolactonase.
The hydroxyl group on carbon 3 of 6-phosphogluconate is oxidized to a ketone group and a second NADP$^+$ is reduced to NADPH. This two step reaction involves oxidation of the secondary alcohol group on C-3 to a ketone followed by a $\beta$ type elimination that expels the carboxyl group on carbon 1 as CO$_2$ (decarboxylation) leaving the product ribulose-5-phosphate. This reaction is catalyzed by 6-Phosphogluconate Dehydrogenase. This reaction marks the end of the oxidative phase.

If the pentose phosphate pathway is performed for the generation of ribose-5-phosphate along with NADPH generation, the ribulose-5-phosphate is converted to ribose-5-phosphate by the action of Ribulose-5-Phosphate Isomerase. This is one of the reactions of the non-oxidative phase and the pathway would end here.

If (excess) pentoses are to be converted to hexoses the entire non-oxidative part of the pathway is required. In this example, to balance the reactions of the non-oxidative phase of the pathway, six pentoses from the oxidative phase of the pathway or from exogenous sources (six ribulose-5-phosphate / 30 carbons) will enter the recovery phase and be converted to five hexoses (30 carbons). Two things to note at this point. First, any multiple of three pentoses (i.e., 3, 6, 9 …) allows us to balance the carbons on paper (e.g., 3 pentoses go to 2 hexoses & 1 triose, etc.). Second, how the cell balances the flow of carbon through the non oxidative phase depends upon cellular conditions at that moment, i.e. what the cell has versus what the cell needs.

The Pentose Phosphate Pathway - Recovery Phase

Two ribulose-5-phosphate are converted to two ribose-5-phosphate by the action of Ribulose-5-Phosphate Isomerase. The other four ribulose-5-phosphate are converted to four xylulose-5-phosphate by the action of
Ribulose-5-phosphate 3-Epimerase.

Carbon 1 & 2 from (two) xylulose-5-phosphate are transferred to (two) ribose-5-phosphate. This reaction produces (two) glyceraldehyde-3-phosphate and (two) sedoheptulose-7-phosphate. This reaction is catalyzed by Transketolase. The enzyme contains Thiamine Pyrophosphate as a prosthetic group. The Transketolase reaction involves movement of ketone groups and reactions that involve ketone functional groups usually require Thiamine Pyrophosphate.

The first three carbons of the (two) sedoheptulose-7-phosphate are now transferred to the (two) glyceraldehyde-3-phosphate to form (two) erythrose-4-phosphate and (two) fructose-6-phosphate. This reaction is catalyzed by Transaldolase.

During the last step of the non oxidative recovery phase carbons 1 & 2 from the remaining (two) xylulose-5-phosphate are transferred to the (two) erythrose-4-phosphate to form (two) glyceraldehyde-3-phosphate and (two) fructose-6-phosphate molecules. This reaction is also catalyzed by the enzyme Transketolase.
Using enzymes from Gluconeogenesis allows the cell to convert the four fructose-6-phosphate and the two glyceraldehyde-3-phosphate into five molecules glucose-6-phosphate. These glucose-6-phosphate molecules can enter any of the pathways of carbohydrate metabolism.

With this pathway the cell can

1. generate 12 NADPH from the complete oxidation of one glucose molecule by using the oxidative phase and the complete recovery phase. \(6 \text{ glucose-6-phosphate} \rightarrow 5 \text{ glucose-6-phosphate} + 6 \text{ CO}_2\)
2. generate 2 NADPH and 1 ribose-5-phosphate from 1 glucose-6-phosphate by using the oxidative phase and part of the recovery phase.
3. convert excess pentoses into hexoses by utilizing the recovery phase.
4. generate ribose-5-phosphate by utilizing the recovery phase in reverse.

Control of the Pentose Phosphate Pathway

The Pentose Phosphate Pathway is controlled at the first step of the oxidative stage. Glucose-6-phosphate Dehydrogenase is an allosteric enzyme. It is allosterically inhibited by NADPH and it is allosterically stimulated by NADP+ and glucose-6-phosphate. When the cell has a full charge of NADPH the pathway is inhibited, when the cell needs NADPH for biosynthesis it is stimulated.

Integration and Control of Carbohydrate Metabolism

The five pathways of carbohydrate metabolism are integrated into a functional whole by reciprocal control of the allosteric enzymes in the pathways and by the action of three hormones; Insulin, Glucagon, and Epinephrine. Before the action of the hormones are discussed, the control of carbohydrate metabolism by the allosteric enzymes will be reviewed.

