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PRINCIPLES OF NUTRITION SCIENCE

DEPARTMENT OF NUTRITIONAL SCIENCES, UNIVERSITY OF MICHIGAN SCHOOL OF PUBLIC HEALTH

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Introduction

These chapters are the lecture notes from NUTR630: Principles of Nutritional Science taught by Drs. Dave Bridges and Olivia Anderson. Each chapter represents in general, one lecture in this course.

Part I

Regulation of Metabolism and Overview of Digestion

Metabolic Control Systems

This lecture will cover the basics of metabolic control, including how enzymes and transport systems control the fate of a metabolite and how systems are integrated to maintain homeostasis. These concepts should be a review of material covered in your Biochemistry classes. For further details please refer to the books on reserve for this course[1,2]

[1] Jeremy M Berg, John L Tymockzko, and L Stryer. *Biochemistry*, volume New York. 2013. ISBN 0-7167-3051-0

[2] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Explain what a rate limiting enzyme is, what a committed enzyme step is and what a reversible reaction is.
- Predict the differences in speed and persistence of allosteric, post-translational and transcriptional regulation of metabolism.
- Describe the role of cellular transport in macromolecular regulation. Understand the differences between active and passive transport.

Key Terms and Concepts

- Activation Energy
- Allosteric Regulation
- Cofactor
- Compartmentalization
- Concentration Gradient
- Enzyme
- Feedback Inhibition
- Isoenzymes
- Post-Translational Modification
- Rate Limiting Enzyme
- Transcription Factor
- Transporters (Active and Passive)

Control of Metabolic Flux

Cells need to control the rates at which nutrients are taken up, stored, or used and there are several ways by which this occurs. Here we will review the biochemistry of both nutrient transport and enzyme function. Understanding these concepts will be very important to understanding how the metabolic pathways we will discuss are controlled.

Cellular Transport Systems

First we will describe the ways in which cells control nutrient permeability. Most of the nutrients we will discuss[3] are unable to pass through the plasma membrane of the cell. Allowing or denying access to a nutrient is one way by which cells can control nutrient metabolism. Without these transport mechanisms we would be unable to absorb digested food, or transport nutrients from cell to cell. While we normally think of transporters as getting nutrients into or out of a cell, they are also important *within* cells, for example getting pyruvate into the mitochondria, or storing calcium in the endoplasmic reticulum.

[3] The exceptions are sterols and some other lipids

Types of Membrane Transporters

Membrane transporters are generally fairly specific for the molecule they transport. For example GLUT4 transports glucose, but GLUT5 transports fructose. Transporters can broadly be separated into two major types, passive transporters and active transporters. These can be differentiated by considering whether they work *with* or *against* the concentration gradient, with active transporters typically working against the concentration gradient.

Passive transporters allow for nutrients to pass down a concentration gradient into the cell. As an example, the liver expresses a glucose transporter named GLUT2. Glucose can either enter the liver (if there is more glucose in the blood than the liver) or exit the liver (if the reverse is true). Passive transporters will only allow a nutrient to enter a cell *if there is less of the nutrient in a cell than in the blood*. This is quite efficient for disposing of excess nutrients, such as after a meal, but is not effective in storing things away against a concentration gradient. It may seem like passive transporters are not regulated, but as we will see in the case of GLUT4, the amount of transporters at the cell surface can be controlled by cell signaling[4]. The rate of a passive transport is defined by three things, the gradient of the transported molecule, the number of transporters at the relevant membrane, and the efficiency of the transporter.

[4] if you want to jump ahead, here is a review on that process [Leto and Saltiel, 2012]

Active transporters can force nutrients into a cell *against* the concentration gradient. These transporters function like pumps and have to use energy of one sort or another to force the molecule into the cell. You may think that this is a bad idea, but there are lots of examples where this matters physiologically. One example is retaining salt. If your kidneys weren't actively retrieving sodim out of

urine and back into the blood, then you would rapidly lose osmotic pressure in your blood. The key is to think about the concentration inside or outside the cell, and if you are pushing against the transport gradient, you need active transport.

Powering Active Transport

Active transport requires energy of some type. This energy can come from several sources such as ATP, other concentration gradients, or even light. Some examples are described in Figure 1. The key to controlling the rate of these transporters is not only the concentration gradient of the transported molecule, but also the levels (or gradient) of the powering force. In the cases where molecules are co-transported they can either be pulled in simultaneously (this is known as a symporter) with the molecule of interest as shown on the left of Figure 1, or can be exchanged where one molecule exits, powering the entry of the molecule of interest (this is known as an antiporter). A classic example of an antiporter is the sodium:glucose exchanger SGLT1, which extrudes sodium down its concentration gradient (into the gut lumen) to force uptake of glucose from the gut into cells. This allows for efficient carbohydrate uptake in a meal[5] even if the gastrointestinal cells have similar or higher glucose levels to the gut lumen.

[5] SGLT2 does a similar thing, retrieving glucose from urine back into the blood. Therefore inhibiting SGLT2 prevents glucose retrieval back to the blood, and is the target of several drugs which try to lower blood glucose in diabetics. The trade names for these drugs include Invokana, Farxiga and Jardiance.

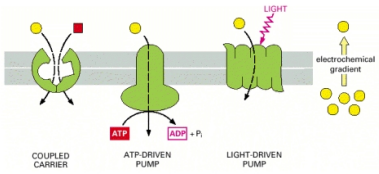

Figure 1: Examples of active transport. Reproduced from Alberts et al. [2002]

Transporters themselves can be regulated. This is often done by changing the number of transporters at the cell surface, or by changing the activity of the transporter. For example, in the case of GLUT4, insulin stimulates the translocation of GLUT4 from intracellular vesicles to the cell surface, increasing the amount of glucose that can be taken up by the cell. This is a common mechanism by which cells can rapidly respond to changes in nutrient availability. Other transporters can be regulated by changing their activity, for example by phosphorylation. See the section below Integrated Control of Metabolism by Regulation of Enzymes for more details on how this happens.

Enzymes

Thermodynamics

Every chemical in the body has a certain amount of energy. When we eat, some of this chemical energy is converted into ATP to allow for function. This is known as catabolism. When we are storing nutrients, we use ATP to generate higher energy molecules

such as fats or glycogen. This is known as anabolism. Every molecule in our body has a set amount of energy and a chemical reaction can be considered endothermic (requiring energy) or exothermic (releasing energy), depending on whether the reactants or products have higher energy. The levels of these metabolites at equilibrium can be calculated with the following equation where K_{eq} is the *Eqiulibrium constant*[6]:

[6] The square brackets mean the concentration of A or B

$$K_{eq} = \frac{[B]}{[A]} \tag{1}$$

The equilibrium constant can be calculated from the *free energy* of the reactants and products.

$$\Delta G_o = G_o^{'} - RT \ln K_{eq} \tag{2}$$

$$\Delta G_o^{'} = G_o^{'}(reactants) - G_o^{'}(products) \tag{3}$$

Some reactions have products with very similar energy levels and the balance between the reactants and the products is based primarily on their concentrations. This is known as an *equilibrium* reaction which would have a K_{eq} of near to 1. If a reaction requires a lot of energy to occur, this is often an *irreversible* or *committed* step[7]. This means that once this reaction happens, there is no going back. If you think about the metabolic pathway in Figure 2, this would mean that once you proceed through step 2 to make C you cannot go back to B. Given the free energy ($G_o^{'}$) and concentration of the reactants and products in a reaction you can calculate the ΔG and equillibrium constant for a reaction and estimate whether it is reversible[8] under normal conditions.

[7] This would have a large, negative G_o

[8] There is a good blog post explaining how the steady state ΔG is determined on this basis at http://sandwalk.blogspot.com/2007/10/aldolase-reaction-and-steady-state.html

Enzyme Kinetics

Without enzymes, many reactions occur very slowly due to the *activation energy* needed for the reaction to occur. Enzymes increase the rate of a chemical reaction by reducing the activation energy required for a reaction to occur. This does not change the equilibrium constant, it just allows the reaction to reach equilibrium faster. This is sketched out in Figure 3, note that ΔG is not changed, but the dashed line has a higher activation energy, and therefore slower reaction rate than the solid line.

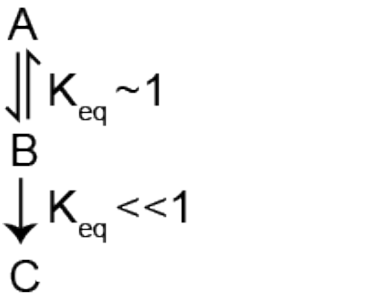

Figure 2: Example schematic of a metabolic pathway.

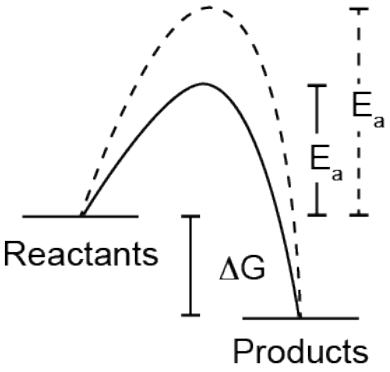

Figure 3: Example schematic of the

Most metabolic pathways are controlled by altering the rates at which metabolites are converted to final products. The overall rate of a metabolic pathway is controlled by the *rate-limiting step*[9]. In a linear pathway, the speed of this step's enzymatic reaction controls the overall rate. Quite often the rate-limiting enzyme is an

important point of regulation, as adjusting the speed of this reaction can speed up or slow down and entire pathway.

Reaction rates increase in rate as the concentration of substrates increase until the enzymes are saturated (see the solid line in Figure 4). This is known as Michaelis-Menten kinetics. The reaction rate constant (k) and rate can be calculated from the activation energy with these equations[10]:

$$k = Ae^{-\frac{E_a}{RT}} \tag{4}$$

$$rate = k\frac{[Reactant][Enzyme]}{[Reactant]+K_m} \tag{5}$$

If products build up the reaction becomes more complex and now looks like this where K_p is the binding constant for the product:

$$rate = k\frac{[Reactant][Enzyme]}{[Reactant]+K_m\left\{1+\frac{[Product]}{Kp}\right\}} \tag{6}$$

[10] A and R are constants, T is temperature and e is Euler's number. K_m is the Michaelis constant for an enzyme.

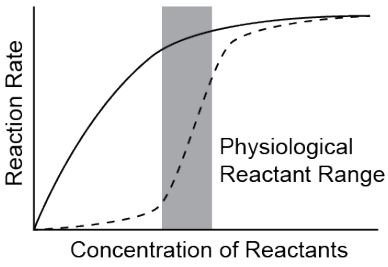

Figure 4: Example of Michaelis-Menten (solid line) and allosteric (dashed line) kinetics.

Allosteric regulation is another way by which enzymes can control reaction rates. Allosteric enzymes are generally multi-subunit enzymes that change their K*m* as more products bind. An example of this is the dashed line in Figure 4. This has several advantages in terms of regulation. One advantage is that the reaction rate can be effectively zero or at maximum in a much narrower range, bracketing the actual range of substrates present physiologically. Another advantage is that allosteric activators or inhibitors can shift the curve to the left or right, to effectively increase or decrease the reaction rate. This is a common mechanism by which the activity of rate-limiting enzymes are regulated.

On the basis of these equations, reaction rates (and the rate of a particular metabolic pathway) can be increased by several things[11]. Try to convince yourselves how this happens based on the equations listed above. Can you think of any other things that would affect pathway flux?

[11] Some examples include: decreasing the activation energy; increasing the amount of the reactants; decreasing the amount of the products; increasing the number of enzymes; decreasing the K_m of the enzyme; or shifting the substrate sensitivity of the allosteric enzyme.

While linear flow through a pathway is important, another aspect of pathway control is how the fate of a particular nutrient is decided. This is illustrated in Figure 5. In the example on top the nutrient would be equally distributed between three products, but in the bottom example, by adjusting the rates of the specific pathways, a nutrient can be directed to a particular product. At several points during this class, we will describe how the *fates* of particular metabolites are controlled by the relative rates of metabolic pathways.

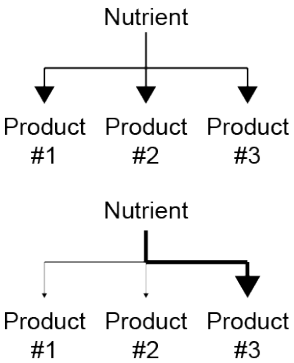

Figure 5: Example of how regulated pathways control nutrient fate.

Cofactors

Many enzymes require non-protein helper molecules to catalyze their reactions. These are known as *cofactors*. Table 1 lists some important cofactors, and the dietary vitamins from which they are derived. Other important roles of vitamins, for example in activating molecules like Co-enzyme A and electron carrier molecules like NADH will be described later in the semester. A lack of a dietary source of a cofactor can often impair the activity of an enzyme.[12]

[12] For example, a lack of vitamin B_1 (thiamine) can impair the activity of pyruvate dehydrogenase, which is important for converting pyruvate to acetyl-CoA. This can lead to a condition known as Wernicke-Korsakoff syndrome, which is characterized by confusion, ataxia and ophthalmoplegia. This is all because pyruvate dehydrogenase cannot function properly.

Table 1: Some examples of cofactors that are important for enzymatic catalysis.

Cofactor	**Source**
TPP	Vitamin B_1
Pyridoxal Phosphate	Vitamin B_6
Biotin	Vitamin B_7
THF	Folic Acid
Iron	Dietary Fe
Selenium	Dietary Se

Isoenzymes, Compartmentalization and Tissue Specificity

Enzymes can be tissue specific. This means that the same reaction can be catalyzed by different enzymes in different tissues. These are known as *isoenzymes* or *isozymes*. An example of this is lactate dehydrogenase, which has several isoforms that are expressed in different tissues. The liver isoform is more active at lower pH than the muscle isoform, which is more active at higher pH. This means that the liver can continue to produce glucose from lactate even when the blood pH is low, such as during exercise. This is an important concept to understand, as it allows for different tissues to have different metabolic priorities, and will be discussed in more detail later in the course.

Cells are not just a bag of enzymes. They are highly organized structures with different compartments that have different functions. This is known as *compartmentalization*. For example, the mitochondria are the site of oxidative phosphorylation, while the endoplasmic reticulum is the site of protein synthesis and lipid metabolism. This allows for different metabolic pathways to occur in different parts of the cell, which can help to control the rates of reactions and prevent unwanted side reactions. Compartmentalization also allows for the separation of metabolic pathways that may otherwise interfere with each other, such as glycolysis and gluconeogenesis.[13]

[13] Try to think of another example of a metabolic reaction and how it might be compartmentalized to a specific tissue or a subcellular location.

Integrated Control of Metabolism by Regulation of Enzymes

What we do (or do not do) with nutrients is largely governed by the activity of transporters and enzymes. There are several ways in which enzymes are regulated, both based on intracellular and extracellular signals. An example might be that a lack of intracellular ATP causes an increase in ATP producing pathways such as glycolysis. On the other hand, low circulating blood glucose levels may work

to stop a glucose consuming process such as glycolysis. We will discuss this in detail throughout the class, but some of the hormones we will discuss in this course that are particularly important are listed in Table I:

Hormone	Main Function
Insulin	Reduces blood glucose and lipid levels
Adrenaline	Increases blood flow, nutrients to muscle
Glucagon	Increases blood glucose levels acutely
Cortisol	Increases blood glucose levels chronically
GH/IGF-1	Promotes protein synthesis and bone growth
Testosterone	Promotes protein synthesis
Leptin, GLP-1	Suppresses appetite
CCK, Gastrin, Secretin	Regulation of digestion

Table 2: Some important metabolic hormones we will discuss in this class.

Hopefully these hormones, how they work and how they are regulated is material you are familiar with from previous classes. If not, or you want a refresher, check out the **Endocrine Regulation of Macronutrient Metabolism** handout also available on Canvas.

Allosteric Regulation

As noted above, one way by which enzymes are regulated is by allosteric regulation. This is a common mechanism by which the activity of rate-limiting enzymes are controlled. Allosteric enzymes have multiple subunits and can change their conformation when a metabolite binds to them. This can either increase or decrease the activity of the enzyme, depending on the metabolite that binds. Allosteric regulation is often rapid, and can be reversed quickly, so it is a good way to control metabolic pathways in response to changes in substrate or product levels.[14] This means that when ATP levels are high, glycolysis is slowed down, but when ATP levels are low, glycolysis is sped up.

[14] An example of this is phosphofructokinase-1, which is allosterically activated by AMP and inhibited by ATP.

Post-Translational Modification

One common way by which enzymes are regulated is by the modification of existing proteins. One common example is protein phosphorylation. In this example a phosphate molecule is attached to an existing protein, which could increase or decrease its activity. This is often reversible, so a good analogy is that post-translational regulation is like flipping a switch for an enzyme on or off. This can occur fairly rapidly, and is not a permanent change.[15] This is a good way to rapidly change the activity of a pathway in response to a stimulus, such as low blood glucose levels or exercise.

[15] An example of this is that in response to glucagon or adrenaline, glycogen phosphorylase is phosphorylated, which increases its activity and allows for glycogen breakdown to glucose.

Transcriptional Regulation

Another way to change the activity of a pathway is to selectively change the number of enzymes. If this is done at the messenger RNA level, it is known as transcriptional regulation. This is because transcription is the process by which new mRNA is made. By increasing or decreasing the rate of mRNA (and eventually protein) production, the cell can respond to a stimulus to make more or less of a particular protein.[16] The regulation of transcription is often controlled by *transcription factors*, a class of proteins that can bind to selective sites of DNA and recruit the machinery to make (or prevent the making) of mRNA. For a really great review with more details on transcription factors, take a look at Lambert et al. [2018]. These kinds of changes are slow, energetically costly and difficult to reverse. They represent a chronic response, and are not appropriate for short term modifications. The relationship between allosteric, post-translational and transcriptional regulation is demonstrated in Figure 6. Reflect on an example of metabolic regulation that you can think of. Then consider the timescale by which the changes happen, and try to think what would be the most appropriate mechanism to alter metabolism.

[16] An example we will discuss in this course is that when stress levels are high, the brain responds by increasing appetite.

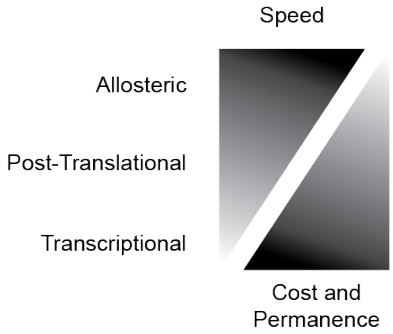

Figure 6: Shematic of the timing and permanence of some forms of enzymatic regulation.

Reflection Questions

1. A drug company develops three candidate compounds targeting the same metabolic enzyme: one is an allosteric inhibitor, one blocks the phosphorylation site used by a kinase, and one suppresses the gene's transcription factor. A patient needs rapid correction of a dangerously elevated metabolite. Analyze which compound would be most appropriate for acute treatment and which would be better suited for long-term management, using your understanding of the timescales and reversibility of each regulatory mechanism.

2. Feedback inhibition most commonly acts at the committed step of a pathway rather than the rate-limiting step, yet these are often the same enzyme. Evaluate why targeting the committed step (rather than any slow step) is the more logical control point for a cell. Use a specific example from carbohydrate or lipid metabolism to support your argument.

3. Fatty acid synthesis occurs in the cytoplasm while fatty acid oxidation occurs in the mitochondria, yet both pathways share several intermediates. Explain how compartmentalization prevents these opposing pathways from running simultaneously in a futile cycle, and identify the transport step that serves as the key regulatory

gate between them.

Endocrine Control of Metabolism

Metabolism of macronutrients occurs in every cell of the body. The integrated control of these separate organs is accomplished by endocrine (and neuroendocrine) signaling pathways. This review material will cover the roles of key metabolic hormones in normal and patho-physiological states, especially diabetes. We will cover the endocrine and neuroendocrine regulation of the gastrointestinal system in a separate lecture. This material should be a review from the endocrine unit in your undergraduate physiology courses, and the course will typically presume these concepts are familiar to you. For more details about the mechanisms of regulation, such as signal transduction, protein phosphorylation and transcriptional regulation, see the Metabolic Control Systems *handout, also on Canvas. If there are concepts or vocabulary in these notes that are unfamiliar to you, please reach out to the instructional team as early as you can so that we can better support your success in this course.*

Key Terms and Concepts

Below is a list of some key vocabulary related to this unit. If any of these terms (or terms not listed here) are unclear or you would like defined, please use the class vocabulary page, available on Canvas.

Metabolic Processes These are metabolic pathways important to this class.

- Anabolism vs Catabolism
- Autophagy
- β Oxidation
- *de novo* Lipogenesis
- Gluconeogenesis
- Glucose Oxidation, including Glycolysis and the TCA[17] Cycle and Oxidative Phosphorylation
- Glucose Uptake, including Insulin Stimulated Glucose Uptake
- Glycogenesis and Glycogenolysis
- Insulin Secretion
- Insulin Sensitivity (or Insulin Resistance)
- Ketogenesis and Ketoacidosis
- Lipolysis
- Proteolysis
- Triglyceride Synthesis

[17] Tricarboxylic Acid Cycle, also known as the Kreb's Cycle or Citric Acid Cycle

Hormones You should be familiar with where these hormones come from, and when they are elevated.

- Adrenaline, also known as Epinephrine[18]
- Cortisol
- Glucagon
- Growth Hormone
- Insulin
- Insulin-Like Growth Factor 1 (IGF-1)

[18] Along with noradrenaline/norepinephrine

Protein Kinases These are proteins that phosphorylate other proteins and lead to post-translational regulation[19]. You should be familiar with what hormones regulate these kinases, and over the course of the semester how they control metabolism.

- Akt — Also referred to as Protein Kinase B. Promotes glucose uptake and glycogen synthesis downstream of insulin.

[19] A *kinase* is an enzyme that adds a phosphate group to a molecule, a *protein kinase* is an enzyme that adds a phosphate group to another protein. These reactions are reversed by enzymes called *phosphatases*

- AMP-Activated Protein Kinase (AMPK) — Activated by low energy levels, promotes catabolism and inhibits anabolism.
- Glycogen Synthase Kinase 3 (GSK3) — Inactivated by insulin, promotes glycogen synthesis.
- Protein Kinase A (PKA) — Activated by adrenaline and glucagon, promotes glycogenolysis and gluconeogenesis.
- Mechanistic Target of Rapamycin Complex 1 (mTORC1) — Activated by insulin, high ATP, and high nutreint status. Promotes nutrient storage and protein synthesis.

Transcription Factors . These regulate gene transcription, or the production of new proteins. This typically is much slower than allosteric or post-translational regulation.

- cAMP-Response Element Binding Protein (CREB) — Activated by PKA, promotes gluconeogenesis.
- Peroxisome Proliferator-Activated Receptor (PPAR) — Sensors of lipids and regulators lipid metabolism.
- Glucocorticoid Receptor — Activated by cortisol, promotes gluconeogenesis and proteolysis.
- FOXO — A family of Forkhead Box proteins. Active during fasting; promotes gluconeogenesis and autophagy.
- Sterol Response Element Binding Protein (SREBP) — A family of transcription factors that regulate lipid synthesis and uptake.

Mechanisms of Glucose Regulation

Glucose is typically maintained in a very narrow range, between 80 to 110 mg/dL. The major tissues responsible for regulating glucose levels are listed in Table 3. Glucose levels need to be re-established after changes in feeding status or energy utilization or elevated during the absence of dietary carbohydrates. In general, when glucose levels decrease, glucagon is released from alpha cells of the pancreas to promote glucose production, either from glycogen breakdown or gluconeogenesis. Alternately, after a meal when glucose levels increase, insulin is secreted from beta cells of the pancreas causing glucose levels in the blood to decrease.

For the purposes of the acute maintenance of glucose homeostasis, four organs are the most important; the pancreas, liver, muscle and adipose tissue. After a meal, muscles are the primary site of glucose disposal [DeFronzo et al., 1981]. The inability to clear glucose from

Table 3: Primary sites for regulation of glucose homeostasis

Process	**Tissue**
Insulin Stimulated Glucose Uptake	Muscle and Fat
Glucose Oxidation	Muscle, Brain
Glycogen Storage/Release	Liver and Muscle
Gluconeogenesis	Liver

the bloodstream and into muscles is one major cause of the hyperglycemia associated with type 2 diabetes[20]. Central to glucose control is the integrated control of multiple organ systems. This is accomplished by *hormones* that orchestrate the actions of these organs.

[20] The other proximal cause is unrestrained gluconeogenesis.

GLUCOSE ENTERS THE CELL, DOWN A CONCENTRATION GRADIENT via passive transport from the blood into most tissues including liver, pancreas, kidneys and the brain. In these cases the passive transport of glucose into the cell is mostly unregulated. However, for glucose to enter into muscle and fat tissue, insulin is required. This is accomplished by moving GLUT4 transporters from intracellular storage sites to the plasma membrane, allowing for glucose influx. Once inside the cell, insulin will promote both glycogenesis and glycolysis.

GLUCOSE CAN BE STORED AS GLYCOGEN[21]. To form glycogen, glucose must first be converted through glucose-1-phosphate into UDP-glucose. This activated form of glucose is then added onto existing glycogen chains through the activity of an enzyme named glycogen synthase. As we will discuss later in the semester, in addition to being regulated by protein phosphorylation and sub-cellular location, glycogen synthase is also allosterically activated by glucose-6-phosphate, promoting increased glycogen synthesis when glucose levels[22] in the cell are high. The combination of the allosteric and post-translational signals mean that when insulin levels are high, glycogenesis is promoted.[23]

[21] This is known as glycogenesis

[22] and therefore G6P levels

[23] How would the effects of insulin on glycogen storage affect people with type 1 and type 2, and their ability to control blood glucose under fasting conditions?

TO LIBERATE GLUCOSE FROM STORED GLYCOGEN, AN ENZYME KNOWN AS GLYCOGEN PHOSPHORYLASE IS ACTIVATED. This enzyme hydrolyses glycogen, releasing glucose-1-phosphate, which can then be dephosphorylated into glucose for glycolysis or release into the blood stream. Hepatic glycogenolysis is the preferred source of short term glucose maintenance. In addition to post-translational modifications and recruitment to the glycogen pellet by accessory proteins, glycogen phosphorylase is allosterically activated by energy stress such as increases in AMP, or negatively by increases in glucose-6-phosphate levels. When adrenaline[24] is elevated, glycogenolysis occurs releasing stored glucose for energy or to maintain normoglycemia.

[24] or for the liver, glucagon as well.

GLUCONEOGENESIS IS THE GENERATION OF GLUCOSE FROM NON-CARBOHYDRATE PRECURSOR MOLECULES. These typically include amino acids, lactate and glycerol[25]. The majority of gluconeogenesis occurs in the liver, and generally is important for glucose production

[25] Along with free fatty acids, the products of triglyceride breakdown, also known as *lipolysis*.

from proteins and lipids after glycogen stores are depleted. This biochemistry of gluconeogenesis is similar to reversed glycolysis though in several cases different enzymes are used. As we will discuss later in the lecture, the rate limiting enzymes in gluconeogenesis are phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase. These enzymes are under both transcriptional and post-translational control as described below. Both the supply of gluconeogenic precursors (via breakdown of triglycerides and proteins), as well as the activity of gluconeogenic enzymes are reduced by insulin. Adrenaline and glucagon both *prevent* glycolysis in the liver (but not the muscle). This is to make sure that the glucose that is produced via gluconeogenesis in liver isn't catabolized back to energy in the liver, but instead is sent to the muscles for uptake and use. Ultimately, the body tries to always ensure that the muscles have sufficient glucose and so it would be counterproductive for adrenaline and glucagon to have a negative regulatory role on muscle glycolysis.

When energy is needed, for example during fasting, the primary source is circulating glucose, followed by the the breakdown of glycogen and then the production of new glucose (gluconeogenesis) from proteins and lipids. At the same time, the unnecessary synthesis of new glycogen is stopped. When glucose levels are too high, the first response is uptake and oxidation by the muscle, followed by glycogen synthesis and then eventually by *de novo* lipogenesis. At this time of excess glucose presence, gluconeogenesis (which is not needed) is stopped. A summary of how insulin and adrenaline function to control these processes is shown in Table 4.

Table 4: Primary functions of insulin and the adrenergic hormones, glucagon and adrenaline on glucose homeostasis.

Process	**Insulin**	**Adrenergic**
Insulin Stimulated Glucose Uptake	↑	—
Glycolysis — Liver	↑	↓
Glycolysis — Muscle/Brain	↑	—
Glycogenolysis	↓	↑
Gluconeogenesis	↓	↑
Lipolysis	↓	↑

Pancreatic Cell Types

In order to balance the energy requirements of all tissues, blood glucose is primarily controlled via endocrine and neuroendocrine mechanisms. The primary mediators are insulin and glucagon which are secreted from the pancreas during times of hyper and hypoglycemia, respectively. These two peptide hormones are released from two cell types in the pancreas, the α cells which release glucagon and the β cells which release insulin. Both cell types are located in the Islets of Langerhans within the pancreas (see Figure I).

Key Mediators of Insulin Signaling

Insulin was discovered by Frederick Banting and his colleagues at the University of Toronto in 1921. They performed experiments in which

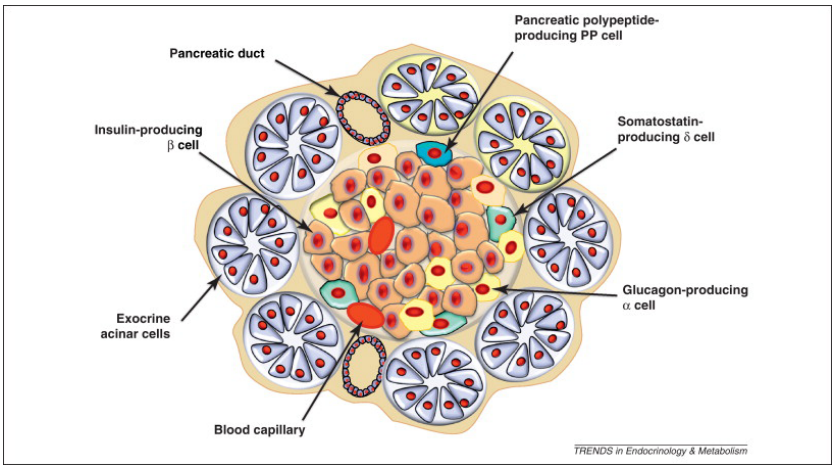

Figure 7: Schematic of a pancreatic islet. Taken from Efrat and a. Russ [2012]

they injected extracts from pancreas fractions into dogs which had their pancreas surgically removed. They showed that a secreted substance from the pancreas lowered blood glucose in these dogs [Banting et al., 1922]. They were then able to confirm that this treatment was also effective in children with type 1 diabetes. This work led to Banting and John Macleod winning the Nobel Prize in Medicine and Physiology in 1923. We now know that type 1 diabetes is caused by autoimmune destruction of the pancreatic beta cells[26]. On the other hand, type 2 diabetes, often associated with obesity, is a *peripheral resistance* to the effects of insulin. This occurs when the muscle, fat tissue and liver stop responding to insulin.[27]

In uncontrolled Type 1 diabetes, insulin is absent. Which processes go unregulated?

[26] At this stage you should be able to predict how the lack of insulin affects glucose disposal, gluconeogenesis, and lipolysis

[27] What do you think would happen if insulin signaling is impaired in the liver, but not the muscle or vice versa?

The majority of the actions of insulin are mediated by a protein kinase called Akt (see [Manning and Cantley, 2007] for more details). This protein kinase is activated downstream of both the insulin and IGF1 receptor. Loss of function mutations in one of the isoforms, *AKT2* in humans results in dramatic peripheral insulin resistance [George et al., 2004][28]. Akt, once activated by insulin or IGF1 can phosphorylate a variety of proteins including key metabolic enzymes, but also activates key signaling cascades. Three important cascades we will discuss that function downstream of Akt are FOXO and GSK3 (which are inactivated by Akt) and mTORC1 (which is activated).

[28] These mutations are quite rare in the general population.

The liver is a key central node of metabolism. The liver is often the first destination for nutrients that are absorbed in the gut and is a key site of storage and interconversion of nutrients. For a more detailed review of liver function we recommend the recent review by Trefts et al. [2017]. Effective liver function is central to many key processes including lipid transport, amino acid oxida-

tion and glucose homeostasis. In concert with increases in obesity, the prevalence of Metabolic Dysfunction-Associated Steatotic Liver Disease disease[29] has been dramatically increasing [Hashimoto and Tokushige, 2011]. Impaired liver function renders us less able to interconvert and dispose of macronutrients and less able to detoxify harmful compounds.

[29] This is often abbreviated as MASLD, but was previously known as non-alcoholic fatty liver disease or NAFLD.

Glucagon Promotes Glucose Elevation

When glucose levels are low, glucagon is released from alpha cells in the pancreas. This promotes the breakdown of glycogen stores in liver and muscle, and the generation of glucose from gluconeogenic precursors in the liver. Glucagon receptors exist mainly in the liver, so glucagon does not exert its main catabolic effects on either adipose or muscle tissue.

The mechanisms which underlie hypoglycemia induced glucagon release are incompletely understood. What is clear however, is that when blood glucose levels decrease, glucagon is released from the alpha cells of the pancreas into the portal vein.

Clinical Note: In untreated type 1 diabetes, insulin is absent, but glucagon secretion is often inappropriately high. This leads to excessive hepatic glucose production—even when blood glucose is already elevated—contributing to hyperglycemia and ketoacidosis.

Glucagon Signal Transduction

Adrenergic-receptor coupled mediated cAMP synthesis was the first example of a hormonal second messenger. Earl Sutherland was interested in the regulation of glycogenolysis, and he noticed that if he added adrenaline to intact cells, he could accelerate glycogen breakdown, but if he added it to lysed cells he could not. In his key experiment he treated one set of livers with adrenaline, then lysed them. He then added that lysate to a second set of livers which had already been broken. He found that there was an internal factor (later identified as cAMP) in the stimulated tissues, that could accelerate glycogenolysis in the other tissues [Rall et al., 1956]. For this work, Sutherland won the Nobel Prize in Medicine and Physiology in 1971.

In metabolism, the main effector of cAMP in cells is Protein Kinase A (PKA). This protein kinase is allosterically activated by cAMP and phosphorylates a wide variety of important metabolic substrates. The identification of PKA and its role in carbohydrate homeostasis led to Fisher and Krebs winning the Nobel Prize in Medicine and Physiology in 1992. The primary role of glucagon is to increase blood glucose, both by mobilizing glycogen stores and inducing gluconeogenesis. The mechanisms for this are identical to those for adrenaline, as both of these hormones activate G-protein coupled receptors [30] and result in PKA activation in the liver.

Clarifying Example: After an overnight fast, blood glucose begins to fall. Glucagon rises and stimulates the liver to break down glycogen and activate gluconeogenesis. Meanwhile, insulin falls, reducing glucose uptake in peripheral tissues, conserving it for the brain.

[30] G protein-coupled receptors are receptors that detect molecules outside the cell and activate internal signals.

The Primary Target of Glucagon is the Liver

As described above, glucagon stimulates the breakdown of glycogen. This proceeds via protein phosphorylation of both glycogen phosphorylase (which activates the enzyme) and glycogen synthase (which inactivates the enzyme). In combination, this leads to a breakdown of glycogen into glucose.

PKA is the primary mediator of the activation of glycogen phosphorylase. Once activated by adrenergic signaling, PKA phosphorylates and activates glycogen phosphorylase kinase. This kinase in turn, phosphorylates and activates glycogen phosphorylase[Krebs and Fischer, 1956]. PKA also directly phosphorylates glycogen synthase, which in concert with the activation of the other glycogen synthase kinases (notably GSK3 and AMPK) leads to increased phosphorylation and inactivation of glycogen synthase.

In addition to the activation of these protein kinases, there is a reduction of glycogen associated protein phosphatase activity. As a balance, this leads to more highly phosphorylated and therefore more glycogenolytic activities.

GLUCAGON ALSO PROMOTES GLUCONEOGENESIS IN THE LIVER. There are both post-translational and transcriptional mechanisms by which adrenergic signaling promotes gluconeogenesis. Similar to glycolysis, the allosteric and post-translational regulation of gluconeogenesis is rapid, while the transcriptional regulation is slower but more stable.

Post-translationally, the best studied route by which PKA activates gluconeogenesis is through inactivation of phosphofructokinase-2. PFK-2 normally generates the carbohydrate Fructose-2,6-bisphosphate which is a positive regulator of glycolysis and a negative regulator of gluconeogenesis. The alleviation of this inhibition allows for promotion of the gluconeogenic metabolism.

Transcriptionally, the transcription factor CREB is phosphorylated by PKA where it plays a role in transcriptionally activating the rate limiting gluconeogenic enzymes PEPCK, FBPase and G6Pase. This is energetically costly, and occurs slowly. Transcriptional changes are therefore often more permanent in nature.

Other Glucoregulatory Hormones

Since glucagon works primarily on liver tissue, different hormonal messengers function to stimulate catabolism of lipid in muscle and fat tissue. A key difference from adrenaline and glucagon, is that adrenaline also has major effects on fat and muscle tissues, as well

as glycogen. Therefore, in addition to simulating hepatic gluconeogenesis and glycogenolysis, adrenaline also promotes lipid release and muscle glucose oxidation. Both adrenaline and glucagon function by stimulating adrenergic signaling and cAMP-dependent PKA activation. Some similarities and differences between glucagon and adrenaline are shown in Table 5.

In adipose tissue, adrenaline induces lipolysis, via phosphorylation and activation of Hormone Sensitive Lipase (HSL), Perilipin and Adipocyte Triglyceride Lipase (ATGL). These proteins function to mobilize triglycerides into free fatty acids for use in other tissues, especially muscle. For more information on the regulation of lipolysis, see [Young and Zechner, 2013]. At an acute level, these do not contribute much to glucose homeostasis but are extremely important for lipid metabolism.

Table 5: Comparison between glucagon and adrenaline. Glucagon does not promote lipolysis, think about why that might be the case.

	Glucagon	**Adrenaline**
Source	Pancreas	Adrenal
Signal	Hypoglycemia	Acute Stress
Receptors	Liver	Widespread
Signaling Pathway	PKA	PKA
Gluconeogenesis	↑	↑
Hepatic Glycolysis	↓	↓
Lipid Oxidation	↑	↑
Lipolysis	—	↑

Longer term glucose control is regulated by two other hormones previously discussed, growth hormone and cortisol. These hormones are elevated during times of growth or stress where it is important to keep circulating glucose available for other functions. During a prolonged fast, both GH and cortisol can be released, causing longer-lasting changes which ensure adequate blood supply to the brain.[31]

[31] How do the timescales of cortisol vs. adrenaline effects differ, and why does this matter clinically?

Incretins enhance insulin release, and are typically released from the gut. They were first described when it was noted that when equal amounts of glucose are provided either through the gut, or intravenously, the gut-supplied glucose leads to a more robust insulin secretion effect. Eventually gut-derived peptide hormones, GLP-1 and GIP-1[32] were described. Both of these peptides are degraded by an enzyme called DPP-4, and inhibitors of this process have provided an exciting new potential therapeutic mechanism for reducing body weight and enhancing glucose control.

[32] glucagon-like peptide 1 and gastric inhibitory peptide respectively

Incretins are discussed in more detail in the gastrointestinal physiology unit, but have several functions. In addition to potentiating insulin response, they reduce food intake via signaling in the brain. This is the primary mechanism by which GLP1 receptor agonists (*e.g.* semaglutide (marketed as Ozempic or Wegovy) or tirzepatide (marketed as Mounjaro)) reduce food intake.

Pathophysiology Related to Glucose Control

Type 2 Diabetes Mellitus

Type I Diabetes is typically caused by autoimmune destruction of pancreatic beta cells. Without these cells, the pancreas is unable to produce insulin and without careful monitoring and exogenous insulin, blood glucose levels will rise. At the same time, lipolysis is very high. The excess flow of fatty acids into a liver which is unable to oxidize them[33] results in the production of ketone bodies, which when uncontrolled can lead to diabetic ketoacidosis.

[33] We will discuss this in much more detail when we talk about lipid oxidation, but because the liver is diverting TCA cycle intermediates towards gluconeogenesis, the TCA cycle is unable to oxidize Acetyl-CoA. This results in Acetyl-CoA being converted into a ketone and released from the cell. The biochemistry is similar to nutritional ketosis, except in that case ketone levels are not typically elevated as high.

Insulin Resistance and Type 2 Diabetes Mellitus

Type II diabetes occurs as a result of a multi-step process starting with negative feedback loops on insulin signaling. As more nutrients are stored, for example in obesity metabolic tissues become resistant to the effects of insulin, likely as a way to protect against excessive lipid storage.

As tissues become more insulin resistant, more insulin must be secreted by the pancreas to maintain normoglycemia. If insulin resistance proceeds, more and more insulin will need to be produced and secreted by beta cells. Eventually the beta cells will be unable to keep up with this demand and glucose levels will rise as the amount of endogenous or exogenous insulin is less and less effective.

Hormonal Regulation of Protein Metabolism

Protein homeostasis is an important ongoing process that is regulated both at the level of *protein synthesis* and *protein degradation*. Protein synthesis is especially important in the context of growth and development.

Endocrine Regulators of Protein Synthesis

There are several hormones that control protein synthesis, often mediated by a protein kinase called mTORC1. This kinase promotes protein synthesis by increasing the rates of initiation of translation, and the rates of peptide chain elongation. For more details about how mTORC1 regulates protein production, we recommend this review[Gingras et al., 2004].

Insulin and Insulin-like Growth Factor (IGF) are potent activators of protein synthesis. Insulin, as described above is secreted by the β cells of the pancreas in response to increased blood glucose. Elevations in amino acids, such as leucine and alanine are also potent activators of insulin secretion [Floyd et al., 1966]. Insulin-like Growth Factor 1, on the other hand is produced in the liver and is regulated both nutritionally, and by Growth Hormone (GH[34]). Both insulin and IGF1[35] activate receptors in peripheral tissues to promote protein production. Insulin and IGF1, along with other signals such as elevations in the amino acids Leucine, Lysine or Arginine lead to mTORC1 activation which promotes the synthesis of proteins.

[34] The GH/IGF1 axis will be described in the next section.

[35] There is very little IGF1 activity on adipocytes, so it does not have a strong anti-lipolytic role.

Another major regulator of protein synthesis is Growth Hormone. Growth hormone is released from the somatotroph cells

in the anterior pituitary. The two primary regulators of GH secretion are the hypothalamic hormones GHRH[36] and somatostatin[37]. Growth hormone is highest during youth while people are actively growing. As a person ages, the amount of growth hormone decreases. Growth hormone also undergoes a normal diurnal rhythm. GH levels are highest shortly after going to sleep and lower during the day. Because of this, most growth occurs during sleeping when nutrients can be used for growth and are not needed for normal activities. This hormone has two main functions:

1. Direct actions on bone, muscle, adipocytes and liver tissue via its own receptor[38].
2. Indirect actions by promoting the release of IGF1 from the liver. This helps to promote anabolism in muscle and bone.

[36] Growth Hormone Releasing Hormone.
[37] Sometimes called growth hormone inhibiting hormone or GHIH.

[38] In general these functions serve to direct substrates (fatty acids, amino acids and carbohydrates) from storage tissues such as liver and adipose towards growing tissues such as muscle and bone.

Hormonal Regulators of Protein Degradation

Protein breakdown occurs via two mechanisms, proteolysis which targets specific proteins for degradation and autophagy[39] which can target entire organelles. Both of these processes result in the liberation of amino acids from proteins. Unlike fatty acids (triglycerides) and glucose (glycogen) there is no standard storage molecule for amino acids, so when the body needs amino acids, a variety of proteins are catabolized.

[39] or self-eating

One major pathway by which proteolysis is suppressed is the Insulin/IGF-FOXO pathway. Akt-dependent signaling insulin and IGF1 *reduces* FOXO activity (see Figure 43). When active, FOXO transcriptionally activates proteolytic genes known as atrogenes to liberate amino acids. These amino acids may be liberated for fuel, or to provide building blocks for gluconeogenesis. Therefore, when insulin/IGF signaling is active, proteolysis is reduced.

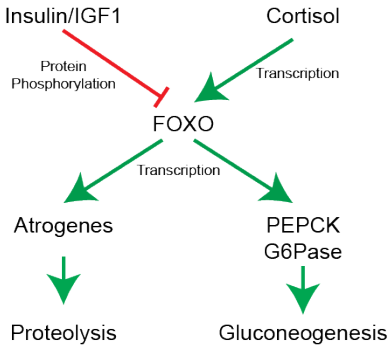

Figure 8: Schematic of regulation of FOXO and its role in proteolysis and gluconeogenesis.

Cortisol functions to make blood glucose available to key organs such as the brain, during times of stress. As such, cortisol promotes gluconeogenesis, by transcriptionally activating several enzymes, including G6Pase, PEPCK and pyruvate carboxylase. At the same time, cortisol promotes delivery of gluconeogenic precursors such as glycerol (from adipocyte lipolysis), lactate and alanine (from muscle tissue) to the liver. Chronically elevated cortisol leads to substantial muscle breakdown and is a major side effect of prescribed glucocorticoids or chronic stress. This is described in Figure 43. Based on this you might be able to make a prediction of how insulin resistance might affect glucose production and proteolysis.[40]

[40] Cortisol increases gluconeogenesis but also has immune-suppressive effects—why might this be a problem in long-term steroid therapy?

Endocrine Regulation of Lipid Metabolism

Lipid synthesis is promoted by insulin and reversed by adrenaline. Triglyceride breakdown can be thought of in two phases, the conversion of triglycerides into fatty acids and glycerol[41] and then the conversion of these fatty acids into energy[42].

[41] This is known as lipolysis and releases fatty acids and glycerol from stored triglycerides.

[42] This is known as fatty acid oxidation or β-oxidation.

[43] This is known as *de novo* lipogenesis.

Insulin functions to promote lipid storage in two ways. It promotes the production of lipids from precursors[43], promotes the esterifictaion of fatty acids and glycerol into triglycerides and prevents the breakdown of triglycerides into fatty acids. We will discuss the mechanisms by which insulin controls fat storage later in the semester.

To liberate and use lipids for fuel first, fatty acids are released from adipose tissue, with the activation of β-oxidation happening concurrently in muscle. There are several hormones that regulate this, but adrenaline, which promotes both adipocyte lipolysis and muscle lipid oxidation is very important as is the energy sensor AMPK As we will learn later in the semester, several key lipolytic and oxidative enzymes are activated by adrenaline (and PKA) mediated protein phosphorylation.

Reflection Questions

1. Compare the hormonal and intracellular signaling state of a hepatocyte during the fed state versus a 24-hour fast. For each state, identify which hormones are dominant, which kinases (PKA, Akt, AMPK) are active versus inactive, and predict the net effect on glycogen metabolism, gluconeogenesis, and de novo lipogenesis.

2. In early type 2 diabetes, skeletal muscle becomes insulin resistant—GLUT4 translocation in response to insulin is impaired—yet insulin continues to effectively stimulate hepatic lipid synthesis via SREBP1c. Evaluate why this selective insulin resistance is metabolically dangerous. Which aspect of insulin signaling remains intact in the liver while glucose disposal is impaired in muscle, and how does this combination accelerate disease progression?

3. A patient undergoes major surgery and is kept NPO (nil per os—nothing by mouth) for 3 days post-operatively while receiving intravenous dextrose. Cortisol and adrenaline are markedly elevated. Predict the combined effects on protein catabolism, gluconeogenesis, and blood glucose. Why might blood glucose remain elevated despite exogenous glucose infusion, and which specific hormonal

signals and downstream kinases explain each component of this response?

Energy Balance and Appetite

This lecture will cover the basics of energy balance, including how we sense and measure energy intake and expenditure. This has very important consequences for understanding weight gain and loss, and understanding how different macronutrients are absorbed, stored and metabolized.

Learning Objectives

- Apply the concept of energy balance to understanding weight gain and weight loss.
- Explain the differences in energy content of various macronutrients.
- Differentiate between the components of energy intake and energy expenditure and evaluate how these contribute to energy balance.
- Interpret how energy intake and energy expenditure are assessed, including the biases and limitations of these methods.
- Understand how energy balance and its various sub-components are changed in response to dieting.

Key Vocabulary

- Energy Balance
- Thermogenesis
- Diet-induced Thermogenesis
- Resting Energy Expenditure
- Exercise Associated Thermogenesis
- Metabolic Inefficiency

Energy Balance and Changes in Body Weight

Obesity is now a major epidemic in most societies, with recent estimates showing that in America 40.3% of adults and 19.7% of children are considered obese [Stierman et al., 2021, Emmerich et al., 2024]. Obesity is estimated to cause 47% of the cases of type-2 diabetes, 25% of cases of cardiovascular disease, and 28% of cases of hypertension [Tanamas et al., 2016]. Fundamentally, people gain weight because of positive energy balance[44]. This means that if energy intake is larger than energy expenditure, weight (primarily in the form of stored fat) will increase. This unit will give an overview as to how we define and talk about energy balance (Figure 9).

[44] This may seem surprising or debatable, but the best evidence now is that the other factors that cause obesity (*e.g.* genetics, food choices, hormonal differences) work by causing positive energy balance.

IT MAY SEEM A BIT ABSTRACT to think of food, exercise and body weight all in terms of energy, but fundamentally every macronutrient has a different caloric content, and when it is catabolized, that energy is released. Often some of that energy is passed to another molecule

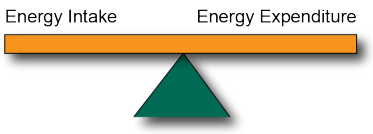

Figure 9: Energy balance, when energy expenditure matches energy intake results in no gain or loss of weight. Positive or negative energy balance occurs when one side increases or the other side decreases.

(for example, to ATP) but ultimately all catabolic processes end up generating heat. The production of heat, known as *thermogenesis*, is an important part of understanding energy balance. We all have intuitive ideas about someone's metabolic rate whether it be how one person can eat a large meal with no consequences, while another seems to restrict their diet but is unable to lose weight. Here we will discuss some of the physiology that makes up that energy balance and how this is modified by changes in diet.

Energy Intake

What is Energy Intake?

Energy intake is the sum of the amount of energy that is taken up by a person. While a major part of energy intake is the amount of food we eat, a less appreciated aspect is the efficiency[45] of nutrient extraction during digestion. As we will cover in her introduction to digestion, food passes through our bodies and efficient digestion involves extracting as many nutrients as possible from the bolus of food. Therefore we can consider energy intake as:

$$Energy_{intake} = Energy_{ingested} - Energy_{excreted} - E_{digestion} \tag{7}$$

This is important to keep in mind, because different foods have both different energy content and different aptitudes towards being absorbed by the body. Different people may also have more or less efficiency of nutrient ingestion. Someone who is malabsorptive, for example may have normal $E_{ingestion}$ but very high $E_{excretion}$ meaning that their E_{intake} is very low. The energy needed for digestion, is part of what we call Diet-Induced Thermogenesis[46], and is the energy that is expended in the process of digestion. This could be the breaking of molecular bonds as foods are broken down, or the heat generated by contraction by gastrointestinal smooth muscle cells.

How do the foods we eat affect energy intake?

In general, we know how much energy is contained per gram of each of the major macronutrients (see Table 6). These amounts, were calculated by Atwater and colleagues in the early twentieth century from determining how much heat was produced by burning pure fat, protein or carbohydrates[47]. These values are listed in the nutritional information for many foods. You might expect that the caloric density of your meal may be calculated by performing indirect calorimetry experiments on these foods. This is not the case, in general food manufacturers use the composition of their food (in terms

[45] In this context, and throughout the course efficiency indicates the percent of energy that is absorbed or used for actions. Efficiency may be important for an athlete, where nutrients are converted effectively into ATP and then motion. By the same token, efficiency is the enemy of weight loss, because if macronutrients are efficiently converted in the simplest way to energy, excess calories may be stored.

[46] It is not all of Diet-Induced Thermogenesis however, which also includes the energy released during storage and transport of foods to their eventual destinations

Table 6: Caloric density of the three major macronutrients and ethanol. These values are known as Atwater's factors

Macronutrient	Energy Density
Carbohydrates	4 kcal/g
Proteins	4 kcal/g
Lipids	9 kcal/g
Ethanol	7 kcal/g
Fiber	2 kcal/g

[47] Generally oxygen consumed is calculated rather than heat generated, a method known as indirect calorimetry

of macronutrients) and Atwater's factors to calculate energy density. This can be misleading, since it refers to the complete energy content of a fuel and not necessarily the amount of calories actually absorbed by a typical person.

How do we assess energy intake?

The FDA suggests that a normal healthy woman eats 2000 kcal per day (2500 for men). The ideal way to assess energy intake might be to determine the caloric content of all the foods eaten by a person, for example by indirect calorimetry of an identical meal and then subtracting the energy remaining in feces, while accounting for heat generated during digestion. This is very difficult to do and is rarely done. More often, to assess $E_{ingested}$ we generally make use of dietary recall surveys, and then compare the amounts and types of foods to a reference database. A commonly used Food Frequency Questionnaire can be found at `https://hsph.harvard.edu/wp-content/uploads/2024/07/FINAL-GRID-2022_Proof-.pdf`. There is substantial debate in the research community regarding the accuracy and biases of the different types of dietary recall assessments.

Energy Expenditure

The other side of the energy balance coin is energy expenditure, or how calories are used. While energy intake is difficult to measure, energy expenditure is even harder to measure accurately without specialized equipment. At a molecular level, increases in energy expenditure mean that heat is generated or an inefficiency is present. We will discuss several examples of this through the course, but Figure 38 illustrates one example. We will discuss this in more detail when we talk about gluconeogenesis, but this cycle is active during exercise where anaerobic glycolysis generates lactate, which is then converted back to glucose for the muscle. Each turn through this cycle has a net loss of 4 ATP, but nothing new is generated. Therefore this pathway is inefficient, in that energy (in this case ATP) is used without anything new being made or stored. If you had a lot of Cori Cycle activity, you would have increased metabolic inefficiency and therefore caused a higher energy expenditure.

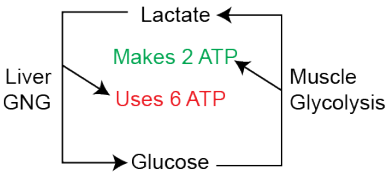

Figure 10: An inefficient metabolic pathway, the Cori Cycle. GNG means gluconeogenesis. Each turn through this cycle uses up 4 ATP (6 ATP used in the liver, 2 generated in the muscle).

What are the components of energy expenditure?

Energy expenditure can be broken down into several components which add up to one's total daily energy expenditure (TDEE). This is shown schematically in Figure I taken from a review on the topic [Tam and Ravussin, 2015]. These subgroups include the basal metabolic

rate (BMR), diet-induced thermogenesis (DIT), non-exercise activity thermogenesis (NEAT) and exercise activity thermogenesis (EAT).

$$TDEE = BMR + DIT + NEAT + EAT \tag{8}$$

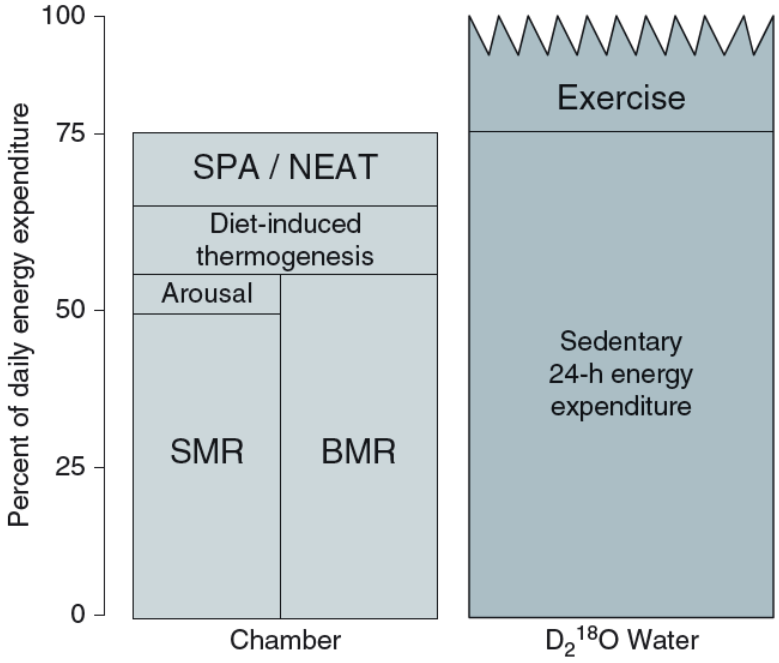

Figure 11: Components of total daily energy expenditure. SMR represents sleeping metabolic rate. SPA represents spontaneous physical activity.

Each of these components may be affected by genetics, diet, activity levels, sex, and other factors. What should be apparent from Figure I, is that contrary to many people's intuition, exercise generally comprises only a small portion of total energy expenditure. To illustrate this, running 5 km may seem like the bulk of your energy expenditure if you went for a run that day, but that expends about 350 Calories, or less than 20% of your total daily energy expenditure.

How do we calculate energy expenditure

The major factors that contribute to the BMR are age (declines with age), sex (approximately 11% higher in males) and body mass, particularly fat-free (or lean) mass. Up to 85% of the variance in BMR can be explained by these factors, with a further 11% being heritable [Bogardus et al., 1986]. Due to this precision, online calculators are reasonably accurate estimators of energy expenditure. There are several online calculators available where you can enter your height weight, age and sex to get an estimate. Try playing around with

those numbers to see how they affect estimated energy expenditure. There are two main ways in which energy expenditure can be experimentally determined.

INDIRECT CALORIMETRY monitors oxygen consumption and carbon dioxide production, typically over several hours or days. This can be done in large rooms called metabolic chambers where an individual can live for several days. The amount of oxygen consumed along with the amount of carbon dioxide produced can be converted into a measure of energy used, in the same way that food combustion can be used to determine the energy content of a meal. This approach has several advantages, including the fact that DIT, EAT and NEAT can separately be calculated based on whether the individual is currently eating, moving, etc. The major disadvantage is that the subject has to remain in the room to be monitored and this may not be representative of their normal behaviors.

DOUBLY LABELED-WATER on the other hand allows the subject to go home and live their normal life, which might be a better approximation of their natural energy expenditure rates. This technique is based on the differential release of isotopes after a subject drinks radio-labeled water ($^2H_2^{18}O$). The hydrogen atoms are released only with water, but the oxygen atoms are released as both CO_2 and water. Because of the slower equilibration process it does not provide the temporal resolution of indirect calorimetry, but rather provides an integrated measure of total CO_2 production or the time period. A third experimental method relies on energy conservation and measures E_{intake} and if the subjects are weight stable, E_{intake} should be equal to $E_{expenditure}$.

How does nutrition affect energy expenditure?

DIET-INDUCED THERMOGENESIS, also known as the thermic effect of food represents the energy that is used in digestion, absorption and storage of food. While it only accounts for 5–15% of TDEE it is the most affected by nutrition choices. Both meal size (larger, less frequent meals have higher DIT) and macronutrient composition (protein exerts a higher DIT) are major factors in DIT, along with genetics, age, activity levels and insulin sensitivity. A summary of things that raise the components of energy expenditure can be found in Table I.

While it was once thought that lower TDEE could be causal of obesity, careful studies in the early 1990s showed that obesity was as-

Component	Increased By
BMR	Young Age, Male, Lean Mass, Weight Above Set Point
EAT	Exercise and Physical Activity
DIT	Protein in Meal, Insulin Sensitivity, Meal Size

Table 7: Components of energy expenditure, and some things that modify their magnitude

sociated with *higher* energy expenditure rather than lower energy expenditure [Ravussin et al., 1982]. This is now thought to be an adaptive response to gaining weight. When excess calories are consumed, the body responds by increasing the metabolic rate to try to maintain homeostasis. Supporting that, during weight loss, the metabolic rate actually *decreases*, likely in an attempt to limit changes in body weight [Leibel et al., 1995]. This is what is known as a metabolic set point. It is a metabolic state that the body adapts tp. Unfortunately for those trying to sustain weight loss, this persistent reduction in metabolic rate lasts for many years [Rosenbaum et al., 2008]. Further complicating efforts in weight reduction, studies that have assessed appetite and appetite-driving hormones in successful dieters have shown that there are chronic feelings of both hunger and elevations in hunger hormones, again many years after weight loss [Sumithran et al., 2011]. These two factors, reduced TDEE and increased drive towards energy intake are major reasons why successful weight loss is so difficult to maintain over time. A schematic of this is shown in Figure 12.

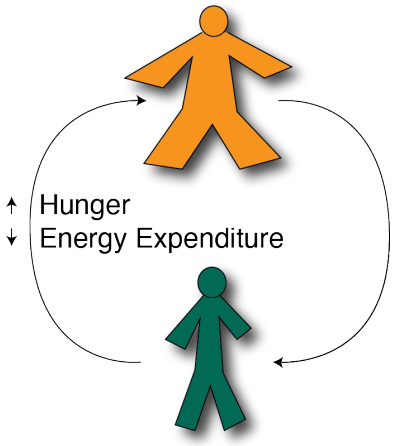

Figure 12: Physiological adaptations to weight loss promote weight regain. An individual loses weight, but compensatory decreases in energy expenditure and increases in appetite often result in weight regain.

Obesity is mostly driven by appetite. While it is true that some people have a higher energy expenditure, the vast majority of people who are obese have high energy expenditure. If you conmpare obesity rates and energy expenditure across cultures and economic strata, energy expenditure does not explain these differences in obesity rates [McGrosky et al., 2025]. It is is predicted that differential obesity rates are is due to differences in energy intake. Another line of evidence is that genetic studies of people with obesity have shown that the majority of genetic variants associated with obesity are related to appetite control, rather than energy expenditure [Jeffery and Harnack, 2007]. Finally the drugs and interventions that are most effective in reducing obesity work primarily by reducing appetite not by increasing energy expenditure. For example GLP-1 Receptor Agonists[48] reduce appetite (particularly towards highly palatable foods) but not energy expenditure [Martin et al., 2025]. This is not to say that energy expenditure does not play a role in obesity, but rather that it is not the primary driver of obesity. The primary driver of obesity is appetite, and the drive to eat more than we expend. In the next unit we will discuss how appetite is regulated, and how this can be modified by genetics, physiology and the environment.

[48] Drugs such as Ozempic or Mounjaro.

Reflection Questions

1. A participant in a 6-month weight loss trial loses 10% of their body weight through caloric restriction. Using your knowledge of homeostatic regulation of energy balance, explain why their TDEE at their new lower weight is disproportionately reduced compared to what would be predicted from their body composition alone, and predict the consequences for long-term weight maintenance if they return to their pre-diet caloric intake.

2. Two individuals consume 500 kcal meals: one consists entirely of fat, the other entirely of protein. Using Atwater's factors and your understanding of diet-induced thermogenesis, compare the net energy available from each meal and explain why the protein meal results in greater DIT. Then evaluate whether substituting dietary fat with protein is a viable strategy for creating a negative energy balance.

3. A clinical researcher argues that reducing sedentary behavior (NEAT) is a more effective long-term strategy for weight management than structured exercise (EAT). Using your knowledge of TDEE components and the adaptive metabolic responses to caloric restriction, evaluate this claim and explain why increasing EAT alone often fails to produce the expected magnitude of weight loss.

Overview of the Digestive System

This lecture will provide an overview of the anatomy and physiology of the digestive tract. Food is digested to release its contained nutrients in order to be absorbed into the body for cellular use. To understand how human bodies utilize macronutrients, it is important to have a clear comprehension of how (macro-) nutrients from food are digested and subsequently absorbed from the digestive tract to circulation (i.e., how do macronutrients get into our bodies).

Learning Objectives

- Identify the organs that make up the digestive tract
- Describe the structural components of the digestive tract organs
- Explain how digestive processes are regulated
- Describe key features of the main organs in the digestive tract
- Understand the roles of digestive secretions from accessory organs on the breakdown of macronutrients
- Identify the unique features of the small intestine and illustrate how they enhance digestion and absorption
- Describe how digested macronutrients are absorbed across thesmall intestine
- Explain the fate of inadequately digested materials
- Examine health conditions related to the digestive tract

Key Vocabulary and Concepts

- Alimentary canal
- Bolus and chyme
- Duodenum, jejunum, and ileum
- Gastrin, Secretin, Ghrelin and CCK
- Glands, including lingual and salivary
- Gut associated lymphoid tissue (GALT)
- Enteric nervous system
- Enterohepatic circulation and bile
- Esophageal sphincters (upper and lower)
- Lumen
- Mucuosa, Submucosa, Muscularis externa and Serosa
- Plexus of Meissner and Plexus of Auerbach
- Sympathetic and parasympathetic nervous system
- Villi, microvilli and brush border

The Digestive Tract Organs

The alimentary canal, the **main organs** that make up the digestive tract, is a continuous, hollow tube. The alimentary canal consists of five main organs (oral cavity, esophagus, stomach, small intestine, large intestine). Each specific organ has unique features to aid in the digestion process. A specific type of muscle called a sphincter connects each consecutive main organ. Sphincters act as a passageway for the digested food product. When food material is not present in the tract, the sphincters constrict closing off the passageways. When food material is present and moving down the tract, a regulatory signal results in the *relaxation* of the sphincter muscle, opening the passageway and allowing the food material to travel down the tract from one organ to the next.

The digestive tract also consists of organs referred to as **accessory organs** (salivary glands, pancreas, liver, gallbladder). Their main role in digestion is to provide, store and deliver secretions to the main organs for digestion and mobilization of food material. Although accessory organs are not in direct contact with the food material as it travels down the tract, the proper functioning of accessory organs is crucial for proper digestive processes.

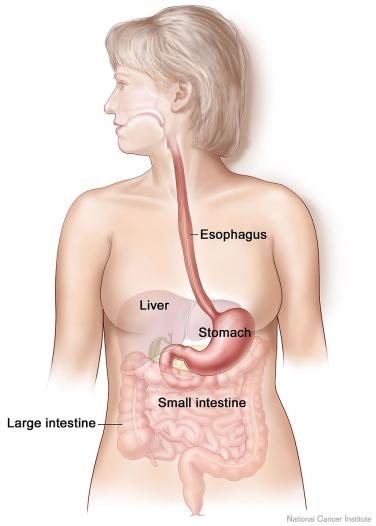

Figure 13: Diagram of the main organs of the digestive tract.

Structure of the Digestive Tract

Remember that the main organs of the digestive tract form a continuous, hollow tube. The hollow space, where the digested food material travels is called the *lumen*. The main organs are made up of four distinct layers (see 8). Each of the four layers has their own unique functions that support proper digestion. The innermost layer is called the mucosa and in itself has three sublayers — the epithelial lining, lamina propria and muscularis mucosa. The epithelial lining is comprised of exocrine and endocrine cells. The exocrine cells' main function is to secrete mucus to lubricate the food material. The endocrine cells secrete hormones into the bloodstream that signal for digestive processes to occur. The second sublayer is the lamina propria. This sublayer of the mucosa contains tissue called *gut associated lymphoid tissue (GALT)*. GALT plays an important immune function in the body by releasing macrophages and lymphocytes that ward off foreign microorganisms that humans ingest. The last sublayer is the muscularis mucosa. This is a thin smooth muscle layer that produces twitch-like movements when it contracts. The main purpose of the twitching is to disengage food material that may have gotten stuck on the digestive tract wall.

Table 8: Layers of the digestive tract.

Layer	**Sublayer**
Mucosa	Epithelia
	Lamina Propria
	Muscularis Mucosa
Submucosa	
	Plexus of Meissner
Muscularis Externa	=Circular Muscle
	Plexus of Auerbach
	Longitudinal Muscle
Serosa	

THE SECOND LAYER IS THE *submucosa*, which is made of blood vessels, elastic fibers and a bundle of nerves. As food is ingested and moves down the tract, the digestive organs can stretch (think of the stomach), the elastic fibers help to expand the organs, but also to get them back to their original size and shape. The submucosa contains a nerve bundle called the *Plexus of Meissner* that plays a role in regulating secretions from accessory organs into the main organs and in controlling blood flow (see more in Regulation of Digestive Processes section). The third layer is the muscularis externa made of inner-circular and outer-longitudinal muscles. These muscles work together to produce a muscle movement called peristalsis. Peristalsis is a wave-like contraction that pushes food material forward through the tract. There is another nerve bundle located in the muscularis externa called the *Plexus of Auerbach*. This Plexus plays a role in controlling muscle movements.

The outermost layer is the *serosa*. The main functioning of the serosa is to provide structural support for the tract and to secrete lubricant to help protect the inner wall of the digestive tract.

Regulation of Digestive Processes

As food material is digested, the tract itself is highly regulated to ensure nutrients are properly digested to the correct molecular form that is suitable for absorption and that digestion occurs in a timely manner (not too fast, not too slow). The digestive tract has two main types of regulation, including neural and hormonal. The neural regulation stems from the peripheral nervous system and includes both sympathetic and parasympathetic control, thus you can think of the regulation from each to have opposite effects. The sympathetic slows digestion while the parasympathetic stimulates digestion.

Reflection: From the information we have discussed thus far, what could be one example of what the sympathetic nervous system might induce? And the same consideration for the parasympathetic?

EXTRINSIC AND INTRINSIC NERVES MAKE UP THE SYMPATHETIC AND PARASYMPATHETIC NERVOUS SYSTEM OF THE DIGESTIVE TRACT. The extrinsic nerves that connect the digestive tract to the spinal cord and brain control the constriction and relaxation of the muscles within the digestive tract as well as play a role in secretion of substances into the tract from the accessory organs. The parasympathetic extrinsic nerve endings that penetrate the digestive tract release acetylcholine resulting in the promotion of digestion, or in other words, increased muscle movements and secretions. The sympathetic extrinsic nerve endings in the digestive tract secrete norepinephrine stimulating decreased secretions and muscle movement and constriction. This muscle control is important for the passage of food material from one consecutive main organ to the next. The intrinsic

nerves include fibers innervating the interior of the digestive tract, and they control digestive secretions (Plexus of Meissner) and peristalsis (Plexus of Auerbach). The intrinsic nerves are often referred to as a nervous system specific to digestive control and are collectively called the *enteric nervous system* (ENS). For more details see Furness [2012].

The central nervous system also plays a role in activating digestion. External sensory stimuli such as thought, smell, and taste of food can trigger the release of gastric juice (the production and composition of gastric juice is described below in Stomach subsection) which stimulates digestion in the stomach. The endocrine cells of the mucosal layer of the tract release hormones that help control digestive processes in addition to the neural control. The list of hormones found to play a role in digestive regulation is exhaustive. For the purposes of this discussion, we only focus on a select four that have been well characterized in their role for digestion (Table 9).

Table 9: Sites of production of key digestive hormones

Hormone	**Site of Production**
Gastrin	Stomach
Secretin	Small Intestine
CCK	Small Intestine
Ghrelin	Stomach

GASTRIN is produced by the stomach (and to a lesser degree in the small intestine) in response to the stomach distending as well as the presence of macronutrients within the lumen of the stomach. It plays a role in stimulating motility and digestive secretions locally. Gastrin also stimulates the emptying of the food material from the stomach to the next main organ, the small intestine. As part of its function, gastrin stimulates hydrochloric acid (HCl) release within the stomach. Increasing HCl initiates the inactivation of gastrin.

SECRETIN IS PRODUCED IN THE SMALL INTESTINE AND RELEASED IN RESPONSE TO THE ACIDIC FOOD MATERIALS ENTERING THE SMALL INTESTINE. Secretin slows down the rate of gastric emptying and gastric digestive secretions. In turn, secretin initiates the release of digestive juices from the pancreas, which is important for digestion within the small intestine.

CHOLECYSTOKININ (CCK) IS ALSO PRODUCED IN THE SMALL INTESTINE. It stimulates the release of a substance called bile that is important for fat digestion and mobilization within the tract. It also stimulates the release of enzymes produced in the pancreas important for catalyzing digestion processes within the small intestine.

GHRELIN IS A HORMONE THAT IS SECRETED IN THE ABSENCE OF FOOD. It stimulates hunger and has been found to initiate the production of gastric juices in the anticipation of food intake [Inui et al., 2004]. Increasing research explores other hormones like Ghrelin that play a role in satiety signaling pathways. For further reading,

Schwartz et al. [2000] provide a nice overview of these satiety signals and how targeting these pathways is a potential treatment strategy for weight control.

Reflection: In general, describe how malfunctioning of the pancreas could affect digestion?

The Main Organs and Their Functions

Again, the digestive tract consists of five main organs. The organs are categorized as the *upper digestive tract* (oral cavity, esophagus and stomach) and the *lower tract* (small and large intestine). In this section we will discuss the main macronutrient digestion events that occur within each organ.

Oral Cavity

The oral cavity is the first main organ food comes into contact with. The oral cavity encompasses the mouth and the pharynx. The features of the mouth that assist initial digestion include teeth, salivary glands[49] and the tongue. The digestion of food can be *mechanical* as well as *chemical* (*i.e.*, enzymatic). Before moving forward, it is important to distinguish between mechanical and chemical breakdown of food. Mechanical digestion is simply breaking down larger food particles to smaller ones without the occurrence of a chemical reaction (*e.g.*, hydrolysis). Mechanical digestion can occur by sheer forces, like the teeth, or by rigorous muscle movements, like peristalsis. Chemical digestion occurs when the food nutrients come into contact with an enzyme or acid and undergo a chemical reaction (likely hydrolysis) forming a simpler nutrient that will eventually be broken down into its absorbable form.

[49] **Definition:** Glands — small organs throughout the body with the main function of secreting substances to other parts of the body.

The teeth in the oral cavity start digestion by the mechanical breakdown of food fibers. *Salivary glands* in the mouth secrete a substance called saliva. Saliva is compromised mostly of water as well as key components that help with digestion and immunity. Salivary alpha amylase[50], released with saliva, is the first enzyme that begins chemical breakdown via hydrolysis of carbohydrates within the mouth. Mucus is also found in saliva, which helps lubricate the food for ease of travel through the tract. One last component of saliva is *lysozyme*. This is an enzyme that attacks foreign bacteria found in food products. This is especially important for oral health, like tooth decay. The tongue combines the food and saliva for chemical digestion and lubrication to occur. Once the food is well mixed with saliva, the food mass is called a *bolus*. Another key purpose of the tongue is to help push the bolus to the back of the mouth to initiate swallowing so the movement of the bolus continues down the tract for further digestion. Another set of glands located on the tongue is

[50] You can tell this is an enzyme since it ends in *-ase*, and it primarily functions to breakdown amylose a sugar we will talk about in a few lectures

called *lingual glands*. These produce an enzyme called *lingual lipase* that is incorporated into saliva. Lingual lipase plays a role in the digestion of certain types of lipids. Lingual lipase travels with the bolus but remains inactive until it reaches the stomach.

Esophagus

If you imagine swallowing a piece of food (technically a bolus), it is initially a voluntary action. It is a conscious effort to chew and get the bolus to the back of the mouth to the pharynx. Following the act of swallowing the digestive process becomes involuntary because of neural and hormonal regulation, as described above. As the bolus moves from the pharynx of the oral cavity, the first sphincter called, accordingly, the *upper esophageal sphincter* (UES) relaxes, thus opening up a passageway for the bolus to enter the esophagus. At the same time as the UES opens, a flap of cartilage called the epiglottis shifts to block the trachea (part of the respiratory tract). The blockage of the trachea prevents food from entering the lungs, which could potentially be life threatening (see Conditions of Interest section below). The bolus travels down the digestive tract by peristalsis. The force of peristalsis is quite strong (have you ever successfully ate or drank something while standing on your head?). Altogether the bolus is in the esophagus for no more than a few seconds. As the bolus approaches the bottom of the esophagus, the *lower esophageal sphincter* (LES) relaxes and the bolus enters the stomach. As we will discuss later, following the relaxation of the UES and LES, the constriction of the UES and LES are of great importance so the bolus does not have the ability to travel back to organs it has already been in but rather continue down the lumen of the alimentary canal to continue proper digestion.

Stomach

The bolus is now in the stomach. At rest the adult stomach is about 1.5 ounces. The stomach has some unique features on the mucosal layer. The mucosa has structural pits along the entirety of it. These gastric pits contain glands with four different cell types. Each cell type secretes certain substances that aid in motility and chemical digestion (Table 10). The secretions from the mucous neck, parietal, chief and endocrine cells make up gastric juice. The mucus in the gastric juice acts as a lubricant to the bolus. Hydrochloric acid (HCl) decreases the pH of the stomach environment. The lower pH from HCl acts as an immune defense to kill off foreign microorganisms and activates enzymes that are also part of gastric juice such as pepsinogen. In addition to pepsinogen, lipase is another enzyme

that is part of gastric juice. These enzymes target protein and lipids, respectively. Two additional enzymes originating from the mouth are also present in the stomach including salivary alpha amylase and lingual lipase. Salivary alpha amylase has traveled with the bolus from the oral cavity to the stomach. Alpha amylase thrives in a more neutral environment so it is inactivated anywhere from a few minutes to a half hour after entering the stomach [Rohleder and Nater, 2009]. Lingual lipase becomes activated in the acidic environment. The bolus is mixed with gastric juice by a churning movement from the oblique muscles of the muscularis externa. Once sufficiently mixed with gastric juice the bolus is now called the *chyme*.

Table 10: Gastric cell types and their secretions

Cell Type	Secretion
Mucous neck	Mucus
Parietal	HCl
Chief	Pepsinogen, lipases, water
Endocrine	Regulatory hormones

There are several points of regulation of digestion activity in the stomach. First, the thought, smell, and taste of food can send a signal to the central nervous system that simulates the production and release of gastric juice. There is hormonal control by gastrin, produced by endocrine cells in the gastric pits. Gastrin will function to release gastric juice from the pits. It will also promote the gastric motility of chyme to continue to move forward through the alimentary canal. Thus, in the stomach, gastrin is promoting digestive processes. Another hormonal point of regulation is by secretin. Secretin is produced by the small intestine. As the chyme is emptied from the stomach to the small intestine (i.e., gastric emptying), secretin will signal the gastric cells to decrease the production of components for gastric juice and the motility in the stomach to slow. Overall, although secretin is produced in the small intestine, it plays a role in regulating digestion processes in the stomach.

Reflection: What was something you learned as you read about the upper portion of the digestive tract?

Small Intestine

The chyme is emptied into the small intestine as the pyloric sphincter relaxes. The small intestine itself is made of three distinct sections, the *duodenum, jejunum, and ileum*. Anatomically-speaking the duodenum is structurally different than the jejunum and ileum whereas the latter two have no real anatomic distinctions, but as chyme moves down the small intestine, the distinct roles each section has in digestion and absorption of nutrients is clear. The duodenum, being the closest in proximity to the stomach receives the chyme, which is highly acid (pH about 2) from HCl containing gastric juice. The duodenum contains Brunner's glands that secrete bicarbonate to neutralize the chyme. The jejunum is where the majority of macronutrient absorption will occur. The ileum is where the minimal absorption of the remaining nutrients (both macro and micro) may occur if they have escaped absorption in the duodenum or jejunum.

To complete macronutrient digestion, the small intestine provides a means of both mechanical and continued chemical digestion. The muscles of the small intestine contract in a way that results in a back and forth movement called *segmentation*. The purpose of segmentation is two-fold: First, it aids in mechanical breakdown of chyme and Second, it increases the amount of contact time that the nutrients have with the surface of the small intestine, increasing the chance of absorption. Structurally, the small intestine is shaped differently than other areas of the digestive tract. If you were to take a microscopic visual of the lining of the small intestine, it would look like there were finger-like projections shooting in towards the lumen. The finger like projections are called *villi* and are lined with *enterocytes* also referred to as intestinal absorptive cells, within the small intestine. The enterocyte has a polarity with an *apical membrane*[51] and a basolateral membrane[52]. If you were to zoom in on one villus you would see that the lining of this finger-like projection contain even smaller finger-like projections called *microvilli*. Altogether, the villi and microvilli are referred to as the *brush border*.

[51] This is the side facing the digested food, or the lumen of the gastrointestinal tract
[52] The side in contact with blood or lymph vessels

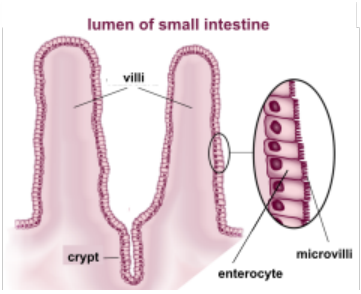

Figure 14: Schematic of the relationship between villi, enterocytes and microvilli. From https://commons.wikimedia.org.

The structure of the brush border allows for a large surface area to permit efficient absorption. The basic absorption pathway of a digested macronutrient in the small intestine would be as follows: lumen → across apical membrane of enterocyte → through the enterocyte → across basolateral membrane of enterocyte → capillaries or lymph vessels.

In addition to a large surface area, the brush border also contains enzymes that catalyze the completion of carbohydrate and protein digestion. Further digestion in the small intestine relies on the pancreas to produce and secrete pancreatic juices. Pancreatic juice contains digestive enzymes that also help complete digestion of carbohydrates, proteins and lipids so they are in a molecular form available for enterocyte transporters allowing for absorption into circulation. The pancreatic juice also contains bicarbonate to neutralize the small intestine environment and maximize digestive enzyme activity. Pancreatic juice is regulated by secretin and CCK, both of these hormones are produced in the small intestine (Table 9).

The liver plays an important role in fat digestion and absorption. The liver is the site for bile synthesis. Bile is a substance comprised of cholesterol, bilirubin, water, phospholipids and bile salts. Once produced, the bile is stored in the gallbladder until chyme is present in the small intestine. The bile travels to the duodenum via the common bile duct. Bile is important first for digestion. The bile salts of bile are amphipathic – the hydrophobic side coalesces to a fat molecule while the hydrophilic side stabilizes the compound in the aqueous environment of the small intestine. As the bile salts are coalesced to the

fat molecule, you can think of them as melting the individual lipid droplets apart from each other so digestive enzymes[53] have access to them. Once the fat molecules are broken down to their simplest digested form, the bile salts come into play again to help with absorption. This time the bile salts will surround a group of free fatty acids (absorbable form of fats). A group of free fatty acids surrounded by bile salts are referred to as *micelles*. The micelles are stabilized as they reach the enterocytes of the small intestine. Once they reach the brush border membrane, the bile salts are released and the free fatty acids are transported across the enterocyte to the lymph vessels of the villi. Once complete, about 90% of bile is recycled from the ileum back to the liver through a cycle called *enterohepatic circulation*.

[53] Quick check-in: where are these enzymes coming from again?

Reflection: Which accessory organs play a role in digestion within the small intestine? Explain one role of each accessory organ.

Absorption and transportation of nutrients. The enterocyte has the microvilli membrane that project towards the lumen of the tract. This membrane is the apical membrane and thus is the first membrane that the nutrients must cross. Once across the apical membrane the nutrients travel across the enterocyte to the basolateral membrane that is in contact with the blood and lymph vessels, thus, the second membrane the nutrients must cross. Macronutrient absorption across the apical and basolateral membranes requires passive diffusion, carrier-mediated transport or pinocytosis depending on what nutrient is being absorbed. The specific type of transportation mechanism will be discussed in the digestion and absorption lectures specific to each macronutrient.

Large Intestine

The digestive process of macronutrients is not perfect. There will be leftover material that is not properly digested or properly digested material that bypassed the absorption within the small intestine due to lack of contact with the mucosa or unavailable transporters. This material will stimulate the relaxation of the ileocecal sphincter connecting the ileum and the large intestine. Muscle movements keep the material in the proximal region of the large intestine to allow time for absorption of water, sodium and chloride. The undigested carbohydrates and amino acids can serve as fuel for the gut bacteria. As the gut bacteria degrades the carbohydrates and amino acids they release byproducts like short-chain fatty acids (SCFAs). SCFAs serve a variety of purposes like cellular proliferation, pH alteration, or they can even be absorbed through the enterocytes in the large intestine for utilization of cells in our body (e.g., brain or liver cells). As the undigested material moves down the large intestine water is continually being drawn from it and absorbed into the body. This

continuous dehydration results in fecal matter.

Pathologies Related to GI Structure and Function

Gastroesophageal reflex disease (GERD)

Almost everyone has experienced 'acid reflux' at one point in their life time. This is when gastric juices and/or food materials flow back into the esophagus and sometimes to the mouth. When this happens on a regular basis or over a long period of time it is classified as gastroesophageal reflux disease (GERD). After repeated exposure to acidic contents from the stomach, the mucosal layer of the esophagus can become irritated and inflamed. Over time fibrotic tissue can form resulting in the narrowing of the lumen. If there is repeated exposure in the mouth, this can lead to dental problems such as enamel exposure.

GERD OCCURS WHEN THE LES HAS NOT PROPERLY CLOSED LEAVING THE PASSAGEWAY FROM THE ESOPHAGUS TO THE STOMACH OPEN. There are common and well-characterized risk factors that leave the LES in its relaxed state. A large, high-fat meal will remain in your stomach for a longer period of time. The high amount of food eaten will stretch the stomach disallowing the LES to close properly and because the bolus is in the stomach for a longer period of time, there will be increased production of gastric acid. Pregnancy can also lead to GERD because all of the internal organs are more tightly packed due to increased size of the uterus creating an increased pressure on the stomach. During pregnancy there is an increased production of progesterone that is meant to be a muscle relaxer to prepare for delivery. This muscle relaxant affects other muscles in the body including the sphincter muscles. Alcohol and nicotine in cigarettes are muscle relaxants. Spicy and minty foods and chocolate contain compounds within them that cause muscle relaxation. Being overweight is positively associated with GERD.@

GERD CAN BE TREATED BY IDENTIFYING THE TRIGGERS AND ADAPTING LIFESTYLE CHANGES TO AVOID THEM. Lifestyle factors that will improve the symptoms of GERD include weight loss, dietary restrictions, cessation of smoking, eating smaller meals and exercise (to lose weight). If lifestyle factors are not working, there are over the counter antacids available. Antacids contain an alkaline like magnesium or calcium that neutralize the stomach environment. Antacids are one of the few approved over the counter medications that are safe and acceptable for pregnant women to ingest.

Aspiration pneumonia and asphyxiation

Remember that the respiratory tract and digestive tract are separate systems in the body but the organs of the tracts are in close contact to one another. The epiglottis works to block the trachea as the bolus is swallowed. There are certain conditions when these systems do not work in concert with each other resulting in the bolus entering the respiratory tract. This has the ability to occur under conditions of low consciousness or those that affect the swallowing center of the brain. Conditions of low level of consciousness include drug overdose, alcohol poisoning or brain injury. Conditions affecting the swallowing control center in brain include Parkinson's disease, tumor, and stroke. When the bolus enters the trachea a couple things may happen. First, the airway becomes inflamed from irritation from the food, liquids, or even vomit (drug overdose or alcohol poisoning). The inflammation of the airway is referred to as aspiration pneumonitis. If an infection occurs it would become aspiration pneumonia, requiring antibiotic treatment.. In a worst case or unmonitored scenario asphyxiation will occur where an individual would not have sufficient oxygen entering the airways leading to suffocation.

Patients who are monitored under these conditions require specialized diets where swallowing becomes an easier process on the muscles that are involved. They will likely be prescribed a semi solid (mechanically altered) diet (*e.g.*, peeled fruits, ground meats, noodle consistency, *etc.*). Or in a worst-case scenario, the patient would require enteral or parenteral feeding dependent on the situation.

Reflection Questions

1. A patient is prescribed a proton pump inhibitor (PPI) that blocks parietal cells from secreting HCl, eliminating gastric acid. Predict at least three downstream consequences for digestion in the stomach and small intestine, explaining the mechanism behind each.

2. A patient undergoes a cholecystectomy (surgical removal of the gallbladder). Bile is still synthesized by the liver, but is now continuously dripped into the duodenum rather than released in concentrated bursts following a meal. Evaluate how fat digestion and absorption would be affected, and explain why bile salt recycling via enterohepatic circulation might be impaired.

3. A patient with untreated celiac disease has extensive villous atrophy — the villi in the jejunum are almost completely flattened. Using your knowledge of small intestine structure and macronutrient absorption mechanisms, explain why this leads to malabsorp-

tion. Which macronutrients would you expect to be most severely affected, and why?

Part II

Carbohydrates

Carbohydrate Structure

For this lecture we will discuss the basic structure of carbohydrates. Understanding the basic molecular components of a carbohydrate will give you the foundation needed to comprehend digestive and absorptive mechanisms. Beyond digestion and absorption having knowledge of the carbohydrate structure is also necessary in understanding metabolism.

Learning Objectives

- Understand the basic structure of carbohydrates
- Distinguish between the three classes of carbohydrates and the relevance to our diet
- Understand the significance of glycosidic bond configuration related to digestive processes
- Describe different storage forms of polysaccharides found in plants and humans

Carbohydrate Basic Structure

Carbohydrate is commonly abbreviated as CHO, giving us insight into its basic structural components. They are made of *carbon* chains with *hydrogen* and *oxygen* groups having the standard molecular formula of C_n $(H_20)_n$. The carbohydrate structure contains two types of functional groups — First, a hydroxyl (-OH) and Second carbonyl. A carbonyl group contains a carbon double bonded to oxygen. There are two types of carbonyl groups that a carbohydrate can contain — either an aldehyde or a ketone. The structure of an aldehyde carbohydrate includes a carbon double bonded to oxygen at the end of the carbon chain, while for a ketone, the double bond is a non-terminal carbon (Figure 15).

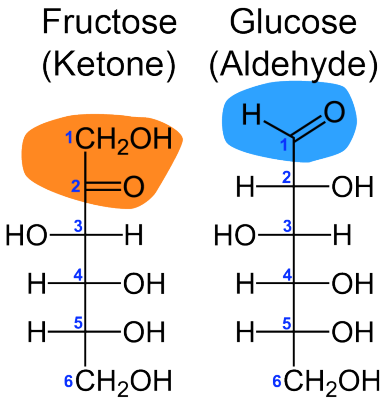

Figure 15: The difference between an aldehyde sugar, and a ketone sugar.

Classes of Carbohydrates

Carbohydrates fall into one of three classes. The first and simplest class is **monosaccharides**, often referred to as **simple sugars**. Monosaccharides include **glucose, fructose** and **galactose**. They are found in food sources such as honey, fruits and corn syrup. Typically carbohydrates are not in this simple form in food sources, but during digestion humans break down larger carbohydrates to simple sugars because they are the form of carbohydrates that can be absorbed at the small intestine. Before discussing the larger carbohydrate classes and digestion of carbohydrates, it is important to review the basic CHO nomenclature, chiral nature of the monosaccharide, and a glycosidic bond.

Nomenclature

The systematic naming of monosaccharides is useful to understand because it can help you visualize the molecular make-up of that car-

bohydrate unit. An aldehyde will contain the suffix "**-ose**", while a ketone will contain the suffix -**ulose**. Remember that a carbohydrate is a carbon chain with anywhere from three to seven carbons. Thus, a six- carbon aldehyde carbohydrate is called a hexose, while a six-carbon ketone would be designated as a hexulose. The nomenclature can help to visualize the structure of the carbohydrate, but for the carbohydrates most nutritionally and metabolically relevant, we tend to call them by their trivial names (*e.g.*, glucose, which is technically a hexose).

Chirality

We must briefly discuss the stereoisomerism of carbohydrates because most digestive enzymes, metabolic enzymes and transport mechanisms are stereospecific. Carbohydrates are molecules that are optically active, meaning that if a polarized light were to be passed through it, the light would either rotate to the right (D designation) or the left (L designation). The light rotation is an effect of a **chiral carbon** atom in the carbon chain. A chiral carbon is carbon with four *different* groups attached to it. Glyceraldehyde, a triose, is an example of a carbohydrate containing one chiral carbon (Figure 16).[54]

It is important to note that a carbohydrate with a chain consisting of more than three carbons will have multiple chiral carbons in which the highest numbered chiral carbon will dictate the rotation of the light, thus the D- or L-designation [55]. In other words, glucose, a hexose, can exist in the D-glucose or L-glucose form dependent on the spatial arrangement of the hydroxyl group on its highest numbered chiral carbon (Figure 17).

Cyclization and Anomeric Forms

Most monosaccharides with five or more carbon atoms do not exist predominantly in their open-chain (linear) form in solution. Instead, they undergo an intramolecular reaction where a hydroxyl group attacks the carbonyl carbon, forming a **hemiacetal** (in aldoses) or **hemiketal** (in ketoses). This cyclization creates a ring structure — typically a six-membered *pyranose* ring (like glucose) or a five-membered *furanose* ring (like fructose).

The carbon that was part of the carbonyl group becomes a new stereocenter called the **anomeric carbon**[56], respectively, of the hydroxyl group (Figure 18).. Two stereoisomers may form at this carbon:

- α**-anomer** — the OH on the anomeric carbon is on the opposite side of the ring (trans) from the CH_2OH group.

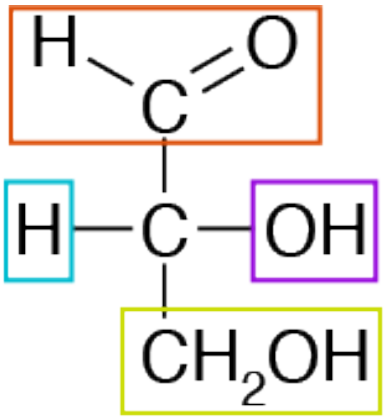

Figure 16: A triose (glyceraldehyde): the chiral carbon is circled and the four surrounding diverse groups are boxed off.

[54] Go back to Figure 15 and try to identify how many chiral centers exist in fructose and glucose.

[55] For purposes of this class we will always indicate to you which carbon is the highest numbered

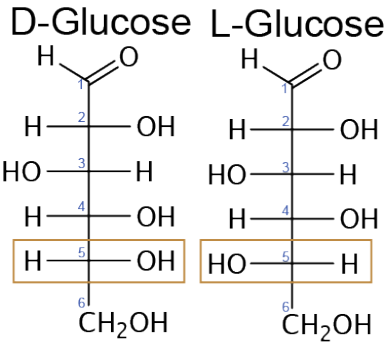

Figure 17: Representation of the chiral designation of glucose given the hydroxyl direction of the highest numbered chiral carbon.

[56] I remember this by alpha → anchor, beta → balloon.

- **β-anomer** — the OH on the anomeric carbon is on the same side (cis) as the CH_2OH group.

These forms interconvert in solution through a process called **mutarotation**.

Understanding the anomeric configuration is essential in nutrition because digestive enzymes such as *α-amylase* are specific to α-1,4-glycosidic bonds (as in starch), and cannot cleave β-1,4 bonds (as in cellulose). This specificity underlies why cellulose is indigestible in humans.

SUGARS THAT CAN HAVE A FREE ALDEHYDE OR KETONE GROUP ARE CALLED REDUCING SUGARS. All monosaccharides can interchangeably cyclize or decyclize. As a result at times the ketone or aldehyde group is free and could react with other food components. One important reaction in cooking is the Maillard reaction where a reducing sugar reacts with an amino group from an amino acid. This is what gives food its distinctive brown color and creates new flavors. Note in Figure 19 that when a sugar is part of a disaccharide, oligosaccharide or polysaccharide the aldehyde or ketone may no longer be free, and it can trapped as part of the glycosidic bond. If both anomeric carbons are part of the glycosidic bond, the compound is no longer a reducing sugar.[57]

[57] For example maltose and lactose are reducing sugars but sucrose is not. Take a look at their structures and try to understand why this is the case.

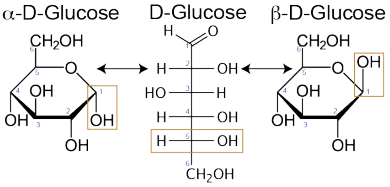

Figure 18: Cyclization of D-glucose to form alpha or beta-D-glucose.

Glycosidic Bonding

GLYCOSIDIC BONDS LINK MONOSACCHARIDE UNITS TO FORM OLIGO- AND POLYSACCHARIDES. The glycosidic bond is named after the direction (alpha- or beta-) of the hydroxyl group attached to the anomeric carbon, the anomeric carbon number and the carbon number of the subsequent monosaccharide linking the units together (Figure 19). To form oligo- or polysaccharides through glycosidic bonding, the monosaccharides undergo a reaction that eliminates a water (H_20) group called **condensation** (Figure 19). Alternatively, the oligo- or polysaccharides can be broken down to their respective monosaccharide units through **hydrolysis**, or the addition of a water molecule. Hydrolysis is the key reaction of digestion that will break apart larger carbohydrate units from food into monosaccharide units that are available for absorption at the small intestine.[58]

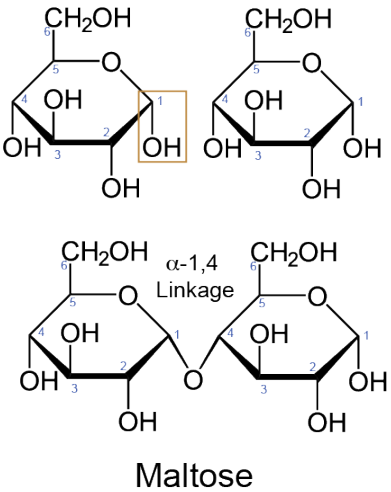

Figure 19: Condensation of two molecules of alpha-D-glucose to form the disaccharide maltose with the glycosidic bond formation designated as alpha 1,4.

Disaccharides

[58] The absorbable unit for carbohydrates in the small intestine is the monosac-

DISACCHARIDES ARE TWO MONOSACCHARIDES LINKED BY A GLYCOSIDIC BOND AND ARE THE MOST COMMON TYPE OF OLIGOSACCHARIDES FOUND IN DIETARY SOURCES. The three major disaccharides in dietary source are maltose, lactose and sucrose[59]. Maltose consists of two alpha-D-glucose units linked by an alpha 1,4 glycosidic bond (Figure 19). Lactose contains a beta-D-glucose linked to a beta-D-galactose by a beta 1,4 glycosidic bond[60]. Sucrose consists of an alpha-D glucose linked to beta-D fructose by an alpha 1, beta 2 glycosidic bond. The anomeric carbons from both the glucose and fructose units form the glycosidic bond of sucrose, thus there is an alpha- and beta-designation in the glycosidic bond name.

[59] If interested, Google dietary sources of maltose, lactose and sucrose. What did you find?

[60] This means that the hydroxyl of the animeric carbon is in the beta (balloon) orientation

Oligosaccharides

The next class of carbohydrates is oligosaccharides. They contain 2–10 monosaccharide units with each unit linked by a glycosidic bond. These are less common in our diets but some examples include raffinose (Gal-Glu-Fru, in brussels sprouts, asparagus and cabbage) and stacchyose (Gal-Gal-Glu-Fru, in beans). We are unable to digest raffinose and stacchyose and therefore are fermented by the microbiota in our large intestine. Fermentable oligosaccharides are one source of irritable bowel syndrome (IBS), a class of triggers known as FODMAPs[61]. FODMAP elimination diets are a common treatment for IBS, but generally only in the short term as they can result in severe nutrient deficiencies [van Lanen et al., 2021].

[61] Also including disaccharides (mainly lactose and sucrose), monosaccharides (primarily fructose), sugar alcohols, and polyols

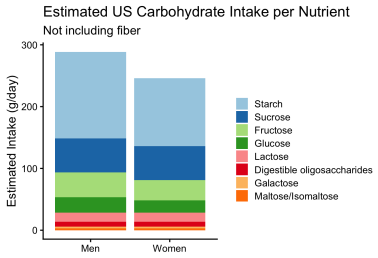

Figure 20: Estimated carbohydrate intake by nutrient, based on data from NHANES and WWEIA

Polysaccharides

Polysaccharides contain 11 or more and up to 10,000,000 monosaccharide units. The monosaccharide units of the polysaccharide can be the same (homopolysaccharide) or can have varying monosaccharide units (heteropolysaccharide). Another characteristic of polysaccharides is their degree of branching. Some are linear and contain zero branching units while others have varying degrees of branching. Typically, the more branching the more water-soluble the polysaccharide is (see Fiber reading for more information on water-solubility and polysaccharides). The branching also becomes important for storage capacity, which we will discuss in more detail when we talk about specific polysaccharides in the following subsections. There are three polysaccharides we will discuss that have relevant implications in human nutrition — starch, glycogen and cellulose. Common among these three polysaccharides is that they are all homopolysaccharides containing only glucose units.

STARCH IS THE MOST ABUNDANT POLYSACCHARIDE FOUND IN PLANTS (*e.g.*, tubers, seeds, roots, some fruits). It is also the most abundantly consumed carbohydrate in American diets (see Figure 20). Starch is found in two forms, **amylose** and **amylopectin**. Amylose is linear, unbranched and made of alpha-D-glucose connected by an alpha 1,4 glycosidic bond. Amylopectin is moderately branched and a homopolymer of alpha-D-glucose linked by alpha 1,4 bonds on the linear chains but by alpha 1,6 bonds at the branching points. The typical length of a linear between branching points of amylopectin is 20–25 glucose units. In uncooked food sources containing starch, the linear structure of amylose allows it to be tightly packed. This creates a structure that is not as readily digestible as the branched, less packed structure of amylopectin. In contrast, the processing and heating of amylose-containing foods (i.e., cooking) can loosen hydrogen bonds within the structure making it more accessible for digestion.[62]

[62] **Reflection**:Have you ever eaten flax seeds? Think of how well your body was able to digest ground ("processed") flax seeds compared to whole flax seeds.

CELLULOSE IS A LINEAR HOMOPOLYMER OF β-D GLUCOSE LINKED BY β 1,4 BONDS. This bond arrangement is significant in the digestive tract because digestive enzymes are not specific to this organization, thus cellulose is non-digestible (further explanation in Digestion section and Fiber reading) and is an insoluble fiber source. Like amylose, the linear structure allows cellulose to be highly packed within plant cell walls, thus cellulose is key for the structural stability of plant cell walls.. Insoluble fibers such as cellulose add bulk to stool and help to regulate bowel movements.

GLYCOGEN IS A HIGHLY BRANCHED, HOMOPOLYMER OF α-D-GLUCOSE. Glycogen is abundant in skeletal muscle and liver tissue of mammals. The glucose units are linked by α 1,4 bonds on the linear chains but by α 1,6 bonds at the branching points (just like amylopectin). The typical length of a linear chain between branching points is 10–14 glucose units producing a higher molecular weight compound compared to amylopectin. The highly branched structure of glycogen allows for increased storage capacity in mammal tissue. More branching also allows for a higher degree of accessibility for metabolic enzymes, thus glycogen is a ready source of energy for humans (and other mammals). [63]. Glycogen as a major storage form of glucose can support blood glucose in a healthy individual for 12-24 hours, depending on the individual and their activity level [Horton and Hill, 2001].

[63] **Reflection**: Pause and summarize (*e.g.*, in a table format) either the make-up of the three disaccharides or the three types of polysaccharides that we discussed. Compare and contrast.

Reflection Questions

1. Glycogen and amylopectin are both branched homopolymers of α-D-glucose using α-1,4 and α-1,6 glycosidic bonds, yet glycogen branches approximately every 10–14 glucose units compared to every 20–25 in amylopectin. Analyze how the higher degree of branching in glycogen affects both the rate of glucose mobilization and overall storage capacity. Why is this relevant to the metabolic demands of mammalian tissues?

2. Cellulose and amylose are both linear homopolymers of glucose, yet one is readily digestible and the other is not. Evaluate the role of glycosidic bond stereochemistry in this difference, and explain why this distinction has direct implications for dietary fiber intake and bowel function.

3. A patient following a low-FODMAP diet is asked to eliminate raffinose-containing vegetables and lactose-containing dairy. Using your knowledge of glycosidic bond specificity and digestive enzyme function, explain why these two structurally different carbohydrates both end up causing similar GI symptoms in susceptible individuals.

Carbohydrate Digestion and Transport

The majority of caloric intake worldwide is carbohydrates, with most people eating about half of their total calories in this form. There is a lot of variation in how we digest and absorb different carbohydrates and this in turn plays a major role in how blood glucose is controlled. In this lecture we will discuss the digestive processes specific to carbohydrates starting in the oral cavity all the way to the end of the tract. We will go over absorption and transportation of simple carbohydrates through the enterocyte to circulation.

Learning Objectives

- Describe digestion of carbohydrates starting in the oral cavity
- Apply knowledge of carbohydrate structure to carbohydrate-specific digestive enzymatic properties
- Compare the enzymatic breakdown of α-1,4 vs. α-1,6 glycosidic linkages.
- Discuss the role of intestinal brush border enzymes in monosaccharide liberation.
- Understand absorption of monosaccharides via glucose transporters
- Explain transportation mechanisms of monosaccharides to target organs
- Define glycemic index and its relation to chronic disease
- Explain the role of insulin in maintaining blood glucose levels
- Explain the history and etiology of lactase persistence

Carbohydrate Digestion

The most common classes of carbohydrates that are in food sources are di- and polysaccharides. The human body must break down these carbohydrates to their respective monosaccharide units because this is the form that can be absorbed across the enterocytes at the small intestine. The enzymes that break down carbohydrates to monosaccharides are collectively called **glycosidases**.

Oral Cavity and Stomach

The first exposure of a carbohydrate to a glycosidase occurs in the mouth. The salivary glands produce the enzyme **alpha amylase,** which is released into the mouth as part of saliva. Alpha amylase targets the oxygen bridges as part of alpha 1,4 glycosidic bonds between glucose units[64]. Its optimal activity occurs in pH around 6.5–7 As food is mixed with saliva and chewed, the carbohydrates are chemically and mechanically broken down. This is a relatively short period of digestion that produces oligo- and shorter polysaccharides and very few monosaccharides. As food travels from the mouth to the stomach, the salivary alpha amylase is still present. Once the bolus reaches the stomach there is a very limited period of digestion because the acidic environment alpha amylase activity[65].

[64] This will be a recurring theme in this lecture, that particular enzymes can only digest specific glycosidic bonds. Keep in mind the limited arsenal of enzymes we have, relative to the very large number of potential glycosidic bonds

[65] Salivary alpha amylase is inactivated at pH less than (more acidic) than 4, so is not active beyond the upper stomach. Think of it as the first leg of a relay, it gets things started but passes the baton to pancreatic alpha amylase in the small intestine.

TASTE OF SUGAR. We have receptors for each of the five tastes; sweet, sour, bitter, salty and umami[66] The sweet receptors can bind to several mono- and di-saccharides including fructose, sucrose and glucose but also other non-caloric compounds such as saccharin, sucralose and aspartame. Interestingly the taste receptors[67] are also expressed throughout the gastrointestinal system suggesting that sugar sensing occurs at more locations than just the tongue[68]. For more about this emerging area check out this review by Janssen and Depoortere [2013]

[66] Though the map you may be visualizing of localized taste receptors on the surface of the tongue is fake news.

[67] Encoded by the genes *T1R2* and *T1R3*

[68] T1R2/T1R3 activation in enteroendocrine cells influences GLP1 release [Jang et al., 2007].

HORMONAL MODULATION AND GASTRIC EMPTYING. The rate at which chyme leaves the stomach and enters the small intestine—called **gastric emptying**—has a profound effect on postprandial blood glucose levels. Faster emptying delivers carbohydrates more rapidly to the absorptive surface of the small intestine, leading to a sharper rise in blood glucose. Conversely, slower emptying blunts the glycemic response.

Several hormones secreted in response to nutrients modulate this process:

- **Gastric Inhibitory Peptide (GIP)** is secreted by K cells in the proximal small intestine. It enhances insulin secretion and slows gastric emptying modestly.
- **Glucagon-Like Peptide-1 (GLP-1)** is secreted by L cells in the ileum and colon in response to carbohydrates and fats. It strongly delays gastric emptying and promotes satiety.
- **Cholecystokinin (CCK)** is released in response to lipids and proteins and also contributes to delayed gastric motility.

These hormones are part of the **incretin effect**—the amplification of insulin secretion when glucose is ingested orally vs intravenously. This effect is partly due to the slowing of gastric emptying, which spreads glucose absorption over a longer time window.[69] **Dietary fiber**—especially soluble fiber—acts synergistically by increasing chyme viscosity, delaying stomach emptying, and slowing intestinal glucose absorption.

[69] GLP-1 analogs (like semaglutide) are used clinically in type 2 diabetes and obesity to improve glycemic control and reduce appetite by mimicking this hormonal regulation.

Small Intestine

When the bolus reaches the small intestine, the salivary alpha amylase is activated again because of the increase in pH. In addition to the salivary alpha amylase, the pancreas releases pancreatic juice that contains pancreatic alpha amylase. Amylose is broken down to maltriose (trisaccharide) and further more to maltose. Alpha amylase can

work on the linear chain of α-1,4 bonds of amylopectin but cannot hydrolyze the branching points that are α-1,6 bonds[70]. The product of amylopectin digestion from alpha amylase is a disaccharide called isomaltose.

The brush border of the small intestine houses glycosidases that help with the completion of carbohydrate digestion. For the purposes of our discussion we will focus on four of these enzymes. **Maltase** is specific to the α 1,4 bond in maltose and maltriose. **Isomaltase** is specific to the α-1,6 (i.e., former branching points) of isomaltose[71]. Then considering the other two disaccharides abundant in our diet, lactose and sucrose, **lactase** and **sucrase** are specific for the β-1,4 and α-1, β-2 bonds of lactose and sucrose, respectively[72].

[70] We do not have enzymes that can digest β-1,4-bonds between glucoses such as those in cellulose, making it a fiber.

[71] Congenital sucrase-isomaltase deficiency (CSID) is a rare genetic disorder that leads to severe carbohydrate intolerance.Patients with CSID cannot digest sucrose or starch properly and often present with chronic diarrhea and failure to thrive in infancy.Treatment often involves a sucrose-free, starch-restricted diet, and in some cases oral enzyme replacement therapy (*e.g.*, sacrosidase). Consider why CSID would impair digestion of starch *as well as* sucrose.

[72] Sucrase/isomaltase is actually one bifunctional enzyme which has two different domains that can catalyze each of these separate reactions.

Monosaccharide Absorption and Transportation

Monosaccharides, like glucose, are impermeable to cellular membranes and require carrier-mediated systems to cross the apical and basolateral membranes of the enterocyte in order to reach circulation. There are two transport systems important for absorption across the enterocyte. The first is an active carrier-mediated transport system called the **sodium-glucose transport 1 (SGLT1)**. To work, this system requires energy through a sodium-potassium ATPase pump (Figure II). The other is a series of carrier-mediated transporters called **Glucose Transporters (GLUTs)** that work by facilitated diffusion. There are 14 GLUT isoforms that have been identified, but the best characterized are GLUTs 1, 2, 3, 4 and 5. Each GLUT has specific tissue distribution throughout the body, for example GLUT 2 and 5 are the isoforms located in the small intestine (see Table II).

Glucose and Galactose

When glucose and galactose are present in lower concentrations in the lumen in comparison to the enterocyte, they require the SGLT1 transmembrane transport protein to cross the apical membrane. This transport system relies on the high sodium concentration in the lumen.

SGLT1 binds to glucose or galactose and to two sodium ions. Once sodium is bound, this allows for passive diffusion through the apical membrane into the enterocyte. Glucose and galactose are then released and carried through the basolateral membrane via GLUT2. To keep the lumen sodium concentrations high and enterocyte sodium concentrations low, the Na/K ATPase pump (energy required) pumps sodium out of the cell and potassium in, thus making this an *active carrier-mediated transport system* (Figure II).

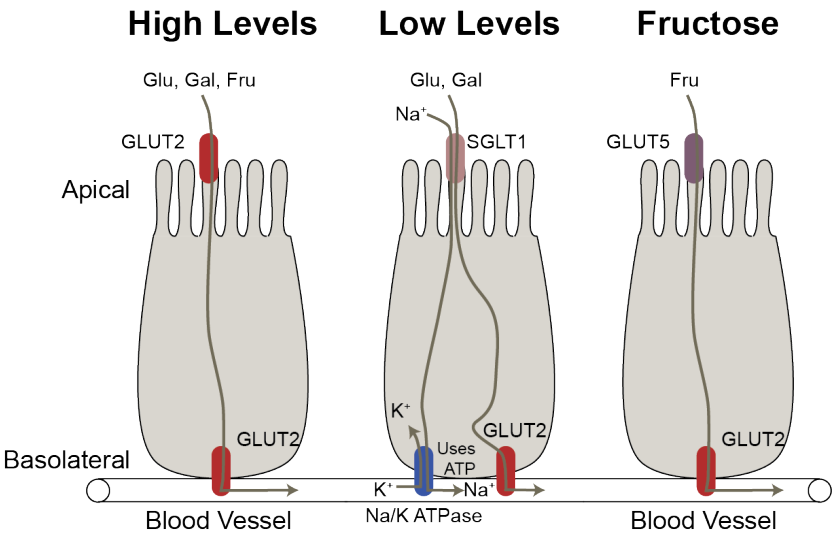

Figure 21: Small intestinal glucose transporters.

Fructose

Fructose is transported by GLUT5 across the apical membrane and by GLUT 2 across the basolateral membrane. Recent work has shown that this system is easily saturable [Jang et al., 2018]. At low levels of fructose, it is often converted to glucose and exported to the blood but at higher levels it can enter the circulation as fructose or stay in the gastrointestinal tract resulting in fermentation by the gut microbiota. That in turn can become acetate and result in hepatic lipogenesis [Zhao et al., 2020].

GLUCOSE, FRUCTOSE AND GALACTOSE — UNDER CONDITIONS OF HIGH LUMEN CONCENTRATIONSFollowing high carbohydrate intake, SGLT1 becomes saturated so GLUT2 is translocated to the apical membrane to assist with transportation across the membrane via passive diffusion [Kellett et al., 2008]. Under this mechanism the three monosaccharides will still cross the basolateral membrane via GLUT2. In addition to SGLT1 saturation, the activation of the "sweet taste" receptors in the enteroendocrine cells of the digestive tract have been shown to play a role in the translocation of GLUT2 to the apical membrane. This is triggered by monosaccharides as well as sugar substitutes [Stearns et al., 2010]. The small intestine is sensitive to insulin, which is released from the pancreas following glucose absorption. The insulin response results in the internalization of GLUT2 (translocation away from the apical membrane) thus decreasing the absorption rate of glucose. Lastly, mRNA expression of GLUT2 is increased after consumption of a high carbohydrate meal [Miyamoto

Isoform	Expression	Substrates
GLUT1	CNS, Placenta	Glu, Gal
GLUT2	Liver, Pancreas, Small Intestine	Glu, Gal, Fru
GLUT4	Adipose, Muscle	Glu
GLUT5	Small Intestine	Fru

Table 11: Summary of major carbohydrate transporters

et al., 1993]. This is not unique to GLUT2, but has also been found for GLUT5 and SGLT1. Thus, there are multiple levels of transporter regulation at the small intestine that influence monosaccharide absorption.

Systemic Carbohydrate Transport

Once the monosaccharides cross the basolateral membrane, they are small enough molecules that they are able to enter the capillary system eventually entering the hepatic portal vein leading to the liver[73]. Galactose and fructose will undergo metabolism in the liver and sm all intestine to be converted into glucose and its derivatives. Glucose can be stored as glycogen, oxidized for energy, broken down for fatty acid or amino acid synthesis or reenter circulation to tissues, all dependent on the body's energy status.

[73] hepatic = liver

Transportation across cell membranes outside of the intestine requires glucose transporters. As stated earlier, the glucose transporters are tissue specific thus the isoform present is dependent upon the tissue type (Table II). Glucose requires insulin signaling to be taken up by GLUT4 at skeletal muscle and adipose tissue.

Insulin Resistance and Diabetes

GLUT4 is a key glucose transporter found on muscle and adipose tissue. This transporter is unique because to function properly it is dependent on proper insulin signaling. Under normal conditions, pancreatic beta-cells respond to an increase in blood glucose levels by secreting insulin into the bloodstream. Insulin receptors are located on skeletal muscle and adipose tissue that bind insulin under these conditions. Once insulin is bound this sends a phosphatidylinositol-3-kinase signal cascade within the cell that releases stored GLUT4 from golgi apparatus in the form of a GLUT4 storage vesicle (GSV). The GSV carries GLUT4 to the cell membrane, releases it and allows for passive diffusion of glucose into the cell.

Insulin insensitivity is a condition that is associated with obesity, Type 2 Diabetes and other chronic conditions [De-

Fronzo, 2010]. It occurs when the muscle and adipose tissue cells become unresponsive to this insulin signal for cellular glucose uptake resulting in elevated blood glucose levels. Because glucose remains in the blood, the pancreas secretes additional insulin to continue to work on getting glucose into cells and also stimulates gluconeogenesis (biosynthesis of glucose by the liver) because it thinks the cells are deprived of glucose. When this is a continuous occurrence, the pancreatic beta-cells become overworked and stop producing sufficient insulin resulting in long-term hyperglycemia leading to Type 2 Diabetes, heart diseaseand other vascular disease. There are several mechanisms that have been hypothesized as playing a role in the mechanism of a cell becoming unresponsive to the insulin signal such as inflammation, genetics and epigenetics. One studied mechanism is **lipotoxicity** in which excess fat buildup within the body results in fat residing on or within cellular space. This can result in disruption of the insulin pathway by cellular damage and inflammation.

Glycemic Index

For individuals who suffer from hyperglycemia, diet can play a key role for controlling their blood level of glucose throughout the day. Carbohydrate-containing food have been categorized by a **glycemic index**. This index is an indication of the absorption rate of monosaccharides from the small intestine after consuming carbohydrate-containing food [Atkinson et al., 2008, Dodd et al., 2011].

The index ranges from 1–100 where 100 is the reference of the fastest absorption potential and often symbolized by white bread. As a rule of thumb, foods higher in fiber and lower in fat have a lower glycemic index and more processed foods (decreased fiber content) have a higher glycemic index (Table II). The glycemic index is a tool that can be used to help educate individuals on proper food choices to help control flux of carbohydrate absorption and to help maintain a steady level of blood glucose when consuming food and throughout the day. The glycemic index is not a perfect tool, however, as it does not take into account the amount of carbohydrate in a food. For example, watermelon has a high glycemic index but low carbohydrate content, so it is not a significant source of glucose. The glycemic load is a more accurate measure of the impact of a food on blood glucose levels as it takes into account both the glycemic index and the amount of carbohydrate in a serving of food [Atkinson et al., 2008].

DIETARY FIBER, PARTICULARLY VISCOUS SOLUBLE FIBER, SLOWS THE RATE OF CARBOHYDRATE ABSORPTION. It forms a gel-like ma-

Glycemic Index	Example Foods
<55 Low	Steel cut oatmeal, fruits, whole wheat bread, corn tortillas, tomato juice
55–69 Medium	Quick oats, brown rice, rye bread, bagels, fruit juices
69–100 High	White bread, instant oatmeal, white rice, popcorn, soda

Table 12: Examples of foods by glycemic index

trix in the lumen of the small intestine, increasing the viscosity of chyme and delaying gastric emptying. This matrix creates a physical barrier that:

A meta-analysis of observational cohort studies shows that for every 10g/day increase in total fiber intake, risk of type 2 diabetes decreased by 9% [Yao et al., 2014]..

- Reduces enzyme-substrate contact (*e.g.*, α-amylase can't reach starch as easily).
- Slows the diffusion of monosaccharides to the enterocyte surface through increased viscosity.
- Delays glucose transporter engagement by reducing the movement of monosaccharides to the enterocyte.

Lactase Non-persistence

LACTOSE INTOLERANCE is a common term used to describe the overarching symptom of the condition of **lactase non-persistence**. This condition results from an enzyme deficiency of the digestive enzyme lactase, described above. In mammals, the lactase gene[74] is highly active in the lactation life stage (*i.e.*, infancy) when the sole food intake is from the mother's lactose-containing breast milk. Following the introduction to other food products, and consequently, exposure to foods containing carbohydrates other than lactose, the production of lactase rapidly declines, as there is no significant need for its activity.

[74] The gene symbol is *LCT*

Because of the innovation of domesticating milk-producing animals, humans are a unique mammal because this allows us the opportunity to continue to drink milk and eat milk-based food products beyond the lactation period [Itan et al., 2009]. Over time some humans developed a genetic polymorphism in the lactase gene resulting in sufficient production of lactase throughout adulthood when there is continuous exposure to lactose-containing foods. Because milk-producing agriculture is a recent event in regard to evolution this polymorphism is thought to arise from a high frequency haplotype due to the benefit of ingesting lactose in cultures that raise milk-producing animals [Harvey et al., 1998, Itan et al., 2009]. Individuals

lacking this polymorphism will have a decline in lactase production and will start to have symptoms of lactose intolerance in early childhood.

Specific symptoms include bloating, cramping and diarrhea. Because the disaccharide lactose will not be properly digested for absorption, it will travel to the large intestine where it can absorb and retain water within the large intestine causing bloating and potentially diarrhea and dehydration. Of note, it has been evidenced that individuals who are **lactase persistence** but do not expose themselves to milk-containing products will have a steady decline of lactase production over time and may also exhibit symptoms of lactose intolerance when reintroducing milk-products [Gerbault et al., 2011].

Reflection Questions

1. A patient with congenital sucrase-isomaltase deficiency (CSID) lacks a functional sucrase-isomaltase enzyme, yet experiences problems digesting both sucrose and dietary starch. Trace the specific glycosidic bonds each enzyme domain targets, and explain mechanistically why loss of a single bifunctional enzyme impairs digestion of two structurally different carbohydrates.

2. GLP-1 receptor agonists (such as semaglutide) are used to treat both type 2 diabetes and obesity. Using your knowledge of the incretin effect, gastric emptying, and glucose transporter regulation in the small intestine, evaluate at least three distinct mechanisms by which enhanced GLP-1 signaling reduces postprandial blood glucose spikes.

3. Two foods both contain 30g of carbohydrate per serving, but one has a low glycemic index and the other has a high glycemic index. Using your knowledge of brush border enzyme activity, glucose transporter regulation, soluble fiber, and gastric emptying, identify at least three structural or physiological factors that could explain the difference in their glycemic response.

4. In normal physiology, insulin released after a meal causes internalization of GLUT2 from the apical membrane of enterocytes, providing a feedback brake on intestinal glucose absorption. SGLT1, by contrast, is not regulated by insulin. In a person with insulin resistance, evaluate how impaired intestinal insulin signaling could contribute to a vicious cycle of worsening postprandial hyperglycemia, and explain why this intestinal mechanism is distinct from the well-known failure of GLUT4-mediated glucose uptake in muscle and adipose tissue.

Fiber

For this lecture we will review the basic structure of fiber and go over several definitions used by the general population. We will discuss the varying types of fibers and their characteristics. The characteristics of each fiber type are indication of how they affect digestive and absorptive processes. The digestive and absorptive effects of fiber can influence several health outcomes in humans.

Learning Objectives

- Review structure of fiber
- Compare various types of fibers
- Describe the various properties of fiber
- Apply the structural properties of fiber to physiological effects on humans

Fiber Basics

We discuss fiber within the carbohydrate unit because its structure is very similar except that the glycosidic bonds are resistant to digestive breakdown and will forgo absorption in the digestive tract. For example, recall the polysaccharide cellulose. It is a linear homopolymer of beta-D glucose linked by beta 1, 4 bonds which are resistant to glycosidases in the digestive tract such as alpha amylase (enzyme specificity to alpha 1, 4 glycosidic bonds). This results in cellulose passing through the digestive tract intact and reaching the large intestine. Fiber is found in plants, specifically within the cellular walls. Common dietary sources of fiber include fruits, vegetables, grains and legumes. Fiber can also be synthetically added to food sources such as cereals, yogurt, juices, and even artificial sweeteners.

MOST PEOPLE DO NOT CONSUME THE RECOMMENDED AMOUNT OF FIBER which is 25g per day for women and 38g per day for men [Institute of Medicine, 2005]. The average American consumes only 16g of fiber per day [Hoy and Goldman, 2010]. The low intake of fiber is concerning because it has been shown to have several health benefits including the prevention of constipation, diverticular disease, and colorectal cancer [Slavin, 2013]. In addition, fiber can help with weight management by increasing satiety and decreasing hunger [Clark and Slavin, 2013].

Consider what you percieve to be some high fiber foods, and look up the amount of fiber per serving. Calculate how many servings of that food you need to achieve 38g, the recommendation for males.

Defining Fiber

The term crude fiber was coined by two scientists in the 1800's who first discovered that there was material leftover from plants after an extraction in an acidic dilute followed by an alkali dilute which mimicked the digestive tract environment. As an understanding of how the digestive tract works and what this leftover fraction was, the definition of crude fiber evolved over time to what we most commonly refer to as dietary fiber or in other words, "the plant polysaccharides

and lignin which are resistant to hydrolysis by the digestive enzymes of a man" [Trowell, 1978]. For research and policy purposes, several definitions beyond dietary fiber have been developed by scientific and regulatory agencies. The definitions either encompass a physiological character of the fiber such as soluble or fermentable or refer to an analytical method (i.e., can be synthetically made) associated with the fiber like functional fiber.

Key Characteristics (and Physiological Effects)

Solubility

As the name of the characteristic suggests, fibers defined as water-soluble will dissolve in water whereas water-insoluble will not. The solubility of a fiber results in unique physiological effects. Fibers with a higher water solubility form a more gel-like substance as they move through the tract. They also tend to have a high viscosity, ability to adsorb and are typically fermentable[75]. Alternatively, insoluble fibers will stay intact as they travel through the digestive tract. Upon reaching the colon insoluble fiber will add to the bulk of fecal matter decreasing its transit time through the large intestine. This property of insoluble fibers helps with the relief of constipation.

[75] See other characteristics sections below for more detail

Fermentable

Whether a fiber is fermentable or not depends on whether the bacteria in our large intestine can ferment it (*i.e.*, metabolize it). Byproducts of fermentation include short-chained fatty acids (acetate, propionate, and butyrate), carbon dioxide and hydrogen. The short chained fatty acids that are produced as by-products can either be used by colon cells for their own energy provision or they can be absorbed at the large intestine entering general circulation and used for energy by non-colon tissue.. Particularly notable among the short-chained fatty acids is butyrate, which is the preferred energy source for colonocytes.

Functionality

The most widely used definition established by the Institute of Medicine is based on functionality [Institute of Medicine, 2005]. Dietary fibers are used specifically for nondigestible carbohydrates that are still intact and intrinsic in plant food sources. Dietary fibers encompass the naturally occurring fibers in food. Functional fibers are non-digestible carbohydrates that have been isolated from plant sources or synthesized in laboratory conditions — according to its

definition, the isolated fiber alone has to provide benefits for human health. Thus, functional fibers encompass either natural fiber that has been separated from the original food or synthetic fiber produced by a human.

Other Characteristics

Water-soluble fibers are typically viscous. The viscosity turns the material more gel-like and slows the movement of food through the digestive tract. Viscous fibers delay gastric emptying leaving chyme in the stomach for a longer period of time and increase the time of feeling full [Willis et al., 2009]. The slower gastric emptying can play a role in the rate of glucose absorption at the small intestine which aides in the well-controlled flux of glucose levels into circulation following food intake. In addition to sequestering carbohydrates, viscous fibers can also sequester proteins and lipids inhibiting their exposure to digestive enzymes. This can impede absorption of these macronutrients at the small intestine. Some fibers have the ability to adsorb (*i.e.*, to bind) to molecules and nutrients. Relevant to human health, some fibers bind fatty acids, cholesterol and bile acids within the digestive tract. Once bound, the material travels to the large intestine, is added to the bulk of fecal matter and is excreted. If it does not get added to fecal matter, bacteria in the large intestine can metabolize the bound molecules. Focusing on the potential of increased bile excretion by fiber via feces, the liver will need to synthesize more bile to keep up with lipid digestion and absorption. Remember that part of the basic structure of bile includes cholesterol, thus LDL cholesterol will be taken from circulation and incorporated into bile ultimately decreasing serum cholesterol [Brown et al., 1999].

Although, this is a well-proposed mechanism, evidence shows that high intake of fiber (equivalent to >3 servings of oatmeal per day) on a regular basis is needed to result in a significant decrease in blood cholesterol. Fiber can also adsorb to specific minerals like calcium and iron. This can have either a positive or negative consequence. If the fiber is also highly fermentable with bound minerals, the breakdown of the fiber by gut bacteria will release the minerals and allow for additional mineral absorption at the large intestine (some minerals actually have efficient transport systems at the colonocytes). On the other hand, if the fiber is poorly fermentable, the minerals will remain intact with the fiber material and be incorporated into fecal matter.[76]

[76] **Reflection:** Use table 13 to fill in the second column based on what you just read.

Table 13: Properties of fiber and their physiological effects

Property	Physiological Effect
Insoluble	
Soluble	
Fermentable	
Viscous	
Adsorbent	

Types of Fiber

The different types of fiber we find in our dietary sources vary greatly in structure and function. Please refer to Table II to see a list of common dietary fibers and what types of food sources they are found in, whether they can be synthesized for consumer products and the types of properties they hold.

Prebiotics

Prebiotics are a subset of dietary fibers and related compounds that selectively stimulate the growth and/or activity of beneficial microorganisms[77] in the gut, conferring health benefits to the host. *Not all fibers are prebiotics;* a compound must show selective utilization by beneficial gut bacteria and a positive health outcome to qualify. Common prebiotics include inulin-type fructans and galactooligosaccharides, but emerging candidates such as resistant starches are also being recognized. The prebiotic effect is central to the relationship between diet, the gut microbiome, and human health outcomes including modulation of inflammation, metabolic health, and possibly mental health through the gut-brain axis [Gibson et al., 2017].

[77] The actual bacteria, when consumed and able to colonize the gut are probiotics

Resistant Starch

Resistant starch (RS) refers to starch and starch-degradation products that escape digestion in the small intestine and reach the colon, where they undergo fermentation by colonic bacteria[Sajilata et al., 2006]. There are five types (RS1—RS5), each with different physical and chemical structures:

- RS1: Physically inaccessible starch (*e.g.*, whole/partially milled grains, seeds)
- RS2: Native granular starch (*e.g.*, raw potato, unripe banana)
- RS3: Retrograded starch (formed during cooking and cooling, *e.g.*, chilled rice)
- RS4: Chemically modified starches (industrial applications)
- RS5: Starch-lipid complexes

Like other fermentable fibers, resistant starch can yield short-chain fatty acids upon bacterial fermentation. Some resistant starch types act as prebiotics. Resistant has been studied for beneficial effects on glycemic response, colonic health, and increasingly, metabolic parameters [Birt et al., 2013].

Table 14: Types of fiber and their properties.

Fiber	Sources	Structure	Properties
Cellulose	Whole grains, root vegetables	beta-1,4 glucose	Insoluble, poorly fermented
Lignin	Wheat bran, nuts, flaxseeds, vegetables, unripe bananas	Complex, irregular polyphenolic polymer	Insoluble, poorly fermentable, provides structural rigidity and increases stool bulk
Hemicellulose	Bran, nuts, legumes	Branched, various units	Depends on branching
Pectin	Fruits	Highly branched, various units	Soluble, fermentable, adsorbent
Gums	Oatmeal, barley, tree	Branched, various units	Soluble, fermentable, adsorbent
β-Glucans	Oatmeal, Rice	beta-1, 3 glucose, with branches	Soluble, fermentable
Fructans	Onions, Artichokes	polyfructose	Soluble, fermentable
Galactans	Chickpeas, Lentils	polygalactose	Soluble
Resistant Starch	Legumes, cooked/cooled starches, unripe bananas	Linear and/or branched glucose	Partially soluble, variably fermentable

Reflection Questions

1. A patient presents with both elevated LDL cholesterol and chronic constipation. They ask whether they should prioritize oatmeal (rich in soluble, fermentable β-glucans) or wheat bran (insoluble, poorly fermentable) in their diet. Analyze the mechanisms by which each fiber type addresses each condition, and explain why a single fiber type may not optimally treat both problems simultaneously.

2. Resistant starch type 3 (RS3) forms when cooked starch is cooled — for example, chilled rice contains more resistant starch than freshly cooked hot rice. A patient with type 2 diabetes consumes equal portions of hot rice and chilled rice. Apply your knowledge

of resistant starch and fiber properties to predict the difference in postprandial blood glucose response, and explain the structural basis for why cooling changes digestibility.

3. A person takes a calcium supplement alongside a high-fiber meal. Using your knowledge of fiber's adsorptive properties and the distinction between fermentable and non-fermentable fibers, evaluate whether the type of fiber consumed with the supplement matters for net calcium absorption, and predict the outcome for each scenario.

The Gut Microbiome

The human body harbors a vast community of microorganisms (collectively the microbiome) that profoundly influence digestion, immunity, and overall health. In this unit we introduce the concept of the human microbiome, survey its distribution across body sites, characterize the composition of the gut microbiome in particular, and critically examine the methods used to study it.

Learning Objectives

- Describe the commensal relationship between humans and microbes, including major location-dependent microbiomes on the human body.
- Evaluate the stability and malleability of the microbiome, in relationship to genetic or life-course dependent changes.
- Consider the environmental factors that shape the gut microbial niche, and how that affects the kinds of bacterial species that flourish. Use this information to predict how bacterial species may be altered by diet.
- Evaluate the role of fiber in providing fuel to the microbiome. Using the physical properties of polysaccharides in fiber, explain why these nutrients are particularly important for microbial function.
- Explain the methods by which the microbiome is assessed, including the strengths and weaknesses of each methodology.
- Describe the relationship between the gut microbiome and the immune system.
- Compare descriptive and interventional studies of microbiota-disease relationships and use this information to consider the strength of the relationship.

Key Vocabulary and Concepts

- Dysbiosis
- Microbiome
- Prebiotics and Probiotics
- Human Milk Oligosaccharides
- Metagenomics
- Pattern Recognition Receptors
- Short-Chain Fatty Acids
- Mucus and Mucins
- Leaky Gut and Intestinal Permeability

What Is the Microbiome?

THE HUMAN BODY IS NOT A SOLO ORGANISM. Every surface exposed to the external environment including skin, mouth, respiratory tract, gastrointestinal tract, and urogenital tract is colonized by a dense community of microorganisms including bacteria, archaea, fungi, and viruses. The term **microbiota** refers to the collection of microorganisms themselves, while **microbiome** more precisely encompasses the microorganisms *and* their collective genetic material and metabolic activity though the two terms are used interchangeably.

For perspective on scale: one estimation placed the number of bacteria in and on the human body at approximately 3.8×10^{13}, roughly equal to the number of human cells (3.7×10^{13}), overturning the older "10:1" ratio that appeared in textbooks for decades [Sender et al., 2016].[78] The collective genome of the gut microbiota alone encodes roughly 150 times more genes than the human genome [Qin et al., 2010].

[78] Most of those bacteria reside in the colon, the rest of the body's surfaces are comparatively sparsely colonized.

A Commensal (and Mutualistic) Relationship

The relationship between host (the human) and microbiota is not parasitic. The host provides a stable, nutrient-rich environment, and the microbiota provide services the host cannot perform alone. Some of these include:

- Fermentation of indigestible dietary fibers to produce short-chain fatty acids (SCFAs) that fuel colonocytes and other tissues
- Synthesis of vitamins, particularly vitamin K and some B vitamins
- Competitive exclusion of pathogens by occupying sites and depleting nutrients
- Maturation and ongoing calibration of the immune system
- Biotransformation of bile acids and xenobiotics

This is best described as a *mutualistic* relationship: both partners benefit. The word *commensal*[79] is often used for microbes that neither help nor harm, but the gut microbiota clearly crosses into mutualism for the functions above. Disruption of the normal microbial community (termed dysbiosis) is associated with a range of diseases discussed later in the course.

[79] eating at the same table

The Human Microbiome Project

The scale and diversity of human-associated microbiota were systematically catalogued by the Human Microbiome Project, a NIH-funded initiative that characterized microbial communities from 18 body sites in 242 healthy adults [Human Microbiome Project Consortium, 2012]. A key finding was that microbial communities are highly *site-specific*: the microbiota of the skin differs dramatically from that of the oral cavity, which differs again from the gut.

Body-Site Microbiomes

Different body sites support distinct microbial communities shaped by local conditions such as pH, oxygen tension, nutrient availability, host secretions, and immune activity. Table II summarizes key features of the major body-site microbiomes.

Site	Dominant taxa	Key features
Oral cavity	*Streptococcus*, *Prevotella*, *Veillonella*	Biofilm (dental plaque); anaerobic niches in gingival sulcus
Skin	*Staphylococcus*, *Corynebacterium*, *Cutibacterium*	Varies by moisture; sebaceous vs. dry sites differ
Stomach	*Helicobacter*, sparse acid-tolerant taxa	Low pH (~2) limits density
Small intestine	*Lactobacillus*, *Streptococcus*	Short transit time; increasing density distal to proximal
Large intestine	*Bacteroides*, *Faecalibacterium*, *Bifidobacterium*, *Ruminococcus*	Highest density (10^{11}–10^{12} cells/mL); anaerobic; primary fermentation site
Vagina	*Lactobacillus* (dominant)	Low pH from lactic acid; highly variable across life stages

Table 15: Major body-site microbiomes and their dominant taxa.

The colon is the most densely colonized site, harboring an estimated 10^{11}–10^{12} bacteria per milliliter of luminal content (roughly the density of a bacterial culture at stationary phase). The low-oxygen environment[80] selects for obligate anaerobes, which

[80] Oxygen tension decreases from the mucosa outward; obligate anaerobes dominate the lumen while aerotolerant species can persist near the epithelial surface.

comprise the vast majority of gut bacteria. These are the organisms responsible for fiber fermentation and SCFA production discussed in later sections.

Composition of the Gut Microbiome

Major Phyla

At the broadest taxonomic level, two phyla dominate the healthy adult gut microbiome: ***Firmicutes*** and ***Bacteroidetes***, together accounting for roughly 90% of gut bacteria in most healthy adults [Eckburg et al., 2005]. *Actinobacteria* (including the genus *Bifidobacterium*) and *Proteobacteria* are present at lower abundances. The ratio of *Firmicutes* to *Bacteroidetes* (sometimes called the F:B ratio) has attracted attention as a potential marker of metabolic health, though its utility as a biomarker is debated.

Taxonomic hierarchy: Domain → Phylum → Class → Order → Family → Genus → Species → Strain. Microbiome studies typically report at the phylum, genus, or species level depending on the resolution of the method used.

Inter-individual Variation and Core Microbiome

Despite a shared set of dominant phyla, the specific species-level composition of the gut microbiome varies enormously between individuals almost certainly more than any other organ system. No single species is universally present across all healthy individuals. This has led to the concept of a *functional core microbiome*: a conserved set of metabolic functions (*e.g.*, SCFA production, bile acid transformation) maintained by different species in different people [Human Microbiome Project Consortium, 2012]. The implication is that functional capacity, rather than species identity, may be the more relevant measure of microbiome "health."[81]

[81] **Quantifying diversity the Shannon index.** Microbial diversity is commonly summarized by the **Shannon diversity index** $H' = -\sum_i p_i \ln p_i$, where p_i is the relative abundance of species i. H' captures both *richness* (number of species present) and *evenness* (how equally abundant they are). A community dominated by one species has a low H'; a community with many equally abundant species has a high H'. Higher diversity is generally associated with a more resilient, functionally redundant microbiome, and reduced diversity is a consistent feature of dysbiotic states such as *Clostridioides difficile* infection.

How Is the Microbiome Measured?

Studying the microbiome requires methods that can characterize microbial communities without necessarily growing each organism in the laboratory. Historically, microbiology relied on culture systems such as growing bacteria on selective media. However it is estimated that fewer than 30% of gut bacterial species can be readily cultured under standard laboratory conditions, because most are obligate anaerobes with complex nutritional requirements. The development of molecular methods has transformed our understanding of microbial diversity.

16S rRNA Gene Sequencing

The 16S ribosomal RNA (rRNA) gene is present in all bacteria and archaea. It contains both highly conserved regions (used for primer design) and hypervariable regions (V1–V9) that differ between taxa and serve as a "barcode" to identify organisms [Woese and Fox, 1977]. The standard workflow is:

1. Extract DNA from a fecal or mucosal sample
2. Amplify the 16S gene (or a hypervariable region thereof) by PCR using universal primers
3. Sequence the amplicons using high-throughput sequencing (e.g., Illumina)
4. Assign sequences to **operational taxonomic units** (OTUs) or amplicon sequence variants (ASVs) by comparison to reference databases

Strengths: inexpensive, high-throughput, well-established pipelines, large reference databases. **Weaknesses:** PCR amplification introduces biases; typically only resolves to genus level; provides no direct information about metabolic function; does not distinguish live from dead cells.

Shotgun Metagenomics

Shotgun metagenomics (also called whole-genome shotgun, WGS) sequences *all* DNA in a sample (both host and microbial) without prior amplification. Reads are assembled or mapped against reference databases to identify organisms and, crucially, to catalog the functional gene content of the community.

Strengths: species- and even strain-level resolution; reveals metabolic potential; no PCR bias; detects viruses and fungi. **Weaknesses:** expensive; computationally intensive; host DNA contamination requires depletion steps; sequencing of DNA does not confirm gene expression.

Metatranscriptomics, Metaproteomics, and Metabolomics

To move from what organisms are *present* to what they are *doing*, complementary approaches are used:

- **Metatranscriptomics**: sequences community RNA (after rRNA depletion) to reveal actively transcribed genes
- **Metaproteomics**: identifies proteins expressed by the community via mass spectrometry

- **Metabolomics**: profiles small-molecule metabolites (e.g., SCFAs, bile acids, tryptophan metabolites) in stool, urine, or plasma, reflecting the net metabolic output of the microbiome

Each layer adds functional resolution but also cost and analytical complexity. Most large epidemiological studies use 16S sequencing; mechanistic studies increasingly combine multiple "-omics" layers.

Table 16: Comparison of microbiome measurement approaches.

Method	Taxa ID	Functional info	Cost	Key limitation
Culture	Low	Limited	Low	Most gut bacteria unculturable
16S rRNA	Genus	No	Low	PCR bias; no functional data
Metagenomics	Species/strain	Gene content	High	DNA only; no expression data
Metatranscriptomics	Species	Active genes	High	RNA unstable; complex analysis
Metabolomics	No	Yes	Medium	Does not identify organisms

Stability and Malleability of the Microbiome

THE GUT MICROBIOME IS SIMULTANEOUSLY STABLE AND SURPRISINGLY PLASTIC. In healthy adults the broad community structure (*i.e* dominant phyla, core functional genes) is relatively consistent from month to month [Costello et al., 2009]. Yet the microbiome is also continuously shaped by genetics, the events of early life, diet, medications, and environmental exposures. Understanding which features are fixed and which are malleable is central to evaluating interventions.

Establishment in Early Life

The gut of a healthy fetus is largely sterile. Colonization begins at birth and the mode of delivery has a marked effect on the founding community. Vaginally born infants acquire microbiota resembling the maternal vaginal microbiome (dominated by *Lactobacillus*), while infants delivered by cesarean section are instead colonized by skin- and hospital-associated bacteria such as *Staphylococcus* and *Clostridium* [Dominguez-Bello et al., 2010]. Breastfeeding further shapes the infant microbiome: human milk oligosaccharides (indigestible by the infant but selectively fermented by *Bifidobacterium*) drive enrichment of bifidobacteria in breastfed compared with formula-fed infants.

C-section delivery has been associated with altered immune development and modestly elevated rates of asthma, allergic disease, and obesity in some cohort studies. However these relationships are confounded by the indications for cesarean delivery.

Over the first two to three years of life, as solid food is introduced, the microbiome increases in diversity and transitions toward an adult-like composition [Bäckhed et al., 2015]. This period of assembly is considered a critical window: perturbations such as antibiotic

use during infancy may have disproportionate and lasting effects compared with equivalent exposures in adulthood.

Genetic Influences

Twin studies have revealed that host genetics contributes to microbiome composition, but that the effect is modest relative to environmental factors. A large study of over 1,000 twin pairs found that roughly one-third of the variation in abundance of certain taxa was heritable, while the majority of variation was explained by shared and non-shared environmental factors [Goodrich et al., 2014]. Importantly, the heritable microbial taxa tended to be those associated with leanness, suggesting a pathway by which genetic predisposition to metabolic phenotypes could be partly mediated through the microbiome. Genes involved in immunity, mucin production, and bile acid metabolism are among the host genetic factors thought to shape the microbial niche.

Antibiotics and Perturbation

Antibiotics are the most potent acute disruptors of the gut microbiome. Broad-spectrum antibiotics can deplete a large fraction of gut bacteria within days, with a characteristic reduction in diversity and expansion of resistant taxa.[82] Recovery occurs over weeks to months but may be incomplete, with some species failing to recolonize at their pre-treatment abundances [Dethlefsen and Relman, 2011]. Repeated antibiotic courses during childhood have been associated with lasting shifts in microbiome composition and with increased risk of inflammatory and metabolic disease in some epidemiological studies, though establishing causality is difficult.

[82] Antibiotic-associated diarrhea, affecting 5–35% of patients depending on antibiotic class, reflects this disruption. In severe cases, depletion of the normal flora allows *Clostridioides difficile* to proliferate and produce toxins, causing potentially life-threatening colitis.

Modifying the Microbiome: Probiotics and Prebiotics

Probiotics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [Hill et al., 2014]. The most commonly used probiotic genera are *Lactobacillus* and *Bifidobacterium*, though *Saccharomyces boulardii* (a yeast) and certain strains of *Enterococcus* and *Bacillus* are also used. Probiotic products are measured in colony-forming units (CFUs); effective doses in clinical trials typically range from 10^8 to 10^{11} CFU per day.

The evidence base for probiotics is strain- and condition-specific. Strong evidence supports the use of specific strains for

prevention of antibiotic-associated diarrhea and for reducing the duration and severity of acute infectious diarrhea, particularly in children [Hill et al., 2014]. Evidence for other claimed benefits (including irritable bowel syndrome, inflammatory bowel disease, and immune modulation) is more mixed, and many trials are limited by small sample sizes, inconsistent strain selection, and short follow-up. A critical limitation is that most orally ingested probiotic bacteria do not permanently colonize the gut; they transit through and their benefits may depend on transient metabolic activity rather than stable engraftment.

Common food sources of probiotics include yogurt, kefir, kimchi, sauerkraut, miso, and tempeh. Probiotic supplements are regulated as food products rather than drugs in the United States, so strain identity, viability, and dose are not guaranteed on the label.

Heating or pasteurization kills live cultures, so the probiotic content of fermented foods varies widely by product and processing method.

Prebiotics

Prebiotics are substrates selectively utilized by host microorganisms to confer a health benefit [Gibson et al., 2017]. In practice, most established prebiotics are non-digestible carbohydrates and fibers [83] which were covered in the Fiber lecture. The key distinction from general dietary fiber is selectivity: a prebiotic must demonstrably enrich beneficial taxa (*e.g.*, *Bifidobacterium*, *Lactobacillus*) rather than simply being fermented by any available organism.

[83] Such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and resistant starch

Prebiotic supplementation reliably increases the abundance of *Bifidobacterium* and raises fecal SCFA concentrations, but whether these changes translate into clinically meaningful outcomes depends heavily on the baseline microbiome composition of the individual, a concept sometimes called **microbiome responsiveness**.[84]. This highlights the need to match prebiotics and probiotics (along with immune tolerance) to allow for long-term stable colonization.

[84] Individuals with low baseline *Bifidobacterium* abundance tend to show larger bifidogenic responses to prebiotic supplementation than those already highly colonized, suggesting a ceiling effect.

Microbial Metabolites and Their Biological Activities

Gut bacteria do not simply consume dietary substrates and disappear. They produce a rich array of small molecules that enter host circulation and exert systemic effects far beyond the colon. The most nutritionally important of these are the short-chain fatty acids, but bile acid transformation products and other microbial metabolites also have significant physiological consequences.

Short-Chain Fatty Acids

When anaerobic bacteria in the colon ferment non-digestible polysaccharides cellulose, hemicellulose, pectin, inulin, resistant starch, and other fermentable oligosaccharides the principal end-products are three short-chain fatty acids: acetate (C_2), propionate (C_3), and butyrate (C_4), produced in an approximate molar ratio of 60:20:20 [Cummings, 1981]. Total luminal SCFA concentrations reach 70–140 mmol/L in the proximal colon, making them quantitatively significant metabolic substrates.

Each SCFA has a distinct metabolic fate:

- **Butyrate** is the preferred energy substrate of colonocytes, supplying roughly 70% of their energy needs via β-oxidation. Very little butyrate escapes the colonic epithelium into portal circulation. Beyond energetics, butyrate is a potent inhibitor of histone deacetylases (HDACs), giving it gene-regulatory and anti-inflammatory properties. Butyrate induces differentiation of colonic regulatory T cells and suppresses pro-inflammatory cytokine production.[85]

- **Propionate** is absorbed and transported to the liver, where it serves as a gluconeogenic precursor. It also acts on free fatty acid receptors (FFAR2/FFAR3) in enteroendocrine cells to stimulate release of PYY and GLP-1, gut hormones that promote satiety and regulate glucose homeostasis.

- **Acetate** reaches the peripheral circulation and is taken up by muscle, heart, and brain. It is the most abundant SCFA in blood and serves as a carbon source for lipogenesis and cholesterol synthesis in peripheral tissues.

[85] The anti-neoplastic properties of butyrate (*e.g.* cell cycle arrest, pro-apoptotic gene expression, reduced proliferation in colorectal cancer cell lines) have generated interest in whether a fiber-rich diet might protect against colorectal cancer partly through butyrate-mediated epigenetic effects. This is an active research area.

Bile Acid Transformation

Primary bile acids (cholic acid, chenodeoxycholic acid) synthesized in the liver are secreted into the small intestine, where they facilitate fat absorption. The fraction that escapes enterohepatic reabsorption reaches the colon, where gut bacteria enzymatically dehydroxylate them to produce *secondary bile acids* (deoxycholic acid, lithocholic acid). Secondary bile acids re-enter circulation and act as signaling molecules at nuclear receptors (FXR) and G-protein coupled receptors (TGR5) that regulate bile synthesis, glucose metabolism, and energy expenditure. Altered microbial bile acid metabolism is implicated in colorectal cancer risk and metabolic disease.

Other Microbial Metabolites

Trimethylamine N-oxide (TMAO) illustrates how microbial metabolism can produce harmful products. Dietary choline and carnitine (abundant in red meat and eggs) are converted by gut bacteria to trimethylamine (TMA), which is absorbed and oxidized in the liver to TMAO. Elevated plasma TMAO has been associated with increased cardiovascular disease risk in observational studies, and germ-free mice fed choline do not produce TMAO demonstrating that the microbiome is required for this pathway.. There are many other microbial metabolites with emerging relevance to human health, including tyrosine and tryptophan metabolites that may also modulate immune responses and have other physiological effects.

The TMAO story is an important example of a microbiome-mediated diet–disease relationship, but causality in humans remains debated: TMAO may be a marker of the dietary pattern rather than a direct mediator of cardiovascular risk.

The Microbiome and the Immune System

The gut is the largest immune organ in the body, containing approximately 70% of the body's immune cells. The intestinal immune system faces a continuous challenge: it must detect and respond to the trillions of commensal bacteria (and dietary antigens) while remaining capable of mounting a response against genuine pathogens. As such, the microbiome is not just a target of immune surveillance, this recognition actively shapes the development and calibration of the immune system.

Immune Surveillance and Pattern Recognition

Intestinal epithelial cells and resident immune cells express **pattern recognition receptors** (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors, that detect conserved microbial structures such as lipopolysaccharide (LPS), peptidoglycan, and flagellin. In a healthy gut, the mucus layer and epithelial barrier physically separate luminal bacteria from PRR-bearing immune cells, maintaining a state of *immune tolerance* rather than chronic activation. Disruption of this separation (as occurs in barrier dysfunction) allows microbial ligands to contact immune cells and trigger inflammatory responses.

Dendritic cells in the lamina propria can extend processes through tight junctions to sample luminal antigens directly, and specialized epithelial M cells in Peyer's patches transport antigens to underlying lymphoid tissue. These mechanisms allow the immune system to "see" commensals without activating a destructive response.

Immune Entrainment: How the Microbiome Educates Immunity

Studies in germ-free animals demonstrate starkly that a microbiome is required for normal immune development: germ-free mice have underdeveloped Peyer's patches, reduced IgA production, fewer intestinal regulatory T cells (Tregs), and dysregulated systemic immune responses [Hooper et al., 2012]. Colonization of germ-free mice with a normal microbiome corrects most of these defects, but the timing matters. Some immune defects induced by germ-free conditions early in life are not fully reversible by later colonization [Olszak et al., 2012].

The Hygiene Hypothesis and Allergic Disease

The hygiene hypothesis proposes that reduced microbial exposure in early childhood due to smaller family sizes, antibiotic use, formula feeding, urban environments, and reduced contact with soil and animals leads to inadequate immune education and a consequent increase in allergic and autoimmune diseases [Strachan, 1989].[86] Epidemiological evidence supporting this hypothesis includes:

- Higher rates of asthma, eczema, and food allergy in industrialized compared to rural populations
- Protective effects of farm exposure, pet ownership, and older sibling order on atopic disease
- Lower rates of allergic disease in children with greater early-life microbiome diversity

[86] The term *hygiene hypothesis* is increasingly considered a misnomer the relevant factor is not personal cleanliness but microbial *diversity* of exposure. The *old friends* hypothesis frames it more precisely: humans co-evolved with specific microorganisms, and their absence disrupts immune regulation.

Food allergies and intolerances may also have microbial roots. Children who develop cow's milk or peanut allergy have measurably different gut microbiome compositions in the first year of life compared to tolerant children, with lower abundance of *Clostridia* and *Bacteroidetes* [Cahenzli et al., 2013]. Mouse models show that germ-free animals are hyper-susceptible to food allergy, and that colonization with *Clostridia* restores tolerance and reinforces barrier function, suggesting a mechanistic link between microbiome composition, barrier integrity, and allergic sensitization.

Allergy vs intolerance: Food *allergy* is IgE-mediated and involves the adaptive immune system; food *intolerance* (*e.g.*, lactose intolerance, non-celiac gluten sensitivity) is typically non-immunological. The microbiome is most clearly implicated in allergy development, though dysbiosis can also affect fermentation-based intolerances.

Gut Barrier Function

The intestinal epithelium is a single-cell-thick barrier separating a lumen containing trillions of bacteria from a sterile internal environment. Maintaining this barrier is essential: its failure

is implicated in inflammatory bowel disease, colorectal cancer, and systemic inflammatory conditions. The barrier has several interdependent layers.

The Mucus Layer

The innermost physical defense is a two-layer mucus gel secreted by goblet cells. The principal structural component is the glycoprotein MUC2 mucin, a heavily O-glycosylated polymer that forms a cross-linked gel. In the colon, the inner mucus layer is dense and largely sterile; the outer layer is looser and constitutes the habitat of many luminal bacteria, which use mucin glycans as a carbon source [Johansson et al., 2008]. *Akkermansia muciniphila* is a specialist mucin-degrading species whose abundance is associated with metabolic health; it illustrates how the host mucus layer itself shapes microbial community composition.

Inflammatory conditions and dietary fiber deficiency both reduce mucus layer thickness, allowing bacteria to contact the epithelial surface. Interestingly, SCFAs (particularly butyrate) stimulate goblet cell differentiation and MUC2 expression, creating a feedback loop in which a fiber-rich diet supports mucus barrier integrity.

Tight Junctions and Epithelial Integrity

Adjacent epithelial cells are sealed by tight junction protein complexes (claudins, occludin, ZO-1) that restrict paracellular passage of luminal contents. Inflammatory cytokines such as TNF-α and IFN-γ downregulate tight junction proteins and increase paracellular permeability a state colloquially termed **leaky gut**. Once permeability increases, bacterial products such as LPS enter the lamina propria, activating TLR4 and driving further inflammation in a self-amplifying cycle.[87]

Butyrate supports tight junction integrity directly: it increases expression of claudin-1 and occludin and reduces epithelial apoptosis, providing another mechanistic link between dietary fiber, SCFA production, and barrier health.

[87] Circulating LPS or its binding protein (LBP) are sometimes used as indirect biomarkers of intestinal permeability in clinical research, though they are imperfect measures.

Antimicrobial Peptides and Paneth Cells

Paneth cells, located at the base of small intestinal crypts, secrete **antimicrobial peptides** (AMPs) including α-defensins and lysozyme. These peptides create an antimicrobial gradient in the crypt, protecting intestinal stem cells from bacterial invasion and helping to modulate the lumenal microbiota. Colonic goblet cells and surface enterocytes produce additional AMPs. Dysregulation of Paneth cell

function is a feature of Crohn's disease, particularly ileal disease, and may contribute to loss of colonization resistance against pathobionts.

Barrier Dysfunction in IBD and Colorectal Cancer

Inflammatory bowel disease (IBD) encompassing Crohn's disease and ulcerative colitis is characterized by chronic, relapsing intestinal inflammation. Both forms show consistent evidence of dysbiosis: reduced microbial diversity, depletion of butyrate-producing *Faecalibacterium prausnitzii* (a key anti-inflammatory commensal), and expansion of adherent-invasive *E. coli* strains. Whether dysbiosis is a cause or consequence of inflammation is difficult to determine from cross-sectional studies, but prospective studies in individuals prior to IBD diagnosis show microbiome alterations preceding clinical disease onset, supporting a contributory rather than purely reactive role.

Colorectal cancer risk is consistently reduced in observational studies among populations consuming high-fiber diets. Mechanistic hypotheses include:

- Butyrate-mediated induction of apoptosis and cell cycle arrest in neoplastic colonocytes, with butyrate derived from fiber fermentation
- Dilution and accelerated transit of potential carcinogens in bulky fecal matter
- Reduced secondary bile acid production (*i.e.* less substrate reaching the colon)
- Anti-inflammatory effects of a diverse, fiber-fed microbiome

Fusobacterium nucleatum, an oral bacterium, has been found enriched in colorectal tumors compared with adjacent normal tissue and in stool of CRC patients [Kostic et al., 2012]. Its presence in resected tumors is associated with worse prognosis and with microsatellite-stable molecular subtypes. Whether *F. nucleatum* is oncogenic or simply opportunistically colonizes tumor tissue remains under investigation.

Reflection Questions

1. A cohort study reports that children born by cesarean section have significantly higher rates of asthma and food allergy at age 5 compared with vaginally born children. A pediatrician concludes that C-section delivery *causes* allergic disease and recommends all C-section newborns receive probiotic supplementation. Critically

evaluate this conclusion. What confounders might explain the association? Using your knowledge of immune entrainment and the hygiene hypothesis, propose a mechanistic pathway by which delivery mode could plausibly influence allergy risk. What type of study would be needed to establish causality?

2. A researcher wants to test whether a high-fiber diet increases the abundance of butyrate-producing bacteria in patients with ulcerative colitis. She is deciding between 16S rRNA sequencing and shotgun metagenomics to characterize the microbiome. Compare the two methods for this specific research question: which would you recommend, and why? What additional measurement would you add to directly assess whether butyrate production actually increased, and what biological matrix would you use?

3. A patient with Crohn's disease has been on broad-spectrum antibiotics for three weeks following a surgical complication. His gastroenterologist now recommends a high-fiber prebiotic supplement to help restore his microbiome. Using your knowledge of the gut microbial niche and fermentation, explain why fiber would be expected to selectively promote the recovery of certain bacterial taxa over others. Given the patient's compromised gut barrier, identify one potential risk of rapidly reintroducing high-fiber fermentable substrates and explain the mechanism.

4. Red meat consumption is associated with elevated plasma TMAO in observational studies, and high TMAO is associated with cardiovascular disease risk. A nutrition advocacy group concludes that dietary choline from red meat raises TMAO and therefore red meat causes heart disease. Identify at least two weaknesses in this causal chain. Design a study that would more rigorously test whether microbiome-derived TMAO mediates the relationship between red meat intake and cardiovascular risk, and describe what result would most strongly support the microbial mediation hypothesis.

5. *Fusobacterium nucleatum* is enriched in colorectal tumor tissue compared with adjacent normal mucosa. A journalist writes that "gut bacteria cause colon cancer." Using what you know about barrier function, the tumor microenvironment, and the distinction between observational and mechanistic evidence, evaluate this claim. What evidence would be needed to conclude that *F. nucleatum* is oncogenic rather than opportunistic? How does butyrate production by a fiber-fed microbiome fit into a more complete model of colorectal cancer risk?

6. A healthy 25-year-old reports that she has avoided all fermented foods and dietary fiber for the past year because she finds them bloating. She asks whether this matters for her health. Using your knowledge of SCFA production, mucus layer maintenance, and immune calibration, construct a mechanistic argument for why chronic low fiber intake might have consequences beyond digestive discomfort. In your answer, identify which properties of dietary polysaccharides make them particularly suitable as microbial substrates, and predict which bacterial taxa would be most depleted by her diet.

7. Twin studies show that roughly one-third of the variation in abundance of certain gut bacterial taxa is heritable, while the majority of variation is environmental. A colleague argues this means the microbiome is "mostly genetic" and dietary interventions will have limited effect. Evaluate this interpretation. How would you use the concepts of microbiome stability, life-course plasticity, and prebiotic responsiveness to argue for or against the potential of dietary intervention to meaningfully shift microbiome composition in adults?

Glucose Transport and Glycolysis

This lecture will cover glycolysis, the backbone of metabolism. This pathway is especially important because it is the root of several pathways of carbohydrate metabolism (gluconeogenesis, glycogenesis, glycogenolysis and the TCA cycle) as well as both the source and result of amino acid synthesis and degradation. An understanding of how glucose flows through glycolysis is also important for understanding how other monosaccharides such as fructose and galactose are metabolized. We will cover the regulation of glycolysis by energy status, key metabolites and hormones. While most of the reactions are listed here, focus on the key steps of control and how they are regulated.

Learning Objectives

- Define the role and relative locations of of glycolysis, gluconeogenesis, the TCA cycle as nodes of carbohydrate metabolism.
- Assess the enzymatic differences and tissue distributions of glucokinase vs hexokinase and explain why this is important.
- Calculate how much ATP is produced from glycolysis, and the relative efficiency of aerobic vs non-aerobic glycolysis.
- Summarize the key points of regulation of glycolysis and what metabolites and hormones regulate these enzymes.
- Evaluate the differences between how muscle and liver glycolysis are regulated. Assess why this is relevant for the functions of these tissues.
- Describe the potential fates of pyruvate, and what enzyme activities dictate the next steps in its metabolism. Given a particular cellular state, you should be able to predict which pathway pyruvate enters.
- Discriminate how non-glucose carbohydrates such as galactose and fructose enter glycolysis, and how their point of entry affects how they are regulated.
- Predict the effects of specific inborn errors in glucose, galactose and fructose metabolism based on the location of the affected enzyme in the relevant pathways. Consider dietary treatments that may be useful in these individuals.

Key Concepts, Abbreviations and Vocabulary

Concepts: Glucose transport, glucose oxidation, negative feedback, feed-forward regulation, inborn errors of metabolism.

Key Enzymes and Proteins: AMPK, PKA, GLUT2, GLUT4, GLUT5, Hexokinase (HK), Glucokinase (GK), Glucose-6-Phosphatase (G6Pase), Phosphofructokinase 1 and 2 (PFK1/2), Pyruvate Kinase (PK), Alanine Aminotransferase (ALT), Pyruvate Dehydrogenase (PDH[88]). You should be able to locate the relative location of these enzymes in glycolysis, and how they are regulated by metabolites and hormones.

[88] covered in more detail next lecture.

Important Metabolites: Glucose-6-Phosphate (G6P), Fructose-1,6-bisphosphate (F16bP), Fructose-2,6-bisphosphate (F2,6bP), ATP, AMP, Alanine.

Glycolysis converts glucose to pyruvate

Glycolysis is the process by which glucose is catabolized to pyruvate. The conversion of glucose to pyruvate can be the first step to full oxidation of glucose to carbon dioxide, or it can end with pyruvate being released as lactate. This is the backbone of metabolism, as most carboydrate, amino acid and lipid metabolic pathways involve glycolysis in one way or another. For a refresher on glycolysis, we recommend the textbooks on reserve at the Shapiro library [Berg et al., 2013, Ferrier, 2017]. We start with glucose, because under most conditions, it is the preferred fuel for most tissues[89]. There are three stages to glycolysis, an energy consuming "charging" steps, a "splitting step", and "energy producing steps" resulting in two molecules of pyruvate for each molecule of glucose. Overall glycolysis to the point of pyruvate follows this stoichiometry:

[89] There are several examples of tissues that prefer other fuels, for example cardiomyocytes tend to use fatty acids and colonocytes tend to use the short-chain fatty acid butyrate.

$$Glucose + 2NAD^+ + 2ADP + 2Pi \rightarrow 2Pyruvate + 2NADH + 2ATP + 2H^+ + 2H_2O \tag{9}$$

ATP can be used directly for energy, while NADH is used for energy (generating 2.5 ATP/NADH in aerobic glycolysis) or for converting pyruvate to lactate (for anaerobic glycolysis).

How does glucose enter cells?

Glucose is impermeable to the plasma membrane of cells. Therefore, in order to enter the cell, specific transporters are required. In the case of glucose, two transporters are the most relevant, particularly GLUT2 and GLUT4[90]. These are passive transporters that only allow glucose to follow its concentration gradient. In the liver, GLUT2 is typically expressed and present at the membrane of the hepatocyte. This allows glucose to enter the hepatocyte (for example after a meal) or to exit the cell (for example during glycogenolysis or gluconeogenesis[91]).

[90] GLUT1 is important for red blood cells (erythrocytes) and the blood–brain barrier, wheras GLUT3 is important for glucose flow into neurons

[91] The production of glucose from precursors such as alanine, lactate or glycerol

GLUT4 on the other hand is the main transporter in muscle and adipocyte cells. GLUT4 is normally present on intracellular vesicles within the cell and is therefore unable to conduct glucose into the cell. When insulin is present, these vesicles fuse with the plasma membrane of these cells, placing GLUT4 transporters on the plasma membrane, and allowing for glucose to enter down its concentration gradient (see Figure 22). GLUT4 trafficking is the first regulated step for glucose oxidation and storage in muscle and fat cells. For more details on how GLUT4 trafficking is regulated see Leto et al. [2013]. GLUT4 translocation in muscle cells is also stimulated by exercise. This is dependent on a protein kinase called AMPK, which is

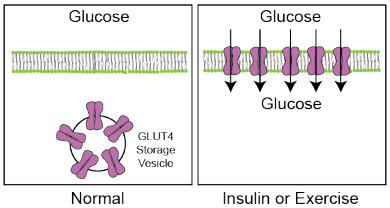

Figure 22: Regulation of glucose uptake in muscle and adipocytes. In these cells, glucose cannot enter unless insulin or AMPK stimulates the translocation of GLUT4 from intracellular GLUT4 storage vesicles to the plasma membrane.

activated when AMP levels are high (or energy is low). Improving glucose disposal rates, by allowing glucose into muscle is one major advantage of exercise in type 2 diabetics, who are resistant to the effects of insulin. For more details on exercise-induced glucose uptake see Richter and Hargreaves [2013].

GLYCOLYSIS OCCURS IN THE CYTOPLASM OF CELLS. All of glycolysis occurs in the cytoplasm of cells, unlike the TCA cycle or the electron transport chain, which require mitochondria. As we will discuss later in the semester, mitochondria are also required for the oxidation of fatty acids and some amino acids. This means that cells with few or no mitochondria (fast-twitch muscle fibers, or red blood cells for example) are highly dependent on glycolysis to generate ATP.@

The conversion of glucose to glucose-6-phosphate

THERE ARE TWO ENZYMES THAT CATALYSE THE FIRST STEP AFTER GLUCOSE ENTERS THE CELL. This step phosphorylates glucose at the 6 position, generating glucose-6-phosphate (G6P). This is the first of two ATP consuming steps in glycolysis:

$$Glucose + ATP \rightarrow G6P \tag{10}$$

In liver cells and pancreas cells, this is done by an enzyme called *glucokinase*. This is a co-operative enzyme[92] that has a very high maximal catalytic rate (V_{max}). This means that at low levels of glucose, very little glucose is phosphorylated but at high levels of glucose, G6P can be produced at high rates. This is especially useful in the liver to prevent glucose oxidation in the liver when glucose levels are low indicating that glucose is needed for peripheral tissue use more urgently. Another way to think about this, is that the co-operativity of glucokinase means that its activity is dependent on glucose levels in the cell. This means that glucokinase can serve as an intracellular glucose sensor.

[92] If you forgot what this means, review the metabolic control systems handout.

HEXOKINASE IS A HIGH AFFINITY ENZYME that catalyzes the same reaction in most other cells, for example muscle and adipose cells. This means that it is very efficient even at low glucose concentrations, but does not have as high of a maximum rate[93]. This is illustrated in Figure 23. This is especially useful in the muscles to ensure they oxidize glucose even at low glucose levels as the muscles always utilize energy. Hexokinase is regulated by negative feedback from its product G6P. Hence, an increase in G6P will downregulate the function of Hexokinase. In contrast to hexokinase, there is no allosteric

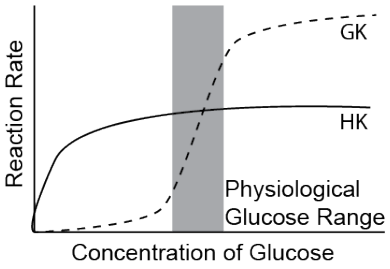

Figure 23: Schematic of the kinetics of glucokinase (GK) and hexokinase (HK). Not the differences in K_m, V_{max} and co-operativity between these enzymes.

[93] It is important that you understand the differences in glucose affinity and V_{max} between glucokinase and hexokinase and the consequences of these differences.

regulation of glucokinase. Think about the differences in how glucose enters these cells, in contrast to liver cells and how the kinetics relate to the regulation of glucose uptake. The differences between glucokinase and hexokinase are summarized in Table II.

Enzyme	Kinetics	Regulation	Tissues
Hexokinase	High Affinity	G6P (-)	Muscle/Adipose
Glucokinase	Co-operative		Pancreas/Liver

Table 17: Differences between glucokinase and hexokinase.

THE REVERSE REACTION OF GK IS THE DEPHOSPHORYLATION OF G6P TO GLUCOSE, AND PRIMARILY OCCURS IN THE LIVER. This is because most cells do not express Glucose-6-phosphatase (G6Pase), the enzyme that converts G6P back to glucose. In the liver, G6Pase allows for dephosphorylated glucose to be released back into the blood, the last step in gluconeogenesis or hepatic glycogenolysis.[94] This is relevant because in non-hepatic cells, the phosphorylation of glucose is *irreversible* and traps glucose within the cell[95].

[94] Which will be discussed three lectures from now.

[95] Think about why the co-operative properties of glucokinase work in tandem with the ability to dephosphorylate glucose in liver cells.

What are the fates of glucose-6-phosphate?

Phosphorylated glucose (G6P) can enter four separate pathways (3 in non-hepatic tissues), depending on the relative activities of the rate limiting steps in these pathways. If glycogen synthase (GS) activity is elevated, glucose can become stored as glycogen. If G6P Dehydrogenase (G6PDH) activity is elevated, glucose will flow through the pentose phosphate shunt (PPS). Glycolysis will proceed if phosphofructokinase-1 (PFK1) is active. These routes are summarized in Figure 24.

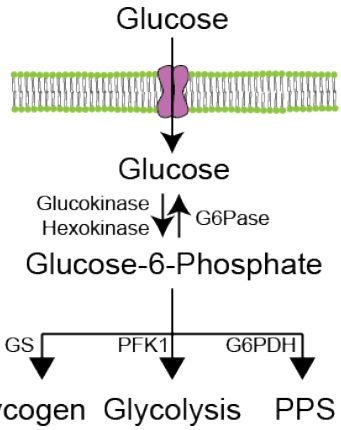

Figure 24: Fates of phosphorylated glucose and key rate limiting enzymes of each pathway. Details of each pathway will be discussed in forthcoming lectures.

The first committed step of glycolysis is catalysed by PFK1

The most important regulatory step that controls flow through glycolysis is catalyzed by PFK1[96]. This is the second ATP consuming step, and the first committed step of glycolysis. [97]:

$$G6P \rightleftharpoons F6P \tag{11}$$

$$F6P + ATP \rightarrow F16bP \tag{12}$$

Because this is such an important regulatory node, there are several facets to the regulation of PFK1. This is accomplished via four allosteric regulators, listed in Table 18. Citrate is a part of the TCA cycle which we will discuss next lecture, and is also an indicator of sufficient fatty acids and acetyl-CoA (as we will discuss in the lipids unit). Elevations in citrate indicate that there are sufficient molecules

[96] For all these reactions G indicates Glucose, F indicates Fructose, GA indicates Glyceraldehyde and PG indicates Phosphoglycerate.

[97] Reaction 11, catalyzed by Phosphoglucose isomerase is a reversible, equilibrium reaction

Table 18: Regulators of PFK1 activity

Molecule	Direction
F2,6bP	Positive
AMP	Positive
ATP	Negative
Citrate	Negative

in the TCA cycle, a condition known as *anaplerosis*[98]. Functionally, this means that when there are sufficient metabolites downstream of glycolysis, glycolysis is impaired at the PFK1 step. This is known as negative feedback and is common in many of the pathways we will discuss this semester.

[98] Anaplerosis is a condition where the TCA cycle intermediates build up due to increased rate of metabolic reactions that feed into the TCA cycle.

ATP AND AMP LEVELS INDICATE THE CELLULAR ENERGY STATUS. In terms of PFK1 regulation, this means that when energy is low (low ATP/high AMP), PFK1 is activated and glycolysis (which is energy generating) proceeds. On the other hand, when ATP levels are high and AMP levels are low (*e.g.* in a liver cell after a meal), PFK1 can be inactivated and G6P will instead be stored as glycogen or enter the PPS. This is one way in which energy can control glycolytic flux.

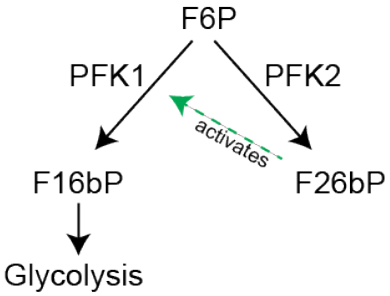

Figure 25: Regulation of PFK1 by F2,6bP and PFK2.

FRUCTOSE-2,6-BISPHOSPHATE IS THE MOST POTENT REGULATOR OF PFK1 ACTIVITY. This molecule is generated from the same Fructose-6-phosphate (F6P) precursor that PFK1 acts on, but instead phosphorylates F6P on the 2-position. This reaction is catalyzed by an enzyme known as PFK2. This mechanism is known as feed-forward regulation, and means that when F6P builds up, it can be converted to F2,6bP, this in turn activates PFK1 to relieve the buildup of F6P in the cytoplasm[99]. The relationship between PFK1 and PFK2 is illustrated in Figure 25.

[99] An analogy for this might be if you are stuck in traffic and honk (to signal the traffic ahead to move faster).

PFK2 IS REGULATED BY REVERSIBLE PHOSPHORYLATION IN THE LIVER. PFK2 activity is *inhibited* by PKA-dependent phosphorylation [Van Schaftingen et al., 1981]. PKA in the liver is activated by hormones such as glucagon and epinephrine. One biological goal of these hormones in the liver is to *promote gluconeogensis*, and therefore it would be counterproductive to have glycolysis occuring at the same time. As such, by reducing PFK2 activity (and reducing F2,6bP levels), PFK1 activity and glycolytic flux is all reduced.[100] As we will discuss a little later on, PKA-mediated inhibition of PFK2 does not occur in muscle cells.

[100] I appreciate that this is a lot of regulation, so I recommend sketching out PFK1/PFK2 and the various positive and negative regulators on your own. Take a step back and think about what would cause these regulators to change, and how this would affect glycolytic flux. Think about whether this would make"sense" based on what glycolysis is doing.

THE NEXT SEVERAL STEPS OF GLYCOLYSIS ARE NEITHER REGULATED NOR RATE LIMITING. In general, the F16bP molecule is broken in two by aldolase, then each part is rapidly converted into phosphoenolpyruvate via the following reactions:

$$F16bP \rightleftharpoons DHAP + GA3P \tag{13}$$

$$GA3P + NAD+ \rightleftharpoons 1,3bPG + NADH \tag{14}$$

$$1,3bPG + ADP \rightleftharpoons 3PG + ATP \tag{15}$$

$$3PG \rightleftharpoons 2PG \tag{16}$$

$$2PG \rightleftharpoons Phosphoenolpyruvate \tag{17}$$

Note that in reactions 14 and 15, there is generation of NADH and ATP respectively[101]. ATP is the primary fuel source in cells, and one molecule of NADH can be converted into 2.5 molecules of ATP via the electron transport chain[102].

The second point, which we will come back to as it relates to lipid synthesis, is that the glycerol backbone, needed to generate triglycerides, can be derived from DHAP[103]. This is important in the context of esterifying fatty acids into triglycerides, as three fatty acids require one activated glycerol backbone. On the other hand, when glycerol is broken down, it becomes DHAP and enters the glycolytic pathway where it can be processed to pyruvate or converted to glucose. Glycerol is a major gluconeogenic substrate, and again enters the glycolytic pathway as DHAP and then is converted back to glucose via mechanisms we will discuss in later lectures.

Pyruvate kinase regulates conversion to pyruvate

$$PEP + ADP \rightarrow Pyruvate + ATP \tag{18}$$

The last step of glycolysis catalyzes the *irreversible* reaction of phosphoenolpyruvate (PEP) to pyruvate, and is catalysed by Pyruvate Kinase. This is the last point of regulation in glycolysis. Fructose-1,6-bisphosphate (F1,6bP) is the product of PFK1, and functions as a *feed-forward* regulator of pyruvate kinase activity (see Figure 26). ATP, similar to its inhibitory role on PFK1, reduces glycolytic flux when energy is not needed and thus inhibits Pyruvate Kinase function. Alanine, on the other hand, is an amino acid that is easily interconverted with Pyruvate by the enzyme Alanine Aminotransferase (ALT)[104]. As a marker for amino acid availability, Alanine reduces liver Pyruvate Kinase activity when there is less of a need to use glucose as fuel[105]. This is because the cell can use Alanine, rather than Phosphoenolpyruvate to generate Pyruvate.

Similar to PFK2, Pyruvate kinase is inhibited by PKA-dependent protein phosphorylation. In the liver, glucagon or adrenaline can inhibit glycolysis at two steps, PFK2 (described above) and also at the Pyruvate Kinase step. In both cases, this is important to prevent glycolysis and gluconeogenesis from occurring

[101] Remember, since glucose was broken in two pieces in reaction 13, one glucose generates two ATP and two NADH at this step.

[102] Discussed next lecture.

[103] This is known as glyceroneogenesis. Alternately, if glycerol levels are abundant, it can be recycled back into triglycerides.

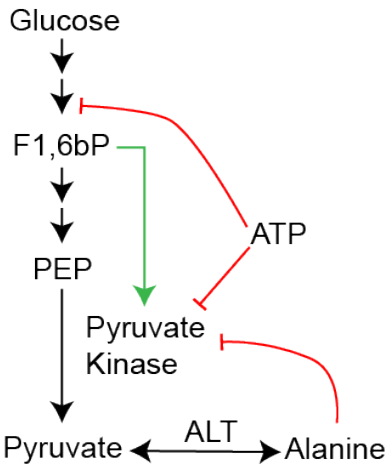

Figure 26: Regulation of pyruvate kinase in the liver. In the muscle, neither ATP nor Alanine play important roles. PKA indicates inhibitory phosphorylation of Pyruvate Kinase in response to glucagon or adrenaline.

[104] This will be discussed in the amino acid catabolism lecture.

[105] The muscle isoforms of Pyruvate Kinase is *not* inhibited by ATP or Alanine, but is still subject to feed-forward regulation by F1,6bP. The adipocyte isoform (PKM2) is also activated by Serine [Christofk et al., 2008] and is expressed in a variety of tumors. It is an emerging anti-cancer target.

simultaneously. Again, muscle cell Pyruvate Kinase is not inhibited in this manner.

The fate of pyruvate

Pyruvate can go in one of four directions in the cell, depending on the activity of Pyruvate Dehydrogenase (PDH) and the relative levels of Alanine in the cell. The regulation of PDH is very important for aerobic respiration, and will be discussed next lecture. These fates are described in Table II. In general, if Alanine is sufficient and PDH activity is low or absent, then pyruvate is converted to lactate via Lactate Dehydrogenase and released from the cell. This is known as anaerobic respiration and is important for fast-twitch muscle fibers and in conditions where oxygen levels are low. Pyruvate can also be easily converted to and from Alanine, via Alanine Aminotransferase (ALT)[106]. Finally, as we will cover in the lectures on the TCA cycle, when acetyl-CoA levels are high[107], Pyruvate can be converted to Oxaloacetate, a process known as anaplerosis.

[106] We will cover how the non-essential amino acids are synthesized later in this course.

[107] Indicating reduced TCA/ETC flux, but sufficient acetyl-CoA production. Think about under which conditions this might occur.

Pyruvate Fate	Conditions	Key Enzyme
TCA cycle	High PDH Activity	Pyruvate Dehydrogenase
Lactate	Low PDH Activity	Lactate Dehydrogenase
Alanine	Low Ala, High Glu	Alanine Aminotransferase
Oxaloacetate	High Acetyl-CoA	Pyruvate Carboxylase

Table 19: Potential fates of pyruvate. While several of these enzymes and processes have not been covered yet, we will discuss all of these later in the semester.

Energy production by glycolysis

Glycolysis occurs in three phases:

1. An investment phase, which uses two molecules of ATP (see reactions 10 and 12). This"charges" the glucose molecule, providing it with enough energy to be split into two 3-carbon molecules. **At this stage there is a net usage of two ATP molecules per molecule of glucose.**

2. The cleavage step, performed by aldolase A, immediately after the highly regulated PFK1 step (see reaction 13). This is a very energetically costly step, as breaking a carbon-carbon bond is quite difficult[108]. This cleavage means that one glucose molecule may eventually generate **two** Pyruvate molecules.

3. The catabolism of each molecule of glyceraldehyde-3-phosphate (GA3P) to pyruvate generates two molecules of ATP via substrate-level phosphorylation[109] (see reactions 15 and 18). There is also

[108] The standard free energy of this step is +28 kcal/mol, making it highly endothermic. For more details on how Fructose-1,6-bisphosphate buildup allows this difficult reaction to occur, see http://sandwalk.blogspot.com/2007/10/aldolase-reaction-and-steady-state.html

[109] Substrate level phosphorylation is the production of ATP by direct transfer from another phosphorylated compound.

the reduction of one NAD+ molecule into NADH (see reaction 14). NADH, as we will discuss in the unit on the electron transport chain is equivalent to an average of 2.5 ATP molecules. Therefore this phase produces a total of 4.5 ATP molecules per GA3P, or **9 molecules per glucose molecule**.

Glycolysis down to the level of pyruvate therefore uses up two ATP molecules, and generates the equivalent of 9 ATP molecules for a **net gain of 7 molecules of ATP per molecule of glucose.** Full oxidation of glucose to CO_2 will eventually produce 32 molecules of ATP/glucose so at this stage there is quite a lot of energy remaining in pyruvate.

Hormonal regulation of glycolysis

INSULIN PROMOTES GLYCOLYSIS BY SEVERAL MECHANISMS. First, in muscle and adipose tissue, insulin promotes the translocation of GLUT4 to the plasma membrane, allowing for glucose entry into the cell. This increases the levels of glucose, and glucose-6-phosphate in the cell. Insulin also promotes the dephosphoryation of both PFK2 and Pyruvate Kinase in the liver [Probst and Unthan-Fechner, 1985]. Recall that in both cases, the dephosphorylated forms are *more active*, so this increases the flux by which glucose gets converted to pyruvate or lactate. The mechanisms by which insulin promotes these dephosphorylation events are still murky.

GLYCOLYSIS IS REGULATED DIFFERENTLY IN MUSCLE THAN IN LIVER TISSUES. There are splice variants[110] of PFK2 that are expressed in a tissue specific manner. In liver tissue the L-PFK2 isoform can be phosphorylated on Serine 32 resulting in its inhibition. This residue is *absent* in the muscle, brain and adipocyte isoforms. Therefore, adrenaline/glucagon-mediated inhibition[111] and insulin-mediated activation (via dephosphorylation) is *only* relevant for the liver isoform of PFK2. The muscle and adipocyte PFK2 isoforms are not regulated by these hormones. This means that glucagon or adrenaline will prevent glycolysis in liver cells but that adrenaline or glucagon will not prevent glycolysis in muscle cells[112]. Similarly, Pyruvate Kinase is inhibited by PKA, ATP and Alanine, only in the liver, but all Pyruvate Kinase isoforms are regulated positively by F16bP. This means that the negative feedback of Pyruvate kinase by PKA, Alanine and ATP is not a factor in the regulation of adipocyte or muscle glycolysis.

[110] Splice variants are different mRNA's which lead to different proteins, transcribed from the same gene.

[111] Via PKA-dependent phosphorylation.

[112] There is a physiological advantage to this. Think about what the consequence would be if adrenaline *prevented* glycolysis in muscle cells, and why it would be advantageous to do this in liver cells.

ANOTHER LEVEL OF CONTROL OF GLYCOLYSIS IS TRANSCRIP-

TIONAL. There are certain conditions where it is helpful to increase glycolysis in a chronic and less rapidly reversible manner. This is typically mediated by transcription factors which synthesize new glycolytic proteins, increasing the capacity by which glycolysis can occur. There are several examples of this such as:

- Hypoxia, or reduced oxygen (via the transcription factor HIF)[113].
- Chronically elevated glucose levels (via the transcription factor ChREBP).
- Chronic glucagon stimulation (via the transcription factor CREB).

[113] Think about why glycolysis may be advantageous during oxygen deprivation.

These transcriptional changes often affect the expression of the enzymes Pyruvate Kinase, Glucokinase and PFK2 [Semenza et al., 1994, Kawaguchi et al., 2001]. These changes are slower and more energetically costly than allosteric or post-translational changes. As such, they are both longer lasting and harder to undo.

Fructose metabolism

Fructose, the other monosaccharide unit in sucrose is largely metabolized within the liver and intestine[114], compared to glucose which is metabolized in multiple tissues. Within the liver, fructose enters the hepatocyte via facilitative GLUT5 channels, which are constitutively present on the plasma membrane. In terms of metabolism, fructose undergoes the following steps, catalyzed by ketohexokinase (reaction 19)[115], aldolase B (reaction 20) then triose kinase (reaction 21)[116,117].

[114] Intestinal fructose metabolism was recently explored in a recent paper by Jang et al. [2018]. There is an *optional* group project on GradeCraft that considers this work. If you (and a couple other classmates) are interested in intestinal metabolism, give that a look.

[115] also known as fructokinase

[116] Be careful here, fructose becomes F16bP then DHAP/G3P whereas glucose becomes F6P then F1P then DHAP/GA3P. These routes can be easily confused.

[117] As an exercise, draw out the pathways by which glucose and fructose become pyruvate, calculate whether the ATP production is similar for these two monosaccharides.

$$Fructose + ATP \rightarrow Fructose - 1 - phosphate \tag{19}$$

$$Fructose - 1 - phosphate \rightleftharpoons Glyceraldehyde + DHAP \tag{20}$$

$$Glyceraldehyde + ATP \rightarrow GA3P \tag{21}$$

Fructose catabolism is independent of PFK1

The products (DHAP and Glyceraldehyde-3-phosphate) are the same metabolites that are produced by the glycolytic step in reaction 13. Importantly this step occurs *after* the two key glycolytic regulatory steps at PFK1 and Glucokinase. Hence fructose feeds into the glycolytic flux past the two regulatory points of PFK1 and Glucokinase. This has very important ramifications for how fructolysis[118] is regulated relative to glycolysis. Since the regulatory steps that can

[118] The breakdown of fructose into pyruvate/lactate.

normally control the flow of glucose are uncontrolled for fructose, fructose is converted rapidly to its end-products, whether there is energy demand or not. This is biochemical basis by which fructose is thought to be more prone to become acetyl-CoA and then fatty acids[119]. Similarly, as we will discuss for unit on gluconeogenesis, fructose can very easily be converted to glucose with little regulatory oversight [Kim et al., 2016].

[119] Think about how citrate can control glucose but not fructose breakdown.

Fructose consumption has been linked to both obesity and liver disease

Fructose is normally present at high levels in fruit, or as the disaccharide sucrose, which has a 1:1 ratio of glucose:fructose. For ease of handling and production, high fructose corn syrup (HFCS) has been in widespread use since the 1970s, particularly in sugar-sweetened beverages. HFCS generally contains 45–60% fructose with the remaining as glucose. While as recently as 2014, the Food and Drug Administration has declared HFCS safe as a food ingredient, epidemiological studies suggested HFCS may be associated with both obesity, and non-alcoholic fatty liver disease[120]. A recent meta-analysis in this area was inconclusive, as it was difficult to separate the effects of added calories due to HFCS from direct metabolic effects of this sweetener [Chung et al., 2014].

[120] A liver disease that starts with lipid accumulation and inflammation of the liver, which can result in impaired liver function or eventually cirrhosis or some liver cancers.

Disorders of fructose metabolism

There are two inborn errors of fructose metabolism, defects in either Fructokinase or Aldolase B[121]. In the case of individuals with Fructokinase deficiency, this is generally not pathological, since Fructose is not phosphorylated, and therefore is not trapped in cells. Patients with Fructokinase deficiency have very high circulating levels of Fructose in their blood, but are otherwise normal. On the other hand, Aldolase B deficiency means that Fructose becomes trapped at the Fructose-1-phosphate step [Cross et al., 1988][122]. This occurs in approximately 1 in 20,000 to 30,000 individuals. This intermediary metabolite builds up, wasting ATP and resulting in liver cirrhosis, hypoglycemia and kidney damage[123].

[121] This is a different enzyme from Aldolase A, which is part of glycolysis and shown in reaction 20.

[122] If you want to explore mutations in any of the enzymes we discuss in more detail, I suggest going to the website `https://gnomad.broadinstitute.org/`, enter the gene name, scroll down, click on LOF (loss of function) then click on any of the variants to see their incidence in various populations. The gene name for Aldolase B for example is *ALDOB*.

[123] Think about, from a dietary perspective how this disease could be managed.

Galactose metabolism

Galactose, the other monosaccharide in lactose is taken up in the liver via GLUT2. In contrast to Fructose, Galactose enters glycolysis near the early steps of glycolysis and is subject to similar regulatory steps. The initial steps of galactolysis are catalyzed by the enzymes galactokinase and GALT[124] or phosphoglucomutase and result in the production of Glucose-6-phosphate:

[124] Galactose-1-phosphate uridylyltransferase, shown in reaction 23. This is *not* the same thing as Gut-Associated Lymphoid Tissue, which is also abbreviated as GALT and was discussed as it relates to the digestive system.

$$Galactose + ATP \rightarrow Gal1P \tag{22}$$

$$Gal1P + UDP - Glucose \rightleftharpoons G1P + UDP - Gal \tag{23}$$

$$G1P \rightleftharpoons G6P \tag{24}$$

Glucose-6-phosphate then proceeds as normal, becoming dephosphorylated to glucose, or processed through glycolysis, glycogenesis or the pentose phosphate pathway (see Figure 24). The UDP-galactose generated in reaction 23 is regenerated back to UDP glucose by another enzyme called UDP-Galactose Epimerase, which catalyzes this reaction:

$$UDP - Galactose \rightleftharpoons UDP - Glucose \tag{25}$$

Inborn errors of GALT or UDP-Galactose epimerase lead to galactose intolerance and a buildup of Galactose-1-phosphate in tissues and the blood.

Reflection Questions

1. A newborn screens positive for hereditary fructose intolerance (HFI), a deficiency of aldolase B. Parents ask whether a diet eliminating only table sugar (sucrose) is sufficient to protect their child. Using your knowledge of fructose metabolism, evaluate whether this recommendation is complete. Which other dietary sources of fructose must be restricted, and at what step would metabolite accumulation cause harm?

2. Epidemiological studies consistently link high intake of fructose-sweetened beverages to elevated rates of MASLD, even in people who are not obese. A colleague argues this is simply because fructose provides excess calories. Using your knowledge of how fructose enters glycolysis relative to glucose, construct a mechanistic argument for why fructose is *disproportionately* lipogenic independent of total caloric load. Your answer should specifically address the role of citrate as an allosteric inhibitor of PFK-1, why this feedback does not apply to hepatic fructose metabolism, and how unregulated carbon flux below the PFK-1 step drives de novo lipogenesis even when cellular energy status is already high.

3. Glucokinase and hexokinase both phosphorylate glucose to glucose-6-phosphate, yet their kinetic and regulatory properties

differ profoundly. Analyze how these differences make glucokinase well-suited for its role as a hepatic glucose sensor, and explain what would happen to postprandial blood glucose control if liver cells expressed only hexokinase instead. How does this compare to the situation in pancreatic β-cells, which also express glucokinase?

Glucose Oxidation and the TCA cycle

The acquisition and utilization of mitochondria during evolution dramatically improved the ability of the cell to generate energy. In the presence of oxygen and ATP demand, the products of glycolysis, amino acid catabolism and lipid oxidation enter the TCA Cycle[125]*. This allows for complete oxidation of metabolites into CO_2 and efficient energy production by the mitochondrial ATP synthase. This unit will describe how the TCA Cycle and Electron Transport Chain are regulated, and how various nutrients interact with this cellular pathway. For more details about the these topics see Chapters 18-20 in Biochemistry: A Short Course*[126]*, available on reserve.*

[125] Also known as the Tricarboxylic Acid Cycle, Kreb's Cycle or Citric Acid Cycle.

[126] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

Learning Objectives

- Evaluate the potential metabolic fates of pyruvate and the signals that control these changes.
- Assess the importance of the mitochondria in glucose metabolism.
- Describe the key regulatory nodes of the TCA cycle.
- Understand the concepts of anaplerosis and cataplerosis and how this can affect TCA cycle efficiency. Predict whether a particular pathway is anaplerotic or cataplerotic.
- Explain the differences in efficiency between anaerobic glycolysis and the TCA cycle linked to the electron transport chain.
- Recall the key functions of the electron carriers NADH, $FADH_2$ and QH_2.
- Calculate ATP production from GTP, NADH and $FADH_2$ equivalents.
- Understand how mitochondria balance nutrient flux with ATP requirements.

Key Vocabulary and Concepts

- Anaplerosis
- Cataplerosis
- Electron Carrier Molecules (including NADH, $FADH_2$ and QH_2)
- Proton Gradients and the Electromotive Force
- Oxidative Stress, Antioxidants, and Reactive Oxygen Species

The Next Steps in Carbohydrate Metabolism Require Mitochondria

While glucose oxidation to the level of Pyruvate generates the equivalent of 7 ATP molecules[127], full oxidation to CO_2 can yield up to 32 molecules of ATP[128]. This process requires transport of pyruvate into the mitochondria where Acetyl-CoA is generated and degraded to CO_2, GTP and reduction of electron carrier molecules. These molecules, NADH and $FADH_2$, are passed to the electron transport chain, generating a proton gradient, which powers the mitochondrial ATPase[129]. Mitochondria are also the only way to catabolize fatty acids, and most Amino Acids. As a membrane-enclosed organelle,

[127] 2 x NADH (5 ATP equivalents at 2.5 ATP/NADH) and 2 x ATP are generated.

[128] The specific amount can vary from 30-32 depending on mitochondrial efficiency.

[129] This is a little confusingly named, as an "ase" generally can break down a molecule. In this case the mitochondrial ATP synthase *generates* ATP from ADP.

many key metabolites are impermeable to mitochondria without transporters. These include cytoplasmic NADH generated during glycolysis as well as Pyruvate[130]. Fortunately there are a variety of transporters and shuttle systems that transport key materiel from the cytoplasm into the mitochondria.

[130] NADPH cannot cross the mitochondrial membrane, so requires shuttle pathways such as the malate–aspartate and glycerol–phosphate shuttles to indirectly share NADPH between compartments. For more detail about these shuttles see McKenna et al. [2006].

Regulation of Mitochondrial Numbers

Before we start with what happens in mitochondria, its worth taking a step back and considering how mitochondrial amounts are regulated. The presence of mitochondria is essential for aerobic metabolism, and muscle fibers that are rich in mitochondria are able to fully oxidize glucose and other fuels. These are known as Type I muscle fibers, or colloquially as slow-twitch muscle fibers[131]. The number and efficiency of mitochondria in muscle is not static, and can be modified by training. For example endurance training dramatically increases the number of mitochondria and the levels of TCA and ETC enzymes in both rodent and human studies [Holloszy, 1967, Gollnick et al., 1972, 1973, Holloszy, 2011]. Understanding the molecular mechanisms by which this happens is a major research area both in terms of human performance, and in terms of modifying energy expenditure and promoting healthy aging (for more details see Cartee et al. [2016]). Some cells have few or no mitochondria. In this case they are entirely dependent on glycolysis for ATP production.

[131] There is another fast-twitch fiber type that contains less mitochondria called IIA fibers

The Possible Fates of Pyruvate

As we discussed in the unit on glycolysis, pyruvate has several possible fates. If Alanine levels are low and Glutamate levels are high, Pyruvate can be converted to Alanine via ALT[132]. If there is energy demand, PDH[133] is activated and pyruvte becomes Acetyl-CoA. If Acetyl-CoA levels are high, Pyruvate becomes the TCA Cycle intermediate Oxaloacetate via the actions of Pyruvate Carboxylase. If none of these enzymes are activated, Pyruvate is converted by Lactate Dehydrogenase and released from the cell as Lactate. The reversible Lactate Dehydrogenase reaction is:

[132] Alanine Aminotransferase.

[133] Pyruvate Dehdyrogenase, discussed in the next section.

$$Pyruvate + NADH \rightleftharpoons Lactate + NAD^{+} \tag{26}$$

Anaerobic Glycolysis

If oxygen is unavailable to the cell, or there are no mitochondria present and lactate is produced, this is the end of anaerobic glycolysis. The reaction to generate lactate uses up the NADH produced

during glycolysis. This means that on net one glucose molecule now generates just two ATP equivalents[134]. The lactate molecule that is released was once thought to be primarily converted back to glucose by a pathway called the Cori cycle[135] but recent experiments monitoring the flux of lactate shows that a large fraction of lactate is anaplerotic, meaning it is used to increase the number of molecules in the TCA cycle [Hui et al., 2017, Ferguson et al., 2018][136].

[134] Walk yourself through this to convince yourself this is correct.

[135] This will be explained in more detail when we discuss gluconeogenesis.

[136] Anaplerosis and its inverse, cataplerosis will be described below, but in general is the removal of TCA cycle intermediates.

Regulation of Pyruvate Dehydrogenase

Pyruvate Dehydrogenase[137] is a mitochondrial enzyme that catalyzes this irreversible reaction:

$$Pyruvate + NAD^+ + CoA \rightarrow AcetylCoA + CO_2 + NADH + H^+ \quad (27)$$

The two most important products here are Acetyl-CoA, which will enter the TCA cycle and NADH which will go to Complex I of the Electron Transport Chain. As one of the most important molecular decision steps, it makes sense that this enzyme is tightly regulated. Intracellularly, PDH activity is turned on when energy is low, and turned off when energy is available. The specific regulators are shown in Table 21 and Figure 27. The first several of these should be familiar to you, as increased substrate, and decreased energy (as sensed by low ATP, high ADP and low NADH, Acetyl-CoA) all promote PDH activity[138]. Ca^{2+} is an activator of PDH activity, and as we will describe, also plays a role in activating several other TCA Cycle enzymes. This is because, when muscles contract they release Calcium. This allows for mitochondria to respond to contraction by generating more ATP.

Table 20: Potential fates of Pyruvate.

Pyruvate Fate	Conditions	Key Enzyme
TCA cycle	High PDH Activity	PDH
Lactate	Low PDH Activity	PDH
Alanine	Low Ala, High Glu	ALT
Oxaloacetate	High Acetyl-CoA	PC

[137] This enzyme uses multiple cofactors, including Vitamin B1-derived TPP, Vitamin B2-derived FAD, vitamin B3-derived NAD and Vitamin B5-derived Coenzyme A. Reduced PDH activity is a major cause of Beriberi and Wernicke-Korsakoff syndrome (which are deficiencies of Vitamin B1.

[138] Now you may be thinking, I thought the key determinant of aerobic respiration was oxygen availability! It may not be clear yet, but once you have read these notes, try to come back to this and think about how low oxygen levels would result in changes in PDH activity

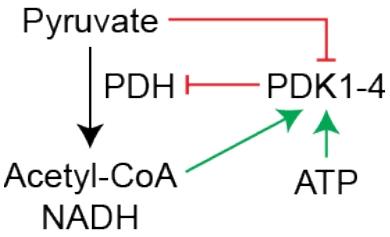

Figure 27: Regulation of pyruvate dehydrogenase.

Much like PFK2 and Pyruvate Kinase, PDH is Inhibited by Protein Phosphorylation. The phosphorylation of PDH is regulated by a specific protein kinase (PDH Kinase or PDK) which adds a phosphate group to PDH and reduces its activity (see Figure 27). The overall mechanism of regulation of PDH therefore is a combination of direct inhibitors of PDH (NADH and Acetyl-CoA), activators of the inhibitory kinase (ATP, Acetyl-CoA, NADH) and activators of the activating phosphatase (Ca^{2+}). Unlike PFK2 and Pyruvate Kinase, PDK activity is regulated mostly at the metabolite level and not acutely by hormonal signals and protein phosphorylation.

Pyruvate Dehydrogenase Kinase is regulated transcriptionally. While the activity of PDK is primarily regulated by metabolites, the number of PDK enzymes can be induced by several signals. Here are a couple, as you read these take a moment to

think *why* this stimuli would alter PDK levels, and what would be the effects on glycolysis, gluconeogenesis and mitochondrial respiration. Remember more PDK means *less* PDH activity:

- Glucocorticoids induce PDK4 transcription via FOXO [Connaughton et al., 2010].
- Starvation induces PDK4 [Wu et al., 1998].
- Insulin reduces PDK4 levels, insulin resistance elevates PDK4 levels [Harris et al., 2001].
- Hypoxia (low oxygen) induces PDK1 via HIF1α [Kim et al., 2006].

Table 21: Regulators of Pyruvate Dehydrogenase.

Regulator	Effect	Mechanism
Pyruvate	Positive	Inactivates PDK
ADP	Positive	Inactivates PDK
ATP	Negative	Activates PDK
Acetyl-CoA	Negative	Activates PDK, Inhibits PDH
NADH	Negative	Inhibits PDH
Ca^{2+}	Positive	Activates PDH Phosphatase

Acetyl-CoA also enters the TCA Cycle after β-oxidation. While we have focused so far on carbohydrate metabolism, now is a good time to talk briefly about lipid oxidation. Unlike carbohydrates, when fatty acids are broken down they produce Acetyl-CoA, not Pyruvate[139]. This means that in humans, fatty acids enter the TCA cycle here. If fatty acids are oxidized in the liver, but the TCA Cycle is not activated, those extra Acetyl-CoA molecules are released as ketone bodies, which can be used by other tissues, after re-conversion back into Acetyl-CoA.

[139] They also generate equal amounts of NADH, $FADH_2$ which we will discuss later

Some amino acids are also converted into Acetyl-CoA. As we will learn in the gluconeogenesis and amino acid catabolism lectures, some amino acids, depending on the circumstances are able to become converted into glucose[140]. Others are converted into Acetyl-CoA and are known as the ketogenic amino acids. These amino acids can only be oxidized into energy by entering the TCA cycle as Acetyl-CoA, but cannot become glucose[141].

[140] These are the glucogenic amino acids.

[141] Later we will learn that the exclusively ketogenic amino acids are Leucine and Lysine. Phenylalanine, Isoleucine, Threonine, Tryptophan and Tyrosine are partially glucogenic and partially ketogenic.

The TCA Cycle Products

The TCA Cycle/ETC completely oxidizes Acetyl-CoA to CO_2. One cycle, using one molecule of Acetyl-CoA generates the following:

Product	→	ATP
3 NADH	→	7.5 ATP
1 $FADH_2$	→	1.5 ATP
1 GTP	→	1 ATP
Total		**10 ATP**

Table 22: TCA Cycle ATP generation. See Table 23 for details on conversion rates.

if we include the NADH generated by Pyruvate Dehydrogenase, that means that Pyruvate oxidation results in 12.5 molecules of ATP.@

Based on what we have discussed in this unit and in the glycolysis unit, try to determine how much ATP is generated from one molecule of glucose[142]. Compare this to the two molecules of ATP generated by anaerobic glycolysis and it should make sense why we breathe heavier when we exercise.

[142] For a slightly bigger challenge, consider that palmitate oxidation generates 8 Acetyl-CoA, 7 NADH and 7 $FADH_2$ molecules, but requires 2 ATP molecules for activation. Consider the energy yield from a C16:0 (Palmitate) fatty acid, compared to glucose.

NADH and $FADH_2$ Are Substrates for the Electron Transport Chain

Once Acetyl-CoA is generated by PDH, or by the breadown of amino or fatty acids, the TCA cycle functions to extract electrons from these substrates and pass them along to the electron transport chain. This is done by transferring electrons from TCA intermediates to electron carrier molecules including NADH and $FADH_2$. These molecules are "charged" by these electrons, and then transfer them to Complex I (for NADH) or Complex II (for $FADH_2$). The electron transport chain takes these charged carriers, and transfers the energy through a series of complexes. After Complex I or II, electrons are next transported to another carrier called Ubiquinone (or Coenzyme Q) which takes them to Complex III.@ Between complex III and IV is a final carrier called Cytochrome c. As electrons are passed between electron carriers, complexes I, III and IV result in proton ions being pumped out of the mitochondrial cristae, resulting in a gradient of protons. The final step at complex IV reduces oxygen into water. Oxygen is the final electron carrier and is essential for the complete oxidation of the electron carrier molecules.

Table 23: ATP producing equivalents.

Molecule	→	ATP
1 NADH	→	2.5 ATP
1 $FADH_2$	→	1.5 ATP
1 GTP	→	1 ATP

The Electron Transport Chain is Coupled to ATP Production

As a result of the reactions in Complex I, III and IV, protons are pumped from the inside of the mitochondria to the inner-membrane space[143]. This generates a proton gradient with more protons on the outside of the inner membrane than on the inside. This gradient drives a proton-coupled pump called ATP Synthase. If the proton gradient is established, and sufficient ADP levels are present inside the mitochondria, ATP Synthase catalyzes the production of ATP from ADP.@

[143] Mitochondria have two membranes an inner membrane and an outer membrane.

The three electron carrier molecules we have described are FAD, NAD and CoQ[144]. These all need to be available in the mitochondria to allow electrons to flow from the TCA cyle through the ETC.@ These molecules are reduced by the actions of the TCA cycle then oxidized back to their original form by the ETC.@ All three of these can be generated endogenously, but NAD and FAD are usually generated from vitamins (see Table 24). It has been suggested

[144] Cytochrome c is a protein not a small molecule.

that as we age, we are less able to generate NAD, and several preclinical trials are underway to test whether NAD supplementation may slow aging in humans (reviewed in Rajman et al. [2018]) .Coenzyme Q is generated endogenously using some enzymes of the steroid biosynthesis pathway, and inhibitors of this pathway (such as statins) have been suggested to result in CoQ deficiency (reviewed in Quinzii et al. [2007]).

Table 24: Electron carrier molecules in the ETC

Carrier	Source
NAD	Vitamin B3
FAD	Vitamin B2
CoQ	Not Considered a Vitamin

THE TCA CYCLE IS BOTH ANABOLIC AND CATABOLIC. While the TCA cycle is primarily thought of as a catabolic pathway, it is also a source of many biosynthetic precursors. For example, Citrate can be exported from the mitochondria and converted into Acetyl-CoA for fatty acid synthesis. Other TCA cycle intermediates are used to generate amino acids, heme and nucleotides. We will discuss this in more detail in the section on anaplerosis and cataplerosis below.

Regulation of the TCA Cycle

The TCA/ETC cycle has to be tightly controlled. This happens in three levels. The first is that the entire system is driven by ATP demand. Think of this aspect of metabolism more as being pulled by the need, rather than being pushed by substrates. The second level of regulation is how many TCA cycle intermediates are present. Since this is a cycle, where the starting substrate (oxaloacetate) is regenerated, having more oxaloacetate molecules in the cycle will increase efficiency. The third level of regulation is regulation of the TCA cycle enzymes themselves. This can be allosteric (increased activity with low ATP/NADH), transcriptional or post-translational.

The ETC is Driven by ATP Demand

Many metabolic pathways are driven by the nutrients that flow in. An example of this is that glycolysis occurs more rapidly when more glucose is present. This is facilitated by feed-forward mechanisms where more glucose results in higher activity of PFK (via F26bP) and Pyruvate Kinase (via F16bP). The electron transport chain, however, is primarily regulated by demand, rather than supply. In this case, demand means high levels of ADP[145]. In the absence of ADP, electrons from $NADH/FADH_2$ do not get transported to the final electron acceptor (O_2). If ADP levels are high, that indicates that ATP needs to be synthesized and the electron transport chain is active.

[145] because of breakdown of ATP to ADP.

Changes in TCA Cycle Intermediates

THE TCA CYCLE REGENERATES OXALOACETATE. Unlike glycolysis, which starts with glucose and ends with Pyruvate, the TCA cycle takes in Acetyl-CoA and after one round of the cycle, is left with Oxaloacetate. That means that other than Acetyl-CoA, the cycle is self-replenishing[146]. As you might suspect, having more Oxaloacetate can mean there is more efficient Acetyl-CoA metabolism. While normal TCA cycle function as we have been describing does not alter these levels, there are several important processes that can affect this. One example is gluconeogenesis, which extracts Oxaloacetate (via the activity of an enzyme called PEPCK) to form glucose. Another example is that biosynthesis of some amino acids uses up TCA cycle intermediates. The process by which TCA Cycle intermediates are removed is known as *cataplerosis*. This process is extremely important for growth as it is a source of many building blocks for cells (for more details see Inigo et al. [2021]).

[146] One analogy for this is that the TCA Cycle is like a subway system, and Oxaloacetate is like a subway car. You need it to get from point A to point B, but you dont use up the car.

THE OPPOSITE PROCESS, IN WHICH TCA CYCLE INTERMEDIATES ARE GENERATED IS KNOWN AS ANAPLEROSIS. These can derive from Pyruvate, or from the breakdown of amino acids[147]. The most important enzyme here is called Pyruvate Carboxylase. This enzyme performs the following irreversible, ATP consuming reaction:

[147] Amino Acid catabolism will be covered later in the course, so here we will focus on anaplerosis from Pyruvate.

$$Pyruvate + Bicarbonate + ATP \rightarrow Oxaloacetate + ADP + Pi \quad (28)$$

There are two important roles of Pyruvate Carboxylase, one of which is to increase TCA Cycle intermediates. The second is to generate Oxaloacetate for gluconeogenesis[148]. The activity of Pyruvate Carboxylase is positively regulated by Acetyl-CoA. Since Acetyl-CoA is not directly anaplerotic, this mechanism balances flow of "passengers" (Acetyl-CoA) to the number of "trains" (Oxaloacetate). Extending the metaphor, if there are too many passengers, Pyruvate Carboxylase results in more trains being put into service. As we will discuss later in the section on gluconeogenesis, Acetyl-CoA (the major metabolite of fatty acid oxidation) is *unable* to become glucose, meaning that while fatty acids can promote gluconeogenesis, the process is still reliant on other precursors such as lactate, alanine and glycerol.

[148] We will discuss this in a couple of lectures.

Allosteric Regulation of the TCA Cycle

As we discussed above, the ETC is inactive unless there is ATP demand. It is therefore imperative that the ETC can feed back to the TCA cycle and stop NADH/$FADH_2$ production if these activated electron carriers are not needed. The primary mechanism by which

this occurs is negative allosteric regulation described in Table II. The signals of high energy availability are ATP, NADH and $FADH_2$.

Enzyme (step)	Activators	Inhibitors
Citrate synthase (1)	OAA, AMP	NADH, $FADH_2$, ATP
Isocitrate dehydrogenase (3)	ADP, Ca^{2+}	NADH, ATP
α-ketoglutarate dehydrogenase (4)	Ca^{2+}	NADH

Table 25: Key regulated steps of the TCA cycle. The step in paranthesis indicates where the enzyme is in the cycle.

You should note from Table II that these enzymes are broadly activated by two things, low energy levels (AMP or ADP) and Calcium increases. Calcium plays a key role here, because in skeletal muscle Calcium release causes muscle contraction. By activating the TCA cycle, Calcium couples the initiation of muscle contraction to the replenishment of ATP levels. By the same token, the TCA cycle is inhibited when there is a buildup of high energy molecues (NADH, $FADH_2$, ATP) either when there is a lack of oxygen or sufficient energy stores.[149]

[149] A high NADH/NAD^+ ratio, such as that as seen in hypoxia or ischemia, inhibits isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. This slows the TCA cycle, reducing NADH generation when the electron transport chain is backed up. The result is accumulation of upstream intermediates and diversion toward anaerobic metabolism (lactate production). In severe ischemia, for example after an ischemic heart attack, this contributes to cytosolic acidification and impaired ATP synthesis.

Anemia and the TCA Cycle

Several enzymes in the TCA cycle as well as the electron transport chain require iron as a cofactor (*e.g.* Aconitase and Complex II). In addition to its role as a cofactor, iron is essential for the delivery of oxygen to cells, which is mediated by iron-containing hemoglobin, and for oxygen transport within muscle cells, which is mediated by iron-containing myoglobin. For these reasons, low iron levels can impair TCA cycle function and aerobic metabolism. This is one of the reasons why anemia can cause fatigue and exercise intolerance. Anemia can also be caused by other chronic diseases such as chronic kidney disease, inflammatory bowel disease and cancer, and this can contribute to fatigue in these conditions. In the United States anemia affects 9.3% of the population, and is 2.4x more common in women than men [Williams et al., 2024]. Globally anemia is the most common nutritional deficiency, affecting 1.62 billion people or about a quarter of people worldwide [World Health Organization, 2025].

Oxidative Damage Can Result from Superoxide Production

The terminal electron acceptor of the TCA cycle and electron transport chain is oxygen. This is the reason why aerobic metabolism requires a supply of oxygen. The oxygen is converted to water by Complex IV of the ETC.@

$$4CytC_{red} + 4H^+ + O_2 \rightarrow 4CytC_{ox} + 2H_2O \tag{29}$$

While this process is efficient most of the time, due to the high reactivity of oxygen, approximately 2-4% of the time, rather than being reduced to water, oxygen can be partially reduced into a superoxide or peroxide molecule. These *reactive oxygen species* such as HO_2^- can cause cellular damage by reacting with proteins and lipids in the cell. This process can also be accelerated when NADH or $FADH_2$ build up. To defend against this, mitochondria express an enzyme known as superoxide dismutase (SOD) which scavenges these superoxides and converts them to hydrogen peroxide and then via an enzyme known as catalase back into water. These reactions are described in reactions 30 and 31 below:

$$2HO_2^- \rightarrow O_2 + H_2O_2 \tag{30}$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \tag{31}$$

Overwhelming this system has been linked to a wide variety of chronic diseases including Alzheimer's, Parkinson's disease, Cancer and Diabetes. One of the benefits of chronic physical activity is to increase the expression of SOD and catalase[150]. By the same token, antioxidant vitamins such as Vitamins C and E can also reduce the potential damage of reactive oxygen species generated by the mitochondria. For more information about reactive oxygen species see Turrens [2003].

[150] For those interested in micronutrients, catalase is an iron-dependent enzyme and SOD is either a mangangese or copper dependent enzyme, and deficiencies in any of these minerals can enhance oxidative damage.

Reflection Questions

1. A marathon runner sustaining aerobic exercise and a sprinter working anaerobically both start with glucose. Trace the fate of pyruvate in each scenario, explaining how PDH activity, PDK regulation, and mitochondrial ATP demand differ between these two contexts. Include the role of calcium signaling in your answer.

2. During prolonged fasting, the body relies heavily on fatty acid oxidation, which generates large amounts of Acetyl-CoA but cannot contribute carbons to glucose. Using your understanding of anaplerosis, cataplerosis, and pyruvate carboxylase regulation, explain how gluconeogenesis can still be sustained during fasting, and describe the specific role Acetyl-CoA plays in coordinating this process.

3. A patient with iron-deficiency anemia reports severe fatigue during exercise. Their physician recommends high-dose antioxidant supplementation (vitamins C and E) to reduce oxidative stress.

Analyze the multiple steps in aerobic metabolism where iron deficiency impairs function, and evaluate whether antioxidant supplementation would be expected to address the primary mechanism of fatigue in this patient.

Oxidative Stress, the Pentose Phosphate Pathway and Alcohol Metabolism

Glucose can enter three pathways; glycolysis, glycogenesis or the pentose phosphate pathway[151]*. This handout will describe the role of this pathway in generating NADPH, nucleosides and the role of reducing equivalents in metabolism. For more details on this pathway, see Chapter 26 in Biochemistry: A Short Course, available on reserve*[152].

[151] sometimes called the pentose phosphate shunt.

[152] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

Learning Objectives

- Understand the role of Glucose-6-Phosphate Dehydrogenase in regulating flow through the pentose phosphate pathway.
- Evaluate the role of glucose derived products in fatty acid and triglyceride synthesis.
- Interpret how the combined regulation of glycolysis, glycogenesis and the pentose phosphate pathway can affect the ability to synthesize lipids.
- Explain how defects in the pentose phosphate pathway can lead to disease.
- Understand the difference between *in vitro* antioxidant activity and *in vivo* effectiveness of dietary antioxidants.
- Explain the role of the liver's cytochrome P450 system, and how this affects detoxification of compounds.
- Analyse how alcohol is metabolized, and how this is different between moderate and heavy drinkers.

Key Vocabulary and Concepts

- NADPH (as opposed to NADH)
- Glutathione and the Glutathione Antioxidant System
- Antioxidants
- Pentoses and Nucleotide Biogenesis
- Reducing Equivalents
- Favism or G6PDH Deficiency
- The Cytochrome P450 System

The Pentose Phosphate Pathway

Recall from the previous lectures, that the flow of glucose between glycogen synthesis, glycolysis and the pentose phosphate pathway is dependent on the rate limiting enzymes of each. The pentose phosphate pathway is a glucose utilizing pathway which runs parallel to glycolysis, taking Glucose-6-Phosphate and converting it into several products, including NADPH Ribose 5-phosphate[153], and several glycolytic intermediates including Fructose-6-phosphate and

[153] which is used to make nucleotides

Glyceraldehyde-3-phosphate. The glycolytic intermediates feed back into glycolysis (see the notes on Glycolysis to see how these pathways reintegrate).

Glucose-6-Phosphate Dehydrogenase

The first enzyme is the rate limiting and irreversible step of the pentose phosphate pathway and is catalyzed by Glucose-6-Phosphate Dehydrogenase (G6PDH). