biochemistry

biochemistry

KULLBERM

Contents

FMR Biochemistry: Chapters and session learning objectives (SLOs)

1. Glycolysis
    - SLO 1. Describe the digestion and absorption of common dietary carbohydrates.
    - SLO 2. Explain how glucose is transported into and out of cells by GLUT transporters including the importance of the transporters' relative affinities.
    - SLO 3. Explain the significance of pancreas and liver producing both hexokinase and glucokinase enzyme isoforms, and why their properties are important for these organs to regulate blood glucose levels.
    - SLO 4. Describe the 3 critical steps of glycolysis that are regulated and explain the significance of these three steps being regulated.
    - SLO 5. Explain how cells cope with the lack of oxygen and inability of pyruvate (end product of glycolysis) to enter the TCA cycle and how the process of anaerobic glycolysis continues.

2. TCA Cycle
    - SLO 1. Explain the significance of the TCA cycle and its biological roles, including its role in catabolic and anabolic pathways.
    - SLO 2. Explain how the irreversible reaction catalyzed by the pyruvate dehydrogenase complex leads to the entry of acetyl-CoA into the TCA cycle and why acetyl-CoA cannot be used as a substrate for gluconeogenesis
    - SLO 3. Outline the co-factor requirements for the pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase enzymes and the biological consequences of thiamine deficiency.

- SLO 4. Explain how the TCA cycle is regulated, including which critical steps are regulated.
- SLO 5. Explain how the TCA cycle can be used for synthesis of intermediates involved in biosynthetic reactions, and how pyruvate carboxylase replenishes oxaloacetate for TCA cycle activity.

3. The Electron Transport Chain and Pentose Phosphate Pathway

    - SLO 1. Outline the process by which ATP is produced as a result of electron flow through the protein complexes of the Electron Transport Chain (ETC).
    - SLO 2. Explain how uncoupler proteins and carbon monoxide (CO) and cyanide (CN) disrupt the ETC.
    - SLO 3. Explain the significance of the pentose phosphate pathway and the different roles served by the irreversible reactions of its oxidative phase and the reversible reactions of its non-oxidative phase.
    - SLO 4. Outline how the oxidative and non-oxidate phases of the pentose phosphate pathway can can be used to either make more NADPH or more ribose-5-phosphate, and what these products are needed for.
    - SLO 5. Understand why red blood cells need the pentose phosphate pathway to detoxify reactive oxygen species (ROS), and explain how glucose 6-phosphate dehydrogenase works in concert with glutathione peroxidase and glutathione reductase to detoxify ROS, such as hydrogen peroxide (H2O2).
    - SLO 6. Determine the consequences of glucose-6-phosphate dehydrogenase deficiency and explain why it is prevalent in regions where malaria is endemic.

5. Gluconeogenesis and Glycogen Metabolism

- SLO 1. Understand the role of gluconeogenesis and glycogen breakdown in maintaining glucose homeostasis, including the role of glucose-6-phosphatase in the liver.
- SLO 2. Outline the 3 bypass reactions of gluconeogenesis that differentiate it from glycolysis.
- SLO 3 .Explain the significance of acetyl-coA not being a substrate for gluconeogenesis.
- SLO 4. Diagram the mechanisms by which glycogen synthesis and glycogen breakdown are reciprocally regulated.
- SLO 5. Correlate clinical presentations of Von Gierke disorder (Glucose 6-phosphatase deficiency) with the biochemical and physiological basis.

6. Metabolism of Fructose, Sorbitol, Galactose and Ethanol

   - SLO 1. Explain the functional consequences of fructose, lactose, galactose, sugar alcohols and ethanol metabolism.

7. Introduction to Diabetes

   - SLO 1. Apply biochemical principles underlying hyperglycemia to clinical tests and physiological consequences in diabetes mellitus.
   - SLO 2. Differentiate between T1 and T2DM in terms of origin, mechanisms of pathology, and treatment options.
   - SLO 3. Explain how signaling transduction is related to T1 and T2D.

8. Cholesterol and Lipid Digestion and Trafficking

   - SLO 1. Outline the pathways of lipid transport in the body, including the roles of chylomicrons, VLDL, LDL, and HDL.
   - SLO 2. Outline pathways of dietary cholesterol uptake and transport.
   - SLO 3. Understand the possible mechanisms of familial

hypertriglyceridemia.
    - SLO 4. Understand the biological basis of familial hypercholesterolemia.
    - SLO 5. Outline the major pathway of de novo sterol synthesis. Understand the biochemical basis of statin action.

9. Lipid Metabolism, Ketone Bodies and Arachidonic Acid and Eicosanoids

    - SLO 1. Analyze the oxidation of fatty acids, including regulation, the carnitine shuttle and generation of ATP.
    - SLO 2. Diagram the pathways for fatty acid storage and release from adipose tissue and their regulation, highlighting the impact of insulin and counterregulatory hormones.
    - SLO 3. Outline the pathway and regulation of de novo lipid biosynthesis
    - SLO 4. Explain how and why ketone bodies are formed and how they are utilized.
    - SLO 5. Understand the biochemical mechanism behind examples of lipid metabolism dysfunction, including carnitine deficiency and medium chain fatty acyl CoA deficiency.
    - SLO 6. Compare the significance of arachidonate derivatives (COX inhibition by aspirin, prostaglandins, leukotrienes).
    - SLO 7. Recognize the roles of eicosanoids in diverse physiological processes and their roles as inflammatory mediators.

10. Fasting and Postprandial State

    - SLO 1. Explain how CHO and lipid metabolism change during acute and prolonged fasting.

- SLO 2. Explain how CHO and lipid metabolism change after a meal, focus on metabolism in liver, muscle, brain and adipose tissue.
- SLO 3. Summarize the actions of insulin, glucagon, and epinephrine in regulating CHO and lipid metabolism.
- SLO 4. Demonstrate the connections between CHO and lipid metabolism, including how CHO carbon molecules become lipids and vice versa.

11. Proteasome and Lysosome

    - SLO 1. Understand the role of protein turnover in generating amino acids, in protein quality control, and in regulating the abundance of proteins.
    - SLO 2. Delineate the two major pathways of protein turnover: the ubiquitin-proteasome pathway and the lysosomal/autophagosome pathway.
    - SLO 3. Outline the genetic basis and pathophysiology of major lysosomal storage diseases.

12. Nitrogen Metabolism

    - SLO 1. Define the concept of nitrogen balance and explain the role of protein degradation in normal nutrition and disease states.
    - SLO 2. Describe the metabolism of nitrogen using aminotransferases, glutamate dehydrogenase, glutamine synthetase and glutaminase.
    - SLO 3. Describe the significance of the urea cycle in removing nitrogen and the presentation of hyperammonemia with defects in urea cycle enzymes.
    - SLO 4. Discuss the diagnostic significance of aspartate aminotransferase and alanine aminotransferase (AST, ALT).

13. Amino Acid Derivatives

- SLO 1. Describe the biosynthetic origin and basic function of the biological mediators histamine, gamma-aminobutyric acid (GABA), serotonin and nitric oxide.
- SLO 2. Describe the biosynthetic origin and basic function of molecules derived from tyrosine: thyroid hormone, melanin, and the catecholamines dopamine, norepinephrine, and epinephrine.
- SLO 3. Discuss the biosynthetic relationship between creatine and creatine phosphate and the use of creatinine as a clinical analyte.
- SLO 4. Describe the biochemical role of reduced and oxidized glutathione.

14. Amino Acid Derivative, Heme and Bilirubin Disorders

    - SLO 1. Discuss the enzymatic defects in, and clinical consequences of, phenylketonuria (PKU) and homocystinuria.
    - SLO 2. Relate the disruption of the heme biosynthetic pathway to porphyrias and lead poisoning.
    - SLO 3. Describe the importance of heme degradation in the development of hyperbilirubinemia.

15. Single Carbon Metabolism

    - SLO 1. Identify the functions of vitamin B12, and explain the role of intrinsic factor in its absorption.
    - SLO 2. Explain the causes and consequences of Intrinsic Factor, B12 and folate deficiencies.
    - SLO 3. Outline the S-adenosylmethionine and folate cycles and explain why B12 deficiency leads to megaloblastic anemia due to a secondary folate deficiency.
    - SLO 4. Illustrate therapeutic uses of pharmaceuticals that disrupt folate metabolism and describe the consequences

16. Purine and Pyrimidine Metabolism

- SLO 1. Identify the key points pertaining to the de novo purine and pyrimidine biosynthesis pathways, emphasizing input metabolites, key regulated steps, and significance for physiology and metabolism.
- SLO 2. Examine the formation of deoxyribonucleotides and the balancing of nucleotide pools.
- SLO 3. Summarize the key aspects of thymidine biosynthesis and describe the mechanism by which inhibitors of this pathway act in cancer chemotherapy.
- SLO 4. Analyze the catabolism of nucleotides and the purine salvage pathway, focusing on its role in the pathophysiology of gout.

PART I

MAIN BODY

I.

FUERSTPG

GLYCOLYSIS

This session introduces the study of metabolism. Metabolism includes the processes by which the body stores and converts ingested food into energy and the many essential chemical building blocks of cells and tissues.

Metabolism also includes how substances are broken down into chemical components that are recycled or excreted. Although much of the material describes detailed enzymatic steps of biochemical conversion of key compounds, it will be equally important for you to recognize and understand a larger picture. The logic and function of the metabolic pathways can be applied to understanding how dysfunction of these pathways could contribute to common chronic diseases.

Glycolysis Learning Objectives and Brief Synopsis:

SLO1. Describe the digestion and absorption of common dietary carbohydrates.

SLO2. Explain how glucose is transported into and out of cells by GLUT transporters including the importance of the transporters' relative affinities.

Knowledge application 1

SLO1 and 2 Quiz 1SL01 and 2 Quiz 2

SLO3. Explain the significance of pancreas and liver producing both hexokinase and glucokinase enzyme isoforms, and why their properties are important for these organs to regulate blood glucose levels.

Knowledge application 2

SLO4. Understand the 3 critical steps of glycolysis that are

regulated and explain the significance of these three steps being regulated.

SLO5. Explain how cells cope with the lack of oxygen and inability of pyruvate (end product of glycolysis) to enter the TCA cycle and how the process of anaerobic glycolysis continues.

Knowledge application 3

SLO4 and 5 Quiz

Definitions:

Metabolism refers to the biochemical processes that occur within a living organism to maintain life.

Catabolism is the breakdown of complex molecules into elementary building blocks.

Anabolism is the synthesis of cellular components like RNA, DNA, proteins, lipids, and carbohydrates from elementary building blocks.

Redox reactions interconvert paired molecules (NAD+ <-> NADH and NADP+ <-> NADPH and FADH+ <-> FADH2) by reduction (gain of electrons) or oxidation (loss of electrons). Note: when NAD+ is reduced to NADH it receives two electrons. You will sometimes see this written as NADH +H, for example in figure 8. This is how NADH is able to pass two electrons when it is oxidized at the onset of the electron transport chain.

https://mediasite.hs.washington.edu/Mediasite/Play/329b89249d1e40c8b4f90719f18bf15f1d

Overview of metabolism

The breakdown (catabolism) and synthesis (anabolism) of biochemical compounds occur through separate enzymatic routes engaging a sequence of enzymes that is called a metabolic pathway.

Catabolism produces cellular energy in the form of ATP and reducing power in the form of NADH, FADH2 or NADPH. Anabolism consumes reducing power and ATP. In the body, there is continual switching between the breakdown pathways and the biosynthetic pathways to maintain homeostasis.

A key element of this switching is a reciprocal regulation of metabolic pathways that catalyze opposed processes. For example, if glucose breakdown (glycolysis) is up-regulated, the opposite pathway of *de novo* glucose synthesis (gluconeogenesis) will be down-regulated, a reciprocal control mechanism that prevents what are called "futile cycles."

Energy metabolism: The main energy currency of cells is ATP, mostly generated by glucose and fat metabolism. ATP can be produced by glycolysis, and, under oxygen-rich conditions, additional ATP is produced by subsequent oxidative phosphorylation of ADP via the electron transport chain (ETC).

Products of glucose and fat metabolism enter the TCA cycle (tricarboxylic acid cycle, also called the Krebs cycle or the citric acid cycle) that generates reducing power that drives oxidative phosphorylation of ADP via the electron transport chain.

A lot more ATP can be generated through aerobic catabolism of glucose through the TCA cycle and ETC (~36 ATPs compared to ~2 ATPs per glucose molecule broken down), BUT oxygen is not always available and can be toxic as it can lead to the production of reactive oxygen species (ROS).

Strategies to make sense of what metabolic pathways mean:

First: *Ask yourself what is the biological role of this pathway and what is the big picture of its activity?*

Example (1): What is the biological function of glycolysis?

Answer: The role of glycolysis is to generate cellular energy in the form of ATP by breaking down glucose (a 6-carbon molecule) into smaller constituents such as pyruvate (a 3- carbon molecule)

Example (2): What is the biological function of the pentose phosphate pathway?

Answer: The role of the pentose phosphate pathway is to generate reducing power (NADPH) and to provide 5-carbon ribose sugar intermediates for nucleotide biosynthesis

Second: Remember that different tissues have different metabolic roles and different metabolic needs.

Example: The liver is an important regulator of blood glucose

levels; to this end the liver can generate glucose through gluconeogenesis or by breakdown of glycogen when blood glucose levels are low. In contrast, the brain uses only glucose for ATP production and is not able to form glucose de novo. The brain is not capable of performing gluconeogenesis.

We will see more of this as we examine different metabolic pathways and processes.

Third: The key to understanding metabolism is understanding how metabolic pathways are regulated and at which levels.

Example: At the cellular level , enzyme activity can be enhanced or suppressed by the binding of enzyme inhibitors or allosteric effectors, or by covalent modifications including phosphorylation and dephosphorylation of enzymes. At the systemic level, metabolicpathwayscanbeup-regulatedordown-regulatedbyactionsofhormones.

Fourth: Remember that metabolic pathways are inter-connected.

Example: Carbons from glucose can be used to synthesize cholesterol and fatty acids. Some amino acid carbons can be converted into glucose as can the glycerol backbone of triglycerides. Other amino acid carbons and 3-carbon fatty acid remnants can enter the TCA cycle. Focus on these connections. Understand the exceptions (e.g., carbons from even numbered fatty acids CANNOT be turned into glucose).

Fifth: You are training to by physicians.

Metabolic diseases range from very common disorders like diabetes to very rare genetic disorders. In either case understanding the relationship between the affected pathway and disease will be very helpful.

METABOLISM CAN BE REPRESENTED BOTH AS BLOCK DIAGRAMS AND AS DETAILED CHARTS

Initially it is appropriate to think of glucose metabolism as

composed of blocks of reactions represented as colored areas in Figure 1.

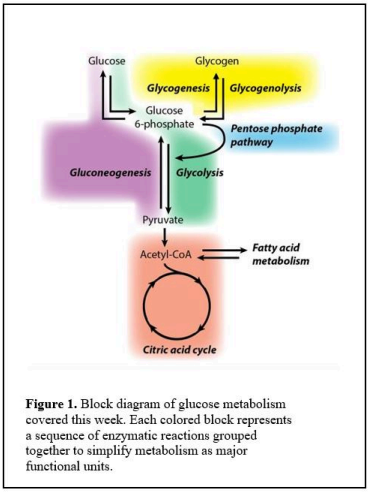


Figure 1. Block diagram of glucose metabolism covered this week. Each colored block represents a sequence of enzymatic reactions grouped together to simplify metabolism as major functional units.

One block produces molecules that are fed into the next. For example, the glycolysis block converts glucose 6-phosphate into pyruvate, which is then converted into acetyl CoA to feed the citric acid cycle.

You should commit to memory the names of the colored blocks and how they relate to each other.

Each of the colored blocks is shorthand for a sequence of enzymatic reactions, a metabolic pathway. For many of these pathways, learning a few of the specific intermediate steps, especially those with important regulation or where an enzymatic deficiency causes a metabolic disorder, will facilitate overall understanding.

As we progress through class sessions, you can find specific reactions that we discuss in this comprehensive diagram. We will "deconstruct" the different metabolic processes and examine their inter-relationships throughout the course.

CARBOHYDRATE DIGESTION

Session Learning Objective 1. Describe the digestion and absorption of common dietary carbohydrates.

Digestive enzymes

As food passes down the alimentary tract, different enzymes are secreted for step wise digestion of carbohydrates (Figure 2 Left Panel):

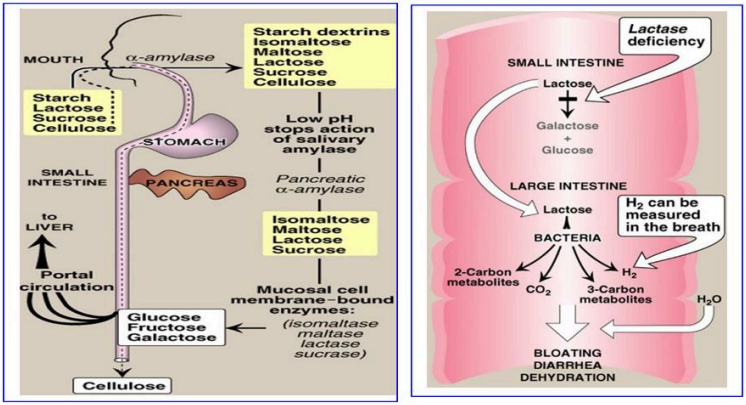


Figure 2. Overview of starch digestion (left). Lactase deficiency leads to abnormal lactose metabolism (right).

1) **Salivary α-amylases** break down dietary polysaccharides in the mouth; salivary amylase can partially digest starch in the stomach until the increased gastric acidity inactivates the enzyme.

2) **Pancreatic α-amylases** catalyzes starch/polysaccharide digestion in small intestine.

3) Additional enzymes break down complex sugars in the intestine into smaller polymers and simple sugars (monosaccharides) like glucose, fructose, galactose. Simple sugars are taken up by gut epithelial cells and rapidly shuttled into capillaries that lead to the portal venous system supplying the liver:

Sucrase: breaks sucrose into glucose and fructose, Glucose is absorbed via secondary active transport into intestinal epithelial cells; fructose enters cells by facilitated diffusion.

Lactase: breaks lactose into glucose and galactose. Lactase deficiency causes bloating and diarrhea with lactose ingestion (Figure 2 right panel). Lactose intolerance develops in > 70% of adults who lose expression of lactase with aging.

Learning objective 2. Explain how glucose is transported into and out of cells by GLUT transporters including the importance of the transporters' relative affinities.

Glucose transport into intestinal cells. Sugars (very polar molecules) cannot cross cell membranes without the aid of transporters. Transport into intestinal cells requires secondary active transport by a Na+-glucose cotransporter (symporter) (Figure 3).

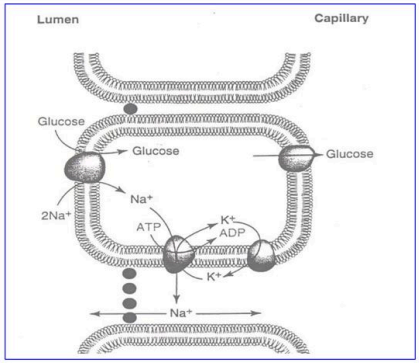


Figure 3. Glucose rapidly crosses intestinal epithelial cells from lumen to capillaries using two transporters, an apical Na^+-glucose cotransporter and a basolateral glucose facilitated transporter.

The flow of luminal glucose into the epithelial cells is driven against its concentration gradient by coupling to the energetically favorable entry of Na+ ions. Since the sodium ion gradient is maintained by the Na+/K+ ATPase pump, the energy needed for concentrating glucose can be traced secondarily to consumption of ATP. Exit of glucose from the epithelial cell into the blood stream is mediated by a glucose transporter (GLUT2) that moves glucose via facilitated diffusion and does not require the Na+ gradient.

Principles: Enzyme Kinetics (Figure 4)

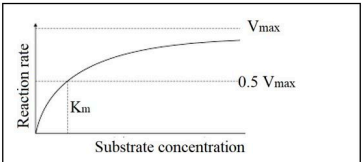


Figure 4. The velocity of enzyme reactions depends on the substrate concentration.

The GLUT transporters we are about to cover have different affinities for glucose.

The velocity of transport depends on the concentration of reactants, i.e., the concentration of the substrate glucose. The velocity rises with substrate concentration but then saturates at a maximum velocity (Vmax) when every transporter becomes occupied by its substrate. We use the term Km for the concentration of substrate that gives the half maximal velocity. If the Km is small, that means that the enzyme has a high affinity for binding the substance, so it can act on its substrate even if the concentration of the substrate is low.

Notice the Km values for GLUT1-4 transporters below. Which of these has the highest affinity for glucose? Does this correlate with a low or high Km? Which transporter has the lowest affinity for glucose? Note: the Km values vary from study to study. The absolute numbers are not important but the relative affinities are (GLUT1 affinity > GLUT2 affinity etc.).

Name	Tissue	Km (mM)	Features
GLUT1	Bidirectional; All mammalian tissues	~5	Basal glucose uptake; especially important in erythrocytes
GLUT2	Bidirectional; Renal tubular, small intestinal epithelial, liver, pancreatic beta cells	~17	Liver: excess glucose from blood used in glycogen synthesis Pancreas: glucose influx involved in signaling insulin release
GLUT3	Bidirectional; All mammalian tissues	~1.5	Basal glucose uptake; Important for glucose transport in neuronal tissues (including brain)
GLUT4	Muscle & adipose tissue	~6.5	Mediates insulin-stimulated glucose uptake in skeletal and cardiac muscle, fat cells

Glucose transporters: Glucose entry into most cells is mediated by multiple glucose transporters that transport glucose via facilitated diffusion. These glucose transport proteins are abbreviated GLUT (Table 1; left). Some key properties of the primary GLUT transporters:

GLUT1: these glucose transporters are found in most cell types. Because they have a low Km/high affinity for glucose, they are responsible for constitutive transport of glucose at even low blood glucose levels. GLUT1 is especially important in RBCs.

GLUT2: mediates entry of glucose into multiple cell types (see Table 1). GLUT2 has a high Km for glucose and is most important with higher blood glucose levels such as after a meal. GLUT2 has a high maximum velocity.

GLUT3: is very important in neurons/the brain. Having the highest affinity glucose transporters made in the brain ensures that glucose is reserved for the brain when glucose levels fall.

GLUT4: is expressed in muscle and adipose cells. GLUT4 regulation is especially important because it is affected by insulin, a hormone secreted by the pancreas when blood glucose is elevated.

Binding of insulin to insulin receptors on muscle and adipose cells recruits intracellular GLUT4 to the plasma membrane where it promotes entry of glucose into the cells.

Knowledge application 1:

1. Describe the importance of Km and Vmax for different glucose transporters.

2. Why is it important that GLUT2 has a low affinity (high Km) for glucose and a high Vmax (see Figure 4 for primer on Vmax and Km)?

3. How does this affect glucose transport into the liver in the fed vs. fasted state? Why would you want neurons to have GLUT3?

4. What two types of transport are required for glucose to enter the bloodstream from digestion? Why are different transporters necessary?

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-6

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-7

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-8

Glucose entry into glycolysis

The first step of glycolysis involves phosphorylation of glucose by an enzyme called "hexokinase" and also in the liver and some other cells such as pancreatic beta cells, by "glucokinase" (Figure 5).

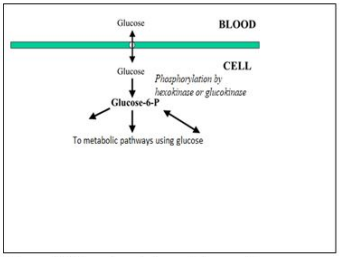


Figure 5. Phosphorylation of glucose by hexokinase or glucokinase ensures that glucose cannot diffuse back out of the cell and lowers the concentration of glucose in the cell so that more glucose is able to enter the cell by facilitated diffusion.

Phosphorylation ensures that glucose cannot diffuse back out of the cell.

Learning Objective 3. Explain the significance of pancreas and liver producing both hexokinase and glucokinase enzyme isoforms, and why their properties are important for these organs to regulate blood glucose levels.

There are two enzyme isoforms we will discuss that can phosphorylate glucose: hexokinase and glucokinase.

The protein isoforms (isozymes) are transcribed from different genes in different sometimes overlapping tissues. Hexokinase is broadly expressed across tissue types, including in the liver. Glucokinase is specific to tissues including the liver and pancreatic beta cells.

Hexokinase has a low Km /high affinity for glucose and is inhibited by its product, glucose-6-phosphate (G6P). Glucokinase has a higher Km for glucose, i.e. lower affinity for its substrate glucose, meaning it is active only when glucose levels inside the

liver cells are high, and is not inhibited by its product, G6P (Figure 6).

Thus, glucokinase is active only when blood glucose levels are high, normally after a meal that promotes influx of glucose into the liver cell. Glucokinase is *not inhibited* by the product of its reaction, glucose-6-P, so it keeps converting glucose to glucose-6-P even as glucose-6-P accumulates in the cell. This allows a heavy glucose load to be trapped inside liver cells, but only when blood glucose levels are high.

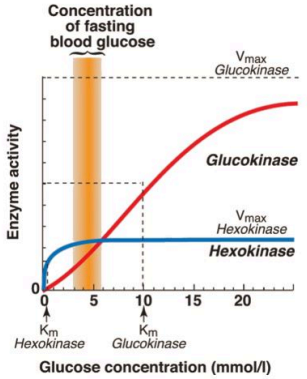


Figure 6. Contrasting substrate-velocity dependency for hexokinase (high glucose affinity, low KM, lower max activity) and glucokinase (low glucose affinity, higher KM, higher max activity).

Glucokinase in pancreatic beta cells functions in a similar manner and results in the production of large amounts of ATP when blood glucose levels are high. The high ATP levels bind to an ion channel and depolarize the cell, ultimately leading to a cytoplasmic Ca2+ spike and release of insulin. Glucokinase in pancreatic cells therefore functions as the body's glucose sensor.

Knowledge application 2:

1. What would happen to an individual who did not have α-amylase enzymes in their saliva, or would lack pancreatic *α*-amylases?

2. Why might it be beneficial for a muscle or an adipose cell to have a glucose transporter, i.e., GLUT4, that is regulated by insulin levels? When could this be important for an organism?

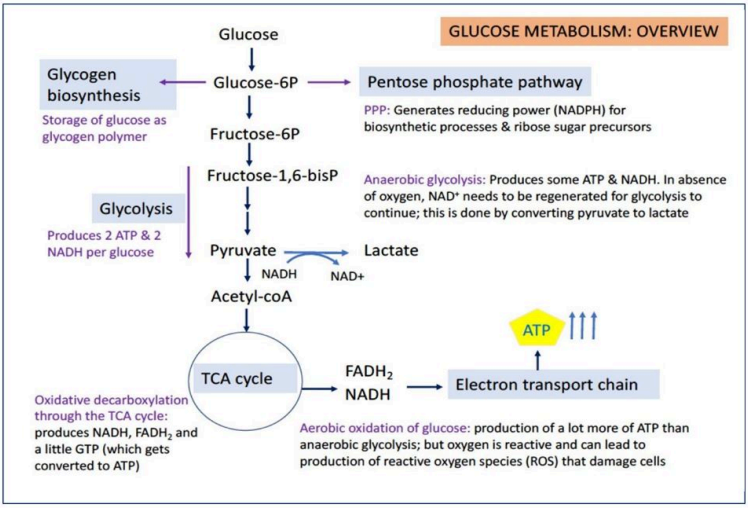
3. A patient with diabetes mellitus has very high blood glucose levels. The glucose enters endothelial cells through GLUT1, but the glucose concentration in the cell exceeds the level at which hexokinase reaches Vmax. What happens to (most of) the glucose?

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-9

Learning objective 4. Describe the 3 critical steps of glycolysis that are regulated and explain the significance of these three steps being regulated.

Overview of glucose metabolism

Once glucose is taken up into cells, it is converted by hexokinase (or glucokinase) to glucose-6-phosphate. Glucose-6-phosphate is a key intermediate that can go in several directions (Figure 7). It can be converted to glycogen, a polymer storage form of glucose in several tissues, including liver and muscle. It can enter into the pentose phosphate pathway (also called the hexose monophosphate pathway), producing NADPH (reducing power for biosynthetic reactions) and ribose sugars (precursors for nucleotide biosynthesis). Lastly, glucose-6-phosphate can enter into the pathway of glycolysis, which is important for energy production in all cells (most active in many cells following a meal).

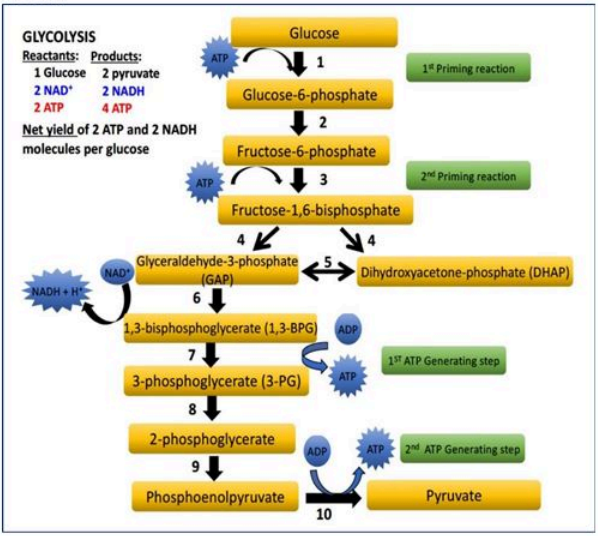


GLYCOLYSIS

Glycolysis is the breakdown of glucose (a 6-carbon sugar) into 2 equivalents of pyruvate (a 3- carbon molecule). By itself, glycolysis can provide energy to the cell. From one molecule of glucose, glycolysis yields 2 ATP, 2 NADH, and 2 pyruvates. Glycolysis is called anaerobic because it does not use oxygen.

Glycolysis consists of 10 reactions (Figure 8), including 3 key irreversible steps. These are ones to remember, so we will be going over them in detail.

Figure 8. The ten steps of glycolysis and its products. Steps 1, 3 and 10 are irreversible and regulated and will be covered in more detail.



Glycolysis starts by phosphorylation of glucose followed by a second phosphorylation to yield fructose-1,6-bisphosphate (referred to as "priming reactions" in the diagram below). These two phosphorylation steps require ATP expenditure. Once fructose-1,6-bisP is formed, glucose is "committed" to break down via glycolysis, although as we will see when we cover gluconeogenesis this can be reversed by activating different enzymes when the cell needs to make glucose.

Question: "*If glycolysis is used to produce energy in the form of ATP, why does the process begin by using 2 equivalents of ATP? Isn't this a waste of energy?*"

Answer:

Actually, it is not such a waste. The multiple steps in some metabolic pathways will maximize their efficiency. Here, glucose

becomes a lot easier to break down into 3-carbon scaffolds if it is phosphorylated at the 1 and 6 carbon positions, therefore harnessing the energy of the cleavage reaction into subsequent ATP production steps. The priming reactions ensure that once the glucose ring is broken open, the energy can be harnessed to generate more ATP and NADH. It is thus a good strategy for the cell to start glycolysis in this fashion.

The reactions of glycolysis are catalyzed by the following enzymes. (You will not need to memorize them, but we may refer to these steps in practice questions). You should know and be able to describe the three enzymes with asterisks and how they are regulated.

***Reaction 1**: **Hexokinase or glucokinase** (regulated irreversible step)

Reaction 2: Glucose-6-P isomerase (catalyzes a rearrangement of atoms)

***Reaction 3**: **Phosphofructokinase-1 (PFK-1)** (regulated irreversible step)

Reaction 4: Aldolase A

Reaction5: Triose phosphate isomerase (another rearrangement of atoms)

Reaction 6: Glyceraldehyde-3-P dehydrogenase: adds a phosphate and reduces NAD+ to NADH

Reaction 7: Phosphoglycerate kinase (confusingly, the enzyme for this reaction takes its

name from the reverse reaction: 3-PG –> 1,3-BPG)

Reaction8: Phosphoglycerate mutase (catalyzes reaction similar to an isomerase enzyme)

Reaction 9: Enolase

***Reaction 10**: **Pyruvate kinase** (again takes its name from the reverse reaction) (regulated; irreversible at physiological conditions).

Note: As we will see, when we cover gluconeogenesis, and in the

glycolysis in class PPT (step 3), the irreversible steps can be reversed by different enzymes to make glucose.

The **three regulated steps** of glycolysis are *reaction steps 1, 3, and 10*. Let's go through them one by one to highlight their significance and how they are regulated.

1st regulated step of glycolysis (reaction 1 of glycolysis): conversion of glucose to glucose-6- phosphate by an enzyme in most tissues by hexokinase and also by glucokinase in some tissues.

Advantage of converting glucose to glucose-6P using the hexokinase enzyme:

Three advantages to phosphorylating glucose by the hexokinase enzyme:

· First, it locks glucose in the cell (negatively charged glucose-6P cannot diffuse or be transported out of the membrane).

· Second, it lowers the concentration of free glucose (unphosphorylated) in the cell and therefore favors diffusion of more glucose into the cell along a concentration gradient.

· Third, this phosphorylation step requires ATP and being irreversible, permits regulation.

Thus, the hexokinase enzyme is *inhibited by its product*, glucose-6-phosphate. Hexokinase has a high affinity for its substrate glucose, meaning that the hexokinase enzyme is active even when glucose levels are low.

2nd regulated step of glycolysis (reaction 3 of glycolysis): conversion of fructose-6- phosphate to fructose-1,6-bisphosphate.

This reaction is catalyzed by an enzyme called **phosphofructokinase-1** (PFK-1).

PFK-1 is allosterically inhibited by ATP and activated by both ADP and AMP. Thus, when ATP levels drop in the cell, and the levels of ADP and AMP concomitantly increase, the rate of this reaction will increase.

PFK-1 is also allosterically activated by an effector molecule

called fructose-2,6-bisphosphate which is made by an enzyme called **PFK-2**.

The activity of PFK-2 is increased when the ratio of insulin to glucagon is high, while the reverse reaction is stimulated by a high ratio of glucagon to insulin. High blood glucose stimulates insulin secretion, low blood glucose stimulates glucagon secretion.

3rd regulated step of glycolysis (reaction 10 of glycolysis): Conversion of phosphoenolpyruvate (PEP) to pyruvate and generating ATP.

This reaction is catalyzed by the enzyme **pyruvate kinase** (PK), which catalyzes the final step of the pathway. PK is inhibited by ATP, and thus activated by a drop of ATP levels. In addition, PK is activated by the product of the PFK1 reaction, fructose 1,6-bisphosphate. Hence, when PFK1 is turned on by falling ATP levels, fructose 1,6-bisphosphate is elevated, which in turn activates PK. When ATP levels are returned to normal, the reverse of this regulatory pattern occurs and the metabolic flux through glycolysis slows down.

Take home message for regulation of glycolysis: The activity of glycolysis is regulated by the energy status of the cell. It is stimulated when ATP levels are low, and down regulated when cellular levels of ATP are high.

In addition to cellular energy status, glycolysis is regulated at the systemic level by hormones such as glucagon, epinephrine, and insulin – we will examine how these hormones regulate glucose metabolism after we have discussed aerobic glucose oxidation, gluconeogenesis, and glycogen metabolism.

Regulation Summary:
Hexokinase: Glucose-6P inhibits activity.
PF1K: F2,6BPG, AMP stimulate. ATP inhibits.
PK: F1,6BPG stimulates. ATP inhibits.
PF2K: Insulin stimulates.Glucagon inhibits.

Session Learning Objective 5. Explain how cells cope with the

lack of oxygen and inability of pyruvate (end-product of glycolysis) to enter the TCA cycle and how the process of anaerobic glycolysis continues.

Glycolysis is the primary mechanism by which ATP is produced in anaerobic conditions.

In addition to generating ATP, glycolysis also generates NADH. Glycolysis cannot continue if there is not an electron acceptor (NAD+).

A reversible conversion of pyruvate to lactate by lactate dehydrogenase can be utilized:

Pyruvate + NADH <——> Lactate + NAD+

NAD+ can permit glycolysis to continue while lactate can enter circulation and be used as a fuel by other tissues that are currently aerobic. The liver can take up lactate and use it as a precursor for gluconeogenesis, thereby returning glucose to anaerobic tissues (typically muscle).

Knowledge application 3:

1. What would be observed in a patient who has a glucokinase deficiency?

2. What is the biological role of glycolysis?

3. What is the major bottleneck of anaerobic glycolysis?

4. What is the advantage of glucokinase having a high KM (i.e. low affinity for its substrate glucose)?

5. What would occur in a person with phosphofructokinase-1 (PFK-1) enzyme deficiency? Into which pathways would glucose be able to flow?

6. What is meant by an allosteric effector molecule?

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-10

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=335#h5p-11

Make sure you know which of the steps in the exercise above are regulated and irreversible!

That's it for now! I know the class is on Monday after your first exam and have worked to make the session a good overview of the material.

2. Tricarboxylic Acid (TCA) Cycle

FUERSTPG

The TCA Cycle
(rev. 10/28/2022)

This session continues our study of metabolism. After the initial catabolism of glucose by glycolysis, what further processing happens? When oxygen is present, how can catabolism capture more energy in the form of ATP? How does metabolism produce more metabolic intermediates essential for synthesizing other molecules (anabolism)?

Session Learning Objectives:

1. Explain the significance of the TCA cycle and its biological roles, including its role in catabolic and anabolic pathways.

2. Explain how the irreversible reaction catalyzed by the pyruvate dehydrogenase complex leads to the entry of acetyl-CoA into the TCA cycle and why acetyl-CoA cannot be used as a substrate for gluconeogenesis.

3. Outline the co-factor requirements for the pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase enzymes and the biological consequences of thiamine deficiency.

4. Explain how the TCA cycle is regulated, including which critical steps are regulated.

5. Explain how the TCA cycle can be used for synthesis of intermediates involved in biosynthetic reactions, and how pyruvate carboxylase replenishes oxaloacetate for TCA cycle activity.

TCA practice Questions
TCA Quiz 1
TCA Quiz 2

TCA Quiz 3

Goals:

To provide an understanding of the catabolism of glucose under aerobic conditions. We will discuss the importance of the pyruvate dehydrogenase reaction as a first step for acetyl-CoA entry into the TCA cycle. We will describe the cycle itself, its regulation, and the shuttling of electrons through the electron transport chain (ETC) to generate ATP.

SLO1: 1. Explain the significance of the TCA cycle and its biological roles, including its role in catabolic and anabolic pathways.

Definitions:

TCA Cycle: tricarboxylic acid cycle. It is also called interchangeably the "citrate cycle" or the "Krebs cycle." It gets these various names because the pathway is cyclic, was described by Sir Hans Krebs, and a key intermediate compound is citric acid, which is a 6-carbon tricarboxylic acid.

Oxidation and reduction. Oxidation is the abstraction of electrons from a molecule, which we refer as the electron donor molecule; and reduction is the acceptance of electrons by a molecule previously in a more oxidized state, which we refer to as the electron acceptor molecule. Oxidation-reduction reactions are always coupled. An oxidizing agent abstracts electrons from an electron-rich reducing agent. Common examples: Oxygen oxidizes elemental iron Fe° by removing electrons to yield Fe2+ (rust) and it oxidizes glucose to CO2 and H2O. Dehydrogenase enzymes catalyze oxidations by removing electrons from their substrates often transferring them to NAD+ or NADP+ forming NADH or NADPH. NADPH is a reducing agent. We say that NADPH has reducing power since it can transfer electrons to a molecule that is oxidized and can accept electrons; in the process NADPH becomes oxidized back to NADP+. A similar situation arises for NAD+ forming the reducing agent NADH. However, remember that NAD+/NADH and

NADP+/NADPH are used for different purposes in metabolic pathways.

What does it do?

The TCA cycle intersects with the electron transport chain (ETC) to make many molecules of ATP from the oxidation of carbon substrates derived from glucose or fat. These processes are aerobic meaning that oxygen is consumed. After glycolysis has generated pyruvate (3-carbons), pyruvate is oxidatively decarboxylated by Pyruvate Dehydrogenase (PDH) to form acetyl-coA which then enters the TCA cycle. Through a sequence of steps, The TCA cycle fully oxidizes acetyl-CoA to CO2 while generating energy in the form of reduced NADH and electrons. The electrons of NADH are then transferred to the ETC through Complex I to generate ATP. In parallel, the electrons of cytosolic NADH are passed on via a series of steps involving the malate-aspartate shuttle to the mitochondrial matrix, generating NADH in the matrix, which is used for ATP production via the ETC.

Several of the molecules generated by the TCA cycle also can be tapped as building blocks for synthesis of other key metabolic intermediates including amino acids. The core enzymes of both the TCA cycle and of the ETC are located inside of mitochondria. Aerobic glucose catabolism is particularly important for Type I skeletal fibers, the heart, and brain function, tissues that consume a lot of O2. Overall, ~ 36 ATPs are produced, and 2 ATPs are consumed per molecule of glucose catabolized under aerobic conditions–including those few from glycolysis. This process involves communication between glycolysis in the cytosol and the TCA cycle, electron transport, and oxidative phosphorylation inside the mitochondrial matrix.

SLO 2. Explain how the irreversible reaction catalyzed by the pyruvate dehydrogenase complex leads to the entry of acetyl-CoA into the TCA cycle and why acetyl-CoA cannot be used as a substrate for gluconeogenesis.

Pyruvate entry into the TCA cycle:

The pyruvate produced through glycolysis is transported into the

mitochondria, where it is oxidized to acetyl CoA, by action of pyruvate dehydrogenase, and then enters the TCA cycle. The PDH reaction is critical, as it is irreversible and ensures that acetyl-coA cannot be used in the reverse reaction for gluconeogenesis. While acetyl-CoA is not a substrate for gluconeogenesis, its oxidation through the TCA cycle and electron flow through the ETC produces the energy (ATP) necessary for gluconeogenesis.

Note that PDH is an enzyme complex, is comprised of three enzyme that catalyze different steps of the reaction. The PDH complex requires 5 cofactors for proper function which are: Thiamine pyrophosphate (TPP); Lipoic Acid; Coenzyme-A; NAD^+/NADH; and FAD/$FADH_2$.

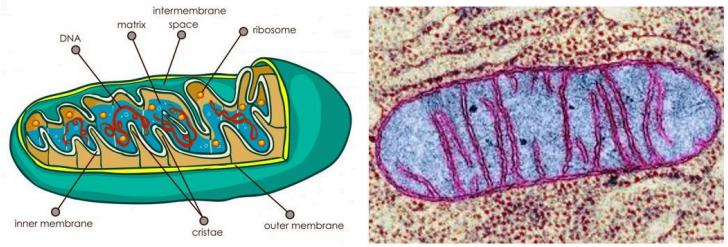
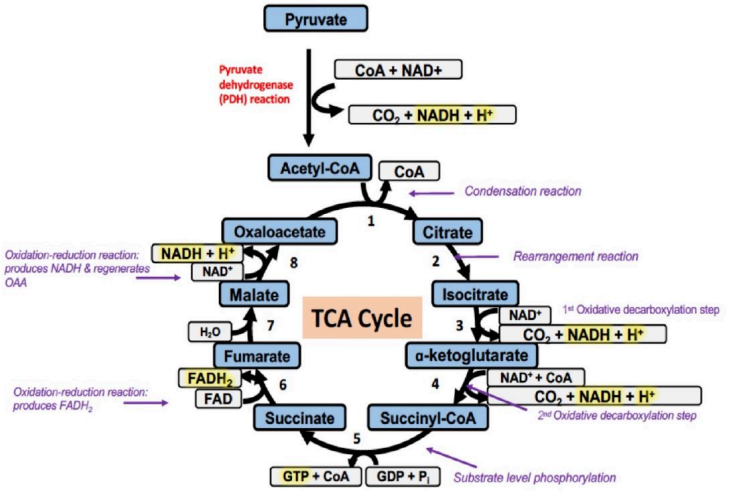


Figure 1. The Mitochondrium. Left, Cartoon of mitochondria with key features and histology. Mitochondria are spatially divided into an intermembrane space and inner matrix by the inner membrane. The matrix contains mitochondrial genomes and ribosomes. Right, pseudocolored electron micrograph of a mitochondrium.

Mitochondria: Mitochondria are cytoplasmic organelles richly endowed with many biochemical pathways. Mitochondria is a plural word. The singular is mitochondrion. The mitochondrial matrix contains the enzymes of the TCA cycle, fatty acid oxidation and the urea cycle, as well as the machinery for replicating and expressing mitochondrial DNA. The outer membrane contains pores which allow free diffusion of small molecules. In contrast, the inner membrane forms a tight barrier and contains specific transporters for small molecules such as pyruvate and malate. The inner mitochondrial membrane contains the electron transport system as well as the proteins involved in oxidative phosphorylation. In

sum, mitochondria are discrete structures dedicated to energy production (ATP), but they are also the sites of other metabolic processes including beta-oxidation of fatty acids, initiation of the urea cycle (part of nitrogen metabolism), and part of the heme biosynthetic pathways.

Figure 2. The steps of the TCA cycle:



The key steps of the TCA cycle are catalyzed by the following enzymes:

Reaction 1: citrate synthase (regulated step)

Reaction 2: aconitase

Reaction 3: isocitrate dehydrogenase (regulated step & generate energy in the form of NADH)

Reaction4: α-ketoglutarate dehydrogenase (regulated step & generate energy in the form of NADH)

Reaction 5: succinyl-CoA synthase

Reaction 6: succinate dehydrogenase (generates energy in the form of FADH2)

Reaction 7: fumarase

Reaction 8: malate dehydrogenase (generates NADH when malate is oxidized to oxaloacetate)

SLO3. Outline the co-factor requirements for the pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase enzymes and the biological consequences of thiamine deficiency.

Definitions:

Oxidative decarboxylation: A complex reaction that cleaves carboxylic acid off a carbon substrate, releasing CO2, and deriving much energy in the form of reduced cofactors. The carbon substrate loses one carbon and is oxidized while the cofactor is reduced.

Pyruvate Dehydrogenase Complex (PDH): A key step prior to entry of acetyl-CoA into the TCA is the oxidative decarboxylation of pyruvate to acetyl-CoA, resulting in the formation of NADH, acetyl-CoA and the loss of one CO2 molecule. Thus the 3-carbon pyruvate molecule is split in a 2-carbon (acetyl) and 1-carbon (CO2) fragment. PDH is a multi-enzyme complex that requires important cofactors for its function. These are thiamine pyrophosphate (derived from thiamine/vitamin B1), lipoic acid, coenzyme-A, FAD/FADH2, and NAD^+/NADH. Like PDH, α-ketoglutarate dehydrogenase in the TCA cycle is a similar multi-enzyme complex requiring the same 5 cofactors for proper function.

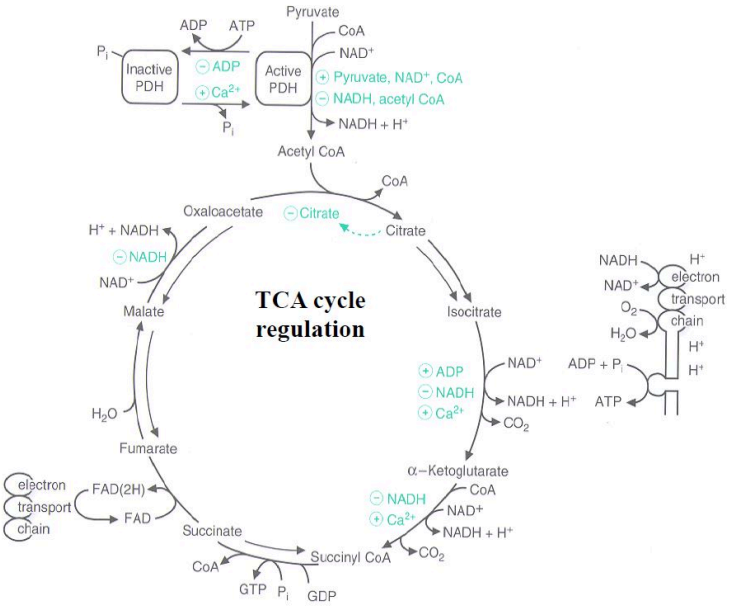
Thiamine Deficiency: Thiamine deficiency causes beriberi, which most dramatically affects the nervous system and the heart. This is because these two tissues are highly dependent on oxidative metabolism for energy production. Beriberi is a problem in those parts of the world where rice is a major food, since rice has a particularly low thiamine content. In the USA, beriberi is most common in individuals with poor nutrition, high ethanol intake (which leads to malabsorption of thiamine),or severe kidney disease. It is characterized by abnormalities in the peripheral nervous system, reflected in abnormal sensation and muscular weakness, and an enlarged heart.

Regulation of PDH. Control of PDH activity is important, because the reaction is irreversible and commits pyruvate either to complete oxidation through the TCA cycle or to the products derived from acetyl-CoA(for example fatty acids, steroids or ketone bodies;

discussed later in the course). PDH is regulated by phosphorylation and de-phosphorylation catalyzed by kinase and phosphatase enzymes, respectively. Formation of the inactive (phosphorylated) state is stimulated by high ATP levels and also by the products of the PDH reaction, acetyl-CoA and NADH. Hence, when energy charge is high and/or the TCA cycle is saturated with acetyl-CoA, the key reaction catalyzed by PDH is turned off.

SLO4. Explain how the TCA cycle is regulated, including which critical steps are regulated.

Figure 3. Molecules that regulate the TCA cycle (ADP, Ca^{2+}, acetyl CoA, pyruvate, NAD^+, CoA, NADH, citrate).



Regulation of the TCA cycle: The TCA cycle is regulated at two steps, catalyzed by isocitrate dehydrogenase (reaction 3) and α-ketoglutarate dehydrogenase (reaction 4). In general, these reactions are regulated by energy charge and by the ratio NAD+/NADH. Because of the tight regulation of PDH, the rate of the citrate synthase reaction can often be limited by the availability of acetyl-CoA. In addition to regulation by the NAD+/NADH ratio,

α-ketoglutarate dehydrogenase is also activated by Ca2+. Calcium activation of this enzyme (together with activation of the PDH reaction) helps drive ATP production at times of increased Ca2+ flux inside the cell, including muscle contraction and hormone secretion which are energetically expensive.

SLO5 Explain how the TCA cycle can be used for synthesis of intermediates involved in biosynthetic reactions, and how pyruvate carboxylase replenishes oxaloacetate for TCA cycle activity.

The TCA cycle also provides intermediates for biosynthetic processes: Besides being the major path-way for the oxidative generation of ATP, the TCA cycle also provides intermediates for biosynthetic reactions. For example, many amino acids can be derived from oxaloacetate and α-ketoglutarate if needed, and many of the carbon atoms of the porphyrin ring are derived from succinyl-CoA (Figure 3). The reverse is also true as these TCA cycle intermediates can be replenished by metabolism of amino acids.

Replenishing the TCA cycle of intermediates – The pyruvate carboxylase reaction replenishes the TCA cycle with OAA (Oxaloacetate)

The reaction catalyzed by pyruvate carboxylase. If intermediates are drained out of the TCA cycle for biosynthetic purposes, how is the oxaloacetate that is required to run the TCA cycle regenerated? This is accomplished by the enzyme pyruvate carboxylase that we will see is also key for gluconeogenesis.

Pyruvate + $C0_2$ + ATP -> Oxaloacetate + ADP +Pi

This enzyme requires biotin (vitamin B7), and the reaction is an energy dependent process needing ATP. The reaction, termed an anaplerotic (replenishing) reaction, is defined as forming an intermediate for a metabolic pathway. Here it replenishes oxaloacetate needed by the TCA cycle. When oxaloacetate is limiting for the first step of the TCA cycle, acetyl-CoA builds up. The elevated acetyl-CoA allosterically activates pyruvate carboxylase, which in turn provides the needed oxaloacetate for the TCA cycle.

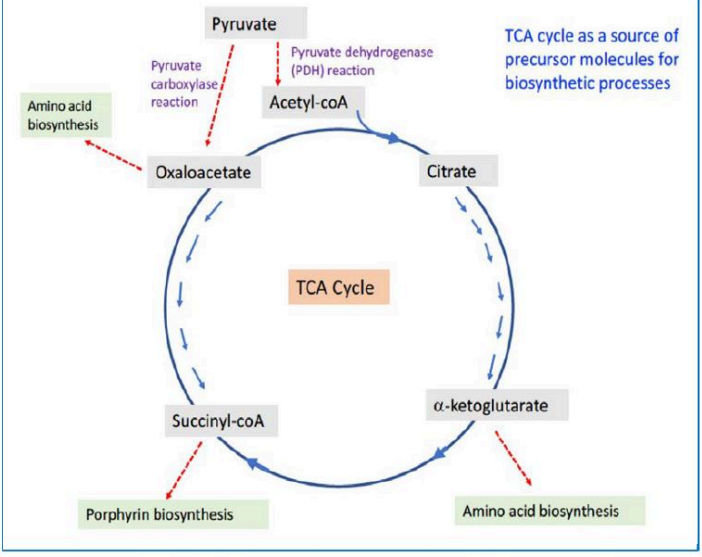


Figure 4. Many biosynthetic precursors are generated in the TCA cycle.

Practice questions to better understand TCA cycle:

1.What is the functional role of the TCA cycle under catabolic (degradation) conditions? **Answer 1.**

2.Which reactions are the most important and need to be regulated? **Answer 2.**

3.What would you expect in a patient who is thiamine (vitamin B1) deficient? (i.e., What symptoms would this patient have and what would happen biochemically?) **Answer 3.**

What would be the consequences of not being able to convert pyruvate to acetyl-CoA as a result of TCA cycle impairment?

4. How are intermediates of the TCA cycle replenished? **Answer 4.**

5. Why does the TCA cycle take so many steps just to decarboxylate acetate (starting with acetyl-CoA), as it would seem that converting the 2-carbon acetyl CoA into two 1-carbon CO2's could be done with fewer reaction steps. **Answer 5.**

TCA Knowledge Quiz 1: Three quizzes in one: Names of key TCA

molecules, products of the TCA cycle and carbon tracking. You do not need to fill every box to check your answers.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=342#h5p-12

TCA Knowledge Quiz 2

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=342#h5p-13

TCA Knowledge Quiz 3

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=342#h5p-14

3.

The Electron Transport Chain

This session continues our study of metabolism. After the initial catabolism of glucose by glycolysis, what further processing happens? When oxygen is present, how can catabolism capture more energy in the form of ATP? How does metabolism produce more metabolic intermediates essential for synthesizing other molecules (anabolism)?

Session Learning Objectives:

SLO 1. Outline the process by which ATP is produced as a result of electron flow through the protein complexes of the Electron Transport Chain (ETC).

SLO 2. Explain how uncoupler proteins and carbon monoxide (CO) and cyanide (CN) disrupt the ETC.

SLO 3. Explain the significance of the pentose phosphate pathway and the different roles served by the irreversible reactions of its oxidative phase and the reversible reactions of its non-oxidative phase

SLO 4. Outline how the oxidative and non-oxidate phases of the pentose phosphate pathway can be used to either make more NADPH or more ribose-5-phosphate, and what these products are needed for

SLO 5. Understand why red blood cells need the pentose phosphate pathway to detoxify reactive oxygen species (ROS), and explain how glucose 6-phosphate dehydrogenase works in concert with glutathione peroxidase and glutathione reductase to detoxify ROS, such as hydrogen peroxide (H2O2)

SLO 6. Determine the consequences of glucose-6-phosphate dehydrogenase deficiency and explain why it is prevalent in regions where malaria is endemic

AEROBIC GLUCOSE CATABOLISM – ELECTRON TRANSPORT CHAIN

SLO 1: Outline the process by which ATP is produced as a result of electron flow through the protein complexes of the Electron Transport Chain (ETC).

The electrons stored in NADH (and FADH2) produced as a result of the TCA cycle activity are transferred through a series of protein complexes of the ETC to oxygen **(Figure 1, below)**. The electron transfer steps proceed down an electrochemical potential energy gradient, resulting in the following net reaction:

$NADH + 1/2\ O_2 + H^+ \rightarrow NAD^+ + H_2O$

As the electrons are being transferred through the ETC, H+ are being translocated across the mitochondrial membrane by complexes I, III, and IV, resulting in the build-up of a H+ gradient across the inner mitochondrial membrane (IM), i.e. higher concentration of H+ in the intermembrane space, than on the matrix side of the IM. The change in membrane potential across the IM and proton concentration gradient provide the energy used togenerate ATP as the H+ are passed back through the ATP synthase complex (see Figure 1).

The electron carriers in the innermitochondrial membrane are oriented such that protons (H+) are pumped out of the mitochondrial matrix as electron transport takes place. The proton electrochemical gradient is used to drive the synthesis of ATP through the ATP synthase complex in the innermitochondrial membrane. Both the electrical potential difference and the proton concentration gradient that are set up provide the energy used to generate ATP as the protons are drawn back, thermodynamically downhill, into the mitochondrial matrix through the ATP synthase complex (Figure 1). Here the electrochemical gradient is harnessed to phosphorylate ATP and store energy for the cell to use. The ATP synthase couples this capture of energy. This unusual coupling of electro-chemical energy across a membrane to phosphorylate ATP is unique to mitochondria (and in plants to chloroplasts).

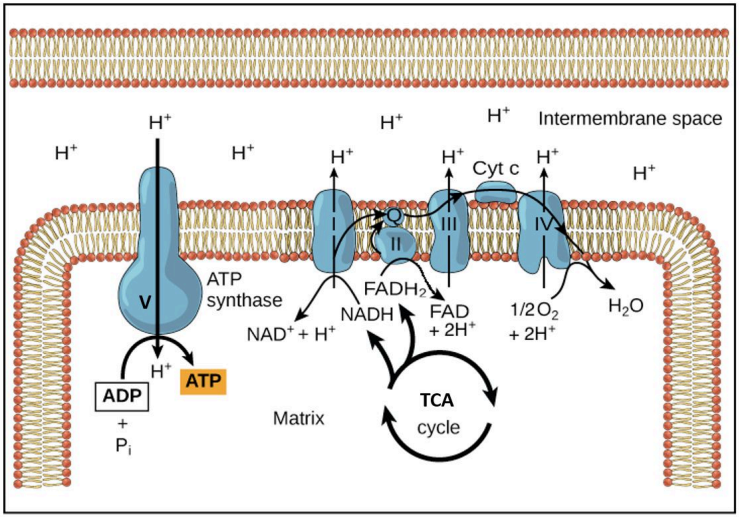


Figure 1. Disposition of ETC complexes in the inner mitochondrial membrane and flow of the reactants, electrons, and products. Complex V, the enzyme ATP synthase, couples the flow of H+ going down its electrochemical gradient to the phosphorylation of ATP. This is like a proton ATPase pump running backwards. Here the mitochondrial matrix is drawn as the top area.

The electrons are transported to oxygen along an organized series of complexes that are located within the inner mitochondrial membrane (Figures 1 and 2). The multiprotein complexes of the electron transport chain correspond to the functional names:

Complex I: NADH dehydrogenase complex
Complex II: Succinate dehydrogenase
Complex III: Cytochrome bc1 complex
Complex IV: Cytochrome c oxidase complex (contains cytochromes a and a3)

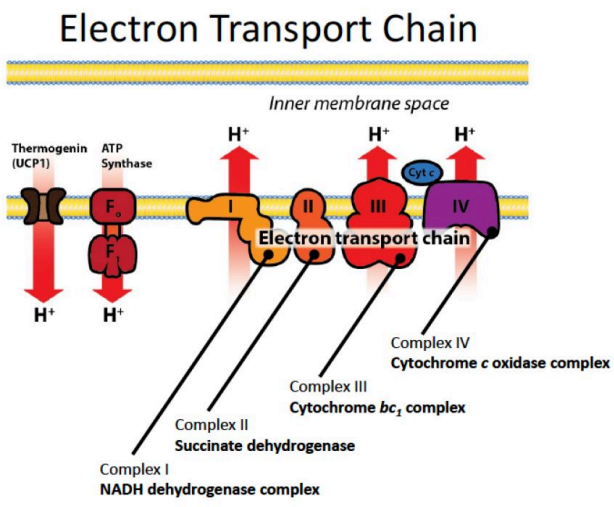
As seen above, the transport of electrons to oxygen is carried out by passage along an organized series of carriers that are located within the inner mitochondrial membrane. Coenzyme Q is lipid-

soluble and transfers electrons from Complex I and II to Complex III; it is not a vitamin. This electron carrier is synthesized from the amino acid tyrosine, which is obtained from our diet. Cytochrome c, a small water-soluble protein in the intermembrane space, transfers the electrons from Complex III to Complex IV. The cytochromes are all heme proteins. Cytochrome a + a3, also called cytochrome oxidase, carries out the final transfer of electrons to oxygen.

SLO2: Explain how uncoupler proteins and carbon monoxide (CO) and cyanide (CN) disrupt the electron transport chain.

Uncoupling electron flow and O2 consumption from ATP production: Under normal circumstances, electron transport, O2 consumption, and ATP synthesis are obligately coupled through the stored proton gradient and will all slow down if the ATP/ADP ratio becomes high. However, chemical uncouplers or uncoupler proteins, disconnect the two processes by dissipating the electrochemical gradient across the mitochondrial inner membrane (Figure 2). Substrate (e.g., glucose) consumption and oxygen consumption continue, and will actually increase, because the protons are not being pushed out against a gradient and just flow right back in without doing any "work" to make ATP. Instead, the energy is released as heat rather than as ATP. In the presence of uncouplers (small molecules or proteins), protons return to the mitochondrial matrix bypassing ATP synthase. This is an important process for the regulation of energy balance and heat generation. Hibernating mammals and newborn humans use uncoupler proteins to maintain body heat.

Figure 2. Sites of action of the small molecules cyanide and carbon monoxide in cytochrome c oxidase and of uncoupler drugs and endogenous uncoupler proteins on the inner mitochondrial membrane. Here the mitochondrial matrix is drawn as the lower compartment.



Molecules that interfere with the proper function of the ETC

Several toxic compounds bind to heme groups and inhibit cytochrome oxidase, including cyanide and carbon monoxide (Figure 3). Similarly, both poisons bind avidly to the heme of hemoglobin in competition to oxygen. Thus, they compromise respiration in several ways. Many other toxins and drugs target different complexes of the ETC. All compounds that inhibit electron transport will prevent the generation of a proton electrochemical gradient and therefore the synthesis of ATP in mitochondria. This will also result in a buildup of NADH in its reduced form, which inhibits the TCA cycle and leaves anaerobic glycolysis as the only source of ATP energy. Even though oxygen may be available, the consequences are similar to those of asphyxiation. Chemical uncouplers such as dinitrophenol increase the number of fatty acids thatmust be oxidized perATPmade, and once were used as diet pills.Natural uncoupling proteins (UCPs) were later discovered and

shown to be involved with regulation of energy balance and heat generation (including in some adults).

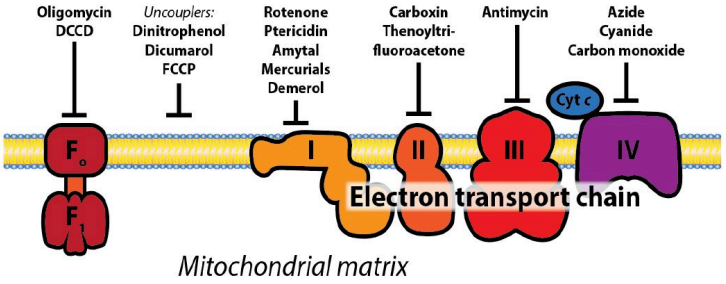


Figure 3: Schematic representation of ETC sites that acted upon by molecules that interfere with the function of the electron transport chain. Some of these are small molecules that specifically inhibit the different protein complexes of the ETC, while uncoupler proteins or molecules create holes in the membrane and destroy the H+ gradient resulting from electron flow through complex I, III, and IV of the ETC. You do not need to know the names of any of these molecules, apart from cyanide and CO.

Thought Experiment: How does the NADH generated during glycolysis enter the ETC since the mitochondrial membrane is impermeable to NAD+/NADH?. What would happen to cellular respiration if all the cytoplasmic NAD+ is trapped as NADH? Can you describe a scenario where this happens? **Thought Experiment Answer.**

MITOCHONDRIAL GENETICS

Mitochondria are thought to have arisen by the entry of a prokaryotic cell as an endosymbiont into an early eukaryotic cell. Mitochondria contain multiple copies of small circular DNA (mtDNA) genomes that are a remnant from this early symbiont; it encodes a few of the mitochondrial proteins involved in electron transport and oxidative phosphorylation, and some tRNA and rRNA

genes. The remaining necessary genes migrated in evolution to the cell nucleus. In humans mitochondrial inheritance is through the oocyte, although there is some evidence supporting extremely rare transmission of some mitochrondria through sperm, resulting in heteroplasmy, or mixed populations of mitochondria in an individual. Mutations accumulate in mtDNA perhaps because the DNA is not packaged by histones and because mtDNA is positioned within the mitochondrion alongside the electron transport system and its byproducts, namely oxygen free radicals.

Different tissues rely on oxidative phosphorylation for energy production to varying degrees. Therefore, as mitochondrial ATP production falls with increasing severity of mitochondrial defects, there is a progressive increase in the number and severity of clinical symptoms. Mitochondrial dysfunction has been linked to numerous diseases, including diabetes mellitus, heart disease, cancer, dementia, among others. The situation is further complicated by the fact that even healthy individuals show a decline of oxidative phosphorylation with age, perhaps due to accumulating damage in mtDNA.

Practice questions on electron transport:

1.What is the biological function of the electron transport chain? **Answer 1.**

2.What are the advantages of respiration compared to anaerobic glycolysis? **Answer 2.**

3.What are the potential trade-offs of using aerobic oxidation and oxidative phosphorylation to generate ATP? **Answer 3.**

4.What symptoms would you expect to see in a patient with hereditary mutations in genes coding for proteins of the electron transport chain, such as hereditary defects in cytochrome c oxidase? **Answer 4.**

5.Would you expect a patient with coenzyme Q deficiency to be able to survive? If yes, what treatment would they need? **Answer 5.**

6.What unpleasant side-effects would you expect to see in a patient taking an uncoupler as a diet aid? **Answer 6.**

7. Assuming a cell makes a mole of ATP before and after an

uncoupling molecule is added, would oxygen consumption be increased, decreased or the same? What about CO2 production? **Answer 7.**

8. Explain why complete oxidative metabolism of glucose is associated with only an approximate number of ATP generated. **Answer 8.**

ETC Quiz (1 True/False, 1 MC, 1 Drag and Drop)

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=372#h5p-18

The Pentose Phosphate Pathway

Definitions:

Pentose: a 5-carbon sugar such as ribose. Pentoses contrast with 6-carbon sugars like glucose and fructose, which are called hexoses.

Reactive oxygen species (ROS): Powerful oxidants that can damage biological molecules by oxidizing them. Examples are ozone (O_3), hydrogen peroxide (H_2O_2) and superoxide ($O^{2.-}$). The cell must detoxify ROS that are produced by metabolism involving oxygen.

SLO3. Explain the significance of the pentose phosphate pathway and the different roles served by the irreversible reactions of its oxidative phase and the reversible reactions of its non-oxidative phase.

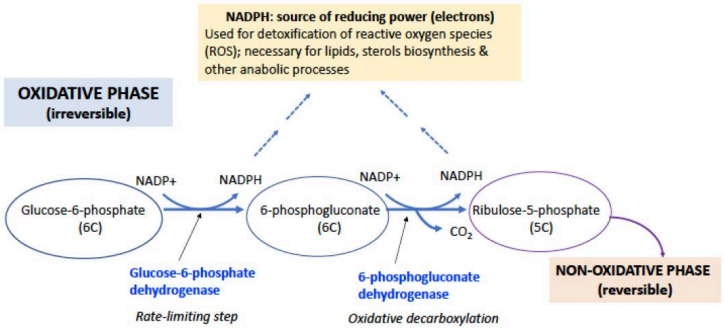
Pathway Overview:

The pentose phosphate pathway (PPP), also called the pentose cycle or the hexose monophosphate pathway (or shunt), operates

in parallel with glycolysis; although they both oxidize glucose, the two pathways have quite different biological roles. The product of the pentose phosphate pathway is not ATP, but reducing power in the form of NADPH which is used as an electron donor in many biosynthetic reactions and in reactions that protect cells from oxidative damage. The cycle also produces five-carbon sugars (ribose units) for biosynthesis of nucleic acids and cofactors such NAD+, FMN, and others (Figures 1 & 2).

PPP activity is minimal in muscle and the brain, where almost all of the glucose is used glycolysis for energy, ATP production. However, PPP activity accounts for a significant portion of the total glucose oxidation in tissues with active fatty acid and cholesterol synthesis, including liver. NADPH is also important for the antioxidant defenses of the body, so PPP is well developed in cells that are exposed to high oxygen levels such as the cornea of the eye, where PPP accounts for 60% of glucose oxidation, and in red blood cells.

Figure 1. OXIDATIVE PHASE OF THE PPP. The PPP has an oxidative phase (irreversible steps), which produces two NADPH, and a nonoxidative phase. PPP is regulated at the first enzyme, Glucose-6-Phosphate dehydrogenase (G6PD), activated when NADPH levels are low. NADPH is necessary for detoxification of reactive oxygen species (see below) to eliminate free radicals and for the biosynthesis of cholesterol, lipids and other products. The product of the oxidative phase, ribulose-5P, a 5-carbon sugar, is then directed to the non-oxidative phase of the PPP, which consists of a series of reversible carbon rearrangement reactions.

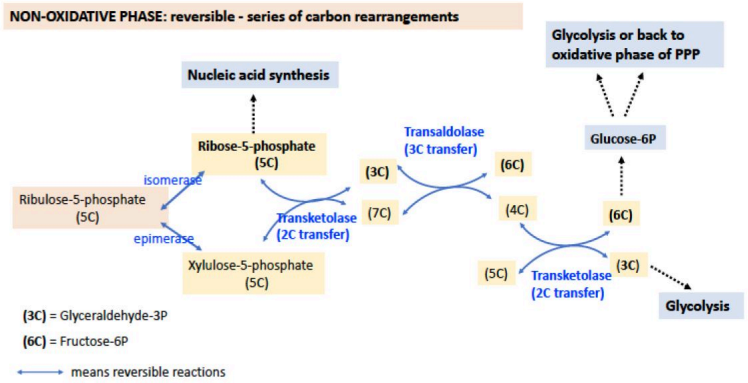


The oxidative phase of the pathway begins with glucose-6-phosphate, which is the product of the hexokinase reaction initiating glycolysis (Figure 1). Glucose-6-phosphate is oxidized to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase (G6PD) with the transfer of two electrons to NADP+ to form NADPH. The lactone is hydrolyzed to 6-phosphogluconate, which is then oxidatively decarboxylated to ribulose-5-phosphate, an isomer of ribose-5-phosphate. This step oxidizes one carbon to CO2 and generates a 2nd equivalent of NADPH, plus one equivalent of a pentose phosphate, which subsequently is available for the nonoxidative phase of the pathway (Figure 1).

The initial oxidation of glucose-6-P to 6-phosphogluconate, catalyzed by G6PD, is irreversible and is the rate-limiting step of the PPP. The ratio of NADPH to NADP+ is the main controlling factor of the reaction rate. When NADPH levels are low, G6PD is activated. The pathway is quite efficient: for example, it maintains the ratio NADPH/NADP+ at ~100 in the liver of a normal healthy individual. Under conditions where both NADPH and pentose phosphate are needed, the pathway may terminate after the oxidative phase.

Figure 2. NON-OXIDATIVE PHASE of the PPP. The non-oxidative phase reactions have double blue arrows, indicating that the reactions are reversible. Many of the sugars are denoted only by their number of carbon atoms rather than by their names here. Transketolases are key enzymes that catalyze the transfer 2 carbons, while the transaldolase catalyzes the transfer of 3 carbons.

Transketolases require thiamine pyrophosphate (TPP), vitamin B1, for proper function.



Note from this diagram how the nonoxidative phase makes several connections with glycolysis through formation of glyceraldehyde-3P, a 3C intermediate), and fructose-6-P, a 6C intermediate. It also generates other useful sugars 4C, 5C, and 7C.

NON-OXIDATIVE PHASE: Generates Ribose-5P and Series of Reversible Carbon rearrangements.

The functional role of the nonoxidative phase of the pathway is to regenerateG-6-P for the oxidative phase by shuffling carbon skeletons, interconverting 3-, 4-, 5-, 6- and 7-carbon sugars, through the action of the enzymes transaldolase (catalyzes the transfer of 3C) and transketolase (which catalyzes the transfer of 2C) (see Figure 2).

An important feature of these reactions is that they are all reversible, meaning that this phase of the pentose phosphate pathway can operate in either direction. Thus, even if there is no demand for NADPH (i.e, .NADP+ is low) and the oxidative phase of the pathway is not active, ribose phosphate can still be generated fromglucose-6-phosphate. Glycolysis and the pentose phosphate pathway share several intermediates, including G-6-P, F-6-P, and glyceraldehyde-3-phosphate. If NADPH is needed, but ribose is not (as in red blood cells), the carbon atoms are used for glycolysis.

Transketolase is one of only a few enzymes in humans that require thiamine pyrophosphate for activity. In normal individuals, a severe

deficiency of thiamine (VitaminB1)will produce beriberi, a disease of diffuse symptoms associated with decreased activity of the 3 thiamine-dependent enzymes (pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase).

SLO 4. Outline how oxidative glucose metabolism and the pentose phosphate pathway are regulated to balance energy needs and NADPH production

MODES OF OPERATION OF THE PATHWAY

The pentose phosphate pathway can operate in several modes, depending on the needs of the cell. When the requirement for NADPH outweighs the need for ribose, for example in adipose tissue where there is a large demand for NADPH for fatty acid biosynthesis, the pathway operates as a cycle, generating NADPH and CO2 and returning 5/6 of the carbon to the glycolysis pathway via fructose-6-phosphate and glyceraldehyde-3-phosphate. As already noted, there are two other modes. If needs for NADPH and ribose are balanced, then only the oxidative phase can operate. If no NADPH is required, ribose-5-phosphate can be made through the nonoxidative phase of the pathway from the conversion of glyceraldehyde 3P and fructose-6P.

SLO 5. Relate consequences of glucose 6-phosphate dehydrogenase deficiency to oxidative stress, red blood cell vulnerability, and possibly malaria resistance.

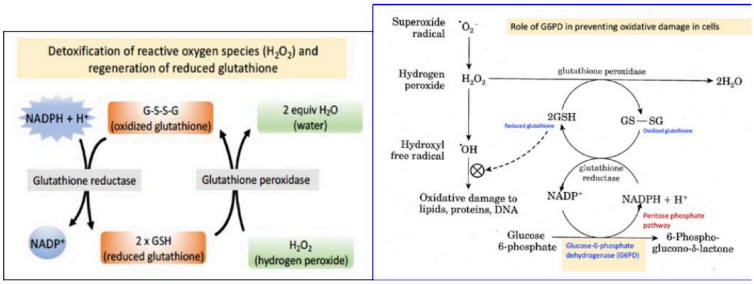
SLO 6. Describe the role of NADPH in detoxifying reactive oxygen species, and explain which cell types depend the most on the pentose phosphate pathway.

Glucose-6-P-dehydrogenase (G6PD) Deficiency.

The first enzyme of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD) plays a role in avoiding oxidative stress. Inherited deficiency of G6PD is an important example of the interplay between genetics and environment. During World War II, American troops were treated prophylactically with antimalarial agents, such as paraquine or primaquine. A significant fraction of those treated suffered severe hemolytic anemia. This was later found to be an X-linked trait associated with mutations

in G6PD gene. These mutations result in a substantial deficiency of the enzyme in red blood cells. Such mutations are surprisingly frequent in the human population, affecting as many as 30% of males in some Mediterranean areas and 11% of male Americans of African descent. The mutations can also result in favism, a condition in which hemolytic crisis is brought on by ingestion of fava beans, the major ingredient of falafel. The antimalarial drugs and divicine, the active ingredient of fava beans, share the common property of exposing the cells of the body to oxidative stress, from the elevated production of reactive oxygen intermediates.

The pentose phosphate pathway is the only source of NADPH in red blood cells, and the major function of NADPH in this cell type is to maintain the tripeptide glutathione (GSH) in its reduced state, which is needed to detoxify reactive oxygen species **(Figure 3)**.



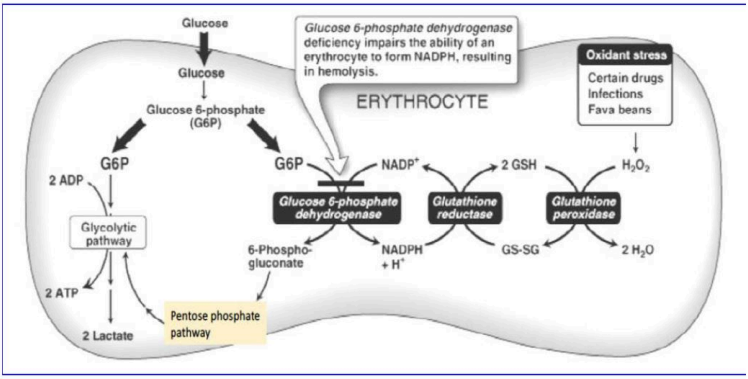
Question: Why are red blood cells especially sensitive to mutations that create lowered levels of G6PD?

Answer: Red blood cells must live for 100-120 days without a nucleus, and therefore cannot regenerate proteins that are degraded over time. Genetic mutations can result in G6PD amino acid changes that yield a protein slightly less stable than the wild type protein. In tissues with nucleated cells, the less stable G6PD can be replenished through synthesis of newG6PDprotein.Replenishment is not an option in red blood cells, so the protein is depleted with time, generating cells that are hypersensitive to oxidative damage.

Role of reduced glutathione (GSH) in detoxification of reactive oxygen species:

GSH uses the sulfhydryl (–SH) functional group of its cysteine residue to protect cells against oxidative damage in at least two ways. First, GSH can react directly with free radicals to destroy them and thus prevent the cellular damage caused by these free radicals. Second, the enzyme glutathione peroxidase uses two electrons from glutathione to reduce hydrogen peroxide to water (Figures 3 and 4). Both of these reactions of glutathione convert GSH to the oxidized, disulfide-bonded dimer, GS-SG. Therefore, in order for glutathione to continue to be effective in combating oxidative stress, GS-SG must be reduced back to GSH. The enzyme glutathione reductase, which is quite active in red blood cells, carries out this reduction, using NADPH as a reducing agent. Therefore, defects in the ability of red blood cells to reduce NADP+ to NADPH compromise this protection system and lead to oxidative damage, ultimately causing rupture of the red blood cell membrane and hemolysis. Approximately 400 million people world-wide are affected by G6PD deficiency. Why should G6PD deficiency be so frequent in the human population? The malaria parasite, Plasmodium falciparum, reproduces in the red blood cells of infected individuals. The parasite requires glutathione for growth. Therefore, G6PD deficiency, like sickle-cell disease, confers resistance to malaria. The geographic distribution of this condition is consistent with positive selection for G6PD deficiency in areas where malaria is common.

Figure 4. Redox pathways in the red blood cell that protect against reactive oxygen species. The key position of G6PD is marked with an arrow.



The relationship between glucose-6-phosphate metabolism and the role of glutathione in red blood cells is summarized in Figure 4. Remember that red blood cells have no mitochondria, so their only source of energy is anaerobic glycolysis.

NADPH beyond glutathione

NADPH is about much more than just reducing glutathione to protect against oxidative damage. In white blood cells NADPH is needed to create superoxide radicals to cause oxidative damage to phagocytosed microbes. All cells rely on NADPH reducing power to produce cholesterol, fatty acids and phospholipids (crucial for the plasma membrane). The liver needs NADPH to neutralize toxins (and hormones) using the cytochrome P450 pathways. Glands used NADPH to make steroid hormones.

Practice questions on the pentose phosphate pathway and reactive oxygen species:

1.What are the two main functions of the pentose phosphate pathway? Answer 1.

2.Why is the PPP so critical to the function of red blood cells? Answer 2.

3.Which tissues have high PPP activity? And what is PPP used for in these cases? Answer 3.

4.What is glutathione and what is it used for? Answer 4.

5.Why is glucose-6-phosphate dehydrogenase deficiency so prevalent in the world? Answer 5.

4. Gluconeogenesis, Glycogenesis, Glycogenolysis

Session Learning Objectives:

SLO1. Differentiate gluconeogenesis from glycolysis, outline 3 bypass reactions that make it energetically favorable, and explain the significance of acetyl-CoA not being a substrate.

SLO2. Diagram the mechanisms by which glucose synthesis and glucose breakdown are reciprocally regulated.

SLO3. Understand how gluconeogenesis in liver helps maintain anaerobic glycolysis in active skeletal muscle through the Cori cycle.

SLO4. Diagram glycogen as a branched polymer. Contrast the use of glycogen in liver and muscle. Understand the pathways by which glycogen is synthesized and broken down.

SLO5. Diagram the mechanisms by which glycogen synthesis and glycogen breakdown are reciprocally regulated.

SLO6. Outline genetic disorders of glucose mobilization (Von Gierke, Pompe, Cori, Andersen, McArdle) including clinical manifestations, lab values and biochemistry.

We start by reminding ourselves of glucose metabolism drawn as a block diagram (Figure 1). We have learned the green glycolysis pathway from glucose to pyruvate making a little ATP, the blue pentose phosphate pathway generating reducing equivalents and various sugars, the orange TCA cycle generating many reducing equivalents and several metabolic building blocks, and finally the grey electron transport chain generating ATP. In this session we will study the purple gluconeogenesis pathway for generating glucose and the yellow glycogenesis pathway for generating glycogen.

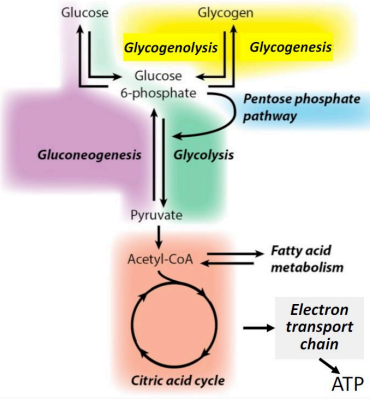


Figure 1. Block diagram of glucose metabolism. Each colored block represents a sequence of enzymatic reactions grouped together to simplify metabolism as major functional units.

SLO1. Differentiate gluconeogenesis from glycolysis, outline 3 bypass reactions that make it energetically favorable, and explain the significance of acetyl-CoA not being a substrate.

Gluconeogenesis is the process of synthesizing glucose de novo from 3- and 4-carbon precursors such as pyruvate, alanine, or glycerol. It is an anabolic pathway. In some ways, gluconeogenesis is very similar to the reverse process of glycolysis (which is the breakdown or catabolism of glucose). However, gluconeogenesis cannot be exactly the reverse of glycolysis for two reasons: (1) it needs to be reciprocally regulated such that when glycolysis is stimulated, gluconeogenesis is turned down and vice versa; and (2) it needs to be arranged as an energetically favorable process. We have learned that glycolysis is an energetically favorable process from a thermodynamic point of view (DG < 0). Now if gluconeogenesis were exactly the reverse of glycolysis, then gluconeogenesis would be highly unfavorable (DG > 0). What we will see is that gluconeogenesis uses 7 of the 10 reactions of glycolysis but has 3 reaction steps that are specific to gluconeogenesis and that make it energetically favorable. We will call these steps "bypasses."

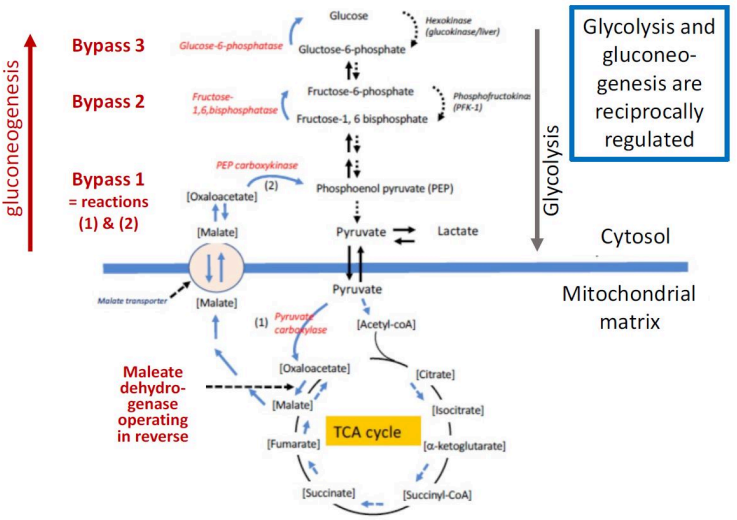


Figure 2. The relationship of liver anabolic gluconeogenesis (left half, red reactions) to the pathway of catabolic glycolysis in the cytoplasm (right half, gray arrow) and the TCA cycle in mitochondria. Bypass reactions listed in red give gluconeogenesis its anabolic direction.

The pathway of gluconeogenesis, from lactate to glucose is shown in Figure 2. Many of the reversible reactions of glycolysis are shared in common between the two pathways. However, three essentially irreversible steps of glycolysis, those catalyzed by pyruvate kinase, phosphofructokinase, and hexokinase are bypassed using different enzymes in gluconeogenesis. The liver, and to a lesser extent the kidney cortex, are capable of converting lactate and a variety of other 3-and 4-carbon molecules to glucose and further to glycogen, as will be discussed next. Gluconeogenesis is important not only in regenerating glucose from the lactate produced by exercising muscle (the "Cori cycle" see below) but also is critical in the maintenance of blood glucose levels (discussed later in the course). Note that acetyl-CoA, the product of fatty acid oxidation is NOT

a substrate for gluconeogenesis. Fatty acid oxidation can be used to generate energy (ATP) for the energy-demanding process of gluconeogenesis, but its metabolic product, acetyl-CoA cannot be used as the carbon precursor. Glycerol on the other hand, which is produced from the breakdown of triglycerides into glycerol + fatty acids can be used as a substrate for gluconeogenesis (it enters the gluconeogenesis pathway at the reversible dihydroxyacetone (DHAP)-glyceraldehyde-3-phosphate step).

A diversion on high-energy bonds and favorable reactions

This note is to help you make mechanistic sense of many reactions and pathways that you have already encountered and will encounter in your study of biochemistry. A fundamental principle in the organization of a biochemical pathway is that to be used by the cell, it has to be "spontaneous" or energetically favorable. As a reminder, in the language of thermodynamics, this concept is expressed by the statement that the ΔG should be negative. Biochemical pathways get some of their complexity just to satisfy the need to gather the energy to make them go forward. They do this by coupling a reaction that is quite favorable to a needed reaction that is not. An example is the phosphorylation of glucose by hexokinase. Addition of a phosphate to a hydroxyl functional group is energetically unfavorable. Indeed, formation of any ester or ether linkage or a peptide bond is energetically unfavorable. Hexokinase makes the glucose phosphorylation reaction go forward by coupling it to cleavage of ATP. We say that ATP has a high-energy bond since cleavage of ATP by water (called hydrolysis) would release a lot of energy and is highly favored thermodynamically. Examples of other energetically highly favored hydrolytic cleavages would be cleavage of Acetyl-CoA and cleavage of UDP-glucose, a molecule we will hear about shortly. They too have a high-energy bond which instead of being wasted, can be coupled to enzymatic reactions to push the reactions forward. This energy allows Acetyl-CoA to transfer acetyl- (2-carbons) to oxaloacetate in the TCA cycle and it allows UDPglucose to transfer glucose making linkages with other glucose moieties. It is useful to think about ATP, Acetyl-CoA, and

UDP glucose as representing "activated phosphate," "activated acetyl," and "activated glucose," respectively in the sense that they can transfer phosphate, acetyl, or glucose to other substrates. Much of metabolism involves using energy to make these high energy bonds and then using them. Now we
can discuss the bypass reactions of gluconeogenesis in these terms.

The bypass reactions of gluconeogenesis

Bypass step 1 of gluconeogenesis is actually a series of steps that bypass the pyruvate kinase step of glycolysis:

Half of these reactions take place in the mitochondria, and the 2nd half takes place in the cytoplasm. The pyruvate kinase step is bypassed in a complicated series of reactions that involve moving molecules into and out of the mitochondrion. Lactate is first converted to pyruvate and after transport into the mitochondrion pyruvate is converted to oxaloacetate by the reaction catalyzed by pyruvate carboxylase as follows:

Pyruvate + CO_2 + ATP -> Oxaloacetate + ADP +Pi

This enzyme requires the vitamin biotin. Since there is no transporter for oxaloacetate, it is converted to malate, which is then transported out to the cytoplasm. In the cytoplasm, the malate is reconverted to oxaloacetate, which is then transformed to phosphoenolpyruvate (PEP) through the PEP carboxykinase reaction as follows:

Oxaloacetate + GTP -> PEP + GDP + CO_2

These two reactions are driven by consumption of the high energy bond of ATP or GTP, respectively.

Bypass reaction 2 bypasses the phosphofructokinase (PFK) reaction of glycolysis.

In gluconeogenesis, fructose-1,6- bisphosphate is hydrolyzed to fructose-6-phosphate by the enzyme fructose-1,6-bisphosphatase (FBPase) as follows:

Fructose-1-6-bisphophate + H_2O -> Fructose-6-phosphate + Pi

In glycolysis, the PFK reaction is driven in the downward catabolic direction in Figure 2 by consumption of ATP, whereas in gluconeogenesis FBPase is driven in the upward anabolic direction

by cleavage of the 1- phosphate. Once fructose-6-phosphate is formed, it is easily converted to glucose-6-phosphate using the reversible glucose-6-P isomerase reaction.

Bypass reaction 3 bypasses the hexokinase/glucokinase reaction of glycolysis.

In the liver, once glucose-6P is formed, it can be converted to glucose by the enzyme glucose-6-phosphatase. This enzyme plays a critical role in the function of the liver in regulating blood glucose levels (discussed in more detail later on). As with bypass 2, in glycolysis the catabolic conversion is driven downward in Figure 2 by consumption of ATP, and in gluconeogenesis, the anabolic bypass 3 is driven upward by cleavage of a phosphate.

Session Learning Objective 2. Diagram the mechanisms by which glucose synthesis and glucose breakdown are reciprocally regulated. Key questions to keep in mind are "when is gluconeogenesis important? Which tissues are most affected by this process?"

Regulation of gluconeogenesis. Gluconeogenesis and glycolysis share many of the same reactions and regulation is clearly required to prevent futile cycles where one pathway is continually undoing the results of the other. This regulation is achieved in two ways. First, gluconeogenesis and glycolysis are reciprocally regulated by the hormones glucagon and insulin as part of the mechanism for controlling blood glucose levels. Second, regulation of gluconeogenesis depends on the energy charge of the cell in a manner that is exactly opposite from regulation of glycolysis, so that when glycolysis is on, gluconeogenesis is off, and vice versa. The interconversion of fructose-6-phosphate and fructose-1,6-bisphosphate is highly regulated. As discussed in the chapter on glycolysis, PFK-1 is stimulated by AMP and inhibited by ATP, therefore, this step in glycolysis is activated by low energy charge and inhibited by high energy charge. In contrast, the reverse reaction, catalyzed by FBPase, is inhibited by AMP. Thus, at low energy charge, when PFK-1 is turned on, FBPase is turned off.

Conversely, when PFK-1 is turned off by high energy charge, AMP is low which allows FBPase to be active.

The interconversion of phosphoenolpyruvate and pyruvate is also precisely controlled. Recall that the pyruvate kinase reaction of glycolysis is also regulated by energy charge. Pyruvate carboxylase, which catalyzes the first step in the conversion of pyruvate to glucose, is activated by acetyl CoA. Elevated acetyl CoA signals the need for more oxaloacetate. When energy charge is high, the TCA cycle is turned off and oxaloacetate flows
in the direction of gluconeogenesis. When energy charge is low, oxaloacetate enters into the TCA cycle by condensing with acetyl CoA, to form citrate.

In the liver, several hormones interact to regulate the transcription of the PEP carboxykinase gene. Expression of this gene is stimulated by glucocorticoids and glucagon, both of which stimulate gluconeogenesis. Insulin has the converse effect on transcription of the PEP carboxykinase gene.

Session Learning Objective 3. Understand how gluconeogenesis in liver helps maintain anaerobic glycolysis in active skeletal muscle through the Cori cycle.

The Cori Cycle: The Cori cycle refers to a process in which lactate derived from glucose in skeletal muscle is delivered to the liver, converted back to glucose, and returned to muscle via the blood. Much of the lactate produced in a 24 hr period is recycled in this manner. The Cori cycle
(Figure 3) illustrates well the way two organs can work together to achieve a biochemical objective. Lactate is produced from glucose (derived from glycogen) by glycolysis in anaerobic muscle and is transported to the liver via the blood stream. In the liver, lactate is converted back again to glucose through gluconeogenesis. The energy for synthesis of glucose in the liver comes from the oxidation of fatty acids.

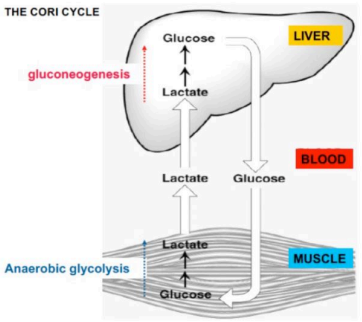


Figure 3. In the Cori cycle glucose is converted to lactate in active muscle, delivered to the liver by the blood stream, converted back to glucose, and then returned to the muscle though the blood stream.

Some medical consequences: The rate of gluconeogenesis is strongly controlled by circulating levels of glucagon, insulin and cortisol. Conditions characterized by imbalances in these hormones can either cause hypoglycemia (insulinomas, ethanol ingestion), or an accelerated gluconeogenesis and accompanying hyperglycemia (diabetes, Cushing syndrome). Van Gierke's disease results from a deficiency in glucose 6-phosphatase, an enzyme of gluconeogenesis. Chronic hyperglycemia leads to degrees of diabetic retinopathy, nephropathy, neuropathy, and sugar cataracts in type 2 diabetes.

Practice questions on gluconeogenesis:

1. What are the key biochemical features of the regulated steps of gluconeogenesis?
2. What is the primary function of gluconeogenesis in the liver?
3. How is reciprocal regulation of glycolysis and gluconeogenesis ensured?
4. What would you expect in a patient who has a deficiency in glucose-6-phosphatase?
5. Why does gluconeogenesis play such a critical role in maintaining blood glucose level homeostasis?

GLYCOGEN METABOLISM

Session Learning Objective 4. Diagram glycogen as a branched polymer. Contrast the use of glycogen in liver and muscle. Understand the pathways by which glycogen is synthesized and broken down.

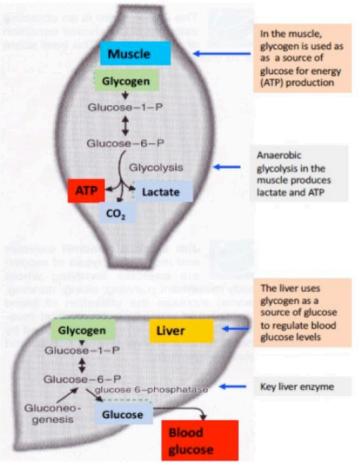
Glycogen metabolism overview:

Glycogen is the main storage form of glucose, and is key to

mobilizing glucose stores in skeletal muscles during vigorous exercise. Glucose molecules are osmotically active particles, thus they cannot be stored in high concentration as monomers. Linking glucose molecules as an enormous polymer such as glycogen circumvents the osmotic pressure issue. The main stores of glycogen are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of ATP during muscle contraction (Figure 4). That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.

Glycogenesis, the process of glycogen anabolism:

Figure 4. Glycogen stores serve different roles in muscle and liver.



–>

Structure of glycogen: Glycogen is a branched-chain polysaccharide made from α-D-glucose. The glucose monomers link to form a primary linear polymer, and after an average of eight to ten glucosyl residues, there is a branch formed by a different glycosidic linkage. Figure 5 diagrams the resulting branched polymer that is connected to a protein at the center. Glucose is added and removed from the many "nonreducing" ends, so there are hundreds of ends available for the reactions of synthesis and breakdown.

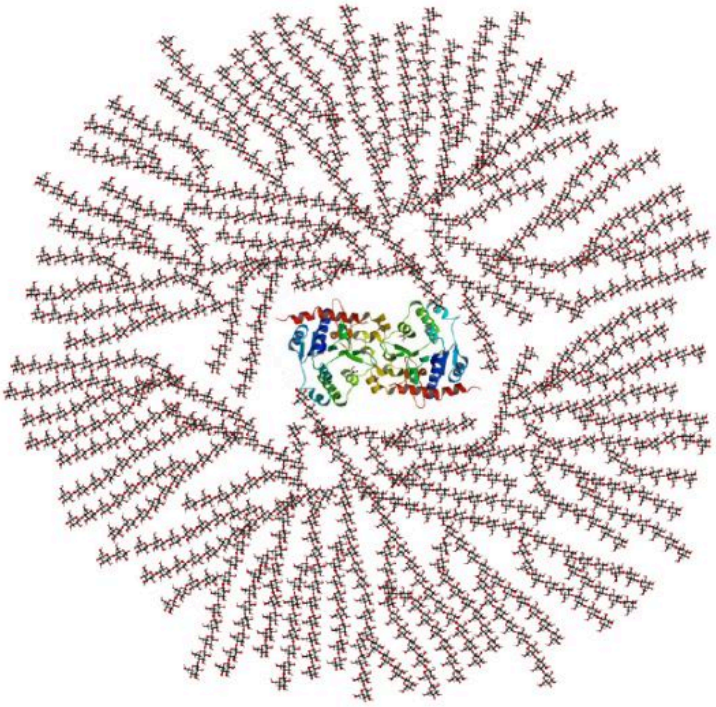


Figure 5. ^ Glycogen is made from α-D glucose. The primary glycosidic bond is an α(1→4) linkage. After an average of eight to ten glucosyl residues, there is a branch containing an α(1→6) linkage. The concept of a branched polymer is important for you to visualize; the chemistry is not.

Glycogenolysis, the process of glycogen catabolism:

During vigorous exercise, the delivery of both oxygen and glucose to skeletal muscle through the blood stream cannot keep up with the demand from the aerobic metabolism of glucose or of fatty acids. Therefore, under these anaerobic conditions muscle becomes dependent on the stored glucose in glycogen, to generate ATP. The breakdown of glycogen is complicated in that the glycosidic linkages of both the linear parts and the branches need to be broken in

order to achieve complete release of glucose residues. Therefore, this process requires two enzymes, glycogen phosphorylase and debranching enzyme.

The enzyme phosphoglucomutase then converts glucose-1-phosphate to glucose-6-phosphate for entry into glycolysis, if needed. Note that one less ATP per glucose molecule is required to enter glycolysis from glycogen, compared to starting with free glucose. Anaerobic glycolysis beginning with glycogen therefore yields a net production of 3 ATP per glucose.

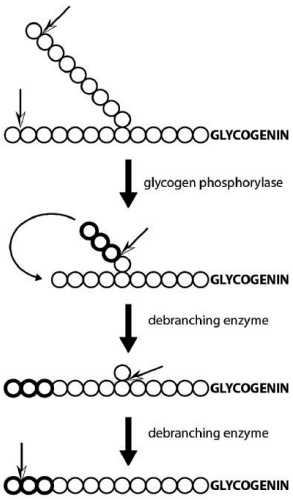


Figure 6. The combined activities of glycogen phosphorylase and the debranching enzyme break down a glycogen molecule. When glycogen phosphorylase get close to a branch point, a debranching enzyme resolves the branch structure and glycogen phosphorylase continues.

Regulation of glycogen phosphorylase in striated muscle. Glycogen phosphorylase is the key regulated enzyme in glycogen breakdown in muscle. Its activity is responsive to: 1) the energy charge of a muscle cell, 2) the contractile state of the muscle and 3) the circulating levels of the hormone epinephrine.

Glycogen phosphorylase can be activated by phosphorylation. In muscle, the phosphorylation state of this enzyme is regulated by at least two signals (Figure 7). Muscle contraction is triggered by an elevation of intracellular Ca2+. This elevated calcium also stimulates phosphorylation of

glycogen phosphorylase through calcium binding proteins that interact with phosphorylase kinase and partially activate it.

Glucose-1-phosphate, is then released from glycogen reserves by the activated phosphorylase and provides energy for contraction. In addition, the phosphorylation of glycogen phosphorylase is stimulated by the "fight or flight" hormone epinephrine in a signaling cascade. Epinephrine from the blood stream binds to beta adrenergic receptors and activates adenylate cyclase, which produces cyclic AMP (cAMP). cAMP, in turn, activates protein kinase A (PKA). PKA then phosphorylates phosphorylase kinase, converting it from the inactive to the active form. The active phosphorylase kinase subsequently phosphorylates inactive phosphorylase, converting it into the active form and thereby stimulating glycogen breakdown. *You might wonder why cAMP doesn't activate phosphorylase directly. The extra steps used allow amplification of the hormonal signal and provide additional regulation points. We will discuss the hormonal regulation of glycogen phosphorylase in the liver in more detail later, in the context of its reciprocal regulation with glycogen synthase.*

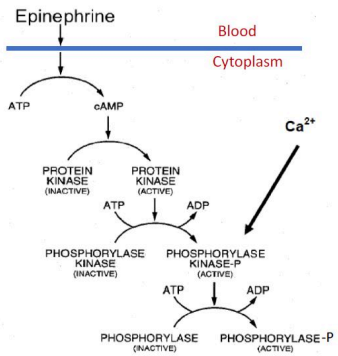


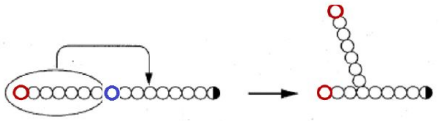
Figure 7. Regulation of muscle glycogen phosphorylase by protein phosphorylation and dephosphorylation.

Glycogen Synthesis:

In resting muscle after exercise, glycogen is resynthesized using glucose delivered to the muscle via the bloodstream. The key enzyme is glycogen synthase, which extends glycogen chains by one glucose molecule at a time. The glycogen synthase enzyme uses an activated form of glucose called uridine-diphosphate (UDP)- glucose.

The high energy UDP-glucose is formed enzymatically by reaction of glucose-1-phosphate with UTP. In addition, synthesis requires a mechanism to make glycogen branchpoints. Introduction of α-1,6 linkages is catalyzed by the enzyme called "branching

enzyme." This reaction creates two ends, both of which can be extended by glycogen synthase (Figure 8 V).



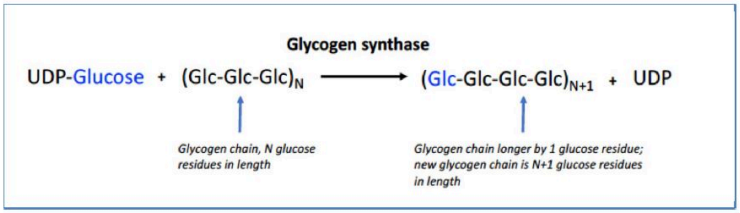


Figure 8. Branching enzyme introduces a branch in an existing linear nonreducing end of glycogen (terminating in the left red glucose residue) by cutting at the blue residue and pasting the cut segment further back in 1-6 glycosidic linkage. This process generates two nonreducing ends (red residues).

Key take home points: Glycogen is the body's way of storing its reserve of glucose as a giant polymer. Glycogen breakdown requires alternating processing by the enzymes glycogen phosphorylase and debranching enzyme. Glycogen synthesis requires alternating processing by the enzymes glycogen synthase and branching enzyme.

Session Learning Objective 5. Diagram the mechanisms by which glycogen synthesis and glycogen breakdown are reciprocally regulated.

Glycogen metabolism – interplay between catabolism (degradation) and synthesis: We have just described glycogen synthesis from glucose-1P and glycogen breakdown to glucose-1P. These steps are encapsulated in reactions 4 &5 and reaction 6, respectively, of Figure 9. Reactions 1, 2 ,

and 3 remind us of the relationship of glucose-1P to glucose and to glycolysis in the liver. Reactions 1, 3, 4 and 5 are necessary to replenish glycogen stores, using blood glucose as a source. Reaction 6 is the glycogen phosphorylase reaction that breaks down glycogen. In the liver, it is coupled to reactions 3 and 2, producing glucose for release into the blood when plasma glucose levels need to be maintained.

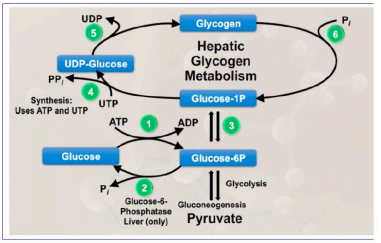


Figure 9. Summary of glycogen metabolism in the liver and its relationship to glucose and glycolysis.

If reactions 4, 5 and 6 are allowed to run in an uncontrolled manner, a "futile cycle" would be formed in which high energy phosphates would be removed from UTP for no productive purpose. As a general rule in metabolism, futile cycles are prevented through reciprocal regulation of catabolic and anabolic processes, our next subject.

Regulation of hepatic glycogen synthase (GS): Like glycogen phosphorylase, glycogen synthase is present in 2 forms (Figure 10), but here, the active form is unphosphorylated, and the inactive form is phosphorylated. This is exactly the opposite from glycogen phosphorylase. To remember which form is active, keep in mind that the hormonal signal to release glucose is epinephrine, which activates pathways that lead to phosphorylation of both proteins. The opposite effects of phosphorylation of these enzymes ensures that if glycogen degradation is up-regulated, glycogen synthesis is down regulated, and vice versa. We will discuss the regulation of hepatic glycogen synthase and glycogen phosphorylase within the context of the feed-fast cycle and the relative levels of insulin to glucagon in another chapter.

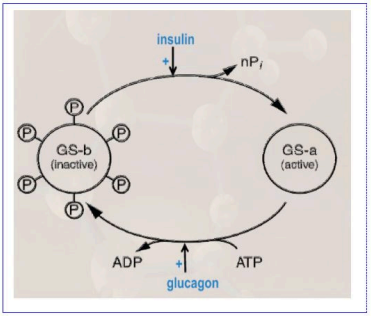


Figure 10. Reciprocal hormonal regulation of glycogen synthase (GS). Glucagon inactivates GS by stimulating its phosphorylation. Insulin activates GS by stimulating its dephosphorylation.

Session Learning Objective 6. Outline genetic disorders of glucose mobilization (Von Gierke, Pompe, Cori, Andersen, McArdle) including clinical manifestations, lab values and biochemistry.

All inborn error of metabolism disorders of glucose mobilization are rare.

Andersen: defect in glycogen branching enzyme. Glycogen has long chains with few branches form inclusions. Hypoglycemia, cirrhosis, death within ~5 years.

Cori: defect in debranching enzyme. Glycogen has short outer branches. Mild hypoglycemia and hepatomegaly.

McArdle: defect in muscle glycogen phosphorylase. Normal glycogen, muscle cramps and weakness, often manifests in teenage years.

Pompe: defect in lysosomal a1,4 glucosidase. Cardiomegaly, weakness, death.

Von Gierke: defect in glucose 6 phosphatase. Sever hypoglycemia, hepatomegaly, lactic acidosis.

Glycogen Storage diseases

Type	Name	Enzyme	Glycogen	Prevalence	Clinical
0		Glycogen synthase	None	Very rare	Hypoglycemia, ketosis
I	VonGierke	Glucose-6-phosphatase	Normal	1/50000	Severe hypoglycemia, big liver, lactic acidosis
II	Pompe	Lysosomal a1,4-glucosidase	Inclusion bodies with glycogen	1/140000	Big heart, weakness, death
III	Cori	Debranching enzyme (a1,6- glucosidase)	Shorter outer branches	1/100000	Mild hypoglycemia, big liver
IV	Anderson	Branching enzyme	Long chains with few branches	Very rare	Cirrhosis, hypoglycemia, death
V	McArdle	Muscle glycogen phosphorylase	Normal	1/100000	Muscle cramps, weakness, teens
VI	Hers	Liver glycogen phosphorylase	normal	1/70000	Mild hypoglycemia, cirrhosis

Figure 11. Clinical correlates of glycogen storage disorders.

Practice questions on glycogen metabolism:

1. What is the biological role of glycogen in striated muscle? How does this differ from its role in the liver?
2. Under low insulin to glucagon ratio conditions in the liver, which enzyme would you expect to be activated, glycogen synthase or glycogen phosphorylase? Can you explain your reasoning? (hint: what are blood glucose levels like when insulin is produced versus when glucagon is produced?)
3. What would you expect in a patient who has a deficiency in debranching enzyme?
4. Why is it important for phosphorylation to have an opposite effect on glycogen synthase compared to its effect on glycogen phosphorylase?
5. Would you expect a person who is not able to synthesize glycogen to be able to survive? Why or why not?

5. Metabolism of Fructose, Sorbitol, Galactose and Ethanol

Session Learning Objectives

SLO1. Outline the biochemical pathways involved in metabolism of fructose, its intersection with glycolysis and consequences of dietary imbalance in fructose consumption and inborn errors of metabolism related to fructose metabolism.

SLO2. Outline the biochemical pathways involved in metabolism of sorbitol, its intersection with glucose and fructose metabolism and consequences of sorbitol accumulation.

SLO3. Outline the biochemical pathways involved in metabolism of galactose, its intersection with glycolysis and consequences of dietary imbalance in galactose consumption and inborn errors of metabolism related to galactose metabolism including lactose intolerance.

SLO4. Outline the biochemical pathways involved in metabolism of ethanol, its intersection with the TCA cycle and damage that can be associated with ethanol metabolism.

FRUCTOSE METABOLISM:

SLO1: Outline the biochemical pathways involved in metabolism of fructose, its intersection with glycolysis and consequences of dietary imbalance in fructose consumption and inborn errors of metabolism related to fructose metabolism.

As a component of common table sugar (sucrose), fructose is a major source of calories in the human diet. Free fructose is also found in honey and in many fruits and soft drinks. Unlike for glucose, the uptake of fructose into cells is not enhanced by insulin, and elevated blood fructose does not stimulate insulin production by the pancreas. (A recent review on studies in the liver and kidneys

reported that: 1) Fructose is converted to glucose to variable extents, depending on exercise condition, gender, and health status (range: 30-50%). 2) A portion of fructose is incorporated into glycogen after conversion to glucose, but the extent is not known. 3) Fructose affects hepatic glucose production and whole-body glucose disposal. Therefore, it is difficult to establish unambiguously how much fructose is processed into lactate or fatty acids. It may vary from one individual to the next).

The pathways of fructose metabolism are summarized in Figure 1. Fructose is phosphorylated by a specific kinase, fructokinase, to yield fructose-1-phosphate (F-1-P). In turn, F-1-P is cleaved by a special aldolase, (aldolase B), that is present only in liver and kidney tissues; it acts efficiently on both F-1-P and fructose-1,6-bisphosphate to yield dihydroxyacetone phosphate (DHAP) and glyceraldehyde. The two molecules of dihydroxyacetone phosphate produced from fructose are then either converted to
glucose (through gluconeogenesis) or further metabolized to pyruvate by way of glycolysis.

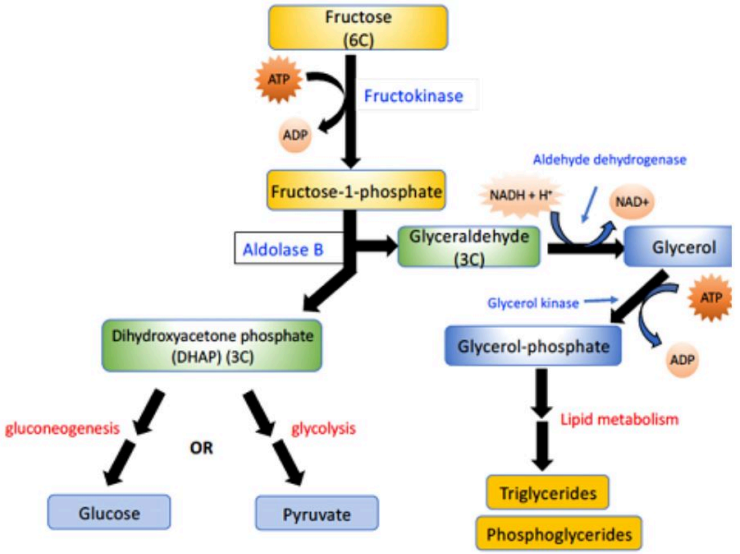


Figure 1. Overview of the metabolic fates of fructose. Fructose (top) can be converted to glucose, or pyruvate, or triglycerides.

The metabolism of fructose presents a particular challenge for the body: Fructokinase (Figure 1) is quite active and is not regulated (unlike hexokinase). The aldolase B reaction that follows is relatively slow. Therefore, on a high-fructose diet, F-1-P tends to accumulate in the liver and kidney, consuming inorganic phosphate (Pi). Pi depletion in turn creates a shortage of ATP, because Pi is required for oxidative phosphorylation. If healthy mammals are kept on a diet with fructose as the only sugar, severe liver and kidney damage can result. An extreme example of this is seen in the condition known as fructose intolerance, which results from a lack of the aldolase B enzyme that cleaves Fructose-1-phosphate. These individuals are normal, except that they become hypoglycemic and nauseous when they ingest fructose (because of inhibited liver function). People with fructose intolerance therefore need to avoid sweetened foods.

Another inherited condition involving fructose metabolism is essential fructosuria, which comes about due to a lack of fructokinase. These individuals are normal, except that they have very high levels of fructose in their urine.

SORBITOL METABOLISM

SLO2: Outline the biochemical pathways involved in metabolism of sorbitol, its intersection with glucose and fructose metabolism and consequences of sorbitol accumulation.

Conversion of glucose to fructose via the sorbitol pathway Most sugars are rapidly phosphorylated following their entry into cells. Therefore, they become trapped in the cytoplasm, because organic phosphates cannot freely cross membranes without specific transporters. An alternate mechanism for metabolizing a monosaccharide is to convert it to a polyol (sugar alcohol) by reduction of the aldehyde group to an additional hydroxyl group.

Synthesis of sorbitol: Aldose reductase reduces glucose, producing sorbitol (Figure 2). This enzyme is found in many tissues. In cells of the liver, ovaries, and seminal vesicles, there also is a second enzyme, sorbitol dehydrogenase, which can oxidize the

sorbitol to produce fructose (these cells use fructose as a major carbohydrate energy source). The pathway from sorbitol to fructose in the liver provides a mechanism by which any available sorbitol is converted into a substrate that can enter glycolysis or gluconeogenesis.

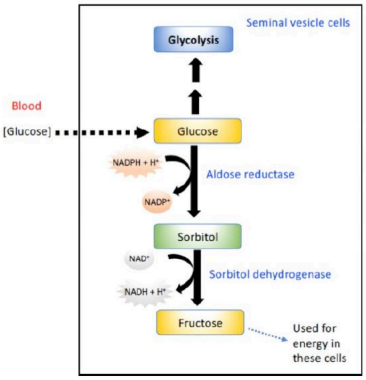


Figure 2. Conversion of glucose to sorbitol and sorbitol to fructose in cells of the seminal vesicle.

Effect of hyperglycemia on sorbitol metabolism: Because insulin is not required for the entry of glucose into the cells listed above, large amounts of glucose may enter these cells during times of hyperglycemia (for example, in uncontrolled diabetes). Elevated intracellular glucose concentrations and an adequate supply of NADPH cause aldose reductase to produce a significant increase in the amount of sorbitol, which cannot pass efficiently through cell membranes and, in turn, remains trapped inside the cell. This is exacerbated when sorbitol dehydrogenase is low or absent (for example, in retina, lens, kidney, and nerve cells). As a result, sorbitol accumulates in these cells, causing strong osmotic effects and, therefore, cell swelling as a result of water retention. Some of the pathologic alterations associated with diabetes can be attributed, in part, to this water retention, including cataract formation, peripheral neuropathy, and microvascular problems leading to nephropathy and retinopathy.

GALACTOSE METABOLISM

SLO3: Outline the biochemical pathways involved in metabolism of galactose, its intersection with glycolysis and consequences of dietary imbalance in galactose consumption and inborn errors of metabolism related to galactose metabolism including lactose intolerance.

The major dietary source of the monosaccharide galactose is from

the disaccharide lactose or "milk sugar". The digestion of lactose takes place in the small intestine, and yields one molecule each of glucose and galactose. Like fructose, the transport of galactose into cells is not insulin dependent.

As with other sugars, the metabolism of galactose must be initiated by phosphorylation, in this case by galactokinase (Figure 3).

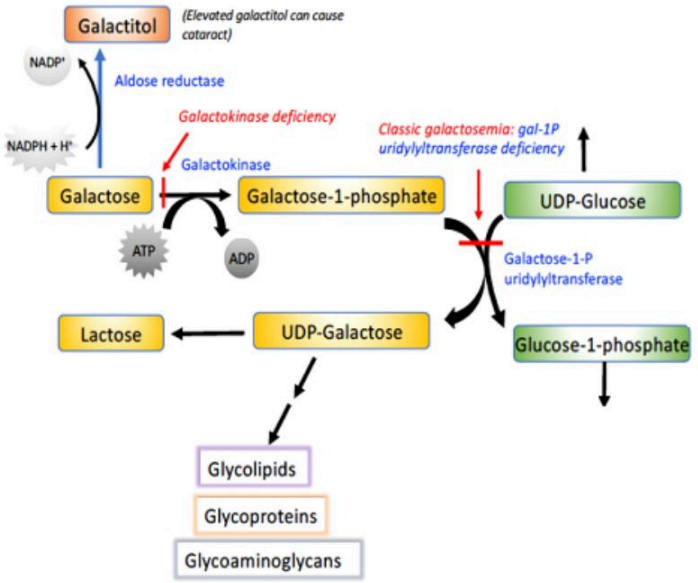


Figure 3. Overview of galactose metabolism. Galactose can be phosphorylated and then activated to be added to glycolipids, glycoproteins, and glycoaminoglycans. When defective, several of the enzymes in this diagram lead to disease.

The resulting galactose-1-phosphate can then be activated and added to glycosylated molecules.

There is an important collection of genetic disorders in humans, known collectively as galactosemia, that are associated with this metabolic pathway. They all involve a defect in galactose metabolism, so that this sugar or its derivatives accumulate in the

blood and tissues of these individuals. The most common form of galactosemia results from a deficiency in the transferase. If untreated, these individuals develop cognitive impairment, enlarged livers and cataracts. The cataracts result from the reduction of galactose to its corresponding alcohol, galactitol. If detected early, galactosemia can be treated by placing the baby on a special diet lacking
lactose.

ETHANOL METABOLISM

SLO4: Outline the biochemical pathways involved in metabolism of ethanol, its intersection with the TCA cycle and damage that can be associated with ethanol metabolism.

Ethanol is metabolized in the liver by two consecutive oxidation reactions (Figure 4). Ethanol is first converted to acetaldehyde through the action of alcohol dehydrogenase, and then acetaldehyde is oxidized to acetic acid by aldehyde dehydrogenase. Note that both of these reactions convert NAD+ to NADH.

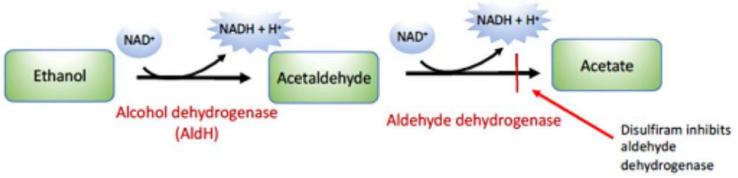


Figure 4. Ethanol metabolism and the site of action of Antabuse (disulfiram).

Alcohol metabolism shifts the redox balance of the cell by increasing NADH relative to NAD+. Therefore, consumption of even a moderate quantity of alcohol can shift the redox balance in the cytosol of liver cells. For example, this increase in liver NADH results in elevation of blood lactic acid levels, which can cause problems in individuals with gout (discussed later in the course). Acetaldehyde is a toxic compound that normally does not normally accumulate to high levels. It is thought to contribute to 1) the effects of a

hangover after drinking, 2) liver damage from chronic high alcohol consumption and 3) fetal alcohol syndrome. Antabuse (disulfiram) acts by inhibiting aldehyde dehydrogenase, thus causing an adverse reaction to alcohol consumption because of acetaldehyde buildup.

Two compounds closely related to ethanol, methanol and ethylene glycol, are quite toxic because of their conversion to corresponding aldehydes. Both alcohols are weak substrates for alcohol dehydrogenase and are converted into aldehydes that damage many tissues, including the eyes. Ethanol competes quite favorably with methanol and ethylene glycol for binding to alcohol dehydrogenase. Therefore, one therapy for poisoning by these alcohols is to continuously infuse ethanol into the patient, controlling the concentration in the bloodstream so that it effectively competes with methanol or ethylene glycol for the enzyme. This slows the formation of the toxic aldehydes and allows time for the alcohols to be excreted harmlessly in the urine.

Alcohol-related hypoglycemia

The ethanol-mediated increase in NADH causes pyruvate, oxaloacetate (OAA), and other intermediates of gluconeogenesis to be diverted into alternate pathways, resulting in the decreased synthesis of glucose. This can precipitate hypoglycemia, particularly in individuals who have depleted their stores of liver glycogen. Hypoglycemia can produce many of the behaviors associated with alcohol intoxication, such as agitation, impaired judgment, and combativeness. Therefore, alcohol consumption in vulnerable individuals (such as those who have fasted or have engaged in prolonged, strenuous exercise) can produce hypoglycemia that may contribute to the behavioral effects of alcohol. It can also increase the risk for hypoglycemia in patients using insulin.

Practice questions on fructose, galactose, and ethanol:

1. What is the significance of fructose uptake and catabolism through the Fructokinase/aldolase B pathway not being regulated by insulin?

2. Which regulatory step of glycolysis is bypassed when fructose is catabolized through Fructokinase/aldolase B? Why is this

important?
3. What is the impact of excess NADH production following alcohol (ethanol) consumption?
4. Which of the following 2 deficiencies would you expect to be more severe for an individual: Galactokinase deficiency or a deficiency in the galactose-1-P uridylyl transferase enzyme? Explain your rationale.
5. How does the polyol pathway play a role in the etiology of Type 2 Diabetes?
6. What are the consequences of NADPH depletion upon production of excess sugar alcohols?

6. Introduction to Diabetes Mellitus

Session Learning Objective 1: Discuss the differences between Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM)

Session Learning Objective 2: Discuss the health risks associated with diabetic ketoacidosis in T1DM

Session Learning Objective 3: Discuss the molecular events associated with the development of T2DM

Session Learning Objective 4: Recall the associated health risks of DM

.

SLO1: Discuss the differences between Type 1 and Type 2 Diabetes Mellitus

Diabetes Mellitus is a very common, very complex disease that you will learn much more about during this block and subsequent blocks. A key feature of both diseases is impaired glucose homeostasis (a key component of carbohydrate metabolism) resulting in hyperglycemia (high serum blood sugar). For both diseases a key cause of hyperglycemia is inadequate insulin signaling through the insulin receptor, but the mechanisms are very different.

Type 1 Diabetes Mellitus (T1DM or DM1)

In patients with DM1, inadequate insulin signaling is caused by an absolute insulin deficiency. Plasma levels of insulin and c-peptide are undetectable or very low due to destruction of pancreatic β-cells. The most common cause of β-cell destruction is autoimmune, and many patients with DM1 will have high levels of autoantibodies such as GAD65, ICA, IAA or IA-2 (you do not need to know these exact tests, just know autoantibody testing is used to diagnose DM1).

The onset of DM1 is commonly very rapid and severe. Symptoms often include frequent urination (polyuria), thirst (polydipsia), weight loss despite an increased appetite (but sometimes nausea, vomiting and abdominal pain), and blurred vision. Many patients initially present in diabetic ketoacidosis (DKA, see below). DM1 is more commonly diagnosed in children or young adults but can occur at any age. The incidence of DM1 is increasing worldwide and the cause of this remains an important research area (eg. Covid-19 infection is now possibly implicated). DM1 is more common in people with first-degree relatives with DM1, but the genetically attributable increased risk is modest (incidence increases roughly from 30/100000 to 400/100,000 people). DM1 accounts for about 5% (~1.3 million) of all patients with diabetes mellitus in the US. Patients with DM1 need insulin therapy to maintain glucose homeostasis. A small fraction of patients with DM1 receive either pancreas transplantation or islet cell transplantation, but these therapies can normalize blood glucose regulation. Stem cell therapy remains an active area of research as a cure for DM1.

Type 2 Diabetes Mellitus (T2DM or DM2)

In type 2 diabetes mellitus (T2DM or DM2), inadequate insulin signaling is caused by insulin resistance and relative insulin deficiency. At the time of diagnosis plasma insulin levels and c-peptide levels are normal or even high, but because of insulin resistance those levels are insufficient and so serum glucose levels become too high. The most common risk factor for insulin

resistance is intra-abdominal obesity which results in an excess of inflammatory signals (see below in SLO3 for cellular details). β-cell damage also contributes to the development of DM2 (and a gradual loss of β-cell function is thought to pre-date the diagnosis by as much as a decade). The mechanism is not usually autoimmune (autoantibody titers are low or negative). Mechanisms include amyloid deposition, inflammation and mitochondrial dysfunction among others, and this remains an active research area.

The onset of DM2 is commonly slow and symptoms (polyuria, polydipsia, weight loss, blurred vision) are milder and progress gradually. Many 'asymptomatic' patients are diagnosed following a routine screening blood test (Hemoglobin A1c >6.5%), and it is estimated that more than 10% of people with DM2 in the US are undiagnosed. DM2 is more commonly diagnosed in adults although the diagnosis in children and young adults is becoming more common. The incidence of DM2 continues to increase worldwide in parallel with increased prevalence of obesity. Patients with DM2 frequently have a positive family history and the disease has strong, complex, multi-gene heritability (non-mendelian). DM2 accounts for about 90% (~ 30 million) of all US patients with diabetes mellitus. Patients with DM2 should be treated with lifestyle measures to aid with weight loss, insulin sensitizers to decrease insulin resistance, insulin secretagogues to help β-cell insulin production increase, medications to help excrete glucose in the urine, as well as medications to help with weight loss. Insulin therapy may be needed as β-cell destruction progresses over years but is often used too early for patients with DM2 when better options exist.

Your math skills should tell you that leaves about 5% of people with diabetes mellitus that do not have classical DM1 or DM2, although the pathophysiology of hyperglycemia involves the same mechanisms. The list of these disorders is very long and includes hormonal disorders (causing excess cortisol, growth hormone or glucagon), pancreatic disorders causing β-cell damage (chronic pancreatitis, hemochromatosis, cystic fibrosis), and monogenic DM disorders (eg. glucokinase mutations).

Diabetes Mellitus and carbohydrate metabolism

Pause. From your previous readings this week you should be able to predict how carbohydrate metabolism is affected by diabetes mellitus

Decreased Insulin Signaling:

1. Glucose uptake (also called disposal). Digestion and absorption of carbohydrate is unaffected (not always true you will learn later about diabetic gastroparesis), but without insulin signaling glucose transport via GLUT4 into adipocytes and myocytes is reduced and blood glucose following a meal is too high. This is called impaired glucose tolerance (IGT). The high glucose after meals (post-prandial) results in increased glucose transport through other GLUT (eg. not insulin-dependent GLUT4) into cells that can be damaged by higher cytoplasmic glucose levels (more detail below).
2. Glycogen storage and gluconeogenesis. Impaired insulin signaling results in decreased glycogen synthesis and increased glycogen breakdown (you should know this mechanism now related to increased phosphorylation (because kinase activity higher than phosphatase activity) of glycogen synthase and glycogen phosphorylase. In the liver this effect on glycogen storage contributes to hyperglycemia because glucose is released into the circulation in excess of what the body requires. The same is true for gluconeogenesis. Insufficient insulin signaling favors increased gluconeogenesis causing the liver (and kidneys) to make glucose from lactate, amino acids and glycerol (you should be able to describe this mechanism related to decreased levels of cytoplasmic fructose 2,6, bisphosphate). When these processes happen overnight the result is impaired fasting glucose (IFG).
3. Glucagon. Glucagon is produced by α-cells in the pancreatic islet. These cells are inhibited by local secretion of insulin by β-cells. When insulin signaling is inadequate glucagon levels

increase and high glucagon levels are common in patients with diabetes mellitus. High glucagon will worsen the effect of low insulin signaling on glycogen breakdown and gluconeogenesis.

4. Other hormones. Epinephrine can increase for a variety of reasons (eg. dehydration) which will also worsen hyperglycemia (though more profound effects are on adipocytes as you will hear later). Cortisol can increase for a variety of reasons (eg. dehydration, infection, nausea) which will worsen hyperglycemia. The adipocyte hormone leptin decreases (from increased lipolysis) in DM1 which causes increased appetite, decreased energy expenditure and changes in non-insulin mediated glucose uptake which all favor hyperglycemia. The adipocyte hormone adiponectin decreases in the setting of obesity and DM2 and contribute to decreased insulin sensitivity

SLO2: Discuss the health risks associated with diabetic ketoacidosis in DM1

While this SLO focuses on DKA, you should understand the following two hyperglycemic emergencies.

1. Diabetic Ketoacidosis (DKA). This emergency occurs most commonly in patients with DM1. Risk factors are inadequate insulin treatment (eg. cost, adherence, equipment failure, failed absorption, etc.) and acute stressors (eg. infections, trauma, etc.) and DKA often occurs in a time period of hours. Blood glucose levels are usually very high (400-600 mg/dL) due to decreased glucose uptake and increased glycogen breakdown and gluconeogenesis (see above). Dehydration from polyuria causes increased glucose reabsorption and worsens the hyperglycemia. Very high plasma glucose becomes osmotically active in plasma which shifts water out of the cellular cytoplasm and dilutes serum sodium (hyponatremia with normal plasma osmolality, which minimizes low sodium

symptoms). Electrolyte abnormalities such as low potassium (hypokalemia) are common. Dehydration and acidosis can cause changes in blood vessels in the brain that cause cerebral edema, especially when rehydration and blood sugar lowering with insulin is rapid. The ketosis comes from uninhibited triglyceride lipolysis in adipose tissue which is converted to ketones by the liver (you will learn about this next week). Emergency treatment focuses on fluid and electrolyte repletion, blood glucose lowering with insulin and correction of acidosis. If the level is low, potassium has to be repleted before insulin is given because the sudden shift of glucose into the cells also shifts potassium into cells (not via GLUT but via a Na^+/H^+ anti-porter and then the Na^+/K^+ ATP'ase pump) which can cause life-threatening hypokalemia (causes arrhythmia).

2. Hyperglycemic Hyperosmolar State (HHS). This emergency occurs most commonly in patients with DM2. Risk factors are stressors (infection, myocardial infarction, stroke, injury, pain, medications) and non-adherence with medications. Blood glucose levels can be extremely high (600-1200 mg/dL) for the same reasons as in DKA, except that HHS usually occurs over a period of days of progressive illness. Patients with HHS are hyperosmolar and the serum sodium may be high (hypernatremia) or artificially normal due to the hyperglycemia (see above). Patients may also be acidotic and this is often a mixed disorder with lactic acidosis and (milder) ketoacidosis. Insulin signaling is not usually low enough to cause unrestrained lipolysis resulting in DKA (more next week), but after days of severe hyperglycemia β-cells can develop glucotoxicity which acutely shuts down insulin production and ketoacidosis can occur. Emergency treatment focuses on fluid and electrolyte repletion, blood glucose lowering with insulin and identification and management of underlying conditions causing the HHS (eg. infection). Morbidity rates and mortality rates for patients with HHS remain high.

SLO3: Discuss the molecular events associated with the development of DM2

Identification of the links between abdominal obesity and insulin resistance and β-cell damage remains a major goal of obesity/ diabetes research. Evidence supports inflammation caused by excessive or ectopic deposition of triglyceride, as well as increased signaling from free fatty acids as playing major roles.

DM2 as a chronic inflammatory disease. Increased abdominal (also called central or visceral) adipose tissue deposition eventually results in macrophage infiltration within adipocytes which then release systemic cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). This low-grade systemic inflammation has several effects.

1. Systemic inflammation activates intracellular inflammatory pathways, such as nuclear factor kappa B (NF-kB). Once activated, NF-kB induces the expression of molecules involved in inflammation and oxidative stress, including JNK which inhibits insulin signaling.
2. Intracellular molecules such as JNK cause increased serine phosphorylation which prevents insulin-mediated tyrosine phosphorylation resulting in insulin resistance. Serine phosphorylation can occur on various components of the insulin signaling cascade, including insulin receptor substrate (IRS) proteins. When IRS proteins are serine phosphorylated this inhibits their ability to activate PI3K, and insulin signaling decreases.
3. Chronically increased serum free fatty acids also stimulate NF-kB, as well as causing endoplasmic reticulum (ER) stress. ER stress can lead to the activation of the unfolded protein response (UPR), which is a cellular defense mechanism that aims to restore ER homeostasis. However, chronic ER stress can lead to the activation of pro-inflammatory pathways contributing to insulin resistance.

4. Altered fatty acid metabolism also increase the production of modified phospholipids (ceramides) that activate specific protein phosphatases. These phosphatases dephosphorylate active tyrosine-phosphorylated IRS proteins and decrease insulin signaling.
5. Adipose tissue inflammation decreases the secretion of the adipocyte hormone adiponectin. This hormone increases glucose uptake and metabolism through the AMP-activated kinase pathways and normally counteracts insulin resistance. Low adiponectin can contribute to hyperglycemia.

Ectopic lipid deposition. In the setting of obesity, triglycerides and free (non-esterified) fatty acids begin to accumulate in other tissues that do not normally store triglycerides. These include skeletal muscle, gonads (contributes to polycystic ovarian disease), pancreas (contributing to β-cell damage), heart (contributing to cardiac dysfunction) and liver (causing hepatic steatosis and steatohepatitis). Triglyceride deposition in these tissues contributes to organ dysfunction and also causes local and systemic inflammation which can worsen insulin resistance.

Genetics and insulin resistance. The risk of developing DM2 can be inherited. The genetics are complex (eg. non-mendelian involving multiple genes that individually convey only low risk) and intricately involved with nutrition, activity and social determinants of health. An enormous amount of research has tried to identify candidate susceptibility genes, and while several genes have been shown to have small effects, there is currently no single defect that explains insulin resistance (eg. if you study 100 patients with the same level of body adiposity and 50 are insulin resistant and 50 are insulin sensitive, there is no single gene mutation that explains the 50 patients with insulin resistance).

SLO4: Recall the associated health risks of DM

Diabetes mellitus is associated with very significant long-term complications that result in most of the morbidity and mortality

from the disease. These include both microvascular and macrovascular complications. The classical microvascular complications are retinopathy (edema and vascular changes in the retina leading to visual impairment), nephropathy (damage to the glomerulus which results in proteinuria and chronic kidney failure), and peripheral neuropathy (results in dysesthesias and eventually sensory loss which predisposes to injury, poor wound healing and lower extremity amputation). Macrovascular complications result from an increased risk of atherosclerosis of vessels of all sizes along the arterial tree. This increases the risk of myocardial infarction (heart attack), cerebrovascular disease (stroke) and peripheral vascular disease. Many studies have shown that improved glycemic control reduces the risk of developing both microvascular and macrovascular disease

Biochemistry of microvascular disease

While this topic remains an active research area there are multiple mechanisms known to link hyperglycemia to microvascular disease.

1. Advanced glycation end products (AGEs) are formed by the non-enzymatic reaction between reducing sugars (eg. glucose) and amino groups of proteins, lipids, and nucleic acids. In DM, hyperglycemia leads to the accumulation of AGEs, which can modify proteins and alter their function. The accumulation of AGEs in the basement membrane of blood vessels can lead to thickening and reduced tissue perfusion.
2. Cytoplasmic hyperglycemia increases oxidative stress from increased flux of electrons through the mitochondrial electron transport chain resulting in enhanced production of superoxide. Oxidative stress can damage cellular components such as proteins, lipids, and DNA. The resulting oxidative damage can lead to endothelial dysfunction, increased vascular permeability, and inflammation.
3. Hyperglycemia can activate protein kinase C (PKC), which is involved in various cellular processes, including angiogenesis

and vascular permeability. PKC activation leads to increased production of cytokines and growth factors, promoting inflammation and inappropriate angiogenesis.

4. Hyperglycemia also leads to intracellular sorbitol accumulation. Sorbitol is normally metabolized to fructose by the enzyme sorbitol dehydrogenase (SDH), but chronic hyperglycemia overwhelms this pathway. Sorbitol accumulation can lead to osmotic stress and cellular damage, particularly in tissues with limited or no SDH, such as the retina, nerves, and kidneys. The accumulation of sorbitol can lead to alterations in cellular function, including impaired mitochondrial function, increased oxidative stress, and reduced energy production.

Hemoglobin A1c is an example of an AGE. Glucose reacts with a valine on the hemoglobin β-chain. Because the lifetime of a red cell is about 90 days, the level of hemoglobin A1c provides an indication of average blood glucose levels over the previous 3 months and is used to diagnose diabetes mellitus and monitor glycemic control in patients with DM1 or DM2.

Biochemistry of macrovascular disease

Atherosclerosis and macrovascular disease is a giant topic and we will only touch on a few mechanisms here related to hyperglycemia. Those mechanisms are largely the same, relying on AGEs, oxidative stress and inflammation except the target tissues now are endothelial cells (the inner lining of blood vessels) and smooth muscle cells (a component of the arterial wall). We will discuss only two specific mechanisms beyond what was described above.

1. In the vasculature, inflammation induced oxidative stress alters low-density lipoproteins (LDL, wait until next week) to form oxidized LDL, which can stimulate the expression of adhesion molecules on endothelial cells and promotes infiltration of monocytes (white blood cells already primed by

the systemic inflammation, called macrophages once they leave the vasculature), leading to the formation of foam cells (dying macrophages) and the development of atherosclerotic plaques.

2. Endothelial dysfunction decreases nitric oxide (NO) production and bioavailability. NO is a potent vasodilator and inhibitor of platelet aggregation, and reduced production leads to vasoconstriction, increased platelet aggregation, and thrombus formation, promoting the development of atherosclerosis.

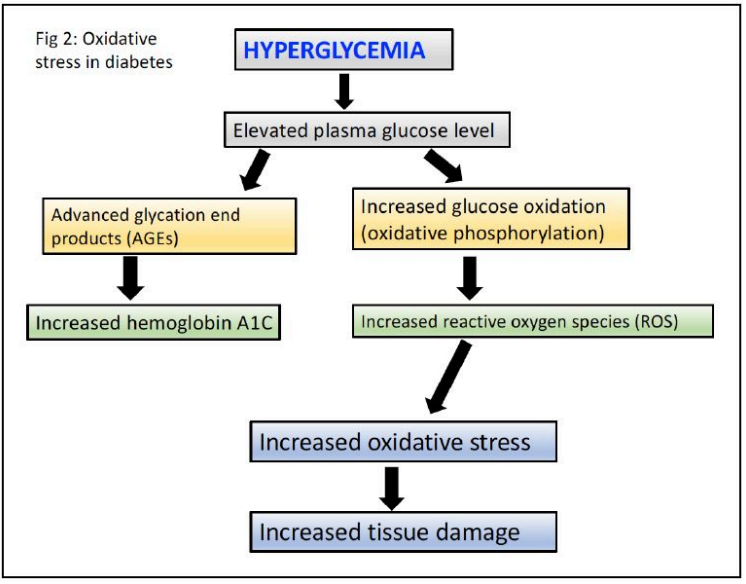


Figure 1.

Practice questions:

1. Can you draw the reaction catalyzed by sorbitol dehydrogenase?

Why is this enzyme important?

3. Explain the interaction between high LDL levels and hyperglycemia in DM2?

4. Explain the differences between insulin levels and insulin signaling in DM1 and DM2?

5. Hypothesize how insulin sensitizers might be used in the treatment of DM2?

7. Cholesterol, Lipid Transport

Introduction: Lipids are commonly referred to as fats. Lipids are large hydrophophobic molecules that do not dissolve in water. Fatty acids (long chain carbon molecules that end in a carboxyl group) are key components of the lipid bilayer of the cell membrane and intracellular organelles. Lipids play a key role in regulating transport of molecules across extracellular and intracellular compartments. Fatty acids and triglycerides (three fatty acids attached to a glycerol backbone) are a major source of energy for the body, including the heart and skeletal muscle. Specialized functions of lipids include the development of surfactant in the lung, formation of bile to facilitate the transport and absorption of dietary lipids in the gut, insulating nerves as myelin, to name only a few. Lipids also serve as signaling molecules, serving as targets of lipid kinases and substrates for eicosanoid signals. The lipid cholesterol (a large molecule with multiple 6 and 5 carbon rings) is a structural component of all cell membranes and is a precursor to bile acids, and steroid (molecules synthesized from cholesterol) hormones including vitamin D. Appropriate cellular levels of cholesterol are essential for normal function. To ensure this, the body obtains cholesterol from the diet and synthesizes cholesterol from other molecules. Dysregulation of cholesterol transport pathways leads to hypercholesterolemia and contributes to atherosclerosis.

This session focuses mostly on lipid physiology outside the cell. We introduce key concepts related to the digestion, absorption and transport of lipids (fats). What are micelles? What is the role of bile salts? What are possible causes of lipid malabsorption? What are the key differences between the exogenous and endogenous pathway of lipoprotein transport? What is a key anatomical difference between lipid and carbohydrate absorption from the gut? How are circulating

triglycerides absorbed? What is unique about peripheral cholesterol metabolism?

This information will connect with subsequent blocks that introduce you to gastroenterology and endocrinology

Session Learning Objectives and Brief Synopses (see main text for detailed explanations):

Session Learning Objective 1. Outline the pathways of lipid digestion and transport in the body, including the roles of chylomicrons, VLDL, IDL, LDL, and HDL.

— lipid digestion is more complex than carbohydrate, involves bile salts and micelle formation to maximize surface area for pancreatic lipases to act in the small intestine. Lipids are transported in the blood within lipoprotein particles that have unique roles and surface protein markers.

Session Learning Objective 2. Outline pathways of dietary cholesterol uptake and transport.

— cells need lipids for maintenance of cell and organelle membranes, fuel, cell division and other specialized functions. Cells take up lipids from circulating lipoprotein particles through various mechanisms. We focus on the lipoprotein lipase pathway which is regulated by insulin, and on the LDL receptor.

Session Learning Objective 3. Understand the possible mechanisms of familial hypertriglyceridemia.

— clearance of triglycerides from plasma usually happens quickly. Understand genetic mutations in LPL, apoE, apoC and how these lead to high serum triglyceride levels.

Session Learning Objective 4. Understand the biological basis of familial hypercholesterolemia.

— uptake of lipoprotein particles by the liver (and other cells) is essential to maintain normal serum lipoprotein concentrations. Genetic mutations in the LDL receptor can impair this process.

Session Learning Objective 5. Outline the major pathway of de novo cholesterol synthesis. Understand the biochemical basis of statin action.— most cells can synthesize cholesterol from Acetyl-CoA. HMG CoA reductase in the cytoplasm is a key enzyme.

Inhibitors of this enzyme ('statins') are commonly used to reduce atherosclerotic heart disease.

Session Learning Objective 1. Outline the pathways of lipid digestion and transport in the body, including the roles of chylomicrons, VLDL, IDL, LDL, and HDL.

Session Learning Objective 2. Outline pathways of dietary cholesterol uptake and transport.

Lipid Digestion and Transport Overview: Dietary (exogenous) lipids (primarily triglycerides) are broken down in the gut because these large molecules cannot cross the cell membrane. Digestion of triglycerides requires bile acids (made by the liver), which act as detergents, and pancreatic enzymes (lipases). The resulting fatty acids are taken up by the mucosal cells of the small intestine, converted back to triglycerides, and secreted into the lymphatic system. Because of poor solubility in lymph or plasma (mostly water), lipids are assembled into soluble lipoprotein particles (see immediately below) for transport and for specific delivery to target tissues. Triglycerides are also synthesized in the liver (endogenous lipids, assembled from serum fatty acids or made from Acetyl CoA) and are secreted into the hepatic vein. Finally, a system exists for transport of extrahepatic lipids (mostly cholesterol) from peripheral tissues to the liver (see legend for a summary).

Lipoprotein Particle Overview: Most lipids are not soluble/miscible in water (aqueous, plasma). To be transported in blood, lipids are assembled into lipoprotein particles (see Table 1). These particles are mini lipid droplets with a surface of phospholipids (the hydrophilic, charged (polar), phospho head groups of the molecules on the outside, the hydrophobic fatty acid tails on the inside – much like the outer leaflet of a cell plasma membrane). Buried inside (the core) are all the hydrophobic triglycerides and cholesterol esters (and a little free cholesterol). Key large proteins (apolipoproteins) serve as scaffolds for assembly and maintaining particle integrity, but also are recognized by cell surface receptors (eg. ApoE receptor and Apo B-100 receptor) and serve to activate key enzymes (Apo C

activates LPL, Apo A activates LCAT, more below). The nomenclature of lipoproteins is archaic based on their separation when centrifuged in a density gradient, while the lipid content and surface proteins are a much more important definition of their role than the density.

Figure 1 legend:
Chylomicron pathway: Exogenous (diet) pathway of lipid transport **VLDL pathway:** Endogenous (liver) pathway of lipid transport

Symbols
LDL: Low density lipoprotein
HDL: High density lipoprotein
VLDL: Very low density lipoprotein
IDL: Intermediate density lipoprotein
TG: Triglycerides
Chol: Cholesterol
CholE: Cholesterol esters
Chylo: Chylomicron
Rem: Chylomicron remnant
LPL: Lipoprotein lipase enzyme

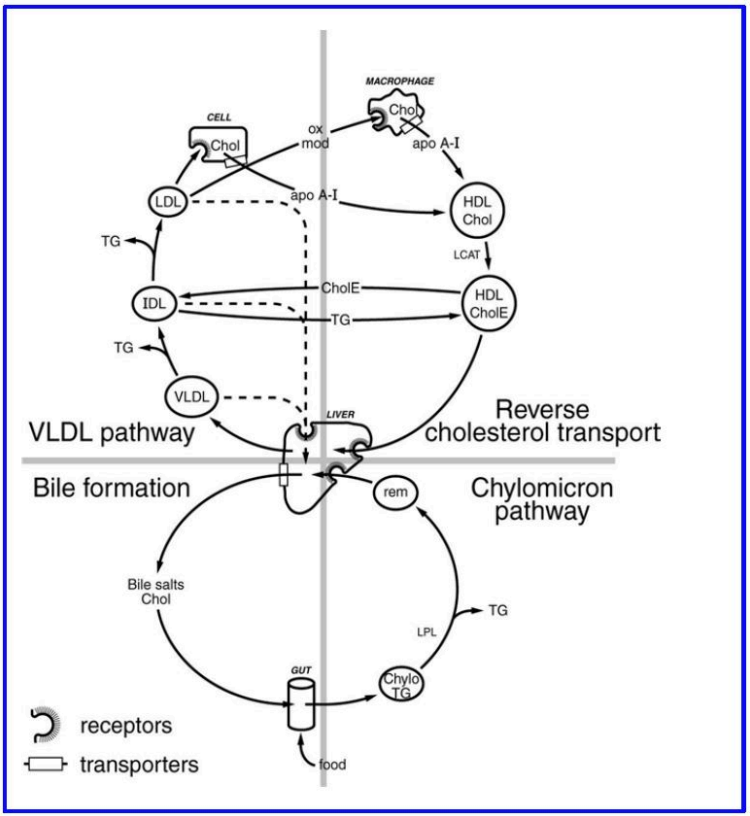


Figure 1: Schematic of Exogenous and Endogenous Lipoprotein Cycles

Clinical Overview: Most clinicians only relate to lipid metabolism in the context of risk of coronary artery disease. LDL ('bad' cholesterol) is the longest lasting lipoprotein particle and if present in excess or modified (eg. oxidation) is deposited within macrophages in the walls of arteries leading to atherosclerotic plaques. Similarly, low levels of HDL ('good' cholesterol) results in atherosclerosis via reduced transport of cholesterol from peripheral tissues back to the liver. Altered lipoprotein metabolism is usually

caused by a complex interaction of nutrition, activity levels, polygenic inheritance and environmental factors. However, rare monogenic disorders increase the risk of cardiovascular disease by altering lipid metabolism at the level of lipoprotein secretion and uptake from plasma. An understanding of lipolysis in white adipose tissue is also important to understanding ketoacidosis, a life-threatening condition most commonly associated with diabetes mellitus.

Board Exam Overview: In addition to the above, there are extremely rare monogenic disorders that impair lipid metabolism at the level of intracellular transport and mitochondrial fatty acid degradation (beta oxidation). These disorders almost always present in childhood as low energy states (weakness, fatigue, failure to thrive, hypoglycemia) due to inadequate cellular fuel/ATP production. It is important for you to be able to distinguish these from glycogen storage disorders with a similar presentation (hint: always look for the ketones! If ketones are present it is NOT a problem with fatty acid transport/oxidation).

TABLE 1	Chylomicron	VLDL	IDL	LDL	HDL
Size	Big (100nM)	Big	Medium	Small (15nM)	Very small
Apolipoproteins	B-48, A, C, E	B-100, C, E	B-100, C, (E)	B-100	A, C, E
TG %	86	55	23	6	5
CE + C %	5	19	38	50	23
Protein %	2	8	19	22	40

Table 1: Important characteristics of lipoprotein particles. These particle vary dramatically in 1) size; 2) the apolipoproteins present on the surface of the particle – important for uptake by specific receptors and activating specific enzymes; 3) the triglyceride (TG) versus the cholesterol ester and cholesterol (CE + C) content inside the particle core, and protein content on the surface.

Dietary Lipids

On average, North American adults consume 150 g of triglyceride,

300mg of cholesterol and 4-8 g of phospholipid daily. Fats aggregate as lipid droplets in water, and your body needs to make these droplets as small as possible to optimize digestion and absorption. Multiple gut specific physical mechanisms contribute (peristalsis, pyloric opening, etc.), but the most important step is chemical. In the small intestine, fats interact with bile salts (produced by the liver, stored in the gallbladder, empty into the gut through the pancreatic duct following fatty meals in response to various gut hormones) which act as powerful detergents and separate the fat droplets into micelles (tiny droplets). Micelles hugely increase the surface area of fat exposed to pancreatic lipases, which digest the triglycerides into fatty acids and monoacylglyerol.

Intestinal mucosal cells absorb fatty acids, cholesterol, monoacylglycerols, and phospholipids from the gut lumen into the cell interior by poorly understood mechanisms (FA and C can diffuse into the cell, but the charged molecules cannot). Within the mucosal cells, triglycerides are synthesized from monoacylglycerols and fatty acids, and cholesterol is re-esterified with a long chain fatty acid by acyl CoA cholesterol acyl transferase (ACAT) (Figure 2). Some short-chain and medium-chain fatty acids (usually fewer than 10 carbons) are secreted directly into the portal system and are not assembled back into triglycerides.

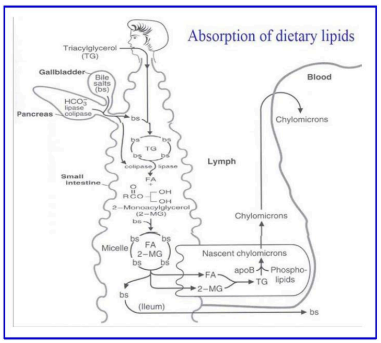


Figure 2: Absorption of dietary lipids

Figure 2 Legend: TG: triglycerides, BS: bile salts, FA: fatty acids, 2-MG: 2-monoacyl glycerol, ApoB: Apo-B48 apolipoprotein, HCO_3^-: bicarbonate.

Clinical questions. What happens if your pancreas is damaged and can't make enough lipase? Can pancreatic enzymes be replaced by medications? What happens if bile salts and ingested lipids don't mix in the right quantity or the right location? Does

gallbladder surgery (cholecystectomy) cause this? Do gallstones (choledocholithiasis) cause this? What is the effect of medications that bind and sequester bile salts (bile acid sequestering resins)? What happens to stool (feces) if fat is not digested and absorbed? Can you prescribe medications that block intestinal fat absorption?

Test your understanding of lipid digestion and absorption with the following exercise. Rouy-en-Y gastric bypass is a common surgical weight loss procedure that bypasses most of the stomach by attaching the jejunum to a limited gastric pouch (see diagram, you will not be tested on any details of this procedure in this block)

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=198#h5p-28

Exogenous Lipid Cycle

Assembly and Secretion

The lipoprotein particle secreted from intestinal cells is called a chylomicron which is how the gut delivers lipids to the rest of the body (see Table 1). The chylomicron is assembled in the rough endoplasmic reticulum starting with the ApoB48 apolipoprotein being imported into the ER. ApoB is a 29 exon gene that makes a giant 4563 amino acid protein. In intestinal cells specific RNA editing creates a premature stop codon so only 48% of the normal ApoB protein is translated (hence ApoB48). Further processing of the chylomicron occurs in the golgi. The entire process takes time (1-4 hours after eating) and costs energy (diet-induced thermogenesis). Chylomicrons are very large (>100nM) and contain mostly triglycerides (86%)(Table 1, Fig 3).

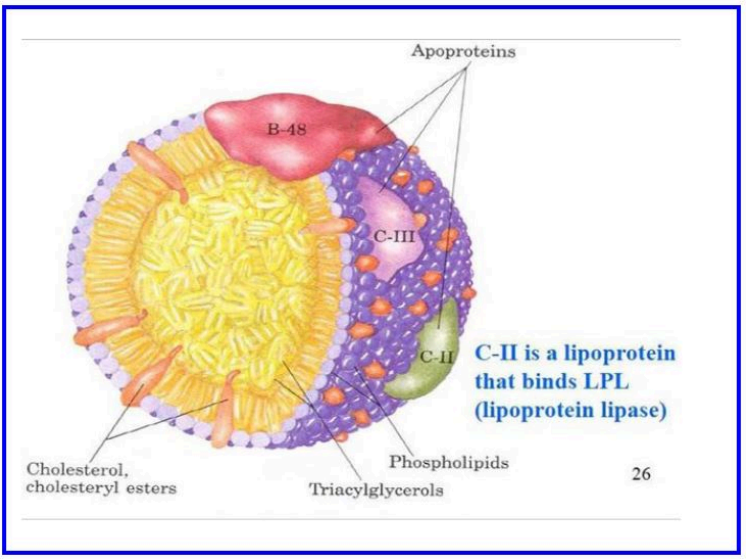


Figure 3: Schematic diagram of a chylomicron

Chylomicrons are secreted from intestinal cells into retroperitoneal lymphatic lacteals that eventually drain into the subclavian vein through the thoracic duct (Fig. 2). This route intentionally avoids the liver initially (unlike carbohydrates and amino acids which enter the portal vein) so other tissues have the first chance to remove triglycerides (and cholesterol and phospholipids) from the chylomicron (Fig. 4). The secreted chylomicron also has Apo A on the surface (mostly transferred rapidly to HDL particles). Once out of lymph and in the blood, chylomicrons rapidly acquire Apo C and Apo E by bumping into already circulating lipoproteins. ApoC activates an enzyme called lipoprotein lipase (LPL) on the surface of endothelial cells and allows for triglycerides inside the chylomicron to be hydrolyzed into fatty acids and monoacylglycerol for cellular absorption. Apo E has high affinity for a hepatic receptor which takes up chylomicron remnants. Because of this chylomicrons are cleared from plasma

rapidly and the estimated residence time in plasma of any individual chylomicron particle is only 5-10 minutes.

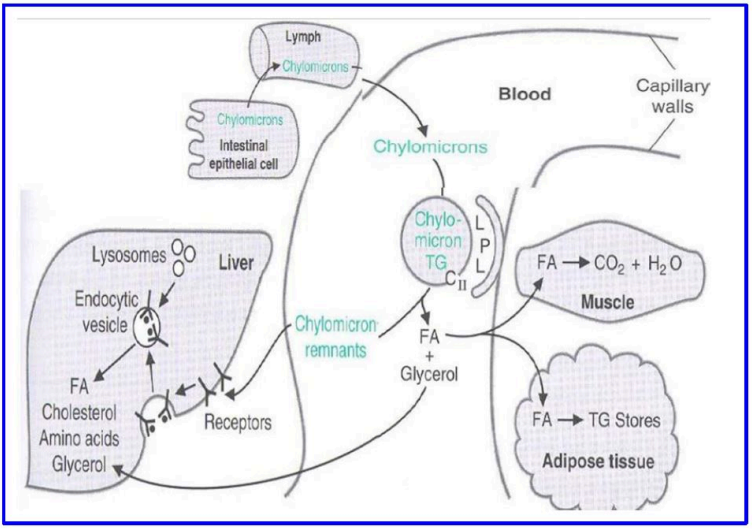


Figure 4: Fate of chylomicrons

Legend. Fig 4 shows the production and fate of chylomicrons and the lipids contained in these gut-derived particles

Delivery of triglycerides. Lipoprotein lipase (LPL) is an enzyme present on the exterior surface of capillary endothelial cells loosely bound to a glycoprotein molecule called heparan sulfate. The enzyme is made by cells within these tissues (eg. myocyte, adipocyte) and secreted into the local capillaries. LPL is most abundant in adipose tissue (including mammary gland), where its synthesis is increased by insulin signaling, but also present in skeletal muscle and heart muscle and at lower levels in many tissues. Triglycerides within circulating chylomicrons is hydrolyzed by LPL and the products (a monoacylglycerol and two fatty acids) (Fig. 4) are taken up by those local cells (eg. adipocytes) and resynthesized into triglyceride, or used as a substrate for energy

metabolism by other tissues such as cardiac and skeletal muscle, or converted to phospholipids to enter a lipid bilayer. One of the apolipoproteins of the chylomicron (APO-CII) is an activator of LPL. The remains of the chylomicron after LPL action to remove triglycerides are called chylomicron remnants.

Cellular Uptake. At least two receptors on the hepatocyte cell surface bind and internalize chylomicron remnants (Fig. 6; below). Binding to both receptors is mediated by interaction with APO-E. The LDL receptor (LDLR, Apo-B100/E receptor) is a major pathway because of its strong affinity for Apo-E containing lipoproteins. However, this is not the only receptor because chylomicron remnants do not accumulate in the plasma of LDL receptor-defective individuals. The second mechanism involves LDL receptor-related protein (LRP). Chylomicron remnants are internalized by receptor-mediated endocytosis. The lipid of the remnant (glycerophospholipid, cholesterol and some triglyceride) is made available for metabolism by the hepatocyte. Chylomicrons also carry dietary lipids such as β-carotene and the fat-soluble vitamins (vitamins A, D, E, and K), which are stored in the liver for systemic use.

Session Learning Objective 3. Understand the possible mechanisms of familial hypertriglyceridemia.

Clinical. Hypertriglyceridemia is a common diagnosis. Mildly elevated TG levels (150-500 mg/dL) can contribute to cardiovascular disease risk. Severely elevated TG (>1000 mg/dL) can cause acute pancreatitis and causes characteristic lipid deposits in skin (xanthelasma (smaller) and xanthomas (larger)). Based on the above you should be able to predict rare monogenic mutations that result in high serum triglycerides (hypertriglyceridemia). Inactivating mutations in the LPL gene present in childhood. Frequency is 1 per million. Mutations in Apo C-II can reduce the ability to activate LPL. Apo C-II deficiency is extremely rare. Three common Apo E alleles are present in all populations (E2, E3, E4). Apo E2 has a single aa substitution (Arg158-Cys) from Apo E3 and has much lower affinity for the LDL receptor. Homozygosity for Apo E2/

E2 is present in about 0.5% of studied populations. Homozygosity results in mild hypertriglyceridemia but can become severe when other conditions are superimposed, including fatty liver, diabetes mellitus, and various medications (estrogen, retinoic acid medications). Fibrates are the most common medication used to reduce triglyceride levels. These medications activate a key family of transcription factors (peroxisome proliferator activated receptors (PPARs)) that reduce triglycerides at multiple levels of lipid metabolism.

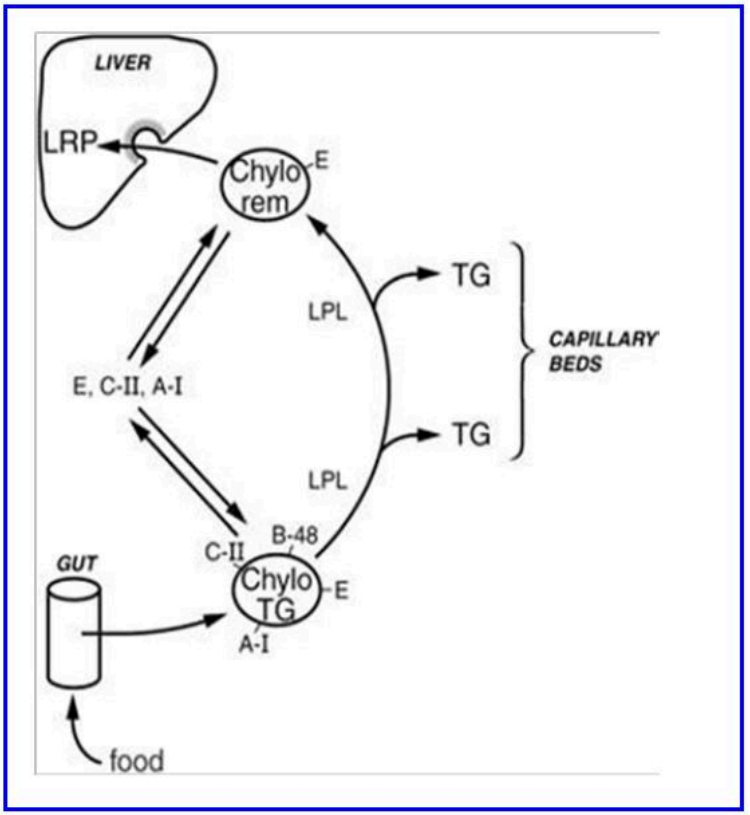


Figure 5

Simplification. As usual the full truth is much more complicated. Lipid absorption and tissue/organelle fatty acid targeting is incredibly complex. Absorbed fatty acids are rapidly and efficiently partitioned and rare fatty acids (will be discussed later) are preferentially turned into phospholipids rather than assembled into triglycerides or metabolized. Specialized plant derived and animal derived fatty acids have unique signaling mechanisms. This point is highlighted by the ability of fish oils (specific omega-3 fatty acids) to affect lipid metabolism and lower serum triglyceride levels. The mechanism is complex, but the best documented is an effect of fish oils to decrease the delivery of free fatty acids to the liver by decreasing triglyceride hydrolysis in adipocytes. This has an effect to decrease VLDL production by the liver.

Endogenous Lipid Cycle

Your 8 year old nephew only eats plain pasta. No butter. His circulating lipids are normal. Why? His liver happily takes all the glucose from pasta (carbohydrate) and turns it into triglycerides and cholesterol (fats) and exports those lipids in periods between meals. The production of very low density lipoprotein (VLDL) by the liver is how hepatic triglycerides and cholesterol is delivered to peripheral tissues. The rates of cholesterol delivery to plasma (VLDL is to the liver what chylomicrons are to the gut) and receptor mediated uptake of VLDL, IDL, and LDL determine the level of plasma LDL cholesterol (Fig 6; below).

Assembly and secretion. The liver has cytoplasmic enzymes that can make fatty acids or cholesterol starting from acetyl CoA (details covered in lipid biosynthesis session). These endogenous fatty acids can be elongated and desaturated if necessary within the ER. Fatty acids are linked to glycerol (3 C sugar) to form triglycerides. VLDL particles are assembled in the ER and golgi (similar to chylomicrons in the intestinal cells) except the major scaffold lipoprotein is the full length ApoB100. The process of hepatic VLDL production is accelerated dramatically by hepatic uptake of fatty acids from adipocytes (released from triglycerides through activation of hormone sensitive lipase in fasting). VLDL is secreted into the

hepatic venous system (not into lymphatics). Once in the blood VLDL acquires Apo C and Apo E. VLDL does not have Apo A to donate to HDL particles. VLDL is secreted from the liver in low amounts continuously, but the rate increases in the post-prandial state (when insulin signaling returns to fasting levels and adipocyte lipolysis increases). This is likely because cells prefer to get their supply of fatty acids for fuel from circulating lipoprotein particles rather than having higher levels of (free, meaning carried by albumin) fatty acids circulating in the blood.

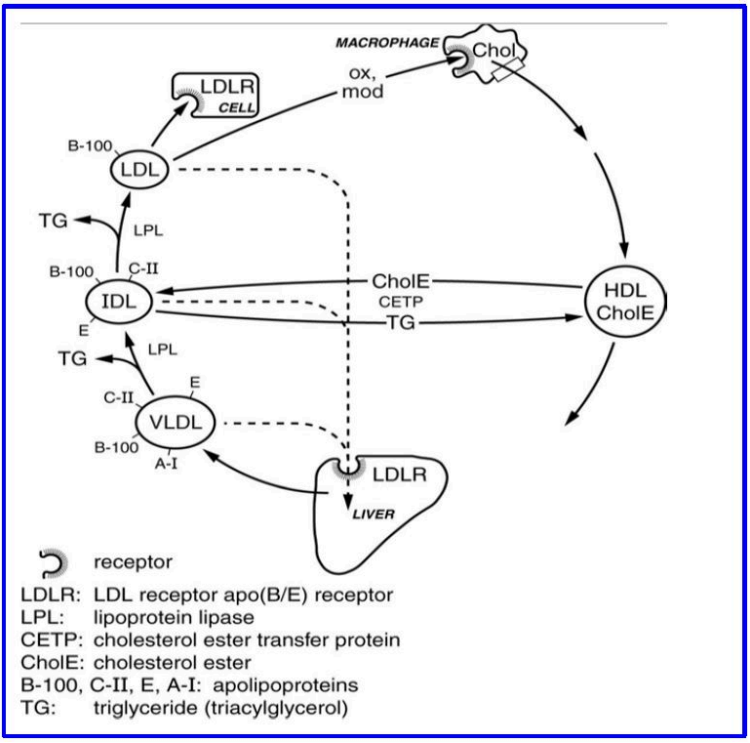


Figure 6: The VLDL transport pathway of endogenous lipids

Delivery of triglycerides. Similar to chylomicrons, lipoprotein lipase (LPL) activity results in the hydrolysis and removal of VLDL

triglyceride in the vascular bed of different tissues. Hepatic lipase, a triglyceride and glycerophospholipid lipase, also plays a role in removal of triglyceride from VLDL and IDL. The resulting smaller lipoprotein, called intermediate density lipoprotein (IDL), can continue to circulate and be metabolized further to an LDL. IDL is also removed from circulation by receptors on the surface of hepatocytes and other cells that recognize Apo E. When IDL loses all the surface ApoE (and most internal triglyceride) it becomes low density lipoprotein (LDL). This particle circulates the longest in plasma, because it only has Apo B100 which has much lower affinity than Apo E for the LDL receptor and so is taken up very poorly by the liver. This long half-life contributes to making LDL the 'bad' cholesterol because it is more likely to become damaged (eg. oxidized) and more likely to be mistakenly taken up by receptors on peripheral cells (scavenger receptors, eg. SRB1, on many cells, but most importantly on monocyte/macrophages).

Cellular uptake. Like chylomicron remnants, receptor-mediated endocytosis is how IDL and LDL are taken up by cells. Uptake by hepatocytes accounts for about half of LDL endocytosis in humans, but most cells express the LDL receptor (at lower levels than hepatocytes) and can take up LDL particles. In plasma, the loss of Apo E from the surface of the particle (IDL becomes LDL) is a critical factor in the rate of uptake of lipoprotein particles by the LDL receptor (lower affinity for Apo B-100 than Apo E). This contributes to a particle half-life for LDL of about 3 days. Once endocytosed the LDL/LDLR complex traffics to the lysosomal compartment. The LDL receptor can be either degraded or recycled to the cell surface (see Clinical content below). The endocytosed lipoprotein contents (phospholipids, cholesterol and (scant) triglycerides) are metabolized or stored by the cell. Complex intracellular signals recognize intracellular cholesterol stores and control LDL receptor gene expression and the quantity of LDL receptors on the cell surface (important for pharmacological therapy).

Exchange of lipids with HDL. In the process of conversion of VLDL to IDL and then to LDL (the archaic nomenclature is confusing

here, IDL is intermediate between VLDL and LDL. Not between LDL and HDL) there is also exchange of cholesterol ester for triglyceride which occurs when these particles bump into HDL lipoproteins (see below). This exchange is mediated by cholesterol ester transfer protein (CETP, a liver protein that circulates mostly bound to HDL) (Fig 6; above). The triglyceride composition of the IDL and LDL decreases and the cholesterol ester content increases as a result of this exchange.

Session Learning Objective 4. Understand the biological basis of familial hypercholesterolemia.

Clinical. The clinical scenario highlighted in this section is familial hypercholesterolemia (FH). This disorder is caused by inactivating mutations in the LDL receptor that decrease the uptake of LDL from plasma (variety of mutations described that affect affinity for Apo B100, receptor production or receptor recycling). Homozygous patients are rare, and present with cardiovascular disease at a very early age (< age 30) and very high plasma LDL levels. Heterozygotes have an intermediate plasma LDL phenotype with early onset cardiovascular disease (age <50). Heterozygote frequency can be as high as 1/250 in some populations. At the end of this chapter, you should be able to describe how treatment with statins (HMG CoA reductase inhibitors) can decrease LDL levels in patients with FH.

Clinical content. A powerful novel treatment for patients with FH are PCSK9 inhibitors. PCSK9 is a hepatic proprotein convertase present in plasma. It binds to the extracellular portion of the LDLR on the cell surface. When bound, PCSK9 targets LDLR for degradation in the lysosome after the LDL/LDLR complex is endocytosed. PCSK9 inhibitors prevent PCSK9 from binding to LDLR and dramatically increase LDLR recycling back to the cell surface – causing an extremely powerful effect to reduce plasma LDL.

Reverse Cholesterol Transport. This is the most complex of the three lipoprotein cycles covered in this course pack. The main lipoprotein for reverse cholesterol transport is high density lipoprotein (HDL). The key apolipoprotein on the surface of HDL

is Apo A. Liver (70%), small intestine (20%) and other tissues (10%) can all make HDL. Cholesterol (an essential part of cell membranes and organelles) is very poorly metabolized by most cells. When cells accumulate too much cholesterol or die (apoptosis, necrosis, etc.), free cholesterol from those cells needs to be removed. HDL does that job.

Assembly and secretion. HDL is secreted as a small flat disk with Apo A and phospholipids (like the very initial stage of chylomicrons or VLDL in the rough ER), but otherwise empty (nascent HDL). The half-life of HDL is up to 24 hours (long by lipoprotein standards). HDL bumps into other lipoproteins and acquires Apo E (but is not taken up by the LDL receptor, mostly exchanges this back to chylomicrons and VLDL) and Apo C (may deliver some TG to tissues by activating LPL, but mostly gives Apo E back to chylomicrons and VLDL), and more Apo A (from chylomicrons).

Delivery of cholesterol to HDL. Very complex. Multiple mechanisms exist. The best described mechanism involves ATP binding cassette proteins (eg. ABCA1, ABCG1) which function as cholesterol pumps from the cell surface into HDL particles. On the HDL particle surface, the enzyme lecithin cholesterol acyl transferase (LCAT, synthesized in liver, bound to HDL) immediately converts free cholesterol to an acylated cholesterol ester. LCAT activity is increased by more Apo A. The cholesterol ester begins to fill up the empty HDL interior. Other mechanisms may involve HDL endocytosis, intracellular cholesterol loading and then reverse endocytosis.

Cellular uptake of HDL. Again, complex. While still circulating, HDL transfers cholesterol esters to IDL and LDL (via CETP activity on the HDL surface, see above). This is one mechanism whereby peripheral cholesterol enters the liver to be recycled (as bile salts or secreted into bile) or re-secreted in VLDL. HDL is also recognized by the scavenger receptor B1 (SRB1) on hepatocytes which (by endocytosis or cholesterol transfer) is another way HDL delivers peripheral cholesterol to the liver. HDL is also taken up by other cells, for example glands that make cholesterol based steroid

hormones (adrenal, ovary, testes) can take up HDL when these glands need cholesterol (they also make their own cholesterol, see below).

Cholesterol Excretion. Most cholesterol recycled from peripheral tissues is excreted into and eliminated by the gut. About 65% comes directly from hepatocytes secreting cholesterol taken up from HDL (directly or indirectly) as free cholesterol in bile. Less well understood, about 35% of cholesterol excretion happens directly in the gut (TICE, trans-intestinal cholesterol efflux). These processes involve two key nuclear hormone receptors LXR (liver X receptor) and FXR (farnesoid X receptor) that transcriptionally control a variety of cholesterol transporters and bile synthesis enzymes. The cholesterol modified into bile salts by the liver and secreted into the bile does not count because bile salts are 90+% reabsorbed in the distal small intestine.

Clinical. Two clinical vignettes relate to this section.

First, Tangier disease is a very rare autosomal recessive disease causing loss of function mutations in ABCA1. This results in extremely low serum HDL levels and impaired reverse cholesterol transport. Cholesterol esters accumulate in the reticuloendothelial system of many cells. Tangier disease causes early cardiovascular disease.

Second, clinical and pre-clinical studies showed that mutations that reduced CETP function were associated with higher HDL levels, increased reverse cholesterol transport, lower LDL levels and reduced cardiovascular events. Pharmaceutical companies developed CETP inhibitors, but multiple clinical trials demonstrated no reduction in cardiovascular disease despite significantly increased HDL levels with these medications.

Session Learning Objective 5. Outline the major pathway of de novo cholesterol synthesis. Understand the biochemical basis of statin action.

Cholesterol Synthesis

This section takes you from lipid particles within plasma back to lipid synthesis inside the cell. We already learned about cytoplasmic

regulation of fatty acid synthesis (from Acetyl CoA indirectly leaving the mitochondria via citrate). Most cells can also make their own cholesterol which is critical because cholesterol is needed for normal plasma membrane function (among other uses). Like fatty acids, production of cholesterol occurs in the cytoplasm.

Biosynthesis of Cholesterol:

The structure and carbon numbering of cholesterol are shown in Fig. 7. The first steps in cholesterol synthesis are called the mevalonate pathway.

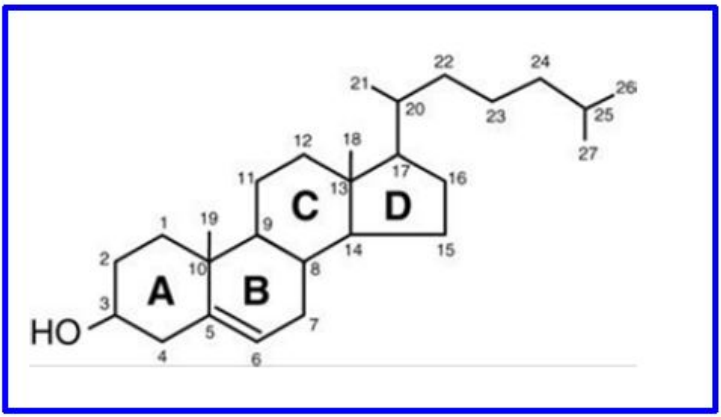


Figure 7. Structure of cholesterol

Mevalonate pathway. This pathway starts with two acetyl-CoA molecules condensing to form acetoacetyl-CoA. In the second step another acetyl CoA is added to form HMG-CoA (C6). The third step is the rate limiting step when HMG-CoA is reduced to form mevalonate. This reduction requires two NADPH and is mediated by the enzyme HMG-CoA reductase. This step is tightly controlled by transcriptional control of the enzyme and by covalent regulation (see below). Finally, mevalonate is decarboxylated and has a pyrophosphate group added in multiple steps requiring 3 ATP to

form the C5 molecule isopentenyl pyrophosphate (isoprenoid subunit).

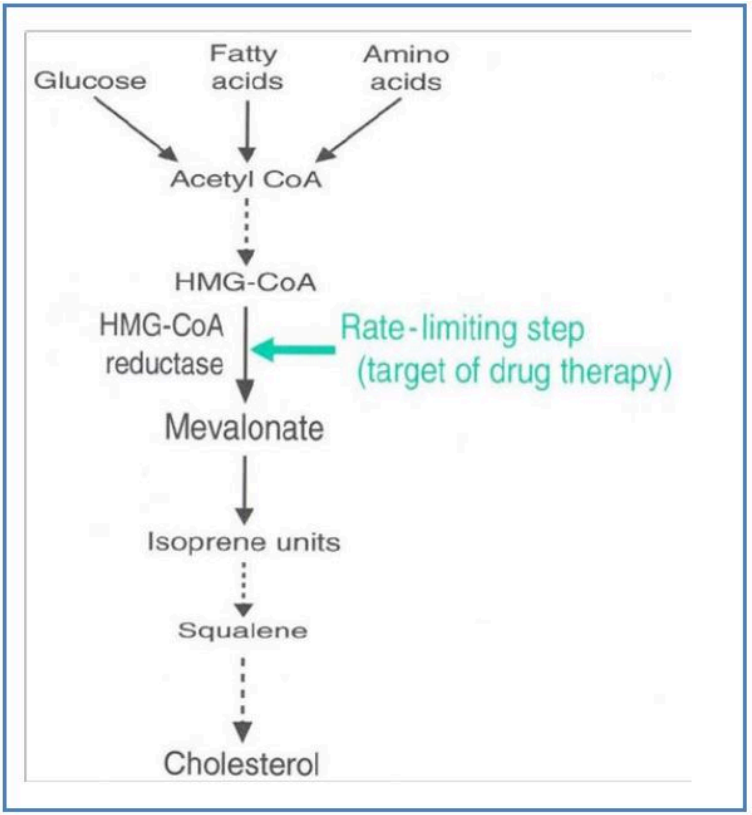


Figure 8. Overview of Cholesterol synthesis

You have already seen some of these enzymatic steps leading to HMG-CoA in hepatocyte mitochondria related to ketone production. The enzymes responsible for HMG-CoA production starting cholesterol synthesis in the cytoplasm are a different set of enzymes.

Regulation of HMG-CoA reductase activity

Covalent Control of cholesterol biosynthesis. HMG-CoA

reductase is inactivated via phosphorylation by cAMP dependent protein kinases; dephosphorylation by protein phosphatases reactivates (Figure 9), making cholesterol biosynthesis subject to hormonal control. In addition, cholesterol binds a nuclear receptor that inhibits transcription of the reductase gene. This is an example of the end product of a biosynthetic pathway inhibiting the key committed step.

Clinical. Statins: HMG-CoA reductase inhibitors are very commonly prescribed medications to lower serum LDL levels and reduce the risk of cardiovascular disease. Common examples include atorvastatin and simvastatin. The medications reduce circulating LDL in at least two ways. First, inhibition of HMG-CoA reductase directly reduces the output of the cholesterol biosynthetic pathway in all cells. In the liver this reduces the release of VLDL into the circulation reducing eventual LDL particles. Second, in addition, decreased cellular cholesterol concentration in all cells upregulates the transcription of the LDL receptor gene, and LDL receptor expression on the cell surface. This increases clearance of LDL from plasma. Side effects of statins include muscle aches, and hepatitis, at least partly related to inadequate intracellular cholesterol levels.

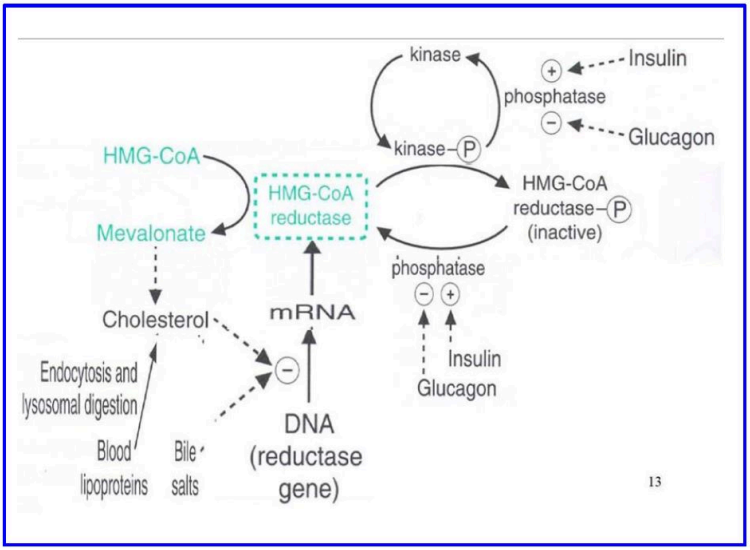


Figure 9 Regulation of Cholesterol Synthesis

Subsequent steps in Cholesterol Synthesis: Multiple complex enzymatic steps result in the polymerization and cyclization of 6 isoprenoid units (C30 molecule called squalene) and then demethylation and migration of double bonds eventually yields cholesterol (C27).

Cholesterol can be esterified at the number 3 carbon with long chain fatty acids (e.g., oleate, linoleate). This molecule is more hydrophilic, easier to transport in plasma, and is not a membrane structural lipid. Note that the planar structure of Fig. 7 does not represent the 3D shape of molecular cholesterol.

Isoprenoid subunits: These C5 building blocks are important for the structure of many molecules including vitamin A and E, the mammalian hormone Vitamin D, as well as carotenes and xanthophylls, which have effects on immune function among other effects.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=198#h5p-30

Review questions:

1. What are chylomicrons used for?
2. How does the pathway for endogenous lipid transport differ from the exogenous pathway?
3. What is the role of lipoprotein lipase? Where is it found?
4. Explain the function of bile salts.
5. Explain the function of the LCAT enzyme
6. What are the functions of the Apo C, Apo E, Apo A and Apo-B100 lipoproteins?
7. What is the rate limiting step of cholesterol biosynthesis?
8. Explain the diverse mechanisms by which the activity of HMG-CoA reductase is regulated.
9. How do statins affect cholesterol biosynthesis?
10. Outline the key steps of the de novo cholesterol biosynthesis pathway

8. Lipid Metabolism, Lipid Disorders, Arachidonic Acid Metabolism

This Pressbook covers three topics, each with its own session learning objectives: and 1) lipid metabolism, 2) ketone bodies and 3) Arachidonic Acid and Eicosanoids.

Fatty acids are important for the formation of cell membrane bilayers and intracellular organelles and are key elements for regulating transport of molecules across extracellular and intracellular compartments. Fatty acids and triglycerides are a major source of energy for the body, including the heart and skeletal muscle. Specialized functions of lipids include the development of surfactant in the lung, formation of bile to facilitate the transport and absorption of dietary lipids in the gut, insulating nerves as myelin, to name only a few. Lipids also serve as signaling molecules, serving as targets of lipid kinases and substrates for eicosanoid signals. The lipid cholesterol is a structural component of all cell membranes and is a precursor to bile acids and steroid hormones including vitamin D. Appropriate cellular levels of cholesterol are essential for normal function. To ensure this, the body obtains cholesterol from the diet and synthesizes cholesterol in a carefully regulated series of steps. Fatty acids and cholesterol are shuttled around the body in large lipoprotein particles. Disorders of lipid metabolism are involved in major clinical disorders including atherosclerosis, diabetes, and obesity.

Session Learning Objectives and Brief Overview

Session Learning Objective 1. Analyze the oxidation of fatty acids, including pathway regulation, the carnitine shuttle and generation of ATP.

— fatty acids enter the mitochondrial matrix through the

carnitine shuttle and are oxidized to acetyl CoA by specific acyl CoA dehydrogenases, NADH is produced directly by the oxidation cycles and through the TCA cycle. ATP production is high compared to carbohydrate metabolism.

Session Learning Objective 2. Diagram the pathways for fatty acid storage and release from adipose tissue and their regulation, highlighting the impact of insulin and counterregulatory hormones (listed twice below- once with storage and once with release).

— white adipose tissue stores triglycerides in large intracellular lipid droplets. Insulin stimulates TG storage. Low insulin levels and counterregulatory hormones stimulate hormone sensitive lipase (HSL) which degrades TG into free fatty acids and glycerol.

Session Learning Objective 3. Outline the pathway and regulation of de novo lipid biosynthesis.

— high cellular energy allows for production of fatty acids in the cytoplasm. Starting from mitochondrial intermediates that need to be shuttled into the cytoplasm.

Session Learning Objective 4. Explain how and why ketone bodies are formed and how they are utilized.

— ketones are produced from acetyl CoA and released by the liver during fasting. Many tissues take up and metabolize ketones for energy.

Session Learning Objective 5. Understand the biochemical mechanism behind examples of lipid metabolism dysfunction, including carnitine deficiency and medium chain fatty acyl CoA deficiency.

— Clinical SLO. Monogenic disorders exist that impair beta oxidation and present as failure to thrive in infants/children.

Session Learning Objective 6. Compare the significance of arachidonate derivatives (COX inhibition by aspirin, prostaglandins, leukotrienes).

— understand the initial steps in the arachidonic acid pathway including the role of phospholipase A2, COX-1 and COX-2 enzymes and the medications that inhibit these steps.

Session Learning Objective 7. Recognize the roles of eicosanoids in diverse physiological processes and their roles as inflammatory mediators.

— understand clinical examples of the roles of specific prostaglandins and thromboxanes in causing symptomatic disease.

Fatty Acid Oxidation Overview. Fatty acid oxidation is a key source of ATP to fuel cellular energy needs. Fatty acids are stored as components of triglycerides (TG) in adipose tissue and released into the blood in response to epinephrine. Most cells (not neurons, not red blood cells) can use fatty acids for fuel. In exercising muscle, fatty acids are oxidized to acetyl CoA, which enters the TCA cycle to generate ATP. In the liver, ATP derived from fatty acid oxidation is used as an energy source for gluconeogenesis. The liver converts fatty acid derived acetyl CoA to ketones, which can be used by many cells (including neurons after a period of adaptation) as a source of ATP production.

Fatty acid synthesis Overview: Fatty acids (mostly C16 palmitate) are synthesized from acetyl CoA in the cytoplasm. The main tissues where fatty acid synthesis occurs are liver and adipose tissue, although synthesis can occur in almost all tissues. Control of the biosynthetic pathway occurs at the first committed step: the synthesis of malonyl CoA, catalyzed by acetyl CoA carboxylase. In addition to its role as a substrate for fatty acid biosynthesis, malonyl CoA plays a key role in coordinating fatty oxidation and synthesis. The synthesized palmitate can be modified within the cell. Enzymes of the endoplasmic reticulum can introduce double bonds and elongate beyond 16 carbons (palmitate). Synthesized fatty acids can be esterified to glycerol and stored as triglycerides. Many tissues also use synthesized fatty acids to make phospholipids. Insulin is the main hormone that promotes synthesis of fatty acids and triglycerides.

Eicosanoid Overview: Arachidonate (a C20:4, Δ5,8,11,14 polyunsaturated fatty acid) is a precursor for a group of complex lipids that mediate numerous biological processes. Collectively, these substances are termed eicosanoids and serve as a

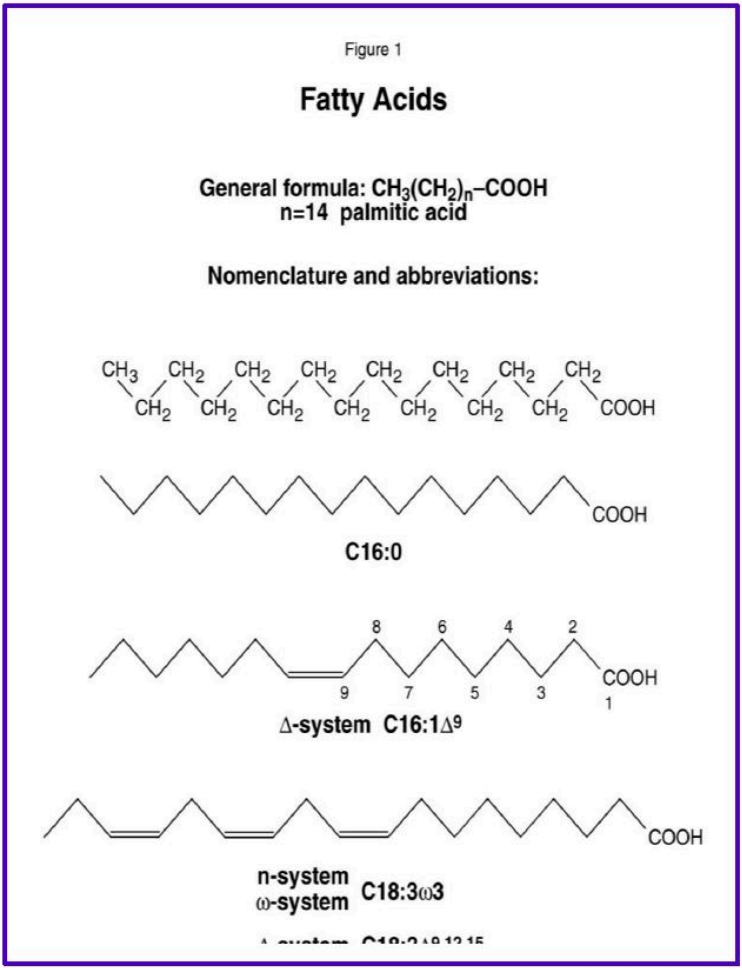
communication pathway between cells that can alert surrounding cells related to cell damage and inflammation. The biosynthetic pathway begins with the release of arachidonate from glycerophospholipids in the inner leaflet of the plasma membrane, catalyzed by the enzyme phospholipase A2. Further metabolism of arachidonate is accomplished by at least different chemical reactions in various tissues resulting in production of prostaglandins, leukotrienes, and lipoxins. Because of their key roles as inflammatory mediators, medications have been developed that alter eicosanoid signaling.

Session Learning Objective 1. Analyze the oxidation of fatty acids, including pathway regulation, the carnitine shuttle and generation of ATP.

Fatty Acid Oxidation

Goals: 1. Appreciate different classes of fatty acids; 2. Analyze the oxidation of fatty acids and explain why it yields more energy than catabolism of carbohydrates or proteins. 3. Explain how fatty acids are transported into the inner mitochondrial matrix and activated for beta-oxidation. 4. Explain how fatty acids are mobilized from adipose stores; 5. Be cognizant of the difference between beta-oxidation of odd carbon versus even carbon fatty acids.

Chemical properties of Fatty Acids: Fatty acids consist of a long hydrocarbon chain and a hydrophilic carboxylic acid group. Systems of nomenclature for fatty acids are shown in Figure 1.

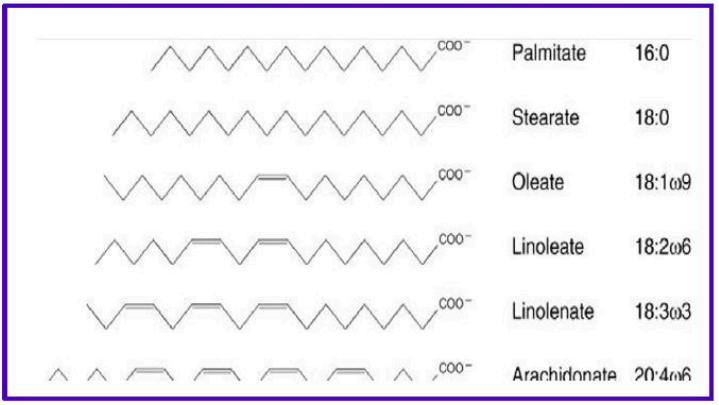


Free fatty acids (FFA or NE(non-esterified)FA) are poorly soluble in water. When present at too high levels their detergent properties cause disruption of cell membranes. FA circulate in plasma bound to the liver protein albumin. Various FA are components of many other molecules including glycerophospholipids, sphingolipids, cholesteryl esters and acylated proteins. The most abundant fatty

acids have 16- or 18-carbon atoms although longer fatty acids (>20 carbons) are present in the lipids of most tissues. Double bonds, when present in a mammalian fatty acid, are always separated by a methylene (-CH2) and are of the cis configuration. Cis double bonds make the carbon chain less straight and cannot be packed as closely together (why butter (saturated) is solid while olive oil (unsaturated) is liquid at room temperature).

Structural representations of some common fatty acids are given in Fig. 2. Fatty acids that are linked to a hydrophilic phosphate containing group are called phospholipids, and are a main component of lipid bilayers. A fatty acid that is part of a different molecule is called an acyl group. Fatty acids are stored in the body as triglycerides primarily in adipocytes, but also in skeletal muscle and in liver. Excess TG storage in cells other than adipocytes eventually harms the cell.

Figure 2: Some common fatty acids



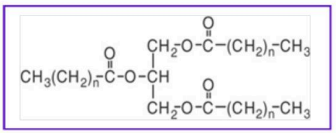


Figure 3: Chemical structure of triglycerides: Glycerol head group + 3 esterified FA

Session Learning Objective 2 Part I. Diagram the pathways for fatty acid storage and release from adipose tissue and their regulation, highlighting the impact of insulin and counterregulatory hormones.

Mobilization and Utilization of fatty acids for energy

Triglycerides are stored in adipose tissue as large fat droplets within adipocytes. In order to be used as fuels, the fatty acids must be released and sent to tissues that require energy (such as liver or exercising muscle), transported, and oxidized. The products of complete oxidation of a fatty acid are CO_2 and H_2O.

Release of fatty acids from triglycerides in white adipose tissue. Release of fatty acids involves the enzymatic hydrolysis of the ester bonds that link them to glycerol by one of several triglyceride lipases (Fig. 4). A major enzyme is hormone- sensitive lipase (HSL). The activity of this enzyme is controlled by phosphorylation through a G protein-coupled receptor (GPCR) cascade (similar to the activation of glycogen phosphorylase in carbohydrate metabolism). The hormone that consistently stimulates lipolysis by activating HSL is epinephrine signaling through β receptors. The roles of glucagon and growth hormone on lipolysis are less clear. The ability of cortisol to stimulate lipolysis vs lipid storage appears to be fat depot specific. The absence of insulin alone also activates HSL. Both fatty acids and glycerol are released from adipocytes into the blood. The free fatty acids immediately associate with plasma albumin. This increases their solubility and limits their deleterious detergent properties.

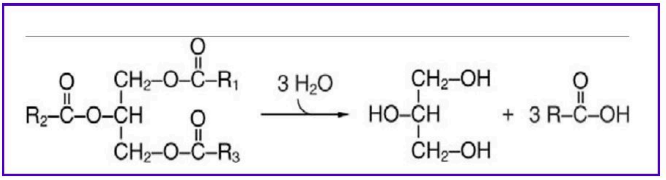
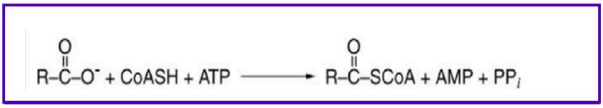


Figure 4: Action of hormone-sensitive lipase: Hydrolysis of TG into glycerol and FA

Uptake and activation of fatty acids and formation of Fatty Acyl-CoA. Fatty acids are taken up by various cells from the blood and

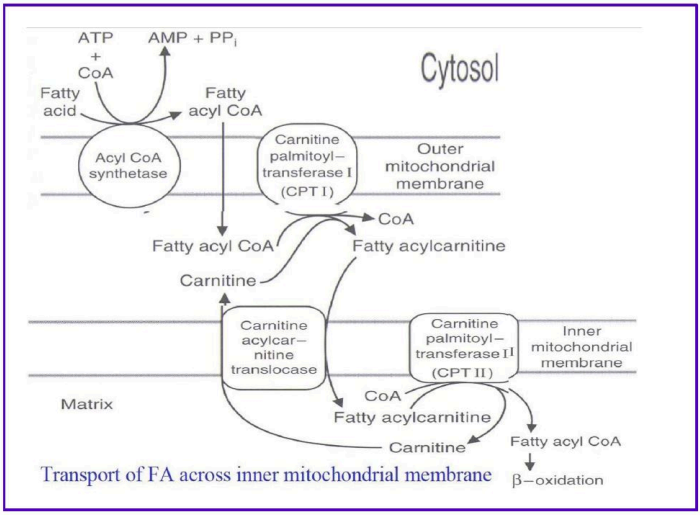
used as fuels for metabolic processes. Uptake of fatty acids by cells is mediated by cellular transporters, and also involves cytosolic fatty acid-binding proteins, which act as intracellular carriers. Specific fatty acids can also bind to receptors (both membrane and cytoplasmic) and alter intracellular signaling and/or nuclear gene transcription. In the endoplasmic reticulum or the outer mitochondrial membrane fatty acids are activated by addition of coenzyme A (CoA), generating fatty acyl-coA derivatives. This reaction requires ATP and is mediated by fatty acyl-CoA synthetase (Fig. 5).

Figure 5: Synthesis of FA-coA derivatives catalyzed by the action of **fatty acyl-coA synthase** enzym



Entry of fatty acids into the mitochondrion. Fatty acid oxidation occurs in the mitochondrial matrix. Acyl-CoA molecules need a transport mechanism into the mitochondria because the inner mitochondrial membrane is not permeable to activated or free long chain fatty acids. The process involves a co-transport system in which a fatty acylcarnitine enters simultaneously with the exit of carnitine (Fig. 6). Synthesis of acylcarnitine in the intermembrane space is catalyzed by carnitine palmitoyltransferase-I (CPT-I), whereas regeneration of acyl CoA on the matrix side of the inner membrane involves an enzyme called CPT-II. Carnitine:acylcarnitine translocase facilitates the exchange of acylcarnitine and carnitine across the inner membrane to complete the process. An important control point is that CPT-I is inhibited by malonyl CoA, an intermediate in fatty acid synthesis (so that fatty acid synthesis and beta oxidation are not occurring in the same cell at the same time).

Figure 6: **Transport of FA across the inner mitochondrial membrane**



Board Focus: Extremely rare disorders of fatty acid mitochondrial transport are a board favorite. Inherited disorders due to CPT-I and CPT-II are described. The key to answering the question will be in the stem to determine where the acyl-CoA is getting stuck. Carnitine deficiency also occurs and can be inherited or acquired. This causes reduced fatty acid transport into the mitochondria. Critical illness is associated with acquired carnitine deficiency and you will commonly see ICU patients receiving carnitine supplementation.

Reactions of fatty acid oxidation. Four different reactions are used to oxidize fatty acids by 2C at a time into acetyl CoA and a shorter acyl CoA (Fig. 7).

Figure 7: β-oxidation of fatty acids in the inner mitochondrial matrix

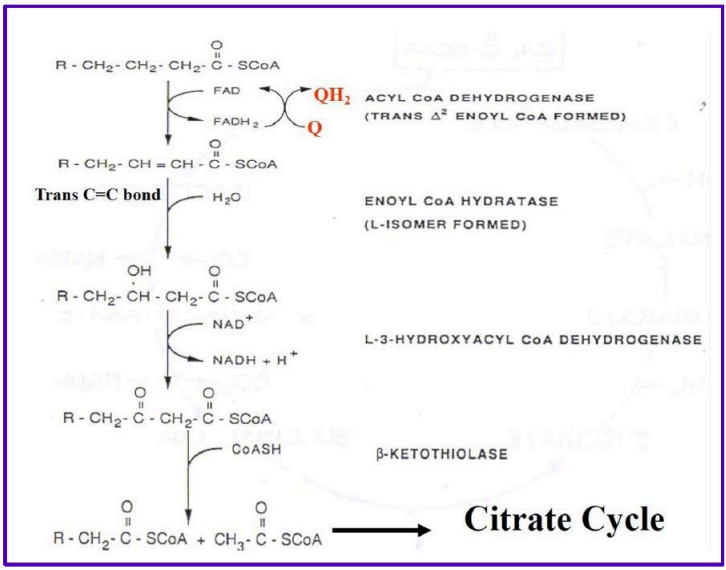


Figure 7: β-oxidation of fatty acids in the inner mitochondrial matrix.

The process is called β-oxidation, because it is the second or β-carbon that is cleaved from the fatty acid. Importantly, the oxidation reactions alone generate FADH2 and NADH in the mitochondrial matrix which can directly enter the electron transport chain to generate ATP. Importantly, this ATP production is not dependent on the TCA cycle and occurs even when the TCA cycle is inactive (eg. during gluconeogenesis). The acetyl CoA enters the TCA cycle as you have already seen in carbohydrate metabolism to generate even more ATP, or is packaged into ketones and released (see below). Additional rounds of acyl CoA oxidation and cleavage continue until the fatty acid is converted completely to acetyl CoA, if the fatty acid has an even number of carbons, eg: C16 palmitoyl CoA. Fatty acids with an odd number of carbon-atoms finish mitochondrial beta oxidation with the 3C propionyl-CoA which is rapidly converted

to succinyl-CoA (4C intermediate) by enzymes used in amino acid catabolism and replenishes the TCA cycle (see Fig. 8).

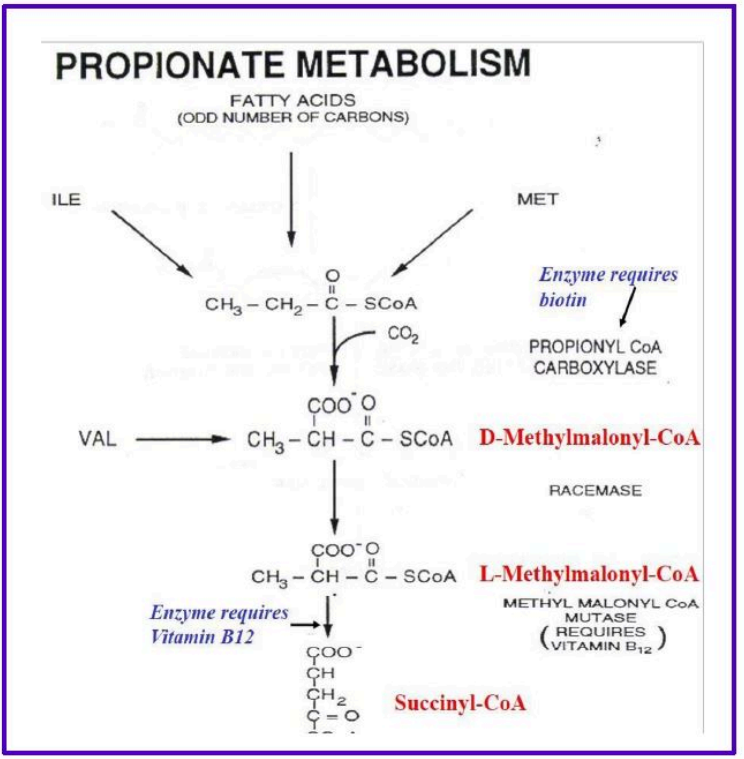


Figure 8. Propionate metabolism.

Fatty acid oxidation in the peroxisome. Peroxisomes take on some of the work of oxidizing fatty acids. These organelles have similar enzymes to oxidize fatty acids, but expressed from different genes. Peroxisomes mostly help metabolize fatty acids that are unsaturated, are longer than 22C, are branched or have an odd number of carbons. Additional enzymatic steps are needed to metabolize unsaturated fatty acids (needs a reductase) and branched chain fatty acids. The FADH2/NADH, propionyl CoA and

Acetyl CoA generated in the peroxisome needs to be transferred to the mitochondria to be fully metabolized into ATP.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-22

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-23

Practice questions:

1. How is fatty acid β-oxidation regulated?
2. What is the function of fatty acyl CoA synthase?
3. Why does the degradation of fatty acids yield more energy than the degradation of glucose?
4. Explain how fatty acids are transported across the inner mitochondrial membrane
5. What are the end products of fatty acid β-oxidation?

SLO3. Outline the pathway and regulation of de novo lipid biosynthesis.

Fatty Acid Biosynthesis

Goals: 1. Define the similarities and differences between fatty acid synthesis and fatty acid oxidation; 2. Understand how fatty acid synthesis is regulated, and how this differs from the pathway of fatty acid degradation.

Cytoplasmic fatty acid synthesis: Fatty acids are synthesized by stepwise addition of 2-carbon units to the carboxyl end of acetate.

The 2- carbon units are derived from malonyl CoA, a 3-carbon compound, generated from Acetyl-CoA and CO_2. This reaction is catalyzed by acetyl CoA carboxylase (ACC) and occurs in the cytoplasm.

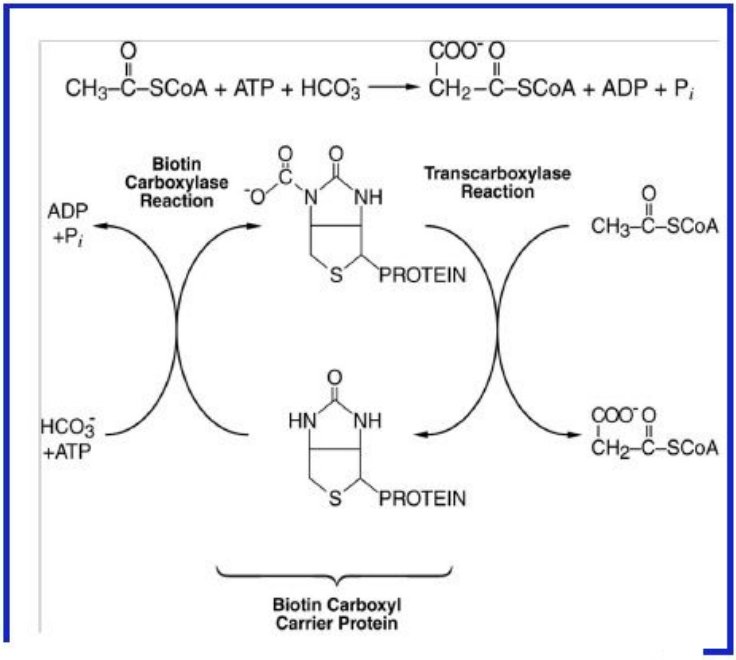


Figure 9: Synthesis of malonyl-CoA from acetyl-CoA, CO2/ HCO3-, and ATP by Acetyl- CoA Carboxylase.

Synthesis of Malonyl-CoA. This reaction occurs in two steps (Fig. 9) and involves a multi-enzyme complex. ATP is required to generate an activated intermediate (carboxybiotin) followed by transfer of the CO2 group to acetyl CoA. This reaction is the rate limiting step of fatty acid synthesis and is the main site of control of the pathway.

Session Learning Objective 2 Part II. Diagram the pathways for fatty acid storage and release from adipose tissue and their

regulation, highlighting the impact of insulin and counterregulatory hormones.

Regulation of ACC

Short term regulation of ACC: Cytoplasmic citrate is an allosteric activator (positive feedback). The enzyme is inactivated by palmitate, the end product of the fatty acid synthase (FAS) reaction (negative feedback). The activity of ACC is also controlled by hormones; epinephrine and glucagon indirectly stimulate phosphorylation of ACC, which inactivates the enzyme, while insulin indirectly causes dephosphorylation and activates the enzyme (similar to how the activity of glycogen synthase is regulated by these hormones).

Long term regulation of ACC: The ACC enzyme is under complex transcriptional control affecting levels in the cell, including by insulin and glucagon. A high carbohydrate and low fat diet also increases ACC gene transcription.

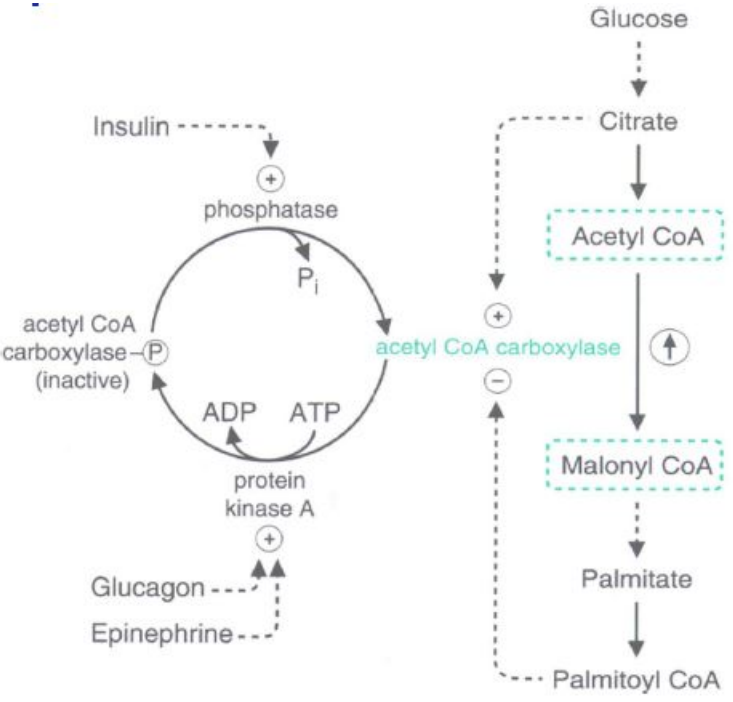


Figure 10: Allosteric and hormonal regulation of acetyl coA carboxylase.

Reactions catalyzed by fatty acid synthase (FAS). FAS consists of two copies of a large protein which has all the activities needed for synthesis of palmitate. Processing of the growing fatty acid by the dimeric enzyme occurs while it remains covalently attached to the enzyme. During biosynthesis, the elongating fatty acid chain and malonyl-CoA are covalently bound to FAS via two different sulfhydryl groups (Figure 11), including one originating from a coenzyme-A like moiety referred to as "acyl carrier protein" (ACP). ACP tagging of fatty acids is a characteristic feature of FA synthesis, in contrast to -CoA tagging for degradation

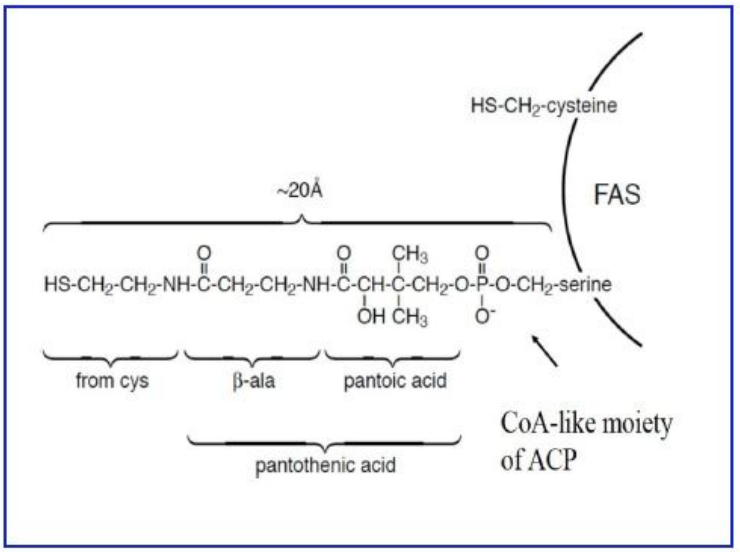


Figure 11.

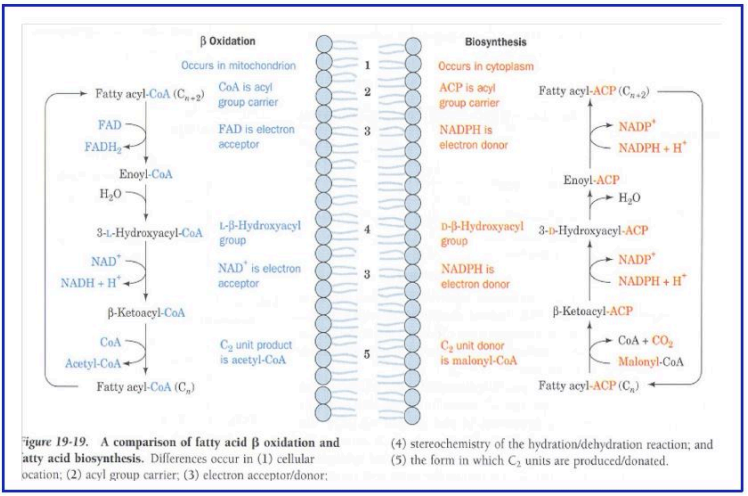


Figure 19-19. **A comparison of fatty acid β oxidation and fatty acid biosynthesis.** Differences occur in (1) cellular location; (2) acyl group carrier; (3) electron acceptor/donor; (4) stereochemistry of the hydration/dehydration reaction; and (5) the form in which C_2 units are produced/donated.

Figure 12: Comparison between fatty acid synthesis and fatty acid oxidation

Transport of acetyl-coA to the cytoplasm for fatty acid synthesis. The coenzyme-A portion of acetyl-CoA cannot cross the inner mitochondrial membrane. Thus, in order for acetyl-coA to be transported from the mitochondrial matrix to the cytosolic side of the inner mitochondrial membrane, acetyl-coA must first condense with oxaloacetate (OAA, also can't cross the inner mitochondrial membrane) to form citrate. Citrate is then transported across to the cytosolic side, where it is re-converted to OAA and acetyl-coA by the action of ATP citrate lyase. Cytosolic OAA is reduced back to malate, which undergoes oxidative decarboxylation by action of the malic enzyme to generate NADPH, CO2, and pyruvate. The NADPH produced is used as reducing power for fatty acid synthesis in the cytosol; pyruvate is transported back to the mitochondrial matrix to be reconverted to OAA by the action of pyruvate carboxylase (Figure 13). It is important to regenerate OAA, as it is a key intermediate of the TCA/Krebs cycle.

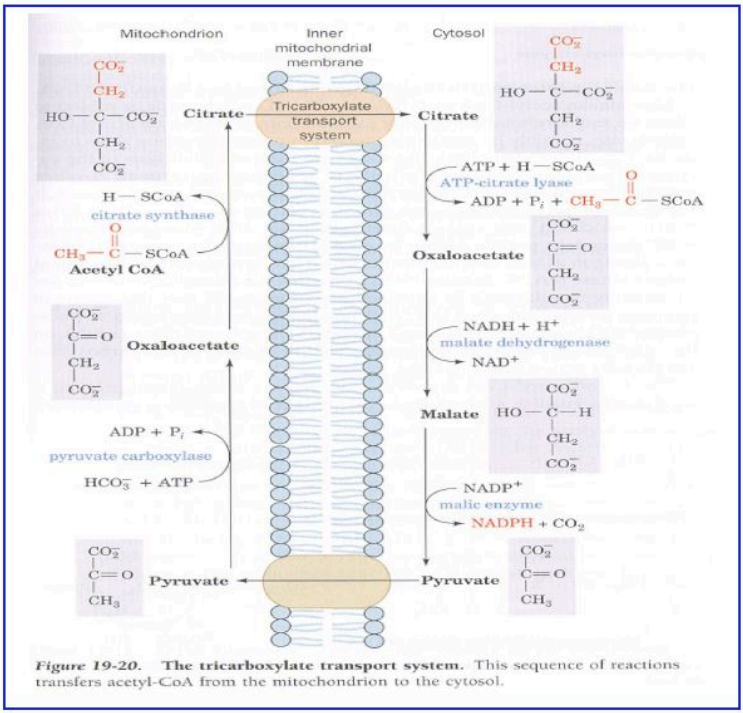


Figure 19-20. **The tricarboxylate transport system.** This sequence of reactions transfers acetyl-CoA from the mitochondrion to the cytosol.

Figure 13: Transport of acetyl-CoA from the mitochondrial matrix to the cytoplasm.

Elongation and Desaturation: The product of FAS (palmitate) has 16 carbons and no double bonds. Palmitate can be elongated and desaturated to produce long chain fatty acids with multiple double bonds. Elongation to 18, 20 and 22 carbon fatty acids occurs in two compartments: the mitochondrion where acetyl CoA is used for elongation, and on the membranes of the endoplasmic reticulum where malonyl-CoA is used. The additional 2-carbon units are added to the carboxyl end of the fatty acid in both cases.

Introduction of double bonds (desaturation): Most organisms possess fatty acyl CoA desaturases that introduce double bonds into fatty acids. Remember the double bonds are always in the cis-

configuration. Most of you have heard of omega-3 fatty acids (from fish oils). This is an older nomenclature and indicates where the first double is located counting from the last carbon (not affected by elongation). A more modern system uses a delta (Δ) and a superscripted number to locate the position of the double bond relative to the carboxyl of the fatty acid (see Fig. 1). Humans have three fatty acyl CoA desaturases with specificities for introduction of a double bond at Δ – 9, Δ -6, or Δ -5 positions. Synthesis of fatty acids generally follows these rules:

1. The first double bond is introduced between carbons 9 and 10 (Δ – 9 position), counting from the carboxyl.
2. If a double bond is already present, the next one is introduced closer to the carboxyl, separated from the first by a methylene (-CH2).

Thus, combinations of desaturation and elongation can lead to synthesis of a variety of unsaturated fatty acids (Fig. 14).

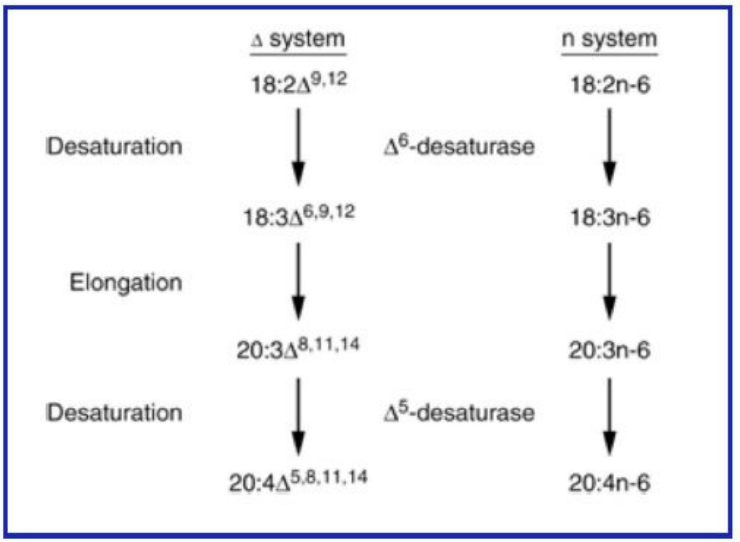


Figure 14: Elongation and desaturation of fatty acids.

However, humans are unable to synthesize fatty acids with double bonds farther from the carboxyl end, eg. Δ12 or Δ15. These fatty acids are considered 'essential fatty acids' because they need to be consumed in the diet to prevent pathology. The main essential fatty acids are linoleic acid (18:3), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:5). All of these fatty acids have double bonds at distant positions (omega 3 or omega 6).

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-25

Practice questions:

1. How is fatty acid synthesis regulated?
2. What is the function of acetyl-coA carboxylase (ACC)?
3. How is the activity of the ACC enzyme regulated?
4. How does fatty acid synthesis differ from beta oxidation of fatty acid
5. Explain how acetyl coA is transported from the mitochondrial matrix and across the inner mitochondrial membrane to the cytosol to be made available for fatty acid synthesis.
6. How are fatty acids longer than 16-carbon chains produced?
7. Now that you understand the role of citrate in catabolism and anabolism, can you explain why citrate inhibits PFK-1, the key committed step of glycolysis?

Session Learning Objective 4. Explain how and why ketone bodies are formed and how they are utilized.

Ketone Synthesis and Metabolism

Goals. 1 Understand where, when and why ketone bodies are synthesized. Identify which tissues can metabolize ketones for fuel. 3. Describe the adaptations that need to occur for neurons to metabolize ketones.

Ketone production and utilization. Fatty acid mobilization and metabolism doesn't always lead to complete oxidation to CO2 in the liver. Acetoacetate and β-hydroxybutyrate, commonly referred to as ketones (also called ketone bodies) are also produced from fatty acids and exported to other tissues (heart, skeletal muscle, others) where they are used as sources of acetyl CoA for energy production. Ketone synthesis (Fig. 15) starts with a thiolase joining two acetyl CoA molecules to form acetoacetyl CoA. Then HMG-CoA synthase (mitochondrial) adds another acetyl-CoA to produce β-hydroxymethylglutaryl CoA (HMG-CoA). This product is cleaved to yield acetyl CoA and acetoacetate by HMG-CoA lyase. β-hydroxybutyrate is produced from acetoacetate by a dehydrogenase requiring NADH. The liver has the largest amounts of HMG-CoA lyase (the cleavage enzyme) and hence is the main site of acetoacetate production. Note the HMG-CoA in the mitochondria is different isozyme from and regulated differently from HMG-CoA in the cytoplasm which is essential for cholesterol synthesis.

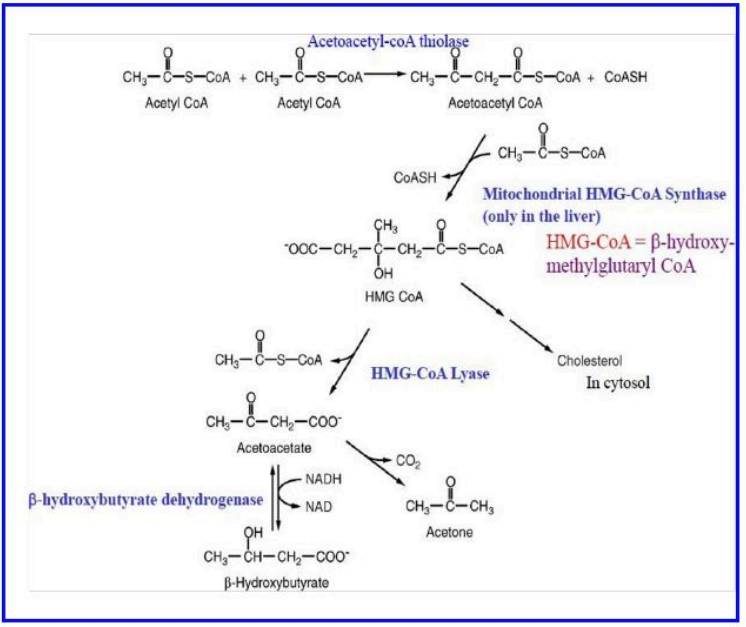


Figure 15: Biosynthesis of ketones (in liver mitochondria).

Ketones are readily taken up from the blood by many tissues via a family of monocarboxylate transporters (MCT). The brain normally does not metabolize ketones for fuel. With prolonged (48-72 hour) starvation both the MCTs and the mitochondrial enzymes are upregulated and neurons begin to use ketones efficiently for fuel. This adaptation by neurons is a critical step in reducing the amount of glucose the liver (and kidneys) need to produce through gluconeogenesis. Metabolism of β-hydroxybutyrate occurs in mitochondria and involves oxidation to acetoacetate, activation by conversion to acetoacetyl CoA, cleavage to 2 molecules of acetyl CoA by β-ketothiolase, and oxidation of acetyl CoA via the TCA cycle. Activation of acetoacetate utilizes succinyl CoA and an enzyme that catalyzes an acyl- transfer reaction (SCOT, succinyl CoA ketoacid CoA transferase) (Figure 16). Non-neuronal cells in the brain, astrocytes being the best example, can metabolize fatty acids (neurons cannot) and can produce ketones in low amounts for neurons to metabolize.

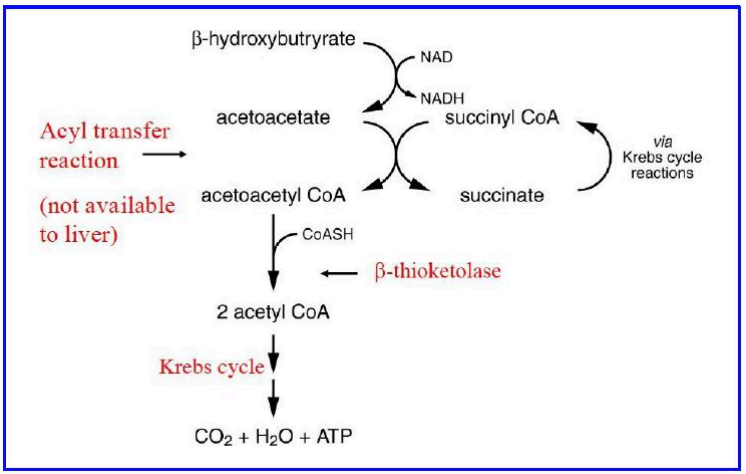


Figure 16: Metabolism of β-hydroxybutyrate in tissues (activation & oxidation of ketones for energy production.

The liver is the most active organ in the production of acetoacetate but lacks the acyl transferase necessary to metabolize ketones, thus ensuring that hepatic ketones exit the cell and are not metabolized by hepatocytes. Ketone metabolism is important in many tissues during fasting as a fuel source. Ketones can accumulate in the blood in various conditions. The most common is Type 1 Diabetes, when very low insulin signaling causes very high rates of triglyceride lipolysis and very high rates of ketone production. This can lead to diabetic ketoacidosis. Other conditions (such as ethanol toxicity) can also cause ketoacidosis. Fig. 16 summarizes the mobilization of triglycerides and subsequent metabolic processing of fatty acids for energy or ketone synthesis.

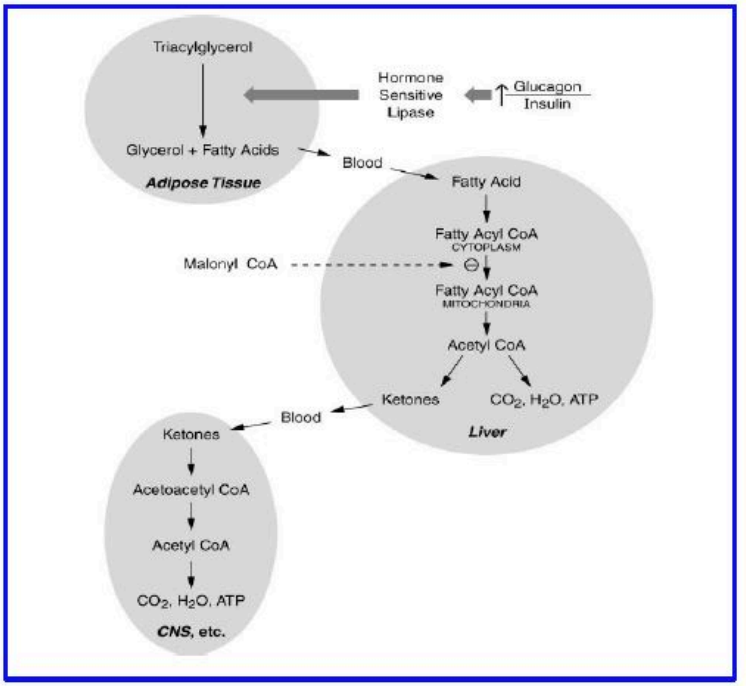


Figure 17: Summary of fatty acid oxidation and ketone body production and utilization.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-24

Session Learning Objective 5. Understand the biochemical mechanism behind examples of lipid metabolism dysfunction, including carnitine deficiency and medium chain fatty acyl CoA deficiency.

Boards Focus: Infants presenting with hypoglycemia and lethargy (failure to thrive) is a common clinical scenario on the boards (not common in practice). Always check the question stem to see if ketones are present. If ketones are present then lipid oxidation is occurring and the diagnosis is likely related to carbohydrate metabolism. If ketones are absent then the problem is with fatty acid transport or fatty acid oxidation (see below).

Inherited diseases of fatty acid oxidation. Several different fatty acyl CoA dehydrogenases catalyze the first step of fatty acid oxidation, including those specific for very long chain fatty acids (VLCAD, very long chain acyl CoA dehydrogenase), long-chain (LCAD), medium chain (MCAD) and short chain (SCAD). Rare genetic disorders cause disorders related to the function of these enzymes. Mutations are most common in the gene for MCAD and result in fasting hypoglycemia. This secondary disturbance in carbohydrate metabolism resulting from a primary defect in the ability to efficiently oxidize fatty acids points out the extent to which fuel metabolic pathways are related to one another. Gluconeogenesis is required for plasma glucose homeostasis during fasting and requires considerable energy (as we have seen earlier). The inability to oxidize fatty acids efficiently reduces the amount of energy that

can be derived from fat metabolism and the diminished gluconeogenesis results in fasting hypoglycemia.

Practice questions:

1. Why are ketone bodies only made in the liver?
2. What are ketone bodies used for in peripheral tissues?
3. Why is ketone body metabolism so relevant to Type 1 diabetes and fasting?
4. Explain how ketone bodies are made.
5. Explain how ketone bodies are activated and oxidized in tissues such as the CNS.
6. In which organelle are ketone bodies synthesized?

Session Learning Objective 6. Compare the significance of arachidonate derivatives (COX inhibition by aspirin, prostaglandins, leukotrienes).

Session Learning Objective 7. Recognize the roles of eicosanoids in diverse physiological processes and their roles as inflammatory mediators.

Arachidonic Acid and Eicosanoid Production.

Goals: 1. Understand the roles of eicosanoids in diverse physiological processes 2. Appreciate the tissue specific pathways for eicosanoid biosynthesis.

Eicosanoids are cellular signaling molecules. These molecules give cells the opportunity to change their own activity (autocrine) or alter the activity of surrounding cells (paracrine), but usually do not cause a signal in distant tissues (endocrine). While endocrine mediators (hormones) such as insulin are produced in large quantities by specialized glands, eicosanoids can be produced in very small amounts in almost all tissues. Eicosanoids are not stored and have a very short half life, being rapidly metabolized to inactive products. Most eicosanoid signals are produced as part of a cellular response to stress. Effects include a wide range range of responses, both physiological/tissue protection and repair, and pathological/ hypersensitivity and allergy responses. Eicosanoids can cause the classic four signs of inflammation, pain (dolor), warmth (calor), redness (rubor) and swelling (tumor), through vasodilation, changes

in vascular permeability and triggering pain sensors. As key mediators of inflammation, there are multiple medications that target eicosanoid pathways.

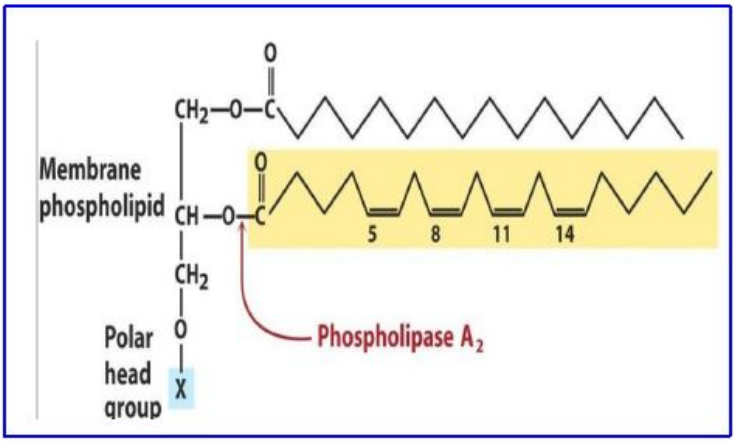


Figure 18. Arachidonic Acid and phospholipase A2.

Release of arachidonic acid. Arachidonic acid is a 20 carbon fatty acid and a precursor for all major classes of eicosanoids, but the free concentration of this fatty acid in cells is very low. Eicosanoid production begins with release of arachidonic acid (Fig. 17). A very specific lipase called **phospholipase A2** catalyzes the release of arachidonic acid from the middle carbon (sn2 position) of a glycerophospholipid (often phosphotidylcholine) in the inner leaflet of a lipid bilayer. Once the fatty acid enters is released, the metabolic pathway that arachidonic acid enters and the eicosanoid end products depend on tissue and cell type (Figure 18). With the exception of red blood cells, nearly all human cells can generate eicosanoid signals. Arachidonic acid is not the only fatty acid used for generating eicosanoids, but is the major metabolite.

Modification of arachidonic acid. We focus on two major pathways that modify arachidonic acid into eicosanoid signals. These are the cyclooxygenase and lipoxygenase pathways.

1. Cyclooxygenase pathway gives rise to the:

• Prostaglandins

• Thromboxanes

2. Lipoxygenase pathway gives rise to the:

• Leukotrienes

• Hydroxylated eicosanoids

• HETE = hydroxyeicosatetraenoic acid; diHETE = dihydroxyeicosatetraenoic acid. Most common: 20-HETE – hydroxyl on C-20 of arachidonate; stimulates vascular constriction (vasoconstriction), except in the lungs where 20-HETE stimulates pulmonary vasodilation.

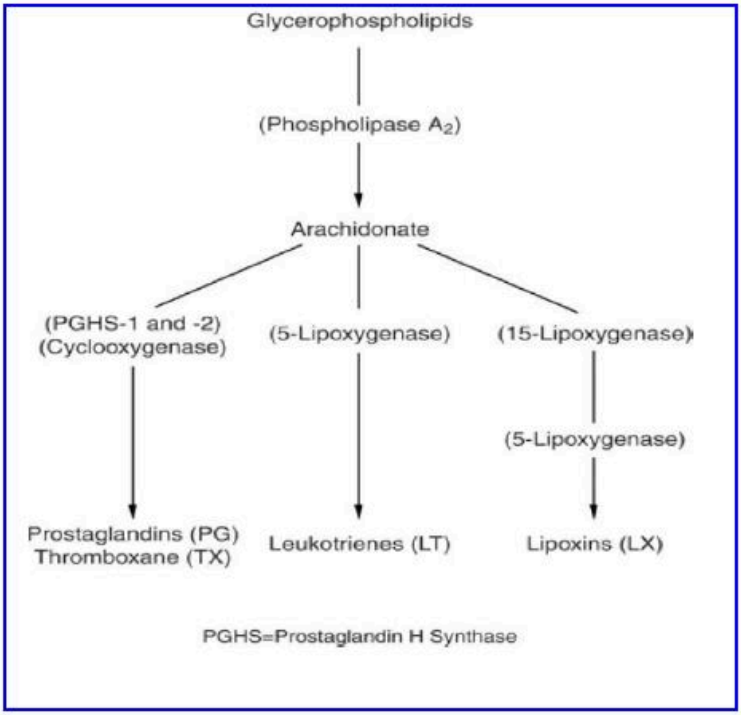


Figure 19: The eicosanoid biosynthesis pathway.
The cyclooxygenase pathway.

After release from a glycerophospholipid, arachidonate can interact with a
membrane protein, PGH2 synthase, prostaglandin H2 synthase, commonly known as cyclooxygenase (COX).

Cyclooxygenase (COX) has two distinct active sites to catalyze the conversion of arachidonate to PGH2 (Fig. 19). The first activity which adds oxygen to arachidonate is the cyclooxygenase reaction (Fig 19). The second activity of the enzyme requires NADPH to provide reducing power.

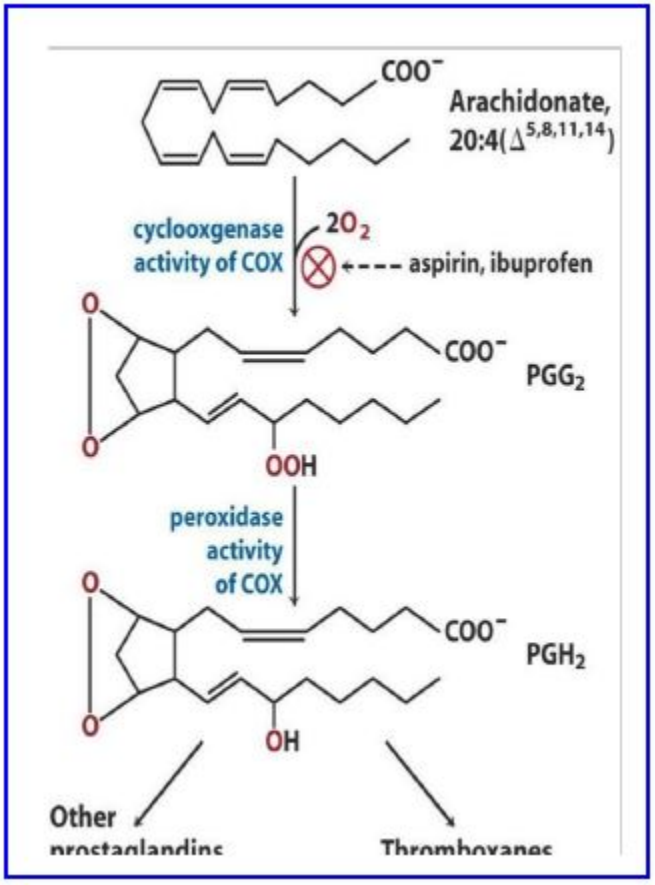


Figure 20. Modifications by cyclooxygenase.

COX isoforms. There are two main COX isoforms. COX-1 is constitutively expressed in most tissues at low levels and is important for key maintenance functions in epithelial tissue including the gut mucosa and the kidney. The expression of COX-2 is more restricted and is induced by inflammatory signals. COX-1 and COX–2 are coded by separate genes.

Tissue specific production of prostaglandins. Most tissues produce a relatively limited set of eicosanoids from arachidonate because of the tissue-specific expression of biosynthetic enzymes. For instance, PGI2 (prostacyclin) promotes vasodilation and decreases platelet aggregation and is a major product of vascular endothelium (in contrast thromboxane A2 increases platelet aggregation and is produced predominantly by activated platelets). PGD is a bronchoconstrictor and is a major product of mast cells.

Modes of action of prostaglandins. In general, eicosanoids are secreted from cells and act on the parent cell (autocrine action) or adjacent cells (paracrine action). Their instability and secretion in low amounts limits the effective range of their action. Prostaglandins exert their effects on cells through interaction with **GPCRs** (G protein-coupled receptors) affecting the activity of adenylate cyclase or PLC (phospholipase C). Those receptors coupled to Gs (stimulatory G-protein) stimulate adenylate cyclase which increases the concentration of cAMP. Those coupled to Gi (inhibitory G-protein) inhibit adenylate cyclase, decrease cAMP concentration, and are antagonistic to the Gs group. Those coupled to Gq stimulate PLC increase IP3 (inositol trisphosphate), which increases Ca2+ concentration. Nine classes of G-protein coupled receptors (GPCRs) have been discovered that mediate the effects of eicosanoids.

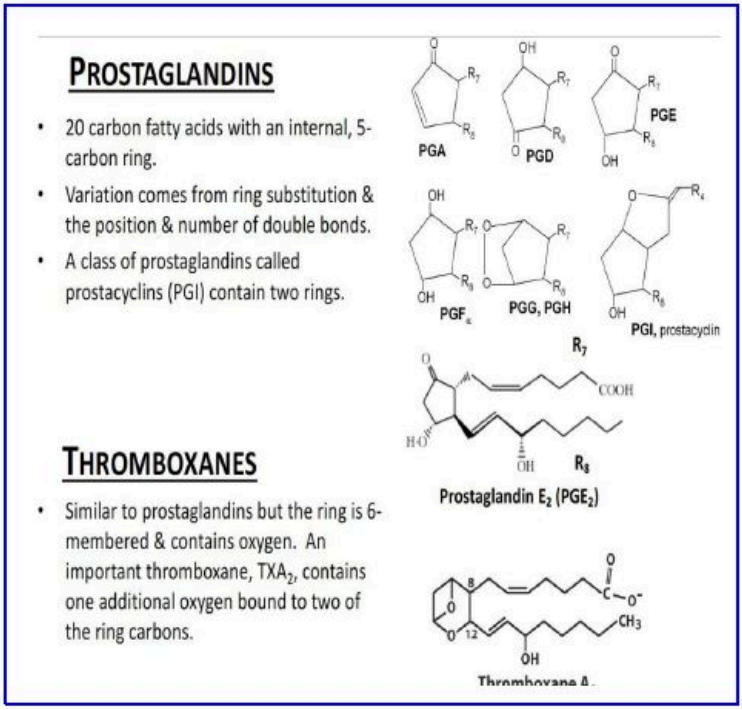


Figure 21. Prostaglandin and Thromboxane Summary.

Physiological roles of prostaglandins. This block will not cover a definitive list of all the physiological processes affected by prostaglandins because their effects are so diverse. The following section touches only on select, well-defined physiological roles.

PGE2; resorption of sodium in the kidney glomerulus; pain pathways via sensitization of neurons; house-keeping functions in gastric mucosa.

PGI2 and TXA2: the opposing roles of these PGs in vascular health has been mentioned above

PGF2α: contraction of uterine smooth muscle

PGD2: release of histamines from sensitized mast cells, vasodilator, bronchoconstrictor.

Pharmacology associated with prostaglandins. Several different classes of drugs affect prostaglandin biosynthesis and/or molecular action.

Glucocorticoids. Prednisone and dexamethasone, are examples of synthetic glucocorticoids and are frequently prescribed for relief of symptoms from inflammatory diseases. One anti-inflammatory effect of glucocorticoids is the inhibition of cytoplasmic phospholipase A1 which decreases the production of eicosanoids by preventing arachidonic acid release from the lipid bilayer.

Glucocorticoids have a wide variety of other anti-inflammatory effects on cytokine (more complex signaling molecules you will hear about later) production and gene expression of pro-inflammatory mediators in many tissues.

Aspirin. Willowbark is a traditional medicine used to alleviate fever and pain. This knowledge led to the discovery of acetyl salicylic acid (ASA or aspirin) as a pain reliever. Aspirin inhibits the activities of COX-1 and COX-2 through irreversible covalent acetylation of the enzyme, which blocks the first active site.

NSAIDs (non-steroidal anti-inflammatory drugs). A number of inhibitors of COXs have been developed by pharmaceutical companies, which collectively are known as NSAIDs to differentiate them from the steroid (glucocorticoid) analogues mentioned above. Examples of NSAIDs include ibuprofen and naproxen. These first generation drugs are relatively non-specific and inhibit both COX-1 and COX-2 to approximately the same extent. Their anti-inflammatory properties are thought to result from inhibition of COX-2 whereas their side effects (gastrointestinal bleeding, prolonged clotting times, renal failure, etc.) are believed to result from inhibition of COX-1. Ibuprofen and NSAIDs other than aspirin bind tightly, but non-covalently to COXs.

COX-2 inhibitors. Specific COX-2 inhibitors were developed in an effort to reduce the side effects associated with use of NSAIDs by not inhibiting COX-1. The medications worked in reducing joint pain without causing stomach or kidney side effects, but two medications were withdrawn from the market because long term studies showed they increased risk of heart attack (myocardial infarction) and stroke (cerebrovascular disease). One selective COX-2 inhibitor with a much shorter half life is still on the market.

Why did COX-2 inhibitors cause cardiovascular disease? The vascular endothelial product PGI2 decreases cardiovascular disease by reducing platelet aggregation, increasing vasodilation, and decreasing proliferation of vascular smooth muscle cells (SMC). In contrast, TXA2 increases platelet aggregation, promotes vasoconstriction, and increases proliferation of SMC. Thus, vessel health is highly dependent on balanced production of these two antagonistic mediators. Aspirin use is associated with reduced risk of myocardial infarctions and embolic stroke because the drug irreversibly inhibits COXs in both platelets and vascular endothelial cells. Endothelial cells, however, can rapidly renew COX levels via protein turnover (transcription and translation) whereas platelets are anuclear and are unable to synthesize new COX. Thus, the PGI2 /TXA2 balance is tipped in favor of PGI2. COX-2 is responsible for PGI2 production in vascular endothelium whereas TXA2 synthesis in platelets is largely via COX 1. Thus, specific COX-2 inhibitors with a long half life might be expected to tip the balance in favor of TXA2 and increase the risk of cardiovascular disease.

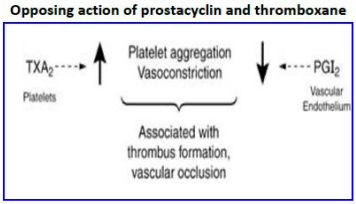


Figure 22. Opposing action of prostacyclin and thromboxane.

Acetaminophen (Tylenol):

This medications is an NSAID that decreases fever and pain through interaction with the endogenous cannabinoid system and does not have significant direct effects on eicosanoids

The Lipoxygenase Pathway:

There are six human lipoxygenase enzymes that are expressed in different tissues and at different levels. Sometimes two lipoxygenases act sequentially on arachidonic acid to generate the final eicosanoid product. Lipoxygenase inhibitors are currently less commonly used than cyclooxygenase inhibitors. A pharmacological lipoxygenase inhibitor is available for the treatment of chronic asthma.

LEUKOTRIENES:

Leukotrienes constitute the second major class of compounds derived from arachidonate. In contrast to the prostaglandins, they are all mediators of inflammation.

LIPOXINS:

These eicosanoids play important roles as anti-inflammatory lipid mediators important in resolution of inflammation and they and their receptors are important targets for pharmacological intervention in chronic inflammatory diseases.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-20

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=221#h5p-21

9. Fasting, Postprandial States Integration

Course pack for Metabolic Integration session focusing on fasting/starvation and post-prandial processes

For this session it is important that you review and understand previously learned material including:

- Glycolysis and gluconeogenesis
- Glycogen synthesis and glycogen degradation
- Fatty acid synthesis (lipogenesis) and fatty acid degradation (lipolysis)
- Ketone production

Feed/Fast metabolism is best thought of as a cycle. You may transition between fed and fasted carbohydrate and lipid metabolism several times a day depending on your meal schedule.

After a meal (post-absorptive) the body relies on glucose for baseline metabolic function in most tissues (including liver, adipose tissue). All tissues that can store fuel (glycogen or triglycerides (TG)) replenish depleted supplies and increase fuel stores. Lipid particles deliver TG and cholesterol to all tissues for repair and cell division. Cells are generally more anabolic and making or replacing proteins as cells take advantage of the abundant amino acids in plasma. Excess glucose enters the pentose phosphate pathway for boosting NADPH levels for subsequent anabolic processes. This time of plenty lasts from 2-6 hours depending on the meal. The liver takes up chylomicron remnants with TG and cholesterol and then secretes synthesized TG and cholesterol as VLDL so that cells can continue to use these molecules as needed for as long as possible after a meal.

As the absorbed nutrients from the last meal are fully metabolized the transition to the fasting state begins, marked by a switch from

glucose metabolism to fat metabolism for many cells (hormonal signals include decreasing insulin and increasing glucagon secretion from the pancreas). However, some cells (eg. neurons, red blood cells) remain dependent on glucose metabolism and would consume enough glucose over time to cause hypoglycemia (low blood sugar) without a new source of glucose (eg. while waiting for the next meal and the gut to deliver more glucose). That source of glucose is the liver and the kidneys which make glucose to prevent hypoglycemia during fasting. Glycogen synthesis is inhibited and glycogenolysis is activated as the liver begins to release glucose to prevent serum glucose from decreasing too much (you should understand how insulin/glucagon regulate this process). Glycolysis slows down and hepatic gluconeogenesis increases (you should understand how insulin/glucagon regulate this process) as TG breakdown in adipose tissue begins to deliver glycerol to the liver (understand how insulin/epinephrine regulate this process). The TG level in the blood decreases (primarily in VLDL and chylomicrons) and the FFA (bound to albumin) levels increase, and FA become the primary metabolic fuel for many tissues. Tissues (that can) switch to using beta oxidation of FA for ATP synthesis instead of glucose (brain, RBCs are important cells that cannot use FA for fuel). Muscle starts to break down and release amino acids to go to the liver as a key source of gluconeogenesis. Gluconeogenesis requires the liver to release Acetyl CoA produced from beta oxidation into the blood as ketones. This occurs because the hepatocyte TCA cycle can not operate while gluconeogenesis from amino acids is active (why is that not true for glycerol?). The ketones are rapidly taken up and metabolized for energy by a variety of tissues so that serum ketone levels in fasting remain below the detection threshold. Some ketones are filtered into the urine and can be measured during fasting (many physicians only associate ketones with hyperglycemia and diabetic ketoacidosis. Not you. You now understand that the liver making ketones is a normal part of the response to everyday fasting that allows the liver to release enough glucose to prevent hypoglycemia).

It is important for you to understand several metabolic adaptations that occur as fasting becomes more prolonged and progresses to starvation.

1. The rate of skeletal muscle catabolism (breakdown) decreases. The mechanism controlling this remains controversial, but is likely mediated by signals from the brain. Skeletal muscle preservation is critical to surviving starvation. Organ failure and death from starvation occur at a critical skeletal muscle mass, usually well before white adipose tissue is fully depleted.
2. Decreased skeletal muscle catabolism decreases hepatic gluconeogenesis which decreases hepatic glucose release into the blood. This would be expected to cause hypoglycemia, but this rarely occur because ...
3. At about this time (roughly 48-72 hours of fasting) the brain and especially neurons have up-regulated their enzymatic machinery to be able to transport ketones across the blood brain barrier and metabolize ketones into acetyl CoA to be used for ATP production. This saves a large amount of serum glucose from being metabolized and can be used by other tissues.
4. The brain also implements a coordinated endocrine and neuronal effort to decrease energy expenditure. This includes decreasing thyroid signaling, shutting off the reproductive axis and decreasing adaptive immune function all of which decrease resting energy expenditure. Activity energy expenditure (the metabolic cost of low power muscle movements) also becomes more efficient. These adaptations save energy and prevent the body from using too much stored fuel (both glucose and TG) too rapidly. These changes are, at least partially, mediated by a rapid and profound decrease in the adipocyte hormone leptin and coordinated by various nuclei in the hypothalamus.

Enzyme activity and hormones that increase with fasting are

inhibited by feeding and vice versa. The reciprocal regulation and coordination of these processes is very careful and efficient. It is important for you to understand the coordinated changes in carbohydrate and lipid metabolism, hormonal signals and enzymatic pathways that change as the fasting state transforms to the fed state following a meal.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=225#h5p-26

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=225#h5p-29

10. Proteasome, Lysosome Function

Session Learning Objectives:

SLO1. Explain the concepts of protein quality control and surveillance.

SLO2. Delineate the two major pathways of protein turnover (1): the ubiquitin-proteasome pathway.

SLO3. Delineate the two major pathways of protein turnover (2): the lysosome/autophagosome pathway.

SLO4. Outline the genetic bases of major lysosomal storage diseases.

Overview of Protein Degradation

SLO1. Understand the concepts of protein quality control and surveillance.

As much as 30% of newly synthesized cellular proteins are discarded without being properly folded (i.e. misfolded, and/or unassembled), even though they may be synthesized normally without mutations of their genes or errors in the translation process. In addition, even if proteins are synthesized and folded accurately as functional proteins with normal tertiary structures, they often undergo damage over time due to various environmental stresses, such as heat, oxidation (i.e. from free radicals), and UV exposure. In healthy individuals, these impaired proteins with non-native or aberrant structures are rapidly degraded. In other words, the cell is fully equipped with a "surveillance system" to rapidly eliminate these defective proteins. The molecular chaperone system recognizes proteins with non-native structures, prevents them from irreversibly aggregating, and facilitates their proper refolding. The ubiquitin-proteasome system (UPS) is responsible for selective destruction of misfolded/unfolded and unassembled proteins which have failed to correctly refold by the chaperone

system. This machinery prevents the accumulation of abnormal proteins and formation of toxic aggregates, as seen in various neurodegenerative diseases (covered in later sessions).

Other proteins need to be removed from the cell when conditions change (e.g. downregulation of receptors) or their task is completed (e.g., proteins involved in cell division). Most proteins are subject to continuous turnover (i.e. a constant process of protein synthesis and degradation). Some individual proteins may have half-lives of only a few minutes, while some last weeks or even years (proteins in tendons and eyes). The concentration of a protein is thus regulated at both the synthesis and the degradation level. Foreign proteins from bacteria or viruses must also degraded, and sometimes proteins must be metabolized as a source of energy or building blocks for other needed molecules. Whatever the purpose, the two major mechanisms by which cells degrade unwanted proteins, depending on their origin or cellular location, are the ubiquitin-proteasome system (UPS) and the lysosomal pathway.

Clinical significance of protein degradation

The UPS is capable of catalyzing rapid, timely protein degradation necessary for a multitude of biological processes, including the cell cycle and programmed cell death (apoptosis). In addition, the UPS plays a major role in the stress response and protein homeostasis.
Proteasome inhibitors have been in clinical use for many years in the treatment of multiple myeloma. They are thought to work by inhibiting the degradation of proteins required for apoptosis. Most cancer chemotherapies trigger apoptosis, and cancer cells can become resistant to these chemotherapies by rapidly degrading proteins in the apoptotic cascade and prolonging the life of the cell.

Proper immune system function relies on the generation of immunogenic peptides by degradation of foreign antigens and their display on the cell surface bound to MHC proteins. Both the proteasomal and lysosomal pathways are involved; this will be discussed more in the I&I block.

SLO2. Delineate the two major pathways of protein turnover (1): the ubiquitin-proteasome pathway.

The ubiquitylation process: tagging of proteins marked for degradation

Ubiquitin (Ub) is a highly conserved small protein, found in all eukaryotes, that acts as a degradation marker for a wide spectrum of cellular proteins. Functional analogs of ubiquitin have been described in some prokaryotes.

Ubiquitylation (also called ubiquitination) involves 3 enzymes: E1 activates the C-terminal end of Ub and transfers it to E2, and then one of many E3 (ubiquitin-protein ligases) transfers the Ub to a Lys sidechain of the target protein. A polyubiquitin chain can be formed using the same set of enzymes, by attaching another ubiquitin through its C-terminal end to a Lys residue within the ubiquitin previously associated with the target protein

A polyubiquitin chain functions as the main marker for proteolysis by the proteasome (a large, ATP-dependent, multi-subunit degradation complex).

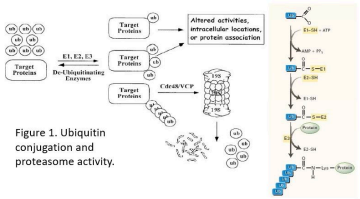


Figure 1. Addition of a single ubiquitin is used to target a protein to a specific location (e.g. the EGFR to lysosomes) or to modify the activity of a protein. Only a chain of 4 or more ubiquitins targets a protein to the proteasome.

Selectivity of protein degradation: Determined primarily at the stage of ligation to ubiquitin

There is a single E1 (activating) enzyme, whereas there are 30 E2s in multiple families and >600 E3 (ligase) enzymes needed to recognize the very diverse target proteins for degradation. Thus, E3 plays a critical role in the selection of the target protein for degradation, because each distinct E3 binds a protein substrate or set of substrates with a high degree of selectivity.

• Primary signal: E3 recognizes small primary sequence motifs on protein substrate

• Secondary signal: the poly-ubiquitin chain is recognized by the

proteasome

• Ub4 is shortest chain that binds to the proteasome

• Cells also have deubiquitylation enzymes (DUBs) to facilitate removal of ubiquitin tags when a target protein enters the proteasome and is recycled (reused)

• DUBs also catalyze reversal of the mono-ubiquitylation reaction when protein is not to be degraded

Why is there a polymeric signal like poly-Ub for protein degradation?

1. Poly-Ub allows the proteasome to interact with vastly different protein substrates.

2. Balance between ubiquitylation/deubiquitylation processes may fine-tune the rate of degradation.

3. Ubiquitin tagging of proteins is not solely to target proteins for degradation. Mono-ubiquitylation is involved in other cellular events, such as: activation of kinases or transcription factors, DNA repair, endocytosis of membrane receptor proteins, and intracellular trafficking of proteins.

Proteasomes: Controlling protein degradation through compartmentalization

Protein degradation poses real hazards to a cell – therefore it is compartmentalized either in proteasomes or in degradative organelles such as lysosomes.

The proteasome is a large ATP-dependent protease complex, consisting of a central catalytic/core particle and two terminal regulatory caps attached to both ends. The "core" of the proteasome is a barrel-like cylindrical particle formed by the axial stacking of four rings, each of which is made up of seven structurally similar subunits. Subunits of each inner ring have the catalytically active residues– with three distinct protease specificities in the different subunits. These active sites face the interior of the cylinder, and substrates gain access to the active sites only after passing through a narrow opening at the center of the outer rings.

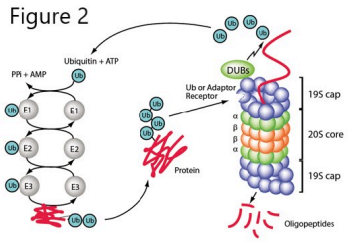


Figure 2. Ubiquitin conjugating enzymes and the proteasome.

The lid complex is involved in the recognition of target proteins, deubiquitylation, and interactions with various proteins, including E3s. As substrate proteins are threaded through the proteasome, they are cleaved into peptides 7-9 residues long. These are just the right size for binding to MHC class I receptors for display to the immune system on the cell surface. Peptides from degradation of both normal cellular proteins and foreign proteins are presented to cytotoxic T-cells in this way. It is up to the T-cells to recognize which are "self" and which are "non-self" (more on that in the I&I block).

Other functions of ubiquitin

The covalent attachment of Ub to a target protein can also serve as a localization signal, rather than a signal for destruction, by acting as a protein–protein interaction domain in endocytosis, membrane fusion and transcriptional control. For instance, mono-ubiquitylation stimulates endocytosis of cell surface receptors and progression through the endosomal sorting complex. Receptors that are not ubiquitylated are recycled back to the cell surface, but those that are ubiquitylated are more likely to be degraded instead. For example, increased stimulation of the epidermal growth factor receptor (EGFR) leads to downregulation by increased EGFR ubiquitylation and lysosomal degradation.

Ubiquitin's involvement in the lysosomal pathway is also exemplified by a familial form of Parkinson disease (PD) that is caused by recessive mutations in PARK2, a gene encoding Parkin, an E3 ubiquitin ligase. Parkin is involved in tagging damaged mitochondria for degradation by lysosomes (mitophagy, described below).

SLO3. Delineate the two major pathways of protein turnover (2): the lysosome/autophagosome pathway.

The endocytic system, autophagy, and lysosomes

This organelle system targets both intracellular and extracellular substrates for destruction in the lysosome. Extracellular substrates are taken up through endocytosis (in clathrin-coated vesicles), pinocytosis, or phagocytosis. The endocytic or phagocytic vesicles containing these substrates are acidified by ATPases that pump protons into the lumen, and then the vesicles dock and fuse with lysosomes. The contents of the hybrid compartment are destroyed by lysosomal hydrolases (proteases, glycosidases, nucleases, etc., that function at low pH). Intracellular substrates, such as complexes or aggregates of proteins, are taken up by autophagosomes, vesicles that wrap around and enclose them. The autophagosome then fuses with a lysosome, allowing it to destroy the enclosed contents. Autophagy can occur in bulk, during starvation. This recycles protein and RNA building blocks for the cell. Autophagy can also function as a quality control mechanism that removes entire damaged organelles, such as mitochondria ("mitophagy").

The lysosome is the terminal compartment in the endocytic pathway. It is also the terminal compartment of the autophagy pathway. The lysosome's lumen (interior) is acidic (pH 4.5-5) and contains a variety of hydrolases that function at low pH and degrade macromolecules. The lysosome also serves as a storage depot for amino acids and metal ions.

Lysosomal hydrolases are synthesized at the rough ER where they are folded and glycosylated. They are then transported to the cis-Golgi where the carbohydrate is terminated by addition of mannose-6-phosphate (M6P). At the trans-Golgi, the M6P receptor is the adapter that picks the hydrolases and places them in a carrier vesicle. The carrier vesicle fuses with the late endosome, which is then delivered to the lysosome. The empty M6PR is then recycled to the trans-Golgi to pick up additional cargo.

Peptides generated by proteolysis in the lysosomes can also be displayed on the cell surface for recognition by the immune system,

just like those produced by the proteasomes. In the phagolysosomes of phagocytic cells, peptides derived from endocytosis and digestion of bacteria or viruses are bound by MHC class II receptors, which are cycled back to the plasma membrane in vesicles with their cargo bound. Once the vesicles fuse with the plasma membrane, the peptides presented on the cell surface by the MHC II proteins are recognized by T cell receptors on helper T cells, and this helps activate an immune response to the pathogen. Again, you will learn more about this in I&I.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://uw.pressbooks.pub/fmrbiochemistry/?p=229#h5p-19

SLO4. Outline the genetic bases of major lysosomal storage diseases.

Lysosomal storage disorders

A variety of inherited lysosomal storage diseases are caused by deficiency of lysosomal hydrolases.

Examples include:

- Gaucher (glucocerebrosidase = β-glucosidase)
- Fabry (α-galactosidase)
- Tay-Sachs (hexosaminidase)
- Nieman-Pick (acid sphingomyelinase)
- Pompe (α-glucosidase).

Some of these diseases can be treated (not cured) through supplementation with the purified enzyme that is endocytosed and targeted by the M6P receptor. For example, Cerezyme (imiglucerase) is a recombinant β-glucocerebrosidase used to treat Gaucher disease. Cerdelga (eliglustat), a small molecule inhibitor of glucosylceramide synthase that reduces the need for lysosomal degradation is also used. Either treatment costs up to $300,000/

yr. Enzyme replacement therapy with recombinant α-galactosidase for Fabry is similarly expensive. On the other hand, there is only palliative treatment available for the autosomal recessive disorder Tay-Sachs. Most babies born with the more severe, infantile form of Tay-Sachs, are homozygous for defective hexosaminidase alleles harboring a 4-base pair insertion in exon 11. GM2-ganglioside accumulates in cells, leading to a mental and physical deterioration that is usually fatal before the age of 5. Clinical trials of gene therapy are underway using injection of an AAV virus carrying the HEXA gene; the first successful results were reported in 2022.

Other lysosomal storage diseases are due to deficiencies in genes encoding proteins needed for protein targeting to lysosomes, or for maintaining the lysosomal ionic environment. One example is I-cell disease, a deficiency of the Golgi phosphotransferase needed to produce terminal mannose-6-phosphate.

Protein turnover and amino acid reutilization

Some proteins are normally rapidly tagged with ubiquitin for degradation and have short half-lives unless this process is inhibited; others are ubiquitylated in response to activation of an E3 ligase. Most proteins are marked for degradation when they lose their native structure due to denaturation, proteolysis, or accumulation of structural modifications. The 20 amino acids are subject to more than 200 different post-translational modifications, some of which signal that a protein has aged and needs to be replaced. These include oxidation of methionine, deamidation of asparagine, and glycation (non-enzymatic attachment of sugar residues) of primary amines (on lysine side chains and terminal amino groups of proteins). This surveillance system helps rid the cell of dysfunctional proteins that might also form toxic aggregates.

Protein degradation is also a source of amino acids for the production of new proteins or as a source of energy. In the latter case, the amino groups must be removed to make urea for excretion, and the carbon skeleton feeds into the TCA cycle in various ways, generating reduced electron carriers for the ETC to generate ATP, or is used to make citrate that is exported from the

mitochondria for fatty acid biosynthesis and energy storage. In a normal 70 kg adult, ~300g protein is synthesized each day. This requires an oral protein intake of just 70g/day, because most of the new protein is made from recycled amino acids. Think about that the next time you eat a 16 oz steak (120 g protein) or drink a "protein shake" (100 g protein/8oz glass!) Any additional amino acids beyond what is needed for protein synthesis must be converted to energy or fat, and in both cases the liver and kidneys must get rid of the nitrogen.

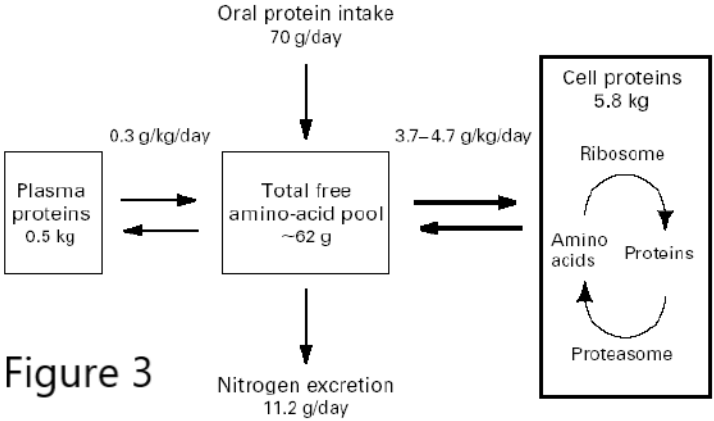


Figure 3. Bulk Protein turnover.

Excess protein degradation, and therefore nitrogen excretion, can become a problem, not just when too much is eaten, but also during prolonged fasting or starvation, when muscle protein must be degraded to provide glucogenic amino acids for gluconeogenesis. It can also occur in certain diseases, like AIDS and cancer, when large numbers of cells are undergoing apoptosis and their proteins are degraded. Clinically, this muscle wasting is called cachexia, and it is seen in the late stages of almost every major chronic illness, affecting many people with heart failure, chronic obstructive pulmonary disease and kidney disease. It is driven in part by inflammatory signals that activate expression of ubiquitin E3 ligases, leading to protein degradation.

11. Nitrogen Metabolism, Urea Cycle

The goal of this chapter is to help students understand the concept of nitrogen balance, and how the body gets rid of excess ammonia (NH3) or ammonium ions (NH4+) when amino acids are catabolized.

Session Learning Objectives:

SLO1: Define the concept of nitrogen balance and explain the role of protein degradation in normal nutrition and disease states.

SLO2: Describe the metabolism of nitrogen using aminotransferases, glutamate dehydrogenase, glutamine synthetase and glutaminase.

SLO3: Describe the significance of the urea cycle in removing nitrogen and the presentation of hyperammonemia with defects in urea cycle enzymes.

SLO4: Discuss the diagnostic significance of aspartate aminotransferase and alanine aminotransferase (AST, ALT). [Covered in Class].

Overview: Nitrogen is consumed mostly as protein in the human diet. The successive actions of proteolytic enzymes in the stomach and small intestine hydrolyze proteins to smaller oligopeptides and amino acids. The intestinal brush border cells contain oligopeptidase enzymes which complete the digestion process. Proteins must be degraded to amino acids or very short peptides before they can be absorbed. After absorption from the small intestine into the blood stream, amino acids have three metabolic fates in the body:

1. Synthesis of tissue proteins
2. Synthesis of other important constituents of the body
3. Catabolism and excretion of the products

SLO1: Define the concept of nitrogen balance and explain the role of protein degradation in normal nutrition and disease states.

Metabolic Fate of Nitrogen: Figure 1 illustrates the metabolic fate of nitrogen ingested by a mammal. Ingested amino acids enter the body's amino acid pool. This pool is rather small because incoming amino acids are rapidly shuffled off to other places. Their principal fate is incorporation into proteins. Amino acids may also contribute their carbon skeletons to synthesis of various TCA cycle intermediates or acetyl CoA, and their nitrogen can contribute either to the synthesis of other nitrogenous compounds (purines, pyrimidines, other amino acids, porphyrins, etc.) or to urea, which is excreted. The excretion of urea is the principal way the human body gets rid of excess nitrogen. A small amount of nitrogen is also lost by excretion of other nitrogenous compounds in the feces, through the skin, and during respiration.

Ammonia has an important place in the flow of nitrogen through the body. Ammonia from catabolites or other sources can be picked up for synthesis of nitrogen-containing molecules, or can combine with CO2 to form urea and be excreted.

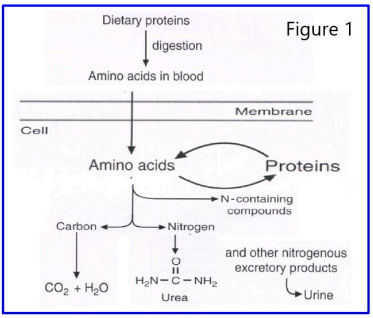


Figure 1

NITROGEN BALANCE

Nitrogenous compounds, such as amino acids, continually enter and leave the human body. The normal, healthy adult maintains nitrogen equilibrium, assimilating about 5 grams of nitrogen per day and excreting the same amount. During infancy and childhood (growth) and during convalescence from certain diseases, the intake of nitrogen exceeds the amount excreted — this is referred to as positive nitrogen balance. During starvation or certain disease states, the output of nitrogen may exceed the amount taken in — a negative nitrogen balance. This represents a net loss of tissue protein due to catabolism of the constituent amino acids.

DEGRADATION OF AMINO ACIDS

There is no storage polymer for excess amino acids like there is for excess glucose or fatty acids. Amino acids in excess of those required for synthesis of protein and other compounds are converted to major metabolic intermediates. The alpha-amino group is removed and the resulting carbon skeleton converted either to TCA cycle intermediates (glucogenic amino acids) or to acetyl-CoA (ketogenic amino acids). All amino acids can contribute to energy production through the TCA cycle or to fatty acid biosynthesis via citrate. Glucogenic amino acids are so named, because they can be used for gluconeogenesis in the liver; the ketogenic amino acids, on the other hand, are used to make ketones under these same conditions. Figure 2 summarizes the fate of the carbon skeletons.

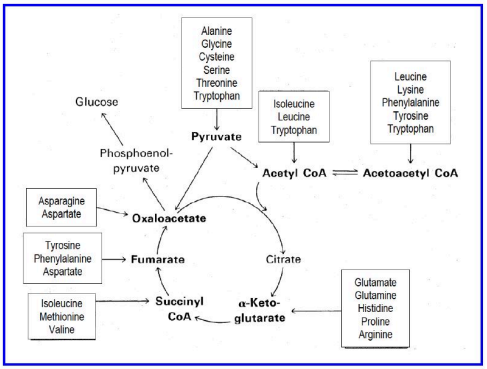


Figure 2: Metabolic fate of the carbon scaffolds of amino acids.

SLO2: Describe the metabolism of nitrogen using aminotransferases, glutamate dehydrogenase, glutamine synthetase and glutaminase.

Transamination Reaction

The major mechanism for removing alpha-amino groups from amino acids is via transamination (Figure 3). Transaminases (aminotransferases) catalyze the transfer of the alpha-amino group of an amino acid to the carbon of an alpha-keto acid. The most common alpha-keto acid used as an acceptor of amino groups in

these reactions is alpha-ketoglutarate, forming glutamate. There are many different aminotransferases, specific for different amino acids, but almost all of these use alpha-ketoglutarate and L-glutamate as one of the substrate pairs.

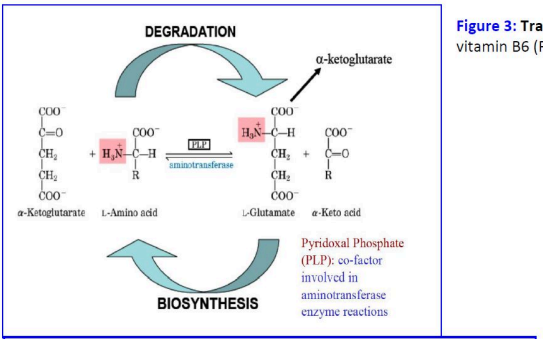


Figure 3: Transamination reactions (requir vitamin B6 (Pyridoxine) as a co-factor)

Reactions catalyzed by glutamate dehydrogenase. This reaction (Figure 4) is fully reversible according to the needs of the cell, although in vivo it probably functions primarily to deaminate glutamate, especially considering that many humans (Americans at least) consume an excess of protein and burn it for energy. Note that ammonia is produced in this oxidation reaction. The reaction, as written, takes place in the mitochondrial matrix and uses NAD+ as the preferred cofactor.

In the cytosol, the enzyme uses small amounts of ammonia present and NADPH as the cofactor for glutamate synthesis (the reverse of the reaction shown). Working together with transaminases, this can yield nonessential amino acids.

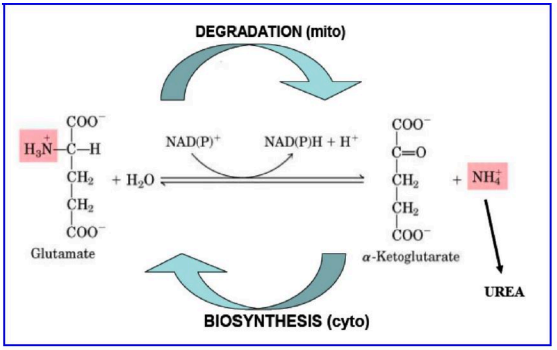


Figure 4: The glutamate dehydrogenase reaction.

Transaminases funnel alpha-amino groups from most amino acids into glutamate. Glutamate is then transported into the mitochondrion and oxidatively deaminated to produce an ammonium ion, which is converted into urea and excreted (Figure 5). Transaminases also function in synthesis of nonessential amino acids, using the amino group of glutamate that is synthesized from alpha-ketoglutarate and ammonia.

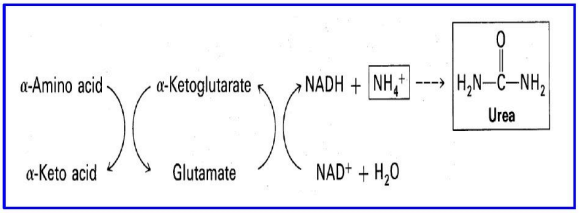


Figure 5: Transfer of nitrogen toward urea production via a series of reactions catalyzed by transaminase and the glutamate dehydrogenase enzyme.

Source of ammonia NH3/NH4+:

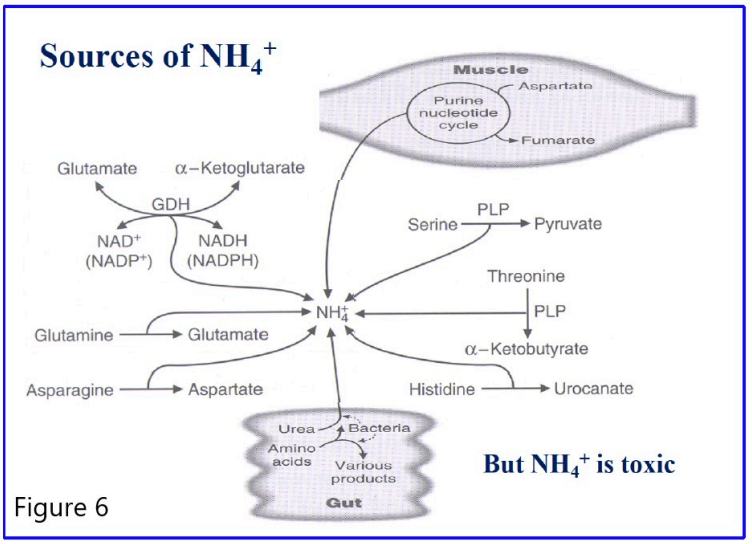


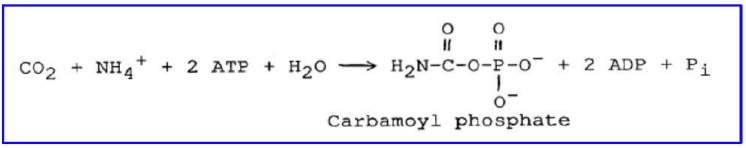
Figure 6: Summary of the sources of NH4+ for the urea cycle. All reactions are irreversible except glutamate dehydrogenase (GDH). Only the dehydratase reactions which produce NH4+ from serine and threonine require pyridoxal phosphate (PLP) as a cofactor. In the liver, NH4+ generated is converted to urea. Note the contribution of the gut bacteria.

SLO3: Describe the significance of the urea cycle in removing nitrogen and the presentation of hyperammonemia with defects in urea cycle enzymes.

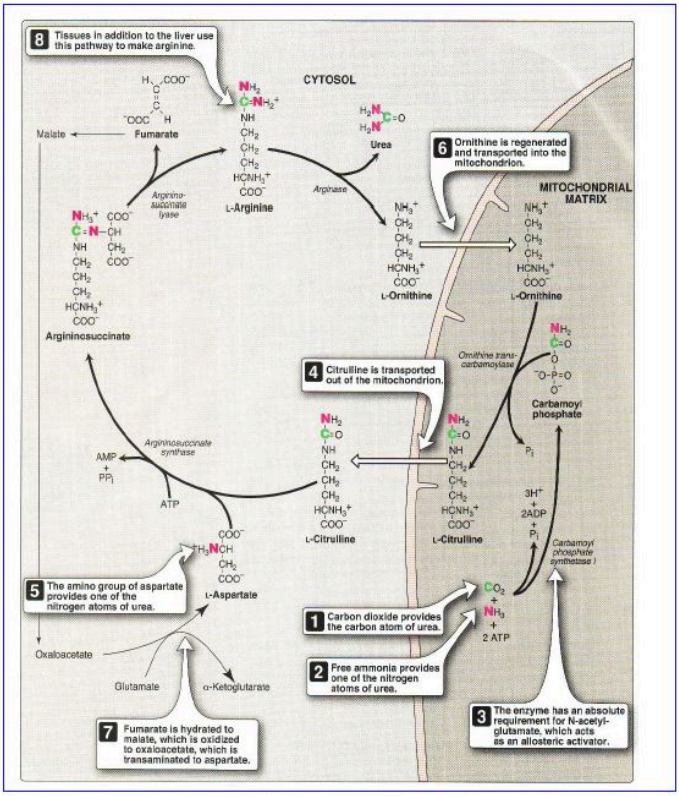
NITROGEN ELIMINATION AND THE UREA CYCLE

Nitrogen is excreted from humans mainly as urea, uric acid and ammonia. Ammonia is toxic at high concentrations (see below), and uric acid is poorly soluble, so nitrogen is mostly eliminated as urea. The urea cycle begins with the incorporation of ammonia into carbamoyl phosphate (Fig. 7).

Figure 7: First step of the urea cycle catalyzed by carbamoyl-phosphate-I (CPS-I)



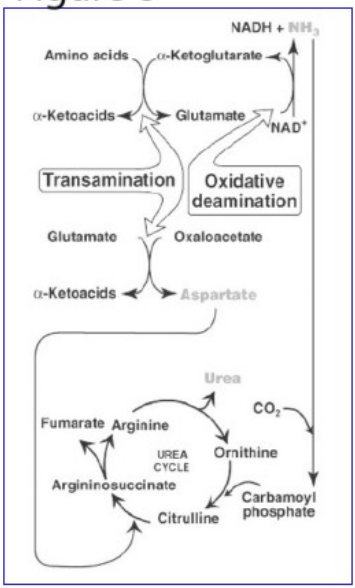
The Urea Cycle (Fig 8): Part of the reaction occurs in the mitochondria, the rest in the cytoplasm. The cycle proceeds through a series of intermediates, resulting in urea being eliminated from arginine.



The Urea Cycle (Fig 8): Part of the reaction occurs in the mitochondria, the rest in the cytoplasm. The cycle proceeds through a series of intermediates, resulting in urea being eliminated from arginine.

Note that the nitrogen atoms of the excreted urea molecule entered the cycle from ammonia and aspartic acid. Thus, in considering the flow of nitrogen from an amino acid catabolized to urea, we have the following scheme in Fig 9.

Figure 9



The reactions of the urea cycle are distributed between the cytosol and the mitochondrial matrix. This sequesters ammonia produced by the glutamate dehydrogenase reaction inside the mitochondrion, where it can immediately feed into the urea cycle. Note that transport systems are present in the inner mitochondrial membrane for moving intermediates between the cytosol and the mitochondrial matrix.

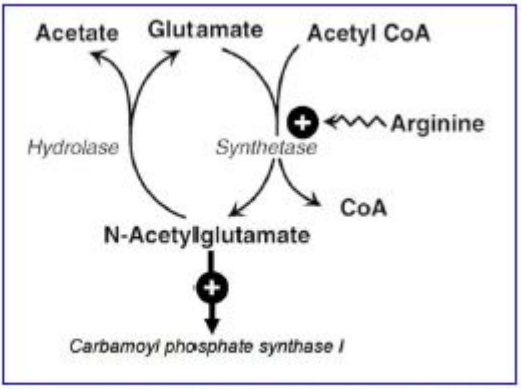


Figure 10: Role of N-acetylglutamate in the regulation of CPS-I activity

REGULATION OF THE UREA CYCLE

Long term adaptive response: A high protein diet leads to elevated serum glucagon, which in turn stimulates transcription of the genes encoding the urea cycle enzymes (Figure 11).

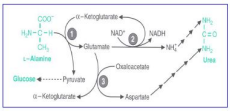


Figure 11: Long-term adaptive response

Short term regulation: Carbamoyl phosphate synthetase I requires N-acetylglutamic acid as an allosteric activator. The activity of the enzyme that catalyzes the synthesis of N-acetylglutamic acid from acetyl CoA and glutamic acid in the liver is stimulated in response to the arginine contained in a high protein meal. N-acetylglutamate signals dietary protein status, controlling the urea cycle through regulation of carbamoyl phosphate synthesis (Figure 10).

High protein diets lead to elevated glucagon Levels that promotes transcription of the genes encoding the Urea Cycle enzymes.

In the scheme above (Figure 11), alanine is converted to glucose and urea via the following reactions:

1. Alanine – key gluconeogenic amino acid is transaminated to form pyruvate which is converted to glucose.

2. The nitrogen now in glutamate can be released as NH4+ (2) or transferred to oxaloacetate to form aspartate (3)
NH4+ and aspartate enter the urea cycle which produces urea.

CONGENITAL HYPERAMMONEMIA

This is a group of diseases characterized by elevated ammonia levels, often accompanied by episodic vomiting, hepatomegaly, intolerance to dietary protein, lethargy, retardation and in severe cases coma and death shortly after birth. Deficiency in any of the enzymes in the urea cvcle can produce these symptoms. Several of the best known examples are discussed below.

Carbamvl Phosphate Svnthetase Deficiency is an autosomal recessive disease with severe hyperammonemia and a rapidly progressive fatal course shortly after birth. Such a complete absence of any urea cycle enzyme is fatal, since there is no alternate pathway for urea synthesis.

Ornithine Transcarbamylase Deficiencv (OTC) is an x-linked disorder with a course similar to the above in males with complete absence of the enzyme; those with reduced activity can be managed with a low protein diet. Heterozygous females can have variable symptoms.

Citrullinemia, argininosuccinic aciduria, and argininemia are all also known; in each case, there is blockage of the step following the accumulated urea cycle intermediate, combined with hyperammonemia.

Ammonia toxicity is speculated to be a common factor to all of these disorders, and is caused by reduced levels of alpha-ketoglutarate inside the mitochondria, due to a shift in the equilibrium of the glutamate dehydrogenase reaction. This results in reduced TCA cycle activity and lowered ATP synthesis. The effects of reduced oxidative metabolism are particularly deleterious to brain function. These diseases have a precipitous course immediately after birth, because maternal metabolism no longer detoxifies the blood of the fetus.

Practice questions:

1. What is the rate limiting step of urea biosynthesis?

2. How is nitrogen originating from the NH2 of amino acids targeted for degradation moved around to be made available for urea synthesis
3. Outline the mechanisms by which urea synthesis is regulated. Why does it make sense for glucagon to activate gluconeogenesis and the urea cycle simultaneously?
4. What are the health consequences of deficiencies in the enzymes of the urea cycle?

12. Amino Acid Derivatives

AMINO ACID DERIVATIVES

WWAMI FMR, *updated* 11/17/2022

Pamela Langer, PhD, WWAMI-WY

Session Learning Objectives:

SLO1: Describe the biosynthetic origin and basic function of the biological mediators histamine, gamma-aminobutyric acid (GABA), serotonin and nitric oxide.

SLO2: Describe the biosynthetic origin and basic function of molecules derived from tyrosine: thyroid hormone, melanin, and the catecholamines dopamine, norepinephrine, and epinephrine.

SLO3: Discuss the biosynthetic relationship between creatine and creatine phosphate and the use of creatinine as a clinical analyte.

SLO4: Describe the biochemical role of reduced and oxidized glutathione.

Amino Acids Derivatives, Overview

In addition to being precursors in protein synthesis, gluconeogenesis, and lipid synthesis, amino acids play important metabolic roles as precursors for other amino acids, nucleotides, and various biological mediators. The physiological and clinical significance of these amino acid derivatives will be discussed briefly in this chapter along with their biosynthesis. For example, creatine is synthesized from two amino acids and the methyl group from S-adenosylmethionine (SAM). *Creatine kinase* catalyzes the conversion of creatine to creatine phosphate, an energy storage molecule, that spontaneously converts to creatinine, an analyte in assessing kidney function.

The 20 proteinogenic amino acids are organized into groups according to properties of their side chains (R groups). Identifying

an amino acid with a group highlights some of its functional properties as well as potential consequences when substituted in a protein because of a genetic change. For example, substitution of an amino acid containing a positively-charged side chain with an amino acid with a negatively-charged side chain may alter protein structure. Substitution of a cysteine with another amino acid may cause the absence of appropriate disulfide bonding critical to formation of a functional protein.

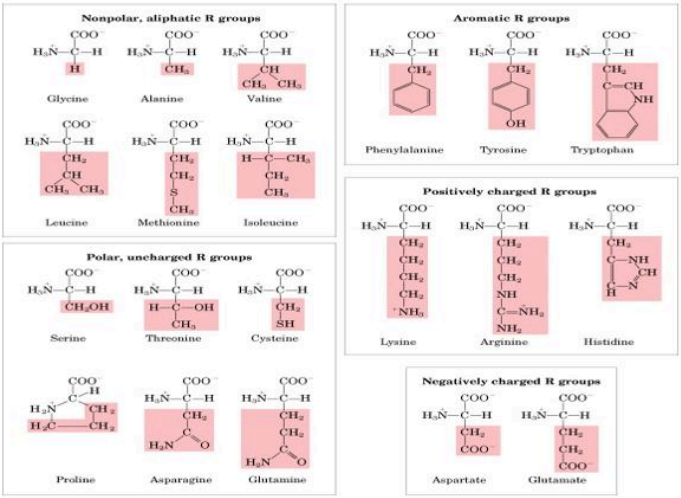


Figure 1 Proteinogenic amino acids.

Knowing the full name, 3-letter, 1-letter, and group designations for the 20 standard amino acids will facilitate recognition of types of genetic changes and comprehension of certain topics in research seminars and papers in medical biochemistry and genetics.

Table 1 Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter abbreviation	Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A	Methionine	Met	M
Arginine	Arg	R	Phenylalanine	Phe	F
Asparagine	Asn	N	Proline	Pro	P
Aspartic Acid	Asp	D	Serine	Ser	S
Cysteine	Cys	C	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	Trp	W
Glutamic Acid	Glu	E	Tyrosine	Tyr	Y
Glycine	Gly	G	Valine	Val	V
Histidine	His	H	Asparagine or aspartic acid	Asx	B
Isoleucine	Ile	I			
Leucine	Leu	L	Glutamine or glutamic acid	Glx	Z
Lysine	Lys	K			

Coenzymes play a significant role in the synthesis of amino acids and their derivatives. Multiple coenzymes are required in some pathways, for example, the synthesis of catecholamines from tyrosine requires tetrahydrobiopterin (BH4), pyridoxal phosphate (PLP, a derivative of vitamin B6), ascorbate (vitamin C) and the co-substrate S-adenosylmethionine (SAM) as the methyl group donor in the final methylation step from norepinephrine to epinephrine. Furthermore, a single coenzyme may be required by multiple pathways. A deficiency in the coenzyme can result in a disorder related to one caused by deficiency of the enzyme using that coenzyme. For example, tetrahydrobiopterin (BH4) deficiency causes a reduction of activity in the BH4-dependent phenylalanine hydroxylase reaction and results in a form of phenylketonuria (PKU). Disorders related to defects in the synthesis of amino acid derivatives will be discussed in more detail in another session.

Amino acid derivatives considered in the first two learning objectives for this session are each derived from single amino acids, with tyrosine-derived derivatives considered separately, followed by those derivatives requiring more than one amino acid for their synthesis. The molecules introduced here will be revisited in other parts of the foundational courses.

SLO 1: Describe the biosynthetic origin and basic function of the biological mediators histamine, gamma-aminobutyric acid (GABA), serotonin and nitric oxide.

Biosynthetic pathways for histamine, gamma-aminobutyric acid (GABA), serotonin, and the catecholamines involve decarboxylation reactions that employ the coenzyme pyridoxal phosphate (PLP), a derivative of vitamin B6. Note that PLP is also used in many other types of metabolic reactions such as aminotransferases and biosynthesis of cysteine or the heme ring.

Histamine:

Histamine, derived from histidine, mediates multiple physiological processes via binding to different histamine receptors that are G-protein coupled receptors (GPCRs), H1-H4 in humans. For example, when hist

Figure 2

amine is released during an allergic response, binding to the H1 receptor results in cell signaling that stimulates mucus secretion, increases vasodilation and vascular permeability and can lead to bronchoconstriction in allergy-induced asthma. In contrast, when histamine is released during the digestive process, binding to the H2 receptor stimulates a different GPCR-mediated cell signaling pathway that results in release of HCl into the gastric lumen. Histamine H2 receptor antagonists such as ranitidine are used clinically to decrease acid production in the stomach.

***Gamma-aminobutyric acid* (GABA)**

GABA, an inhibitory neurotransmitter in the central nervous system, is synthesized from the amino acid glutamate (one of the main excitatory neurotransmitters) using pyridoxal phosphate, a derivative of Vit B6. Underproduction of GABA is associated with epileptic seizures, and GABA analogs (e.g. barbiturates) are used in the treatment of epilepsy.

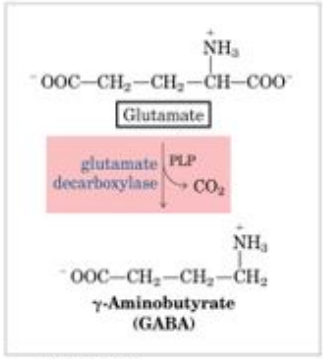


Figure 3

In addition, Gabapentin, a GABA analog, is used in treatment of neuropathic pain with diabetic neuropathy. GABA levels can also be raised by giving inhibitors of the enzyme that degrades GABA (GABA *aminotransferase*), resulting in an increase in activity of inhibitory neurons.

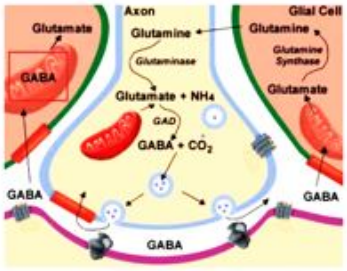


Figure 4

Serotonin

Tryptophan is a precursor in the synthesis of serotonin, a monoamine neurotransmitter involved in mood regulation.

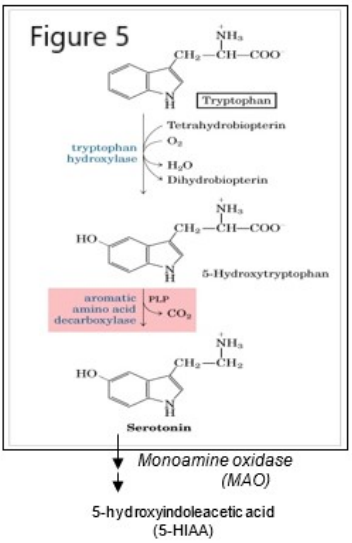
Serotonin is in turn a precursor for melatonin, a hormone involved in sleep regulation and synthesized mainly in the pineal gland. Tryptophan is also used in the synthesis of niacin (nicotinic acid, a form of vitamin B3), and therefore the precursor for synthesis of nicotinamide adenine dinucleotide (NAD+) and associated forms NADH, NADP+ and NADPH. A chronic, severe deficiency of tryptophan or niacin can lead to pellagra, a disease affecting the skin, digestive and nervous systems.

Serotonin is synthesized in a two-step pathway that utilizes the coenzyme tetrahydrobiopterin (BH4) in and pyridoxal phosphate (PLP). Serotonin is degraded to 5-hydroxyindolacetic acid (5-HIAA) via the action of *monoamine oxidase* (MAO), an enzyme also involved in degradation of catecholamine neurotransmitters.

Serotonin levels may be assessed by measuring the level of 5-HIAA, for example for a case where there is a suspected serotonin-secreting tumor.

Inhibition of serotonin reuptake into a presynaptic neuron may be used to increase the level of serotonin in the synapse in the treatment of depression.

Although the names are somewhat similar, melatonin is derived from tryptophan and melanin is a pigment derived from tyrosine. Melatonin and melanin have very different functions.



Nitric oxide

Nitric oxide (NO) is a short-range, short-lived signaling molecule. NO is synthesized from arginine via a *nitric oxide synthase* (NOS)-dependent pathway yielding citrulline and nitric oxide. A NOS-independent pathway, involving nitrate (NO3-) and nitrite (NO2-), may also generate nitric oxide from food rich in nitrates, such as leafy green vegetables and beets. Many different physiological responses are mediated by different NOS isozymes such as eNOS, a vascular endothelial isoform, nNOS, a neuronal isoform, or iNOS, an inducible isoform that plays a role in immune defense mechanisms.

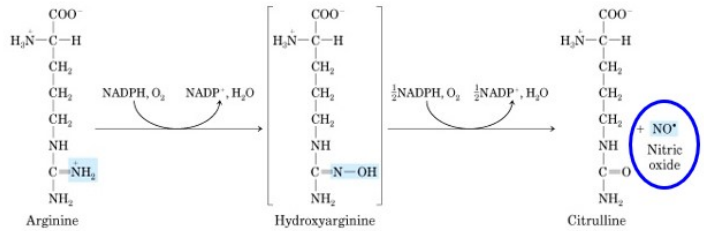


Figure 6

Nitric oxide diffuses readily through membranes, however its high reactivity limits diffusion to about 1 mm from the site of synthesis.

Soluble NO binds the heme iron of *guanylyl cyclase* that catalyzes conversion of GTP to cyclic GMP (cGMP), a powerful biological

mediator in signaling pathways. NO has several effects, but it may be considered as a smooth muscle relaxant that promotes dilation of blood vessels. The mechanism by which cGMP promotes vasodilation may involve reduction in cytosolic Ca2+, resulting in less Ca2+ available to promote smooth muscle contraction. Drugs such as nitroglycerin have been used to relieve angina, as the slow release of NO upon degradation of nitroglycerin causes increased cGMP, resulting in reduced myocardial wall tension, dilation of epicardial coronary vessels, and increased blood flow in collateral vessels.

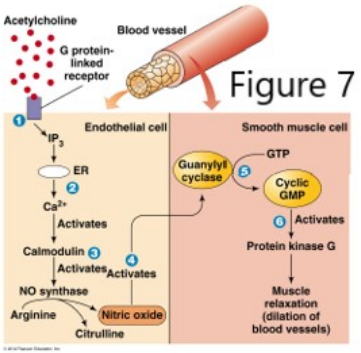


Figure 7

Cyclic GMP is degraded by phosphodiesterases, so if the goal is to increase cGMP, phosphodiesterase (PDE) inhibitors can be used. For example, cGMP levels are increased by inhibiting the breakdown of cGMP with the drug sildenafil, a PDE5 inhibitor that enhances the effect of NO, resulting in penile smooth muscle relaxation and increased blood flow to erectile tissue. Details of the effect of NO on smooth muscle and the heart will be discussed elsewhere in the curriculum.

SLO 2: Describe the biosynthetic origin and basic function of molecules derived from tyrosine: thyroid hormone, melanin, and the catecholamines dopamine, norepinephrine, and epinephrine.

Thyroid hormone

Thyroid hormone (TH) plays an important role in growth and metabolism. TH refers collectively to two forms: T4 is tetraiodinated thyronine, a prehormone that is deiodinated to the active form T3 (triiodothyronine). Note that calcitonin, made in the thyroid parafollicular cells (C-cells) is involved in calcium and phosphate homeostasis but is not included in the designation "thyroid hormone."

Over- or underproduction of thyroid hormone (hyper- or hypothyroidism) will be discussed in detail in other foundation blocks, however it is important to connect the biochemistry and physiology with clinicalapplication. For example, in treatment of hyperthyroidism, thyroid hormone synthesis may be reduced by inhibiting *thyroid peroxidase* (TPO) with methimazole or propylthiouracil (PTU). Because the majority of serum iodide is concentrated by the thyroid, thyroid cells may be selectively destroyed by administering the 131I isotope that will be concentrated in the thyroid.

Thyroid hormone is synthesized by the thyroid in response to hormonal regulation and availability of iodide. Thyroid hormone biosynthesis involves the following steps.

- **Serum iodide (I-)** enters the follicular cell via a **Na/I symporter** against a concentration gradient, using the Na/K ATPase to generate the sodium gradient to drive "iodide trapping."
- **Thyroglobulin (TG)** is exocytosed, and iodide is transported via the apical membrane anion transporter **Pendrin**, to the amorphous acellular colloid portion of the thyroid.
- ***Thyroid peroxidase* (TPO)** oxidizes I- to Io, used to iodinate tyrosines within thyroglobulin.
- The side chain of either a monoiodinated (MIT) or diiodinated (DIT) tyrosine is conjugated with a diiodinated tyrosine in thyroglobulin to form triiodothyronine **(T3)** or tetraiodothyronine (thyroxine) **(T4)** residues in thyroglobulin.
- In response to signals, colloid containing iodinated thyroglobulin is endocytosed by the follicular cell, and proteolysis of thyroglobulin releases MIT, DIT, T3 and T4.
- T3 and T4 are secreted from the cell and are bound to serum carrier proteins.
- T4 (pre-hormone) is converted peripherally to T3 (active) by ***iodothyronine (T4) deiodinase***, a selenoprotein, or to inactive reverse T3 (rT3), depending on physiological conditions.

- Conversion to rT3 reduces the reservoir of T4 that can be converted to T3.
- In the serum, thyroid hormone is transported by **thyroxine binding globulin** (TBG), **transthyretin (TTR)** (previously called thyroxine binding pre-albumin) and **albumin**. Thyroid hormones must be free of carrier proteins (unbound) to be active (e.g. free T3).

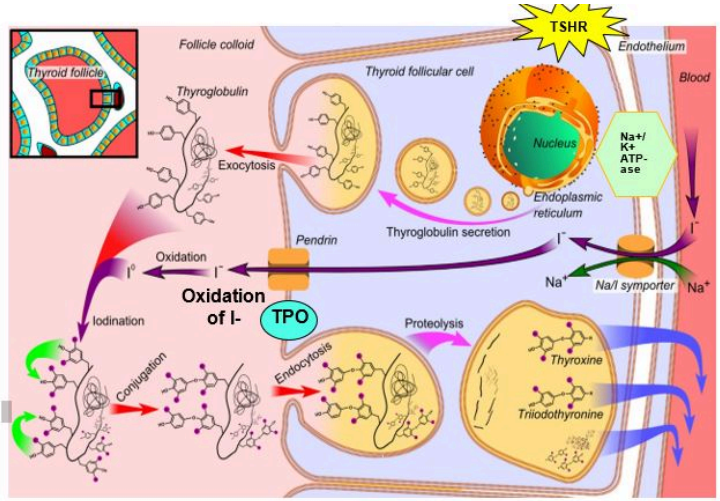


Figure 8 Modified from: http://en.wikipedia.org/wiki/File:Thyroid_hormone_synthesis.png TSHR, thyroid stimulating hormone receptor; TPO, thyroid peroxidase; Na+ gradient from ATP-dependent Na+/K+ ATPase drives I- transport by Na/I symporter (NIS)

Melanin

Various forms of melanin are synthesized from tyrosine, with the enzyme *tyrosinase* catalyzing the first step. Skin color results from pigment (melanin granules) in keratinocytes and melanocytes in superficial skin layers. Relative proportions of melanin variants determine the color of skin and hair. Dark hair contains predominantly eumelanin while blond hair results from a small amount of eumelanin production. Red hair results from production of pheomelanin with very little eumelanin production. Binding of melanocyte stimulating hormone (MSH) to its receptor (melanocortin 1 receptor or MC1R) promotes eumelanin production by activating enzymes in the eumelanin biosynthetic pathway. However, MC1R variants do not bind MSH well, and consequently the cell makes pheomelanin and produces only a small amount of eumelanin. People with red hair may be homozygous for a MC1R allelic variant, however many other genes affect the amount of expression of the enzymes involved in eumelanin and pheomelanin production.

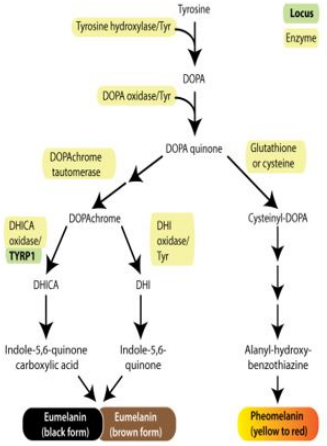
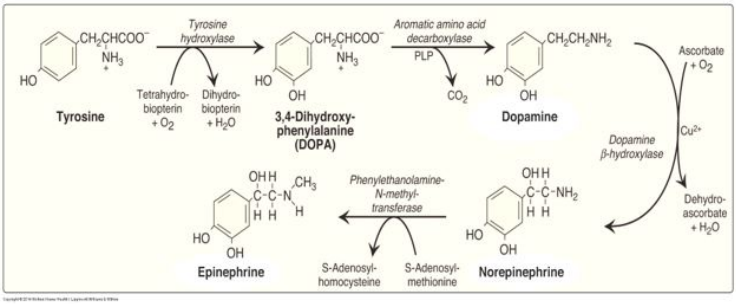


Figure 9

Catecholamines

Three monoamine neurotransmitters are synthesized from tyrosine in the catecholamine pathway: dopamine, norepinephrine (NE, noradrenaline) and epinephrine (Epi, adrenaline). The pathway involves the coenzymes **Tetrahydrobiopterin (BH4); Pyridoxal phosphate (PLP)**, derived from Vit B6; **Ascorbate** (Vitamin C, used in dopamine to NE reaction); and **S-adenosylmethionine (SAM)** (methyl group donor in NE to E reaction).

Figure 10



Monoamine oxidase (MAO) and *catechol-O-methyltransferase* (COMT) are involved in the degradation of dopamine, norepinephrine (NE), and epinephrine (E).

The catecholamine degradation products may be assessed to determine neurotransmitter levels. For example, degradation of NE results in 3-methoxy-4-hydroxy-phenylglycol (MHPG) in the CNS. *Monoamine oxidase* inhibitor drugs (MAOIs) may be used in the treatment of certain depressive disorders.

Patients with Parkinson disease may exhibit a dopamine deficiency, providing the basis for treating these patients with the drug L-DOPA, which crosses the blood brain barrier.

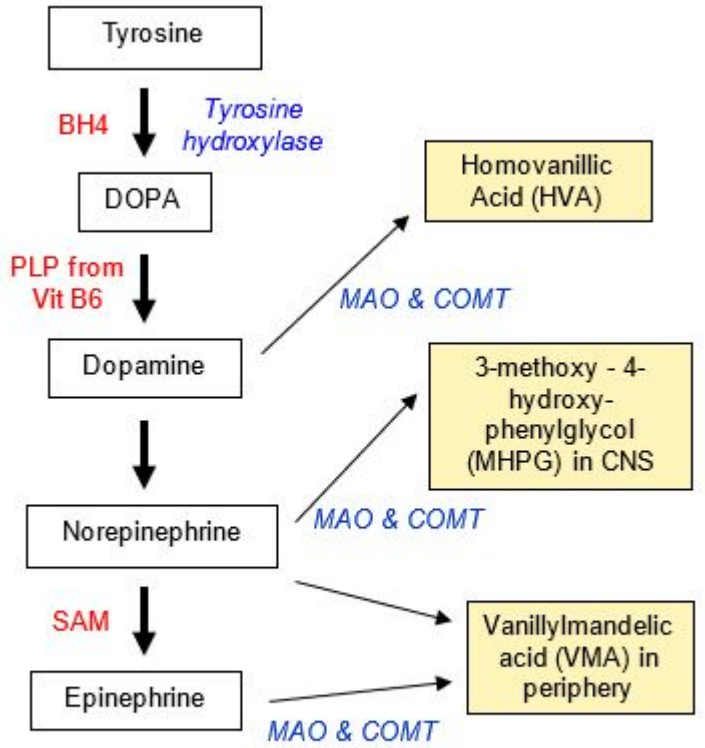


Figure 11.

SLO 3: Discuss the biosynthetic relationship between creatine and creatine phosphate and the use of creatinine as a clinical analyte.

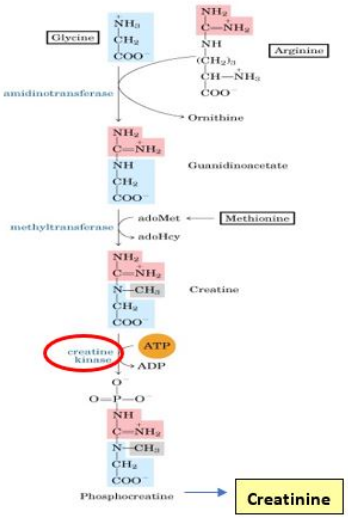
Creatine, creatine kinase, creatinine phosphate, creatinine

Three different amino acids are used to synthesize **creatine**. Phosphorylation of creatine by *creatine kinase* produces **creatine phosphate** (phosphocreatine), an important reservoir for a high energy phosphate bond and used in the conversion of ADP to ATP.

***Creatine kinase* (CK)** is an important enzyme for regeneration of ATP. Serum levels of various CK isoforms have been used

diagnostically during suspected acute myocardial infarction (AMI). However, assessment of changing levels of total CK and CK isozymes in an AMI has been largely replaced with the use of cardiac-specific forms of troponin subunits (cTnI or cTnT).

Figure 12



Creatinine is synthesized via a spontaneous, nonenzymatic dehydration of creatine phosphate, mostly in skeletal muscle. Creatinine is an important clinical analyte for two main reasons: (i) creatinine levels are a function of muscle mass and show little response to dietary change; (ii) creatinine is freely filtered by the kidney (i.e. not reabsorbed from the renal filtrate). Serum creatinine is used as first test for the capacity of the kidney to filter substances from the blood (glomerular filtration) and is used before surgical procedures to assess the patient's ability to clear the anesthetics. A creatinine clearance test is a more detailed measure of renal filtering capability, namely the glomerular filtration rate (GFR); however, this test is rarely done currently.

SLO 4: Describe the biochemical role of reduced and oxidized glutathione.

Glutathione (GSH) acts as a redox buffer, serving as a reducing agent, playing a role in toxin removal, and protecting against oxidative stress. Superoxide radical and hydrogen peroxide are generated when the cell is exposed to stressors such as certain drugs, radiation or normal mitochondrial respiration.

Glutathione synthesis

Glutathione, is synthesized from 3 amino acids (Glu, Cys, Gly), and its reduced form GSH protects cells from oxidative damage during normal cell processes and contributes to the processing of certain xenobiotics including toxins and drugs. In an energy-requiring process, the glutamate of the tripeptide is attached via the gamma-carbon of its R group. Consequently, glutathione is also designated as Gamma-Glu-Cys-Gly. Glutathione cycles between the reduced thiol form (GSH) and the oxidized, disulfide bonded form (GSSG), also called glutathione disulfide. The enzyme *glutathione peroxidase* catalyzes conversion of hydrogen peroxide (H2O2) and GSH to water and GSSG.

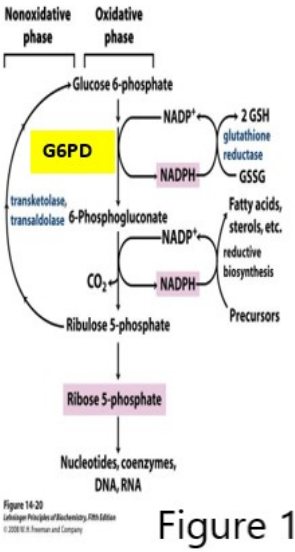


Figure 13

GSH regeneration is catalyzed by *glutathione reductase* using GSSG and NADPH generated by the pentose phosphate pathway (PPP, also called hexose monophosphate shunt, which also produces pentose phosphate sugars used in nucleotide synthesis.)

The first reaction of the PPP, catalyzed by *glucose 6-phosphate dehydrogenase* (G6PD), is critical for producing an adequate amount of NADPH used in reductive biosynthetic pathways and importantly in the generation of GSH. Deficiency in GSH regeneration may result in elevated reactive oxygen species (ROS) and oxidative damage. Individuals deficient in G6PD and exposed to high oxidative stress, may present with serious clinical issues because of the deficiency of NADPH and consequently, deficiency of GSH.

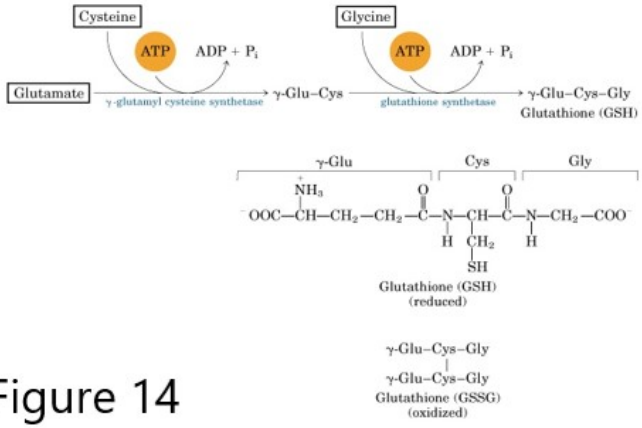


Figure 14

Decreased glutathione in G6PD deficiency

As the only source of NADPH in the RBC is the pentose phosphate pathway, a G6PD defect causes decreased NADPH and therefore low GSH production in the RBC. RBCs carry high levels of O2 and are susceptible to oxidative stress. Furthermore, RBCs are unable to regenerate enzymes because they have no nucleus. Although some people with a G6PD deficiency never exhibit symptoms, G6PD deficiency symptoms are worsened with high oxidative stress caused by taking certain drugs (e.g. the anti-malarial primaquine), having certain infections, eating fava beans, or being exposed to other environmental conditions that increase oxidative stress. In situations of high oxidative stress with low GSH, the most common manifestation of G6PD deficiency is hemolytic anemia.

X-linked recessive G6PD deficiency is the most common genetic deficiency, with ~400 million cases worldwide and highest prevalence in people of African, Asian and Mediterranean descent. In the US, 1 in 10 males of African American descent have a G6PD deficiency, and females are usually affected only if they have 2 defective G6PD alleles.

G6PD deficiency confers partial protection against malaria, a reason promulgated to explain the high prevalence of G6PD defects in the world. There are over 300 variants of G6PD alleles, resulting in a polymorphic clinical presentation, from mild to severe. Screening for G6PD deficiency is not typically part of newborn screening panels.

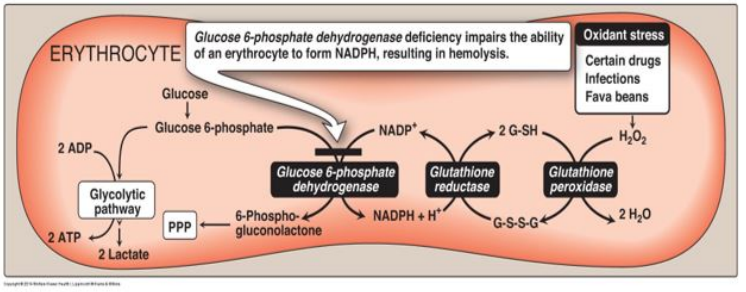


Figure 15

Glutathione in toxin removal

GSH is essential in normal metabolism as well as for the processing of toxins. For example, acetaminophen is processed via multiple pathways, including glucuronidation or sulfation, or to a lesser extent, via oxidation by P450 enzymes, generating a toxic catabolite NAPQI that is detoxified by conjugation with GSH, spontaneously or catalyzed by *glutathione S-transferase* (GST).

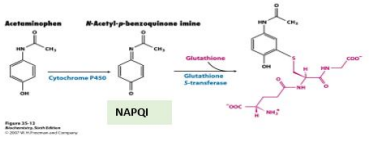
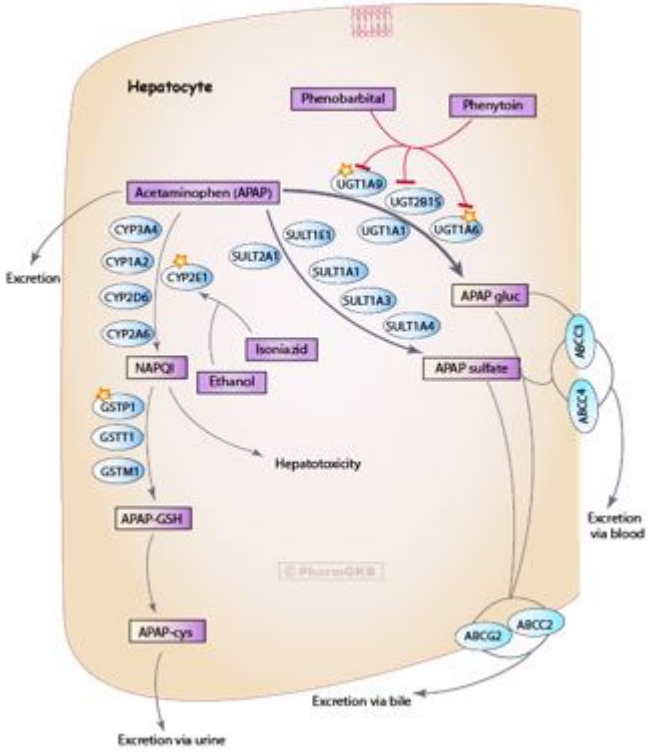


Figure 16

In cases of overdose, high levels of acetaminophen can cause hepatocellular necrosis and liver damage from alteration of the cellular redox potential and damage from the NAPQI toxin, especially if the GSH levels are decreased in starvation. However, toxicity may be exacerbated if the level of activity of certain cytochrome P450 (CYP) enzymes is increased. For example, ethanol will increase the activity of and stabilize CYP2E1, causing

increased activity in the pathway from acetaminophen to NAPQI. Furthermore, genetic polymorphism in CYP2E1 allelic variants can cause individual variation in drug metabolism and susceptibility to liver damage. Consequently, it would be difficult to predict which individuals might have a serious adverse drug reaction if ingesting ethanol and acetaminophen.

Figure 17



13. Amino Acid Derivative, Heme and Bilirubin Disorders

WWAMI FMR Block 1

Pamela Langer, PhD, WWAMI-WY, *Updated 6/22/2022*

Session Learning Objectives

SLO 1: Discuss the enzymatic defects in, and clinical consequences of, phenylketonuria (PKU) and homocystinuria.

SLO 2: Relate the disruption of the heme biosynthetic pathway to porphyrias and lead poisoning.

SLO 3: Describe the importance of heme degradation in the development of hyperbilirubinemia.

Amino Acid, Heme, Bilirubin Overview

An imbalance in the amino acid pool, causing either excesses or deficiencies of amino acids, can result from genetic disorders, such as phenylketonuria (PKU) and homocystinuria. Other disorders addressed in this chapter are associated with either the synthesis or degradation of heme, the prosthetic group for hemeproteins such as hemoglobin. Note that there are many other genetic disorders related to amino acids and amino acid derivatives that are important but are not covered in this session only because of time constraints.

SLO 1: Discuss the enzymatic defects in, and clinical consequences of, phenylketonuria (PKU) and homocystinuria.

With genetic defects causing a pathological consequence, the severity of the disorder depends on the specific mutation. For example, a mutation that causes the complete absence of a protein (from a null mutation) is likely to cause a more severe phenotype than a missense mutation, especially one that causes a conservative amino acid substitution. Clinical symptoms in genetic disorders may be the result of a deficiency of an amino acid, an accumulation of substrates of a defective enzyme, or the utilization of minor

metabolic pathways causing an abnormal excess of certain products.

Phenylketonuria (PKU)

Most cases of PKU result from autosomal recessive defects in the gene encoding *phenylalanine hydroxylase* (PAH), that catalyzes hydroxylation of phenylalanine (Phe) to tyrosine (Tyr). Over 1000 mutations have been identified in the PAH gene, and the worldwide prevalence of PAH is estimated at about 1/10,000 in populations with European ancestry. Milder, rarer forms of PKU occur if there is a deficiency in regeneration of the coenzyme for PAH, tetrahydrobiopterin (BH4), and treatment may be different than for PKU. PAH deficiency is associated with a range of intellectual disabilities depending on the severity of the defect and the effectiveness of treatment. Mechanisms underlying development of intellectual disabilities are incompletely characterized, but several potential mechanisms related to excess Phe have been proposed.

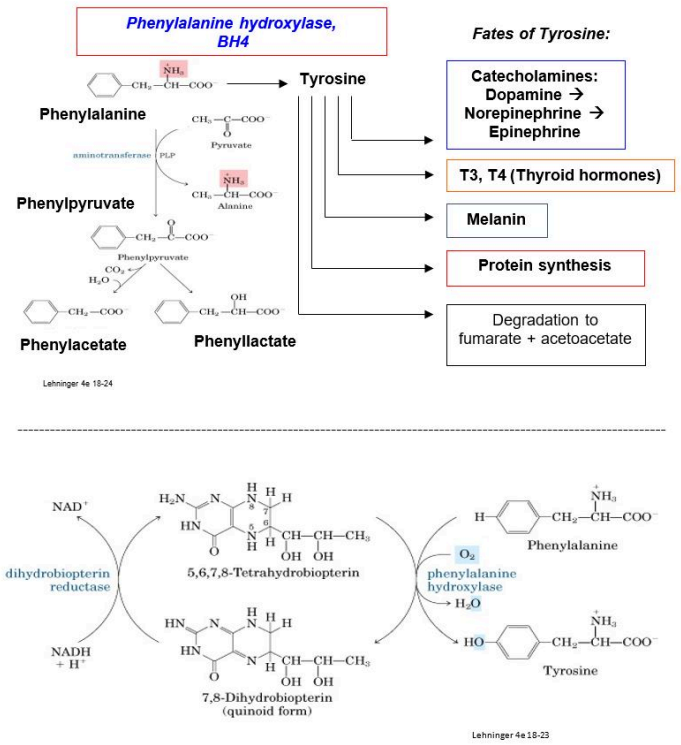


Figure 1.

Metabolic consequences of PKU include elevated blood and urine concentrations of Phe and its derivatives phenylacetate and phenyllactate. Although endogenous synthesis of tyrosine may be deficient, tyrosine is also obtained from the diet, and its physiological level may be normal or low-normal. Products synthesized from a tyrosine precursor are not necessarily deficient in PKU, e.g. hypothyroidism is a rare presentation for PKU. However, *tyrosinase*, an enzyme in the melanin biosynthetic pathway, is inhibited with high Phe. Consequently, individuals with PKU typically present with hair and skin with very little pigmentation.

There are many hypotheses for the connection between

biochemical abnormalities and development of intellectual disability, but the mechanism is incompletely understood. One (controversial) hypothesis involves the Large Neutral Amino Acid (LNAA) transporter. This LNAA transporter is the only transporter for large neutral amino acids, such as Phe, Tyr and Trp, across the blood brain barrier. Inhibition of this transporter with elevated Phe in PKU may cause a deficiency in transport of Tyr and Trp across the blood brain barrier (precursors for catecholamines and serotonin, respectively.)

Although a person may become more tolerant of dietary Phe with increasing age, women with PKU are advised to follow a Phe-restricted diet for at least three months before becoming pregnant and throughout pregnancy. The synthetic sweetener Aspartame, aspartyl-phenylalanine methyl ester, should be avoided at all times.

If a mother with PKU on a Phe-unrestricted diet becomes pregnant, elevated serum Phe levels can cause phenylalanine embryopathy (maternal PKU syndrome) regardless of the genotype of the fetus. Phe levels are higher in the fetus than the mother and may reach a concentration that is teratogenic. However, the mechanism of the teratogenic potential of elevated Phe and/or the Phe derivatives phenylacetate and phenyllactate, is not known. Unmanaged maternal PKU syndrome is at high risk for birth of a baby with intellectual disability, microcephaly, congenital heart disease, and low birth weight. All newborns in the US should be screened for PKU as part of a newborn screening panel, as impaired brain development may be minimized in a newborn by initiating a low-Phe diet at the earliest possible time after birth and within the first week of life.

Although it is beyond the scope of this brief discussion of PKU, various treatment strategies for PKU have been developed, including administration with a synthetic BH4. The newest addition to the list is a phenylalanine-degrading enzyme called *phenylalanine ammonia lyase*, originally derived from a prokaryote and produced as a recombinant form conjugated with polyethylene glycol (PEG) to minimize immunogenicity. Pegvaliase, considered to be the first

in its class as a new medication and approved by the FDA for use in 2018, has the goal of degrading Phe to lower the level of Phe in the blood. Unfortunately, severe allergic reactions are a common side effect of the drug, so the first dose must be administered in a healthcare setting in case of an anaphylactic response.

Homocystinuria

The predominant autosomal recessive defects associated with homocystinuria are found in the gene encoding *cystathionine beta-synthase* (CBS), a pyridoxal phosphate (PLP)-requiring enzyme in the cysteine biosynthetic pathway. The biochemical consequence of this deficiency is an increase in homocysteine (Hcys) and its disulfide-bonded form, homocystine. The side chain of homocysteine (-CH2-CH2-SH) is longer than that of cysteine (-CH2-SH). Homocysteine is an essential part of the folate and S-adenosylmethionine (SAM) cycles and is a substrate for the shared reaction catalyzed by *methionine synthase* at the intersection of these two cycles.

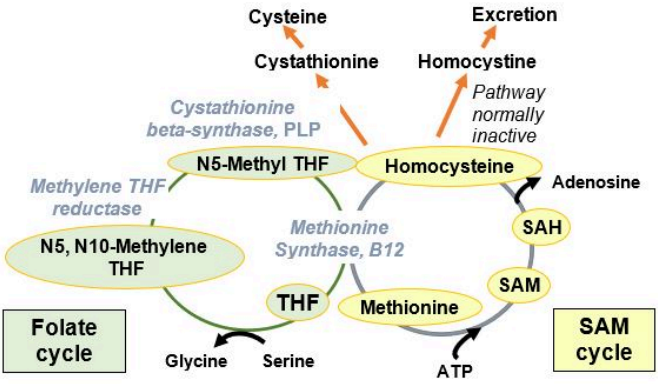


Figure 2

Worldwide prevalence of homocystinuria is reported as 1/200,000 to 1/335,000, but higher in certain populations (Ireland,

Germany, Norway, Qatar). Patients develop hyperhomocystinuria, hyperhomocysteinemia, cysteine deficiency, and physical abnormalities that could result from a number of biochemical consequences. One of the biochemical consequences of elevated Hcys is the inhibition of production or activity of *lysyl oxidase* (LOX), an enzyme important in collagen cross-linking. Elevated Hcys may also promote the SAM cycle and thus methylation affecting transcription.

A newborn may first appear normal but later present with symptoms including developmental delay, Marfanoid appearance, osteoporosis, ocular abnormalities, thromboembolic disease, severe premature atherosclerosis and extreme elevations in plasma Hcys. Elevated Hcys is considered an independent risk factor for increased risk of coronary heart disease (CHD).

SLO 2: Relate the disruption of the heme biosynthetic pathway to porphyrias and lead poisoning.

Heme is the prosthetic group for diverse hemeproteins, including hemoglobin, myoglobin, cytochrome P450 enzymes, cytochrome c in the electron transport chain (and apoptosis), *catalase* acting as an anti-oxidant catalyzing the degradation of hydrogen peroxide (H2O2) to water, and *thyroid peroxidase*, essential in thyroid hormone biosynthesis.

Starting with the amino acid glycine and succinyl CoA, heme is synthesized via the porphyrin biosynthetic pathway, a multi-step pathway distributed between the mitochondrion and cytosol and operating in most cells (see figure for comments by enzyme numbers). ALA *synthase* (5-*aminolevulinic acid synthase*-1, ALAS-1), the first enzyme of the porphyrin pathway, is the rate-controlling step. ALAS-1 is highly inducible and responds to changes in nutritional status (e.g. glucose levels) and use of certain drugs. Normally, heme causes feedback inhibition of ALA *synthase*. When the porphyrin pathway is compromised, porphyrin pathway intermediates are elevated, and oxidized colored porphyrin intermediates can be detected clinically.

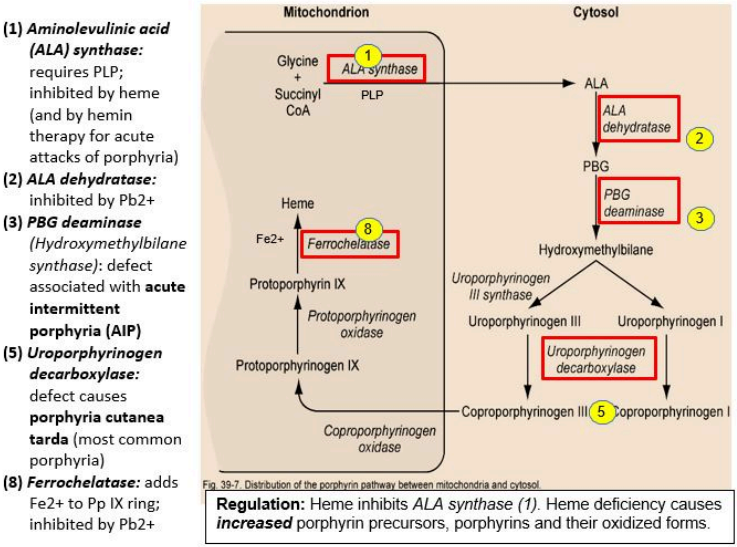


Figure 3: Regulation: Heme inhibits ALA *synthase* (1). Heme deficiency causes *increased* porphyrin precursors, porphyrins and their oxidized forms.

Enzyme defects in porphyrin biosynthesis or inhibition of the pathway can lead to heme deficiency and neurological/ neuromuscular and cutaneous porphyrias. Secondary disorders of porphyrin metabolism are caused by lead poisoning or iron deficiency.

Neurological porphyria, such as an acute hepatic porphyria (e.g. acute intermittent porphyria, AIP) may present with symptoms that include abdominal pain and neuromuscular and psychiatric disturbances. Photosensitization in cutaneous porphyrias results from accumulated, photo-excited porphyrins that are reactive. Cutaneous porphyrias are blistering cutaneous porphyria (e.g. porphyria cutanea tarda, PCT, the most common porphyria) and acute nonblistering cutaneous porphyria. Genetic testing is typically used in diagnosis of porphyrias.

Lead (Pb) poisoning

Lead (Pb^{2+}) inhibits two enzymes in the porphyrin pathway: ALA *dehydratase*, the second step, and *ferrochelatase*, catalyzing the addition of Fe^{2+} to protoporphyin IX. Lead is a neurotoxin that accumulates in soft tissues and bones by binding to sulfhydryl groups, normally bound by Ca^{2+}, Fe^{2+}, Zn^{2+}. It has been observed that Pb^{2+} concentrated in bones may be released later in life as bone is remodeled. Symptoms of lead poisoning include irritability, loss of appetite, weight loss, lethargy, abdominal pain, vomiting, constipation, and learning difficulties. Although the nervous system and kidney are more commonly affected, anemia may result, but only with high lead levels. In treating lead poisoning, it is imperative to remove the source, which is often chips of paint containing lead. It may be more practical, economical and safer to paint over old paint rather than try to remove it. With high dose lead exposure, a chelating agent such as EDTA (ethylenediaminetetraacetic acid) is used to sequester divalent Pb^{2+} cations.

Acute intermittent porphyria (AIP)

AIP is the most common acute hepatic porphyria, with a prevalence of approximately 5/100,000 in the US, including individuals who are pre-symptomatic, and a higher prevalence in Sweden, because of a founder effect. AIP is caused by an autosomal dominant defect in the *HMBS* gene encoding *Porphobilinogen (PBG) deaminase* (also called *Hydroxymethylbilane (HMB) synthase*). The genetic deficiency alone is not sufficient to cause a clinical presentation, and there is a low penetrance of the genetic disorder. AIP rarely presents before puberty and often remains in a latent phase such that many patients never have symptoms.

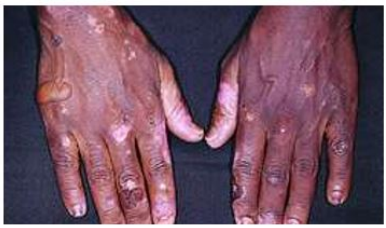
The first sign of an AIP attack is often an intense, diffuse abdominal pain that is constant. Some people have psychological manifestations including insomnia, anxiety, depression, hallucinations and other states of altered consciousness. AIP attacks range from mild to potentially life-threatening acute attacks that include severe abdominal pain accompanied by nausea, vomiting, tachycardia and hypertension.

Although AIP is an autosomal dominant disorder, presentation of AIP is intermittent in that symptoms may occur only after a patient with AIP experiences stresses such as inadequate caloric intake (e.g. with fasting or heavy exercise), excess alcohol intake, menstrual hormonal changes, illness, surgery, or if they are exposed to substances that stimulate porphyrin biosynthesis or the cytochrome P450 enzymes.

Certain prescribed and illicit drugs detoxified by hepatic cytochrome P450 enzymes, or drugs that induce *ALA synthase-1* expression, may also induce an attack, e.g. barbiturates, anti-epileptic drugs, sulfa-containing antibiotics, progestogens, and synthetic estrogens. In these cases, the porphobilinogen (PBG) substrate for the deficient *PBG deaminase* is elevated in urine, and porphyrin concentrations in plasma and stool may be tested to exclude other porphyrin pathway disorders.

***Porphyria cutanea tarda* (PCT)**

PCT is a chronic porphyria of the skin, associated with blistering cutaneous photosensitivity. Photosensitization results from accumulated, photo-excited porphyrins that are reactive. It is important to shield dark and light-pigmented skin from sunlight, as sunscreen does not protect from damaging wavelengths in patients with PCT. The familial form results from a genetic defect in *uroporphyrinogen III decarboxylase*. Patients with monoallelic or biallelic defects in the HFE gene (associated with the iron overload disorder hemochromatosis) may also show symptoms of PCT. Acquired PCT is associated with alcohol, smoking, hepatitis C, estrogens, and HIV. A patient may be initially asymptomatic but develop skin lesions when they take drugs that induce porphyrin synthesis or drink excessive alcohol. Porphyrin intermediates accumulate and react with oxygen to produce reactive oxygen species (ROS) that cause skin damage.



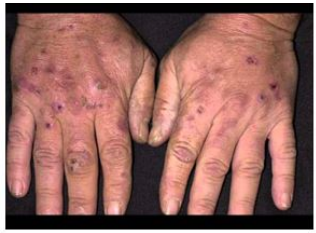


Figure 4.

Photodynamic diagnosis and therapy (PDT)

Properties of porphyrins have been exploited in photodynamic diagnosis (e.g. to reveal location of cancer cells) and photodynamic therapy (PDT), where porphyrin photosensitizers are activated to promote a selective cytotoxic effect on cancer cells. PDT is used commonly to treat pre-cancerous skin conditions. In "blue light therapy," aminolevulinic acid (ALA) is first applied topically to affected areas. Abnormal skin cells (to a much greater extent than surrounding normal cells) convert ALA to protoporphyrin IX (Pp IX), the final intermediate in the porphyrin pathway to heme. When skin is subjected to blue fluorescent light of appropriate wavelength, the Pp IX absorbs energy and produces oxygen free radicals that damage and kill the abnormal skin cells.

SLO 3: Describe the importance of heme degradation in the development of hyperbilirubinemia.

Heme is catabolized first to biliverdin, a green pigment, which is converted to bilirubin, a yellowish red pigment that must be excreted to avoid toxic effects of hyperbilirubinemia. Bilirubin is processed in the liver for eventual excretion via the intestine or kidney. This section discusses localized heme catabolism in skin with bruises, basics of hepatic bilirubin conjugation to facilitate excretion, and lastly, consequences of elevated bilirubin levels in utero and in the newborn.

Bruises

When capillaries in the skin are damaged, blood extravasates, hemoglobin is released from damaged red blood cells, and white blood cells eventually clear the damage. In tissue macrophages, globin is degraded by proteolysis, and heme is catabolized to

biliverdin by *heme oxygenase*, which opens the heme ring, releasing ferric iron (Fe3+) and carbon monoxide. Biliverdin is then converted to bilirubin by *biliverdin reductase*, and the iron is taken into ferritin, an iron storage protein that may become part of hemosiderin, contributing to a yellow-brown color in bruises. The presence of hemoglobin (red-blue) and the heme catabolites biliverdin (green) and bilirubin (yellow-red) typically follows the color-changing sequence: pink/red -> purple/blue/black-> violet/green/dark yellow -> pale yellow.

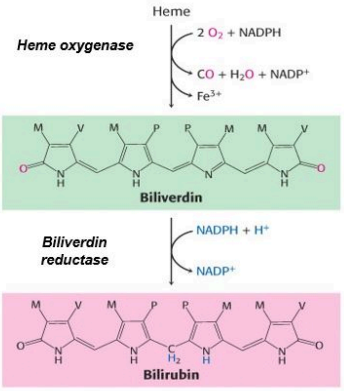


Figure 5

Sometimes blood extravasating from a damaged capillary can leak into tissue near the site of trauma, causing discoloration. Hemorrhagic "blotching" greater than 1 cm, from blood pooling under the skin or mucus membranes, is called ecchymosis. An example of this is periorbital ecchymosis, which may appear as if the person has a "black eye."

With intravascular lysis of red blood cells, the **hemoglobin (Hb) tetramer** binds a **haptoglobin (Hp) tetramer**. The Hb/Hp complex protects the body against potentially harmful oxidative damage by free hemoglobin, heme and iron until globin is degraded and heme is converted to bilirubin, which is then processed in the liver for excretion. Haptoglobin is depleted when excess hemoglobin is released during red blood cell lysis in hemolytic anemia. Consequently, haptoglobin is one of the analytes assayed in screening and monitoring for intravascular hemolytic

anemia. Haptoglobin levels may also increase during inflammation and infection, warranting its designation as an acute-phase reactant.

Hepatic bilirubin metabolism

Bilirubin is transported in the blood by serum albumin. Bilirubin enters the hepatocyte via a form of organic anion transporter polypeptide (OATP) and binds *glutathione S-transferase* (GST, formerly called ligandin), which concentrates the unconjugated, hydrophobic bilirubin in the cell. Conjugation of bilirubin with glucuronic acid via ***UDP-glucuronosyl transferase* (UGT)**, results in the more hydrophilic bilirubin glucuronide and bilirubin diglucuronide that are refered to as conjugated bilirubin. Conjugation of bilirubin facilitates its transport into the biliary canaliculi via multiple drug resistance associated protein-2 (MRP2) pump. The related MRP3 on the sinusoidal membrane pumps conjugated bilirubin back into the blood in times of conjugated bilirubin excess, for example when there is a defect in MRP2 that prevents efficient excretion of conjugated bilirubin from the hepatocyte into the bile.

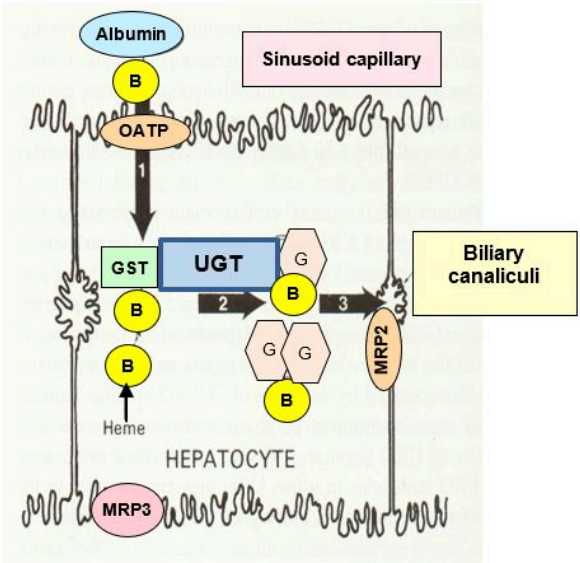


Figure 6.

Defects in the ***UDP-glucuronosyl transferase* (UGT)** gene can cause elevated bilirubin. A relatively common defect is associated with reduced UGT expression, frequently caused by a promoter mutation in the UGT1A1 gene. Patients with this defect have Gilbert syndrome and may be asymptomatic or present with occasional periods of mild hyperbilirubinemia. Children with the rare genetic disorder Crigler-Najjar syndrome, either do not have a detectable level of UGT (Type 1, very severe) or have very low levels of UGT (Type 2). Reduced transport of conjugated bilirubin into the bile is caused by a defect in the gene encoding MRP2 (Dubin-Johnson syndrome). Processing and excretion of bilirubin, relevance to disorders, and changes in analytes will be revisited in more detail in the liver section of another foundations course.

Note that UGT is not a cytochrome P450 enzyme, it has multiple isoforms, and is a Phase 2 enzyme involved in processing several endogenous metabolites and xenobiotics.

Neonatal jaundice

In utero, fetal bilirubin is processed by the maternal liver, so under normal circumstances, toxicity from bilirubin is avoided. However, after birth, processing of bilirubin by the newborn is dependent on the expression of ***UDP-glucuronosyl transferase*** (UGT), which is one of the last enzymes to be expressed in the developing fetal liver. Until UGT is produced adequately in a newborn (especially if premature), they will present with jaundice. Newborn infants with jaundice may be treated by exposure to bili-lights within a certain range of wavelengths, in order to isomerize bilirubin to a more hydrophilic form that is excreted more easily. If bili-lights are unavailable in a healthcare facility, limited exposure to sunlight may be recommended. In addition to the UGT deficiency described above, there are other causes of neonatal jaundice, such as dehydration and breastfeeding failure jaundice.

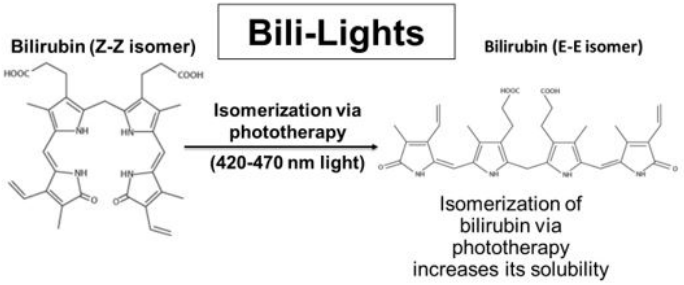


Figure 7.

Hemolytic disease of the fetus and newborn

The most common situation where fetal hemolytic anemia may arise is with Rh incompatibility (also called "Rh disease"). This disorder can occurs if the mother lacks the RhD red blood cell antigen (RhD negative) and the father and fetus are RhD positive. The Rh-negative mother is at risk of exposure to the fetal RhD antigen during her first pregnancy with an RhD-positive fetus, especially during the birthing process. The mother mounts an immunological response to fetal RhD antigen, and her subsequent RhD-positive pregnancies will be at risk for damage to the fetus.

During pregnancy, if maternal IgG antibodies against fetal red blood cell antigens cross the placenta, this can cause lysis of circulating fetal red blood cells, releasing large amounts of fetal hemoglobin. The excess bilirubin from heme degradation, may exceed the level that the mother can process and excrete, thereby exposing the fetus to a toxic level of bilirubin. In addition, as a consequence of a fetal red blood cell deficit, immature fetal red blood cells called erythroblasts may be present in the fetal circulation, a condition called erythroblastosis fetalis. Severe cases may result in fetal death in utero, however fetal hyperbilirubinemia may lead to a range of consequences in the future child, from mild hearing loss to severe intellectual disability. A rare but serious form

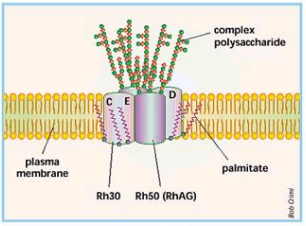
of brain damage called kernicterus, results from the chronic and permanent consequences of bilirubin-induced neurologic dysfunction (BIND).

As a preventive measure, an anti-RhD antibody drug (Rhogam) is given to the mother during her first and subsequent pregnancies to inhibit the mother's immune system from being stimulated with the RhD fetal antigen.

Note that the Rh protein complex is unrelated to the glycosphingolipid ABO blood group antigens.

Rh factor: D subunit of Rh complex

- Rh complex contains several transmembrane proteins, including RhD
- "Rh negative" denotes the absence of the RhD antigen, usually from deletion of the RhD gene
- Note that the Rh protein complex is unrelated to the glycosphingolipid ABO blood group antigens.
- Other types of blood group antigen incompatibilities exist in addition to RhD incompatibility.



Glycosphingolipids in ABO blood group antigens

Glycosylase A or B variant modifies sphingolipid.

- **O antigen:** glycosylase is non-functional and only core oligosaccharide is on lipid
- **A antigen:** A glycosylase variant transfers GalNac to core oligosaccharide
- **B antigen:** B glycosylase variant transfers Gal to core oligosaccharide

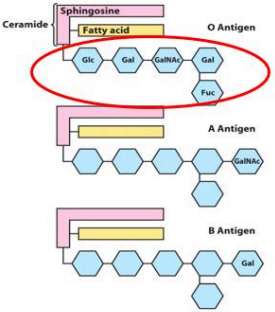


Figure 8.

14. Single Carbon Metabolism

SINGLE CARBON METABOLISM

WWAMI FMR Block 1

Updated 6/20/22, Pamela Langer, PhD, WWAMI-WY

Session Learning Objectives

SLO 1: Identify the functions of vitamin B12, and explain the role of intrinsic factor in its absorption.

SLO 2: Explain the causes and consequences of Intrinsic Factor, B12 and folate deficiencies.

SLO 3: Outline the S-adenosylmethionine and folate cycles and explain why B12 deficiency leads to megaloblastic anemia due to a secondary folate deficiency.

SLO 4: Illustrate therapeutic uses of pharmaceuticals that disrupt folate metabolism and describe the consequences.

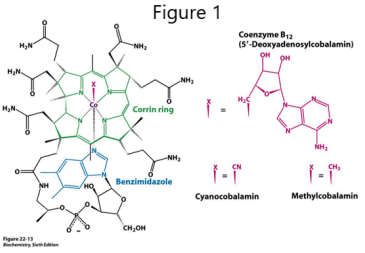
SINGLE CARBON METABOLISM OVERVIEW

One-carbon fragments participate in many metabolic reactions. Single carbon donors are especially important in the biosynthesis of nucleic acid precursors, which in turn are required for cell growth and division. Because of this role in cell proliferation, single-carbon metabolism is a prominent target for chemotherapeutic drugs.

This review of single carbon, one carbon or 1C metabolism discusses the roles of vitamin B12 (cobalamin), S-adenosylmethionine (SAM), and folate derivatives as donors of 1C groups transferred in different oxidation states. For example, methyl groups are transferred from SAM and methylcobalamin; and derivatives of tetrahydrofolate (THF) are used in the synthesis of purines, deoxythymidylate (dTMP), and methionine. For this course, it is not necessary to know the specific names of THF derivative forms or which is used in each reaction. Because of the interconnected relationship of the roles of Vitamin B12, SAM and folate metabolism, a short

introduction to each will begin the discussion.

Vitamin B12 (cobalamin, B12) Figure 1



The corrin ring system of B12, related to the iron-containing porphyrin ring in heme, contains cobalt, and so, another name for B12 is cobalamin. B12 is not made by plants or animals and is synthesized only by a few species of microorganisms. B12 is derived from food sources that have harbored or eaten bacteria that make B12. Intrinsic factor (IF), a glycoprotein made by gastric parietal cells, is required for receptor mediated endocytosis of B12 during absorption in the terminal ileum of the small intestine.

Different B12 vitamers are used in the two B12-dependent reactions in mammals. Other forms of interest are hydroxocobalamin and cyanocobalamin. The low concentration of cyanocobalamin, as the B12 vitamer in many multivitamin pills, is nontoxic. Cyanide (CN) is found in insecticides, tobacco smoke, almonds, apricot kernel, apple and orange seeds, and cassava root, but symptoms appear only with high doses or long-term exposure. In CN poisoning, CN inhibits mitochondrial cytochrome C oxidase, which is part of the electron transport chain, causing a deficit of ATP along with seizure, apnea and cardiac arrest. In addition to giving a patient oxygen, hydroxocobalamin may be used in treating CN poisoning. CN displaces the hydroxyl group in hydroxocobalamin and cyanocobalamin is excreted.

Folate (Vitamin B9), Tetrahydrofolate (THF, FH4)

Animals synthesize the pteridine ring of folate but cannot conjugate it to para-aminobenzoate acid (PABA) and glutamate, so they must obtain folate from their diet or from intestinal bacteria. Folate deficiency is a common vitamin deficiency, particularly during pregnancy with the increased demands of fetal growth, and in alcoholism, with a diet often deficient in folate. However, in many (but not all) places in the world, grains and other food are now supplemented with folic acid.

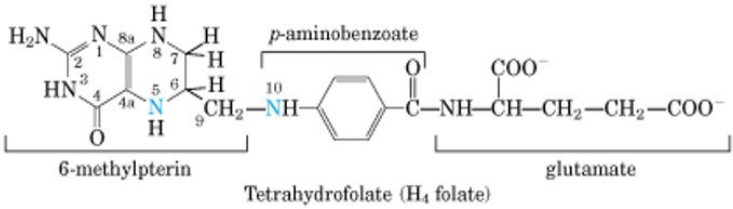


Figure 2.

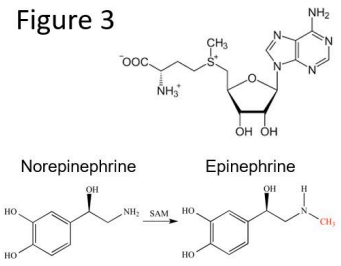
***Dihydrofolate reductase* (DHFR)** catalyzes reduction of folate to dihydrofolate (DHF) and DHF to tetrahydrofolate (THF). Single carbon (1C) units, in different oxidation states, are then carried on N5, N10, or both positions in derivatives of THF.

S-*adenosylmethionine* (SAM)

SAM (Figure 3; Top) is synthesized from methionine and ATPs. SAM contains a methyl group attached to a sulfonium ion and is a highly reactive methyl group donor. After transfer of the methyl group, SAM is regenerated in the "methyl" or SAM cycle. SAM is used by:

- **Methyltransferases,** in DNA methylation and synthesis of creatine, homocysteine, phosphatidylcholine, carnitine, melatonin, and catecholamine neurotransmitters (e.g. the methyl group of SAM is used in the synthesis of epinephrine from norepinephrine)
- **Polyamine synthesis**, utilizing a product of SAM decarboxylation, produces spermidine and spermine from putrescine.

Figure 3



The generation of SAM and regeneration of tetrahydrofolate (THF) are connected in a folate and SAM "bicycle" with a shared reaction catalyzed by the B12 coenzyme-dependent ***methionine synthase***. In the absence of B12,

dietary methionine can still be used to make SAM, but homocysteine and N5-methyl THF will accumulate. Because the functioning of both the folate and SAM cycles is B12-dependent, B12 deficiency may result in a secondary THF deficiency. THF derivatives are required to produce precursors for DNA synthesis and repair. Consequently, a B12 deficiency can result in megaloblastic anemia. The development of megaloblastic anemia will be discussed in more detail later.

This Folate-SAM bicycle figure includes details that will be addressed throughout the discussion of B12, folate and SAM metabolism as well as in other topics in this course.

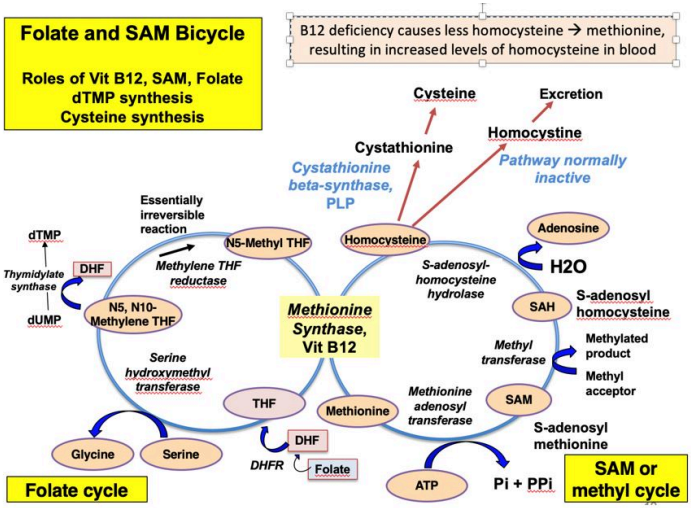


Figure 4. The Folate SAM bicycle- this one only goes forward if you pop a wheelie!

SLO 1: Identify the functions of vitamin B12, and explain the role of intrinsic factor in its absorption.

Vitamin B12 function

Vitamin B12 is used as a coenzyme in only two reactions in

humans. In the B12-dependent ***methionine synthase*** reaction, a methyl group is first transferred from N5-methyl THF to cobalamin and then from methylcobalamin to homocysteine to form methionine in a so-called 'ping pong reaction.' The significance of this reaction is that it: (1) produces methionine, that goes on to become the important methyl donor SAM, (2) regenerates THF to sustain the folate cycle, and (3) frees the methyl group from N5-THF that would otherwise be "trapped" since the folate cycle proceeds in one direction because of the essentially irreversible reaction that generates N5-THF.

Two Vit B12-requiring reactions in mammals:

Methylation of Homocysteine to form Methionine

(*Methionine synthase* uses **methylcobalamin**)

Methylmalonyl CoA à Succinyl CoA

(*Methylmalonyl-CoA mutase* uses **5'-deoxyadenosylcobalamin**)

Secondly, another B12 vitamer (5'-deoxyadenosylcobalamin) is required for the ***methylmalonyl CoA mutase*** reaction in the metabolism of odd chain fatty acids. Propionyl CoA arises from the breakdown of certain amino acids, cholesterol and odd numbered fatty acids. Propionyl CoA is converted to methylmalonyl CoA which in turn is converted by ***methylmalonyl CoA mutase*** to succinyl CoA, which enters the TCA cycle. A deficiency in vitamin B12 would lead to a buildup of methylmalonyl CoA, which is hydrolyzed to methylmalonic acid (MMA) and excreted in the urine of patients with vitamin B12 deficiency.

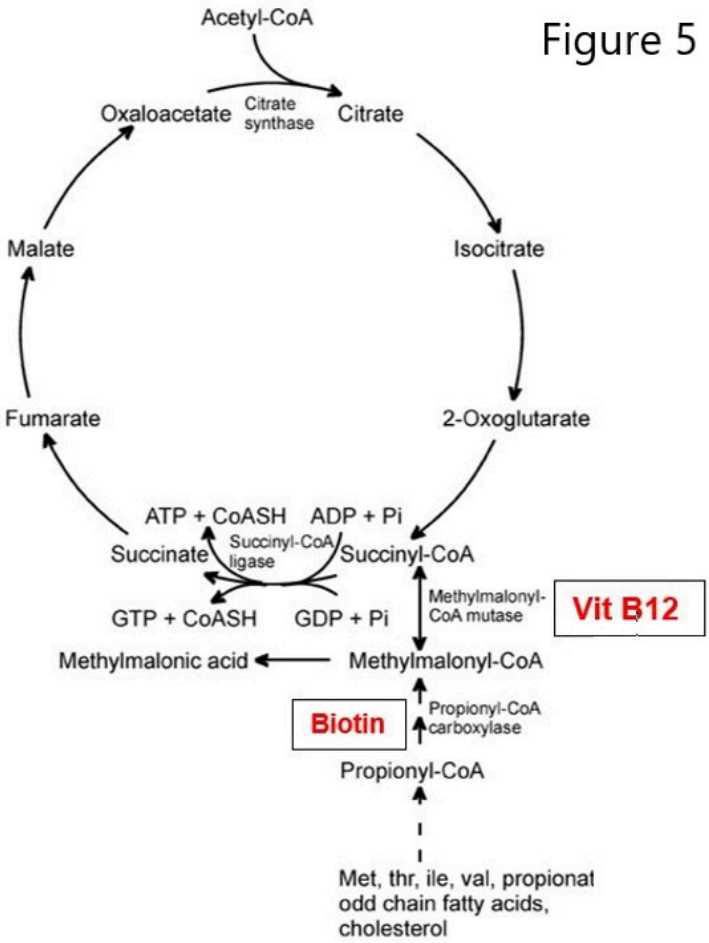


Figure 5. Methylmalonyl-CoA -> Succinyl-CoA requires B12.

People with a rare defect in the ***methylmalonyl CoA mutase*** gene present with mild to life-threating methylmalonic acidemia and very low blood pH. Because early dietary intervention may help with this genetic disorder, it is a disorder on newborn screening panels.

Role of intrinsic factor in Vit B12 absorption

- Dietary B12 is released from proteins in the presence of increased acid and proteases in the stomach.
- HCl from gastric parietal cells is necessary for production of the *pepsin* protease from pepsinogen.
- B12 binds an R-Protein (e.g. haptocorrin made in the salivary gland) in the mouth but mainly in the stomach, once B12 is released from food.
- Then, B12 is released from haptocorrin by pancreatic proteases in the small intestine.
- Intrinsic factor (IF), a glycoprotein secreted by gastric parietal cells, next binds B12 in the intestine.
- The IF/B12 complex goes into the enterocytes lining the ileum via receptor-mediated endocytosis.
- Once inside the enterocyte, IF is degraded, releasing B12 that will exit the cell and bind to other B12-binding proteins (e.g. transcobalamin) in blood.
- The transcobalamin/B12 complex will travel in the blood to cells where B12 functions as a coenzyme for two metabolic reactions.

SLO 2: Explain the causes and consequences of Intrinsic Factor, B12 and folate deficiencies.

SLO 3: Outline the S-adenosylmethionine and folate cycles and explain why B12 deficiency leads to megaloblastic anemia due to a secondary folate deficiency.

Decreased Vit B12 levels: some causes

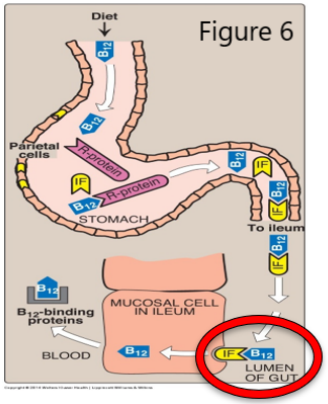
A low Vit B12 level may be caused by decreased intake or availability or decreased absorption of Vit B12, for example:

- **Geriatric decrease in intrinsic factor (IF):** Elderly people and those with certain GI disorders may not produce sufficient intrinsic factor and therefore not absorb B12.
- **Long-term reduced gastric acidity** inhibits dissociation of

protein bound B12

- o **Proton pump inhibitor** (e.g. Omeprazole) causes gastric pH ≥ 5 for 24h; *pepsin* is inhibited at pH 5.
- o **Autoimmune attack on parietal cell H+/K+ ATPase** causes reduced acid secretion. (H+/K+ ATPase pumps H+ into the gastric lumen).

- **Atrophy of gastric mucosa** may cause a parietal cell deficiency (e.g. with ulcers, cancer), resulting in reduced HCl and intrinsic factor (IF) deficiency
- **Autoimmune attack on parietal cell intrinsic factor** results in low IF, causing pernicious anemia, a type of megaloblastic anemia
- **Celiac disease** causes damage to intestinal lining, sometimes affecting area of B12 absorption
- **Intestinal resection** may involve lower ileum where B12 is absorbed



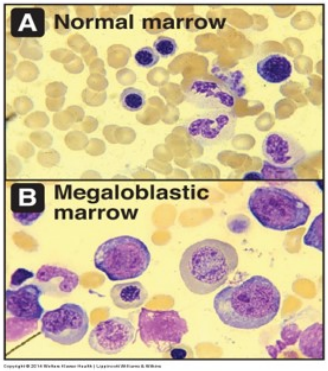
Consequences of Vit B12, folate and intrinsic factor deficiencies

Decreased release of B12 from food because of reduced acid secretion usually results in a mild B12 deficiency that may not have clinical consequences or can be treated easily with B12 injections. However, an intrinsic factor deficiency, or damage to or resection of the terminal ileum site of B12 absorption, can result in severe B12 deficiency leading to megaloblastic anemia.

Tetrahydrofolate (THF) derivatives are required for synthesis of purines and thymidylate. Synthesis is compromised when the methyl group is "trapped" in N5-methy-THF and THF regeneration

is decreased. Thus, the deficiency of the THF derivative (N5, N10-methylene THF) required for dUMP -> dTMP synthesis by ***thymidylate synthase*** causes a decrease in dTTP and therefore decreased DNA synthesis and repair. The cell cycle cannot progress from G1 to S phase, resulting in continuing cell growth without division and accumulation of large nucleated RBC precursors called megaloblasts. The RBC and hemoglobin deficiencies result in reduced O2 transport and megaloblastic anemia. Other consequences of B12 deficiency may include a mild hyperhomocysteinemia associated with increased risk of cardiovascular disease, alteration of methylation reactions from SAM deficiency, and effects stemming from decreased activity of the B12-dependent ***methylmalonyl CoA mutase*** reaction.

Figure 7



The etiology of neurological damage associated with B12 deficiency is likely complex. Patients may present with demyelination, peripheral neuropathy, fatigue, depression and difficulty with balance. While B12 deficiency inhibits ***methylmalonyl CoA mutase***, as well as ***methionine synthase*** at the intersection of the folate and SAM cycles, hypotheses for the mechanisms underlying neurological damage have included consequences of compromising either or both of the B12-dependent reactions. Disturbances in malonyl CoA and methylmalonyl CoA metabolism may alter fatty acid synthesis, affecting regeneration of the myelin sheath. A deficiency in SAM causes a decrease in methyltransferase reactions, potentially affecting DNA methylation and synthesis of creatine, homocysteine, catecholamines, phosphatidylcholine, carnitine, melatonin and polyamines.

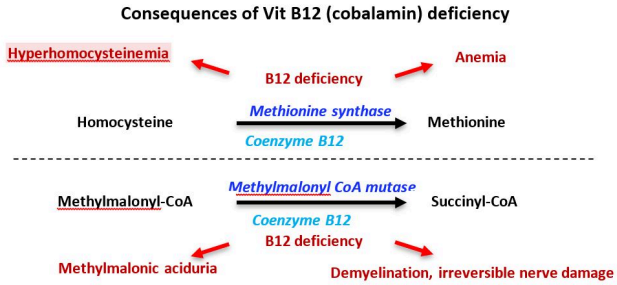


Figure 8. B12 deficiency summary

Folate deficiency in pregnancy

Increased folate is required to meet the demands of cell division, fetal growth, and altered metabolism during pregnancy. Folate deficiency can lead to birth defects such as spina bifida, a defect in closure of the neural tube. Sickle cell anemia causes an even greater need for folate. In the US, folate is added to flour and processed foods to prevent spinal tube defects caused by folate deficiency. Additional supplementation with folic acid, especially prenatally and in the first trimester, has further contributed to the decrease in neural tube defects. As a result of added folate in foods, B12 deficiency in the general population rarely causes megaloblastic anemia. However, once neurological symptoms of a B12 deficiency appear, they are irreversible. Therefore, it is important to check the B12 levels of patients at risk for other causes of a B12 deficit, such as loss of intrinsic factor.

Nitrous oxide toxicity

Nitrous oxide (N2O) oxidizes cobalt in the corrin ring, inactivating vitamin B12 but not reducing B12 levels. Chronic N2O exposure can occur from occupational, recreational or repeat (or even single) exposures in hospital procedures. Toxicity may present with unexplained neurological complications including sensorimotor peripheral neuropathy, megaloblastic changes and myelopathy such as subacute combined degeneration (SACD). For recreational users of N2O (laughing gas/hippy crack, stored in cannisters called

whippets), development of symptoms depends on the number of cannisters used per day, chronicity of exposure, and if the individuals are taking Vit B12 supplements. Intermittent abuse may require several months for neurological symptoms to appear, and rarely are there hematological changes. With respect to hospital procedures, even with a B12 deficiency, N2O anesthesia will rarely result in neurological or hematological complications. With respect to clinical analytes, homocysteine and methylmalonic acid (MMA) levels may be elevated, but B12 levels may be low, normal or high. The reason for this B12 level variance is that N2O inactivates B12, but B12 level determination includes inactive and active B12.

SLO 4: Illustrate therapeutic uses of pharmaceuticals that disrupt folate metabolism and describe the consequences.

Methotrexate (MTX) is a folic acid analog that competitively inhibits ***dihydrofolate reductase*** (DHFR). DHFR normally catalyzes two reactions, involving conversion of folic acid to DHF and DHF to THF. Consequently, many metabolic processes requiring THF derivatives as coenzymes are inhibited with use of methotrexate:

- Synthesis of purine ring on ribose
- Pyrimidine biosynthesis (dUMP -> dTMP)
- Synthesis of methionine from homocysteine, via methionine synthase, and subsequently the production of S-adenosylmethionine (SAM)

Methotrexate is used as an anti-cancer drug to inhibit quickly dividing cells and also commonly used as a disease-modifying antirheumatic drug (DMARD) for rheumatoid arthritis or psoriasis. As DMARDs suppress the immune system and reduce inflammation on a time scale of weeks to months, nonsteroidal anti-inflammatory drugs (NSAIDS) are often used for more immediate pain relief. Methotrexate is sometimes used in combination with other DMARDs as the treatment strategy for rheumatoid arthritis is tailored to be the most effective for the patient. Methotrexate is

on the World Health Organization (WHO) Model List of Essential Medicines.

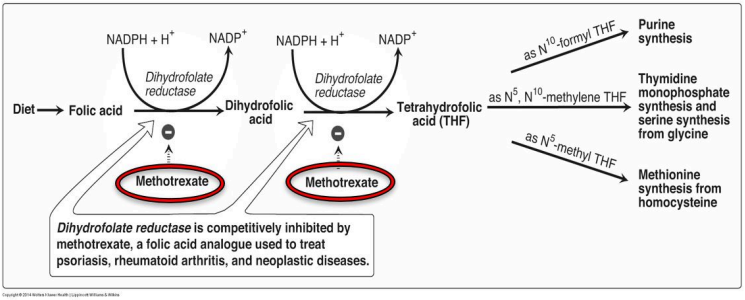
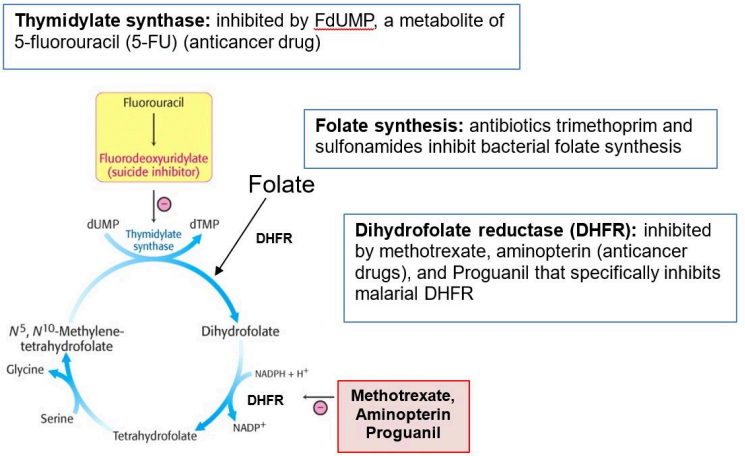


Figure 9. Methotrexate activity.

Aminopterin, another anti-cancer DHFR-inhibitor drug, was originally used to treat pediatric leukemia, but eventually its use was replaced by methotrexate, a safer, inexpensive drug. Some other DHFR inhibitors are anti-pathogen drugs, based on the principle that the drug inhibits the pathogen's DHFR to a greater extent than human DHFR. One example of this is Proguanil that acts to specifically inhibit malarial DHFR and thus inhibit parasite replication within the red blood cell (the intra-erythrocytic stage).

Figure 10. Drug Summary



5-fluorouracil (5-FU) is used in the treatment of several cancers. This pyrimidine analog is converted to fluoro-dUMP (FdUMP,

fluorodeoxyuridylate) which is an irreversible inhibitor of ***thymidylate synthase***. The resulting deficit in dTMP leads to a deficiency in DNA synthesis and repair, as explained previously in the discussion of Vitamin B12 and folate deficiencies. The consequence of this drug therapy is inhibition of quickly dividing cells, with the target being cancer cells. However, because individuals detoxify 5-FU with different efficiencies, depending on the catabolic enzymatic variant they express, 5-FU treatment can lead to an adverse drug reaction that in some cases can be lethal. This consideration is discussed further in the sessions on purine and pyrimidine metabolism and pharmacogenomics.

15. Purine, Pyrimidine Metabolism, Disorders

Updated 6/14/22, Pamela Langer, PhD, WY-WWAMI

Session Learning Objectives:

SLO1. Describe the difference between bases, nucleosides and nucleotides.

SLO2. Outline major steps and precursors utilized in the purine synthesis pathway including PRPP, IMP, AMP and GMP and regulatory steps.

SLO3. Outline major steps and precursors utilized in the pyrimidine synthesis pathway including PRPP, UMP, CTP and dTMP, and the genetic origins of orotic aciduria.

SLO4. Explain how ribonucleotides are reduced to deoxyribonucleotides with a focus on regulation of ribonucleotide reductase.

SLO5. Describe the purine salvage/recycling pathway and the connection between HGPRT and Lesch Nyhan syndrome.

SLO6. Outline the degradation of purines to uric acid, the origins of the disorder gout and relevant treatments, and how a deficiency in adenosine deaminase results in increased dATP and inhibition of the ribonucleotide reductase enzyme resulting in SCID.

SLO7. Describe and provide examples of how knowledge about nucleotide metabolism can be utilized to treat disorders such as cancer.

Overview: This session covers the metabolism of nucleotides and covers the following primary topics:

- The structures and nomenclature of nucleotides and their constituent parts.
- De novo synthesis of purines on PRPP

- De novo synthesis of pyrimidines and conjugations to PRPP
- Reduction of nucleotides to deoxynucleotides
- Recycling of purine bases
- Degradation of purines to uric acid

As you read through the course packet and work through the SLOs make note of genetic disorders and drugs to treat disorders of nucleotide metabolism or to inhibit nucleotide metabolism as a treatment for cancer and other conditions.

SLO1. Describe the difference between bases, nucleosides and nucleotides.

Nucleotides

Nucleotides are used as substrates in the biosynthesis of the nucleic acids RNA and DNA, in regulation of enzymatic reactions, and as a source of energy. The bases within nucleotides are either purines or pyrimidines, which are heterocyclic compounds whose rings contain carbon and nitrogen. The planar character of purines and pyrimidines facilitates their close association or stacking, which stabilizes double stranded DNA.

Guanine, adenine and cytosine are used in RNA and DNA while thymine is used in DNA and uracil in RNA. Sometimes uracil can appear in DNA as an error that is removed during DNA repair. Guanine (G) base-pairs with cytosine (C) and adenine (A) base-pairs with thymine (T) in DNA and with uracil (U) in RNA.

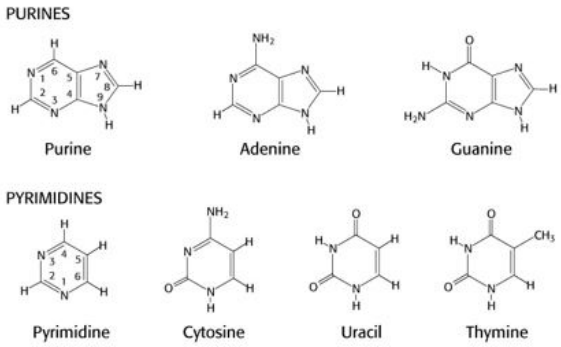


Figure 1.

It is not necessary to memorize the exact structures or atom numbering of these nitrogenous bases. However, you should be aware of:

- the use of base analogs that can be incorporated into DNA and used experimentally in the lab or therapeutically as anti-cancer drugs, such as 8-azaguanine (N at position 8 in the ring instead of C), 6-thioguanine (S bound to C6 instead of O) or 5-fluorouracil (F bound to C5 instead of H)
- processes that modify bases, such as methylation of cytosine to 5-methyl-C or the deamination of adenine to inosine or cytosine to uracil. The biological consequences of these base-modifying processes are discussed in other sections of this course.

Nucleosides and Nucleotides

When a purine or pyrimidine base is attached to a ribose, it is called a **nucleoside**. The addition of a phosphate to the ribose changes its designation to **nucleotide.** Nucleosides and nucleotides have different names listed in the table below, but in common usage the names are often used inaccurately and interchangeably, and the shortcut is to use the 1-letter designation.

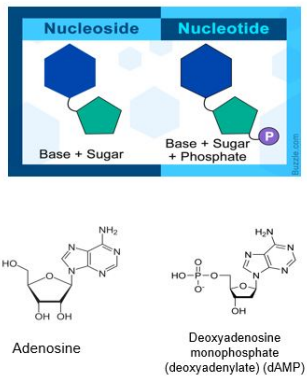


Figure 2

A deoxyribonucleotide is a ribonucleotide reduced at the 2' position of the ribose and is designated with a "d" in front of the name. An exception to this convention is thymidylate (TMP), which is already a deoxyribonucleotide, however thymidylate is often written as "dTMP" for clarity. The structures of adenosine (a nucleoside) and

deoxyadenosine monophosphate (dAMP, a deoxyribonucleotide) are shown here.

RNA BASE	RIBONUCLEOSIDE	RIBONUCLEOTIDE (with 5'-monophosphate)
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
DNA BASE	**DEOXYRIBONUCLEOSIDE**	**DEOXYRIBONUCLEOTIDE (with 5'-monophosphate)**
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Thymidine	Thymidylate (TMP, dTMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)

Table 1. Modified from Biochemistry, 6th edition, Freeman and Co. (2007)

Purine and pyrimidine pathways compared

The ribose for both purines and pyrimidines originates from the pentose phosphate pathway (hexose monophosphate shunt) and is used as 5-phosphoribosylpyrophosphate (PRPP) in the biosynthetic pathways for purines and pyrimidines.

Ribose 5-P + ATP à PRPP + AMP

Phosphoribosylpyrophosphate synthetase

De novo purine (Pu) and pyrimidine (Py) biosynthetic pathways

are highly conserved in nature and proceed via two conceptually different biochemical strategies:

- The purine ring is built on PRPP.
- The pyrimidine ring is synthesized first, and PRPP is the source for the ribose 5-P added to the pyrimidine ring.

While both pathways involve several enzymatic steps, multi-functional enzymes with more than one enzymatic activity and multi-enzyme complexes in a cell promote increased efficiency of the pathways.

The final catabolite of purines in humans is uric acid that is excreted or may precipitate as sodium urate in excess. Pyrimidines are catabolized to CO2, H2O and the nitrogen is excreted via the urea cycle.

SLO2. Outline major steps and precursors utilized in the purine synthesis pathway including PRPP, IMP, AMP and GMP and regulatory steps.

Purine biosynthesis, de novo pathway

The atoms in the purine ring originate from amino acids (aspartate, glycine and glutamine), CO2 and a folate derivative (N10-formyl-THF). Normal folate metabolism is required for synthesis of purines and formation of dTMP (a pyrimidine), needed as a precursor for dTTP used in DNA synthesis. Disruption of purine and pyrimidine biosynthetic pathways is used as a strategy for inhibiting the propagation of quickly dividing cells in cancer.

At the end of the so-called common pathway for de novo biosynthesis of purines, inosine monophosphate (IMP) is generated and acts as a precursor for the synthesis of AMP and GMP. Balance in the ribonucleotide pool is regulated in part by feedback inhibition by on the first two common steps in the de novo pathway and the dedicated reactions for synthesizing AMP or GMP. The availability of ribose 5-P and PRPP also has an effect on the functioning of this pathway. *Glutamine-PRPP amidotransferase* catalyzes the

committed step and may be inhibited by the anti-cancer glutamine analogs azaserine and acivicin.

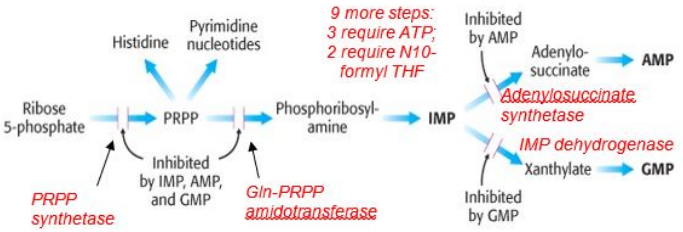


Figure 3.
Key points for de novo purine biosynthesis

1. Precursors for the purine ring include amino acids and a derivative of tetrahydrofolate (THF); deficiency in folate metabolism can compromise purine biosynthesis.
2. De novo purine biosynthesis is an energy-requiring process involving ATP.
3. The purine ring is built on the ribose-containing substrate phosphoribosyl pyrophosphate (PRPP) synthesized from ribose 5-P, a product of the pentose phosphate pathway.
4. Purine biosynthesis employs a common pathway to IMP, is activated by PRPP, and is feedback inhibited by IMP, AMP and GMP.
5. AMP and GMP inhibit their own respective pathways, regulating the balance of AMP and GMP production.

SLO3. Outline major steps and precursors utilized in the pyrimidine synthesis pathway including PRPP, UMP, CTP and dTMP, and the genetic origins of orotic aciduria.

***Pyrimidine* biosynthesis**

The mammalian pyrimidine biosynthetic pathway starts with synthesis of carbamoyl phosphate by the cytosolic *carbamoyl*

phosphate synthetase II using glutamine as a nitrogen donor. This is in contrast to the mitochondrial *carbamoyl phosphate synthetase I*, which uses ammonia and is part of the urea cycle. PRPP is the source of the ribose and phosphate in the synthesis of UMP. The UMP product of pyrimidine biosynthesis is the substrate for multistep processes to produce dTMP and CTP.

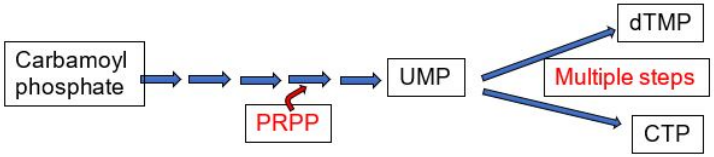


Figure 4.

In eukaryotes, *carbamoyl phosphate synthetase II* is part of a trifunctional enzyme ("CAD") catalyzing the first three steps of pyrimidine biosynthesis. Intermediates remain bound to the enzyme, increasing the catalytic efficiency of the enzyme. The trifunctional enzyme is regulated in humans through inhibition by UTP and activation by PRPP. The final two steps of the common pathway leading up to the UMP product are catalyzed by a bifunctional enzyme UMP synthase, further increasing catalytic efficiency. Thus, the entire pathway of 6 enzymatic activities utilizes trifunctional, monofunctional, and bifunctional enzymes.

Genetic defects in the gene encoding the bifunctional *UMP synthase* will cause an excess of orotate, resulting in the disorder orotic aciduria.

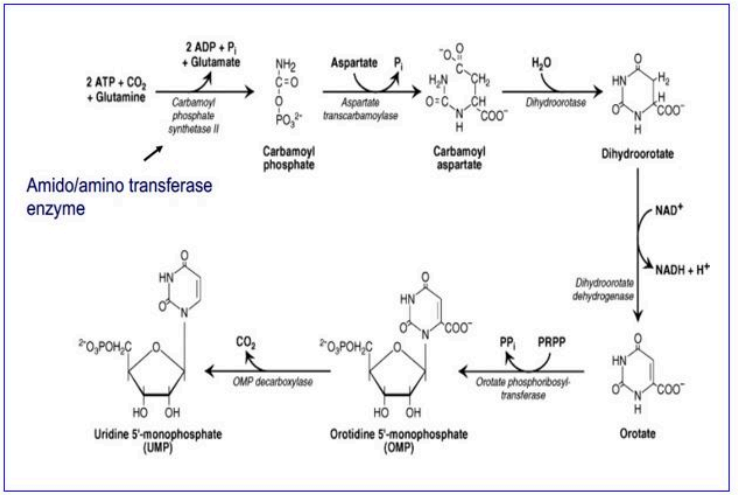


Figure 5.

The production of dTMP, as a precursor for DNA synthesis and repair, is required for cell division. Consequently, *thymidylate synthase* (TS), catalyzing dUMP -> dTMP, is a target for anti-cancer drugs. The *thymidylate synthase* reaction requires a tetrahydrofolate derivative for methylation of dUMP to form dTMP, so a deficiency or inhibition of folate metabolism can compromise production of dTMP and in turn, cell division.

Another enzyme, *thymidine kinase* (TK) catalyzes the phosphorylation of two deoxynucleosides, deoxyuridine or thymidine, to produce dUMP and dTMP, respectively. Viral thymidine kinase (TK) is not as fastidious as mammalian TK and is capable of using different substrates. Consequently, toxic nucleotide analogs (e.g. 8-azaguanine) can be phosphorylated and incorporated into viral DNA when viral TK is present and may be used in some anti-viral strategies. The basic pathways catalyzed by these two enzymes is shown below.

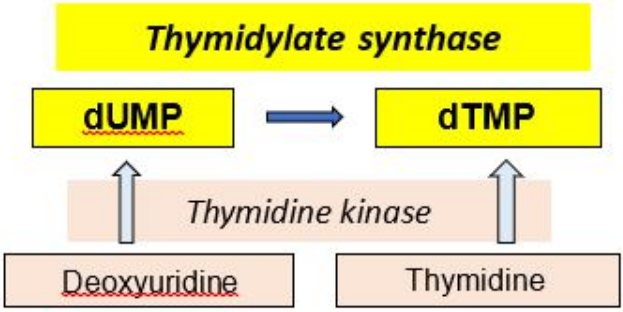


Figure 6.
Key points for pyrimidine biosynthesis

1. In an energy-requiring pathway, amino acids are used in the synthesis of the pyrimidine ring, followed by the addition of the ribose from phosphoribosyl pyrophosphate (PRPP), synthesized from ribose 5-P, a product of the pentose phosphate pathway.
2. Enzymatic efficiency: trifunctional enzyme (CAD) catalyzes first 3 steps and bifunctional *UMP synthase*, the last 2 steps.
3. Common pathway until UMP production, then different multi-step pathways are required to produce CTP and dTMP
4. A derivative of tetrahydrofolate is required for methylation of dUMP in the *thymidylate synthase* reaction catalyzing dUMP -> dTMP.
5. Importance of *thymidylate synthase* (TS), *thymidine kinase* (TK)

- TS may be inhibited by an anti-cancer drug or a deficiency in folate metabolism; viral TK can use toxic base analogs that the human enzyme cannot

SLO4. Explain how ribonucleotides are reduced to

deoxyribonucleotides with a focus on regulation of ribonucleotide reductase.

Deoxyribonucleotides lack a hydroxyl group at the 2' position of the ribose. *Ribonucleotide reductase* catalyzes reduction of a ribonucleotide diphosphate (NDP) to a deoxyribonucleotide diphosphate (dNDP). Without *ribonucleotide reductase* there would be no DNA!

The coenzyme thioredoxin is a reducing agent in the reaction catalyzed by *ribonucleotide reductase*. Reduced thioredoxin is regenerated with *thioredoxin reductase*, using NADPH.

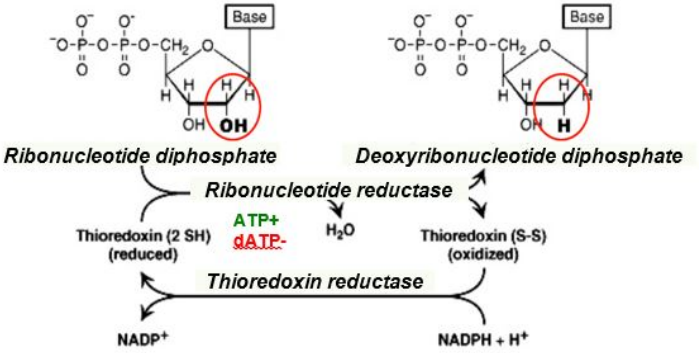


Figure 7.

Ribonucleotide reductase is regulated at two levels and is directed at maintaining balance in the nucleotide pool. The enzyme activity *and* substrate specificity are regulated allosterically. For example,

- **Activity sites:** ATP binding increases the net rate of enzymatic activity and dATP binding inhibits the overall catalytic activity
- **Substrate specificity:** Specific nucleotides regulate enzyme substrate specificity, allowing reduction of specific ribonucleotides and contributing to the balance of nucleotides in the cellular pool.

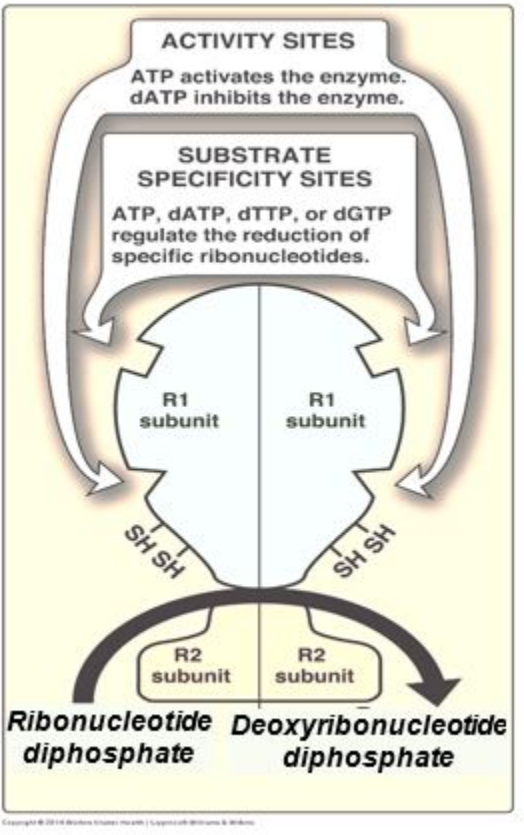


Figure 8.

Without balance in the nucleotide pool, the excess of some nucleotides could potentially drive incorporation of the incorrect nucleotide during DNA synthesis and result in increased errors.

Thymidylate synthase (TS), with substrates dUMP and a THF derivative, catalyzes the synthesis of the deoxyribonucleotide thymidylate, also called dTMP. This reaction also yields dihydrofolate (DHF) subsequently reduced by *dihydrofolate*

reductase (DHFR). Thus, TS and DHFR act in concert to regenerate THF, thus maintaining availability of THF for use in the folate cycle.

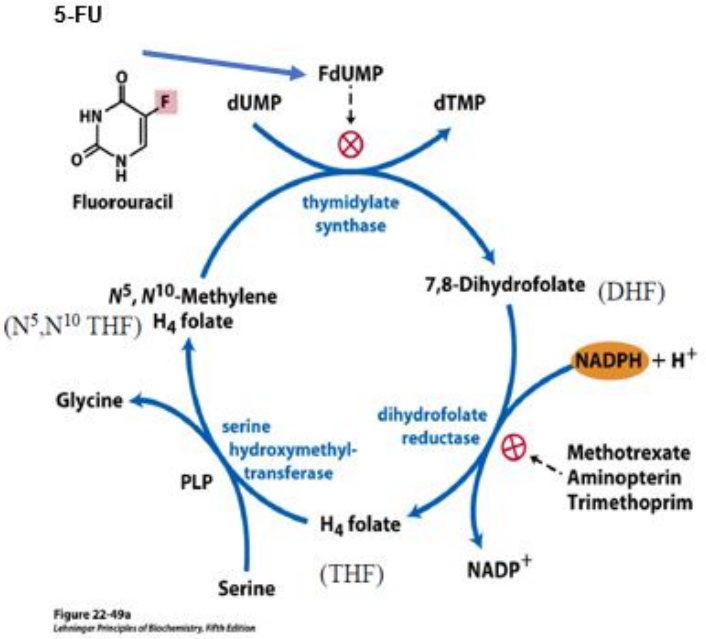


Figure 9.

Intentional disruption of folate metabolism is part of several anti-cancer or anti-microbial strategies. For example, DHFR is inhibited by several drugs including methotrexate, used in therapies for cancer and rheumatoid arthritis. Trimethoprim is an antibiotic that binds bacterial DHFR with an affinity that is orders of magnitude greater than its affinity for human DHFR. Aminopterin was previously used as an anti-cancer agent but is now used predominantly as a selective agent in experimental protocols in the laboratory.

5-fluorouracil (5-FU) is a pyrimidine analog commonly used in combination chemotherapy for several different cancers. 5-FU is

also formulated as a topical cream for treating skin cancer and certain pre-cancerous lesions. 5-FU is converted to Fluoro-dUMP (FdUMP), which binds irreversibly to the active site of *thymidylate synthase*, resulting in deficiency of dTMP, and therefore reduced dTTP for DNA synthesis.

There is significant genetic variation in the population in the DPYD gene encoding *dihydropyrimidine dehydrogenase* (DPD), catalyzing the first step in the degradation of 5-FU. Normally the majority of 5-FU is catabolized to its inactive form by the DPD enzyme. However, depending on the allelic variant present, a DPD deficiency or DPD enzyme that is a poor metabolizer of 5-FU can lead to severe toxicity and cause a serious adverse drug reaction that can be lethal. Diagnostic kits are available for genetic testing prior to a potential use of 5-FU.

SLO5. Describe the purine salvage/recycling pathway and the connection between HGPRT and Lesch Nyhan syndrome.

SLO6. Outline the degradation of purines to uric acid, the origins of the disorder gout and relevant treatments, and how a deficiency in adenosine deaminase results in increased dATP and inhibition of the ribonucleotide reductase enzyme resulting in SCID.

While pyrimidine salvage is not considered biochemically significant, purine salvage pathways contribute to replenishing intermediates in the purine biosynthetic pathway. Purine salvage pathways are catalyzed by phosphoribosyltransferases that recycle purine bases into the de novo purine biosynthetic pathway. *Hypoxanthine-guanine phosphoribosyltransferase* (HPRT or formerly called HGPRT), using PRPP as the ribose 5-P donor, recycles hypoxanthine to IMP and guanine to GMP, and *adenine phosphoribosyltransferase* (APRT) recycles adenine to AMP.

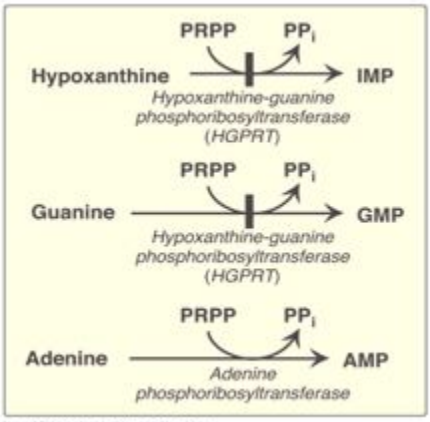
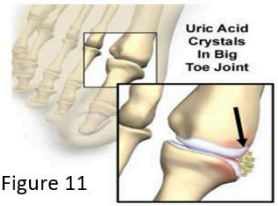


Figure 10.

A deficiency in a salvage pathway enzyme causes overproduction of purines. This counter-intuitive result is best explained by the model where a decrease in PRPP usage by a salvage pathway provides more PRPP to stimulate *de novo* purine biosynthesis, overriding negative feedback regulation by IMP, AMP, GMP.

X-linked genetic defects in the HPRT gene cause a spectrum of neurological symptoms. The most severe forms cause Lesch Nyhan syndrome, associated with excess production of purines and uric acid, resulting in gout. Neurological symptoms may include intellectual disability and self-injurious behavior (self-mutilation), managed with physical restraints as well as pharmaceutical interventions. A partial deficiency of HPRT may cause a Lesch Nyhan variant syndrome where patients present with hyperuricemia and gout but milder neurological manifestations.



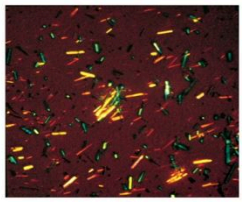
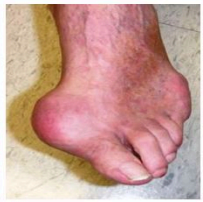



Figure 11

Gout may result from overproduction or underexcretion of uric acid. In Lesch Nyhan syndrome or other situations causing elevated uric acid, such as from increased cell lysis during trauma or chemotherapy, sodium urate crystals (tophi) deposit in a joint and cause an inflammatory reaction. White blood cells phagocytose the tophi and crystals disrupt lysosomes in the cells, causing cell damage and release of toxic products into the joint. Joints and kidneys may be damaged by the sodium urate crystals. Excretion of excess urate is also inhibited by high lactate levels, for example the elevated lactate associated with the increase in NADH from metabolism of ethanol in alcoholism.

An acute attack of gout is treated with anti-inflammatory drugs, such as NSAIDS, glucocorticoids or colchicine, or drugs to inhibit renal reabsorption of urate. Allopurinol is ineffective in treatment of an acute attack but is used as a long-

term therapy for gout. Allopurinol is an irreversible inhibitor of *xanthine oxidase*, which catalyzes two final steps in the catabolism of purines to uric acid. Allopurinol treatment lowers uric acid synthesis while increasing excretion of earlier intermediates in the pathway. However, as allopurinol does not inhibit uric acid production completely, a mixture of hypoxanthine, xanthine and uric acid is excreted but in concentrations that are low enough to reduce the chance of sodium urate crystal formation.

Adenosine deaminase (ADA) deficiency is a rare autosomal recessive disorder. *Adenosine deaminase* catalyzes deamination of adenosine to inosine. Clinically ADA deficiency manifests as a severe

combined immunodeficiency disorder (SCID). ADA deficiency was the subject of the first successful human somatic gene therapy trial reported in 1990. A retroviral vector was used to carry the normal ADA transgene intended to be expressed in lymphocytes. However, participants in the trial continued receiving exogenous ADA protein for ethical reasons, in case the gene therapy trial was unsuccessful.

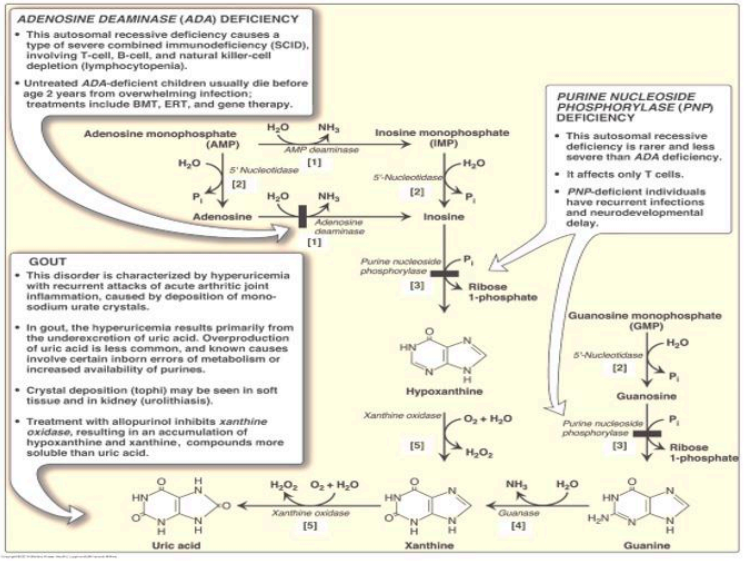


Figure 12.

A deficiency of ADA results in excess production of adenosine, causing a downstream effect of a 100-fold increase in dATP levels. Elevated dATP levels inhibits *ribonucleotide reductase*, leading to compromised production of deoxynucleotides and inhibition of proliferation of B and T lymphocytes in SCID. Immune dysfunction results from impairment of lymphocyte differentiation, function or viability. While this inhibition of ribonucleotide reductase in ADA deficiency contributes to inhibition of DNA synthesis and repair, the overall etiology of ADA deficiency-associated SCID involves many processes.

Purine nucleoside phosphorylase (PNP) deficiency, a rare autosomal recessive disorder, is characterized by severe to non-severe SCID. PNP deficiency, while less severe than ADA deficiency, is associated with neurological symptoms and increased risk of autoimmune disorders. PNP-catalyzed reactions utilize phosphate in removal of ribose from inosine or guanosine, generating ribose 1-P and either hypoxanthine or guanine, respectively.

SLO7. Describe and provide examples of how knowledge about nucleotide metabolism can be utilized to treat disorders such as cancer.

Key points in purine and pyrimidine metabolism disorders and therapies

1. Inhibition of *thymidylate synthase* with F-dUMP (from 5-FU) deprives the cell of a precursor for DNA, resulting in inhibition of DNA synthesis and repair.
2. In Lesch Nyhan syndrome, *HPRTase* deficiency is associated with neurological dysfunction and excess de novo synthesis of purines causes increased uric acid and gout.
3. Inhibition of *xanthine oxidase* with allopurinol reduces production of uric acid and is used in the long-term treatment of gout.
4. *Ribonucleotide reductase* is required for making deoxyribonucleotides from ribonucleotides; inhibition of this enzyme will inhibit synthesis of new DNA.
5. *Adenosine deaminase* (ADA) deficiency leads to excess dATP that inhibits *ribonucleotide reductase*. The severe combined immunodeficiency (SCID) that results in these patients is only partially explained by this mechanism.