Glycolysis is the breakdown of glucose for energy, 2 ATP are obtained from this pathway. Starting with lactate or pyruvate Gluconeogenesis requires 6 high energy phosphate molecules (4 ATP & 2 GTP) and two three carbon fragments to synthesize one glucose. Glycogenolysis is the breakdown of glycogen. Glycogenesis is the synthesis of glycogen. To prevent futile cycles the cell needs to control these pathways. When glycolysis is turned “on”, gluconeogenesis should be turned “off”; when glycogenolysis is turned
"on", glycogenesis should be turned "off"; etc. The allosteric enzymes and allosteric regulators of these pathways function to assure that futile cycles do not occur.

Integration by Allosteric Control
Glycogenolysis vs. Glycogenesis

Glycogen Phosphorylase B has, for the most part, no measurable activity. When phosphorylated by Phosphorylase Kinase it is converted to Glycogen Phosphorylase A and becomes active and under differential allosteric control depending on the tissue. Glycogen in muscle fuels contraction. Phosphorylase A in skeletal muscle is allosterically activated by Ca$^{2+}$ and AMP and allosterically inhibited by ATP. Ca$^{2+}$ signals that the muscles are contracting and AMP signals a low energy state. ATP signals that the muscle has sufficient energy. Liver glycogen is used to raise and/or maintain blood glucose concentrations. In the liver Glycogen Phosphorylase A is allosterically inhibited by glucose, indicating that blood glucose levels have returned to normal. When glucose is bound to Glycogen Phosphorylase A it causes a conformational change in the enzymes exposing the phosphorylated serine residues and Phosphoprotein Phosphatase 1 catalyzes the hydrolytic removal of phosphate from the active Phosphorylase A converting it into the inactive Phosphorylase B.

Glycogen Synthase D is allosterically activated by glucose-6-phosphate. When glucose and glucose-6-phosphate levels are high in the cell glycogen synthase is stimulated and glycogen phosphorylase A is inhibited. In addition Phosphoprotein Phosphatase 1 catalyzes the hydrolytic removal of phosphate from the inactive (dependent) Glycogen Synthase D converting it into the active (independent) Glycogen Synthase I.

Glycolysis vs. Gluconeogenesis

Pyruvate Kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP. This allosteric enzyme is inhibited by ATP, acetyl-CoA, alanine, and fatty acids. It is activated by fructose-1,6-phosphate and AMP. Pyruvate Carboxylase is one of the enzymes necessary for the conversion of pyruvate or lactate to phosphoenolpyruvate in gluconeogenesis. This allosteric enzyme is activated by acetyl-CoA and ATP. When acetyl-CoA levels are elevated glycolysis is inhibited at Pyruvate Kinase and gluconeogenesis is stimulated at Pyruvate Carboxylase. When acetyl-CoA levels are low glycolysis is stimulated (inhibition of Pyruvate Kinase removed) and gluconeogenesis is inhibited (stimulus of Pyruvate Carboxylase removed).

Phosphofructokinase-1 catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate in glycolysis. This allosteric enzyme is inhibited by ATP and citrate and is activated by AMP and fructose-2,6-bisphosphate. In gluconeogenesis Fructose-1,6-Bisphosphatase catalyzes the reverse reaction, the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. This allosteric enzyme is inhibited by fructose-2,6-bisphosphate and AMP. When cellular concentrations of fructose-2,6-bisphosphate and AMP are elevated, glycolysis is inhibited and gluconeogenesis is stimulated. When cellular concentrations of fructose-2,6-bisphosphate and AMP are low, glycolysis is inhibited (stimulus of Phosphofructokinase-1 removed) and gluconeogenesis is stimulated (inhibition of Fructose-1,6-Bisphosphatase removed).

Phosphofructokinase-2 is the bifunctional enzyme that catalyzes either the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate (kinase activity) or the conversion of fructose-2,6-bisphosphate to
fructose-6-phosphate (phosphatase activity). Phosphofructokinase-2 is under allosteric control. Its Kinase Activity is stimulated by PO$_4$$^-$$^3$ and inhibited by citrate; whereas its Phosphatase Activity is inhibited by PO$_4$$^-$$^3$ and stimulated by citrate. This pair of allosteric modulators of Phosphofructokinase-2 assures that Glycolysis and Gluconeogenesis are reciprocally controlled. When the cell is energy poor the concentration of PO$_4$$^-$$^3$ is high and the concentration of citrate is low stimulating the kinase activity resulting in an increase in fructose-2,6-bisphosphate that stimulates Glycolysis and inhibits Gluconeogenesis. When the cell is energy rich the concentration of PO$_4$$^-$$^3$ is low and the concentration of citrate is high stimulating the phosphatase activity resulting in a decrease in fructose-2,6-bisphosphate that inhibits Glycolysis (stimulus removed) and stimulating Gluconeogenesis (inhibition removed).

There is cross talk between Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway. Xylulose-5-phosphate one of the intermediates of the pentose phosphate pathway stimulates the activity of Phosphoprotein Phosphatase 2A. When blood glucose concentrations are high, cellular glucose-6-phosphate concentrations in the liver are high. Glucose-6-phosphate is the starting material for the energy generating reactions of Glycolysis, the TCA Cycle, & Et/OxPhos and it is the starting substrate for the pentose phosphate pathway. The pentose phosphate pathway generates NADPH for lipid biosynthesis and pentoses
for nucleotide biosynthesis. As Xylulose-5-phosphate from the Pentose Phosphate Pathway builds up it allosterically stimulates Phosphoprotein Phosphatase 2A which removes phosphate from Phosphofructokinase-2 stimulating its kinase activity. With the kinase activity stimulated, the concentration of fructose-2,6-bisphosphate increases which increases the rate of glycolysis and ATP generation assuring that the cell has the energy it needs for lipid biosynthesis and nucleotide biosynthesis.

Hormonal Control

Insulin, glucagon, and epinephrine also play a role in controlling the pathways of carbohydrate metabolism.

**Insulin**

- Insulin
- Receptor
- Receptor phosphorylation
  - IRS-1
  - Grb2+Sos+Ras
  - Raf-1(a)
  - MEK(a)
  - MAPK(a)
  - Elk1(a)
  - Elk1(a)+SRF

- Phosphorylates
- PKB(a)
- PI-3K
- PKB(i)+PDK-1

- Glycogen Synthase Kinase(a)
- PO₄–3–Glycogen Synthase Kinase(i)
- GluT4 Transporter Insertion

- ATP
- ADP
- Gα Protein
  - Site 1
  - Site 1–PO₄–3

- Phosphoenolpyruvate Carboxykinase
- Fructose-1,6-bisphosphatase
- Glucose-6-phosphatase
- PO₄–3–Glycogen Phosphorylase A
- PO₄–3–Glycogen Phosphorylase B
- PO₄–3–Glycogen Synthase D
- PO₄–3–Glycogen Synthase I

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Insulin via its tyrosine kinase receptor stimulates glucose transport into insulin dependent cells by stimulating the translocation of glucose transporters (GluT4) from storage vesicles in the cytoplasm to the plasma membranes. It stimulates Glycolysis by inducing the synthesis of several important glycolytic enzymes (Hexokinase, Phosphofructokinase-1, Phosphofructokinase-2, and Pyruvate Kinase). Insulin also stimulates Glycogenesis by inhibiting the action of Glycogen Synthase Kinase 3. The insulin activated kinases phosphorylates the $G_M$ protein at site 1. ($G_M$ stands for muscle-specific glycogen-targeting subunit, there is a $G_L$ protein that performs the same function in the liver and probably similar proteins in other tissues.) $G_M$ protein phosphorylated at site 1 stimulates Protein Phosphatase 1 to removed phosphate from Glycogen Synthase D rendering it active and from Glycogen Phosphorylase A rendering it inactive. Insulin inhibits gluconeogenesis by repressing the synthesis of Phosphoenolpyruvate carboxykinase, Fructose-1,6-bisphosphatase, and Glucose-6-phosphatase.
The liver and adipose tissue are the only “organs” with significant numbers of glucagon receptors. With respect to carbohydrate metabolism, the major effects of glucagon are on the liver, where it functions to increase blood glucose levels. Glucagon in the liver stimulates glycogen breakdown and gluconeogenesis and inhibits glycolysis and glycogen synthesis. Glucagon activates protein kinase A by the Gs effector system. The active protein kinase A phosphorylates four proteins in the liver; Glycogen Phosphorylase Kinase, Glycogen Synthase, Pyruvate Kinase, and Phosphofructokinase-2.

**Glycogen Phosphorylase Kinase (Phosphorylase Kinase)** when phosphorylated is converted from an inactive protein kinase to an active protein kinase. Phosphorylase kinase is a relatively specific enzyme. It has only two substrates. The active Phosphorylase Kinase phosphorylates, transfers phosphate from ATP to Glycogen Phosphorylase and it transfers phosphate from ATP to Glycogen Synthase.

When **Glycogen Phosphorylase Kinase** transfers phosphate from ATP to **Glycogen Phosphorylase**, it is converted from the inactive “B” form to the active “A” form. Phosphorylated Glycogen Phosphorylase
actively catalyzes the breakdown of glycogen to glucose-1-phosphate. The glucose-1-phosphate is converted to glucose-6-phosphate by the action of Phosphoglucomutase, the glucose-6-phosphate is dephosphorylated by Glucose-6-phosphatase, and the glucose is released into the bloodstream.

Glycogen Synthase when phosphorylated (by Protein Kinase A and/or the other protein kinases{see above}) is converted to the “D” form that is inactive and dependent upon positive allosteric regulators for activity. With glycogen synthase inactivated, glycogen synthesis is inhibited. Hence, the action of Glucagon inhibits glycogen synthesis and stimulates glycogen breakdown. The glucose released by glycogenolysis is used to increase blood glucose concentrations.

Pyruvate Kinase, when phosphorylated is inactive. This stops glycolysis at phosphoenolpyruvate. The phosphoenolpyruvate present in the cell is then used for gluconeogenesis, rather than ATP generation.

When Phosphofructokinase-2 is phosphorylated the kinase activity is inhibited and the formation of fructose-2,6-bisphosphate from fructose-6-phosphate is inhibited. Phosphorylation stimulates the phosphatase activity and the hydrolysis of fructose-2,6-bisphosphate to fructose-6-phosphate is stimulated. The decrease in fructose-2,6-bisphosphate concentrations removes the major stimulus of Phosphofructokinase-1 activity. By removing a stimulus, the enzyme is inhibited and consequently glycolysis is inhibited. The decrease in fructose-2,6-bisphosphate concentration removes the major inhibitor of Fructose-1,6-bisphosphatase activity. By removing an inhibitor the enzyme is stimulated and consequently gluconeogenesis is stimulated.

Glucagon by way of the Gs transduction system activates Protein Kinase A. The action of Protein Kinase A results in the phosphorylation of Pyruvate Kinase and Phosphofructokinase-2 effectively inhibiting glycolysis and stimulating gluconeogenesis. The glucose-6-phosphate from gluconeogenesis is converted to glucose by the action of Glucose-6-phosphatase, and this glucose is released from the liver cell to maintain blood glucose levels.

Some of the catalytic subunit of PKA activated by Glucagon moves to the nucleus where it phosphorylates CYCLIC AMP RESPONSE ELEMENT BINDING PROTEIN (CREB). CREB when phosphorylated is an active transcription factor that binds to Response Elements in the promoter region of several genes. With respect to carbohydrate metabolism, it binds to Response Elements in the promoter region of Phosphoenolpyruvate Carboxykinase, Fructose-1,6-bisphosphatase, and Glucose-6-phosphatase to stimulate their synthesis. {More detail on Response Elements will be forth coming in the Control of Gene Expression section.}

Epinephrine
The major effects of epinephrine are on skeletal muscle. It readies the muscle for the “Fight or Flight” response. In skeletal muscle, epinephrine inhibits glycogen synthesis and stimulates glycogen breakdown. Epinephrine functions via a serpentine membrane receptor coupled to the Gs protein, adenylate cyclase, and protein kinase A (cyclic AMP dependent protein kinase or PKA). Activated PKA phosphorylates three enzymes and one non-enzyme protein in skeletal muscle. The enzymes phosphorylated are Glycogen Phosphorylase Kinase, Glycogen Synthase, and Protein Phosphatase Inhibitor 1. The non-enzyme protein phosphorylated is G_{M} protein at site 2.

Glycogen Phosphorylase Kinase (or Phosphorylase Kinase) when phosphorylated is converted from an inactive protein kinase to an active protein kinase. Phosphorylase kinase is a relatively specific enzyme. It has only two substrates. The active Phosphorylase Kinase phosphorylates, transfers phosphate from ATP to Glycogen Phosphorylase and it transfers phosphate to Glycogen Synthase.

When Glycogen Phosphorylase Kinase transfers phosphate from ATP to Glycogen Phosphorylase, it is converted from the inactive “B” form to the active “A” form. Phosphorylated Glycogen Phosphorylase actively catalyzes the breakdown of glycogen to glucose-1-phosphate. The glucose-1-phosphate is converted to glucose-6-phosphate by the action of Phosphoglucomutase, and the glucose-6-phosphate enters Glycolysis to generate ATP to fuel continued muscle concentration.
Glycogen Synthase when phosphorylated (by Protein Kinase A and/or the other protein kinases{see above}) is converted to the “D” form that is inactive and dependent upon positive allosteric regulators for activity. With glycogen synthase inactivated, glycogen synthesis is inhibited. Hence the action of Epinephrine increases the concentration of metabolizable glucose within skeletal muscle by stimulating glycogenolysis and inhibiting glycogenesis.

The response is prolonged by the phosphorylation of G_M protein at site 2 and Protein Phosphatase Inhibitor 1. Site 2 phosphorylated G_M causes Protein Phosphatase 1 to dissociate from the glycogen granule preventing it access to Glycogen Phosphorylase A (phosphorylated, active form) and Glycogen Synthase D (phosphorylated, less active dependent form).

Protein Phosphatase Inhibitor 1 is activated when it is phosphorylated. Only the active, phosphorylated form can bind to the enzyme Protein Phosphatase 1. Protein Phosphatase 1 hydrolytically removes phosphate from the proteins phosphorylated by protein kinase A and other serine / threonine protein kinases. When phosphorylated Protein Phosphatase Inhibitor 1 binds to the dissociated Protein Phosphatase 1 and the activity of Protein Phosphatase 1 is inhibited. The effects of Epinephrine on skeletal muscle are prolonged since Protein Phosphatase 1 is dissociated from the glycogen granule and it is bound by an inhibitor protein. To reverse the effects a different protein phosphatase (?) must remove the phosphate from site 2 of the G_M protein and from Protein Phosphatase Inhibitor 1.

Epinephrine also stimulates the release of glucagon from the pancreas.
Cori Cycle

Gluconeogenesis is an important source of glucose for muscle cells during times of prolonged muscle contraction, during anaerobic metabolism. The muscle cell contains enough ATP for 1.5 to 2 seconds of maximal muscular contraction. CREATINE PHOSPHATE, stored in muscle cells, can supply the energy of another 2 seconds of contraction. Muscle cells store glycogen and this stored glucose can supply the energy for 5 additional minutes of muscular contraction. After this time the muscle’s glucose and glycogen will be exhausted. Muscles can contract for longer than 5 minutes, from where do they obtain the energy.

During prolonged muscular contraction, lactate is produced in the muscle. This lactate is released into the blood stream and travels to the liver where it is absorbed. Some of the lactate is converted to pyruvate and then completely oxidized to CO$_2$ and H$_2$O by the TCA CYCLE and ET/OXPHOS to generate ATP. Liver is always well oxygenated so the TCA CYCLE and ET/OXPHOS is always running at peak efficiency. The remainder of the lactate is converted to glucose by liver gluconeogenesis using the ATP generated by aerobic metabolism. The liver releases this glucose into the blood stream, the muscle absorbs it, and uses it in anaerobic glycolysis to generate ATP in order to sustain muscular contraction. This process, this cycle, is called the CORI CYCLE.

With continued muscular contraction blood glucose levels begin to drop because the skeletal muscle is utilizing more glucose from the blood than the liver can supply from the CORI CYCLE. The decrease in blood glucose levels simulates the release of glucagon. This hormone stimulates the liver to raise blood glucose concentrations and stimulates the adipose tissue to release triacylglycerols. The fatty acids of the
triacylglycerols are used for ATP generation in the liver and other tissues, and the 3 carbon glycerol is used by the liver as a carbon source for gluconeogenesis. A large amount of acetyl-CoA is generated from the released fatty acids, more than what is needed or can be used by the liver. The excess acetyl-CoA is converted to KETONE BODIES. Ketone bodies are small acidic molecules synthesized from excess acetyl-CoA. Eventually you have to stop, or the muscular pain and cramps forces you to stop: “you hit the wall”. Although the liver is very efficient at converting lactate to glucose, the muscle while contracting anaerobically is able to convert glucose to lactate at a fast rate. An athlete, and individual, is forced to stop due to the decrease in cellular pH caused by the generation and build up of lactic acid (lactate) and the acidic ketone bodies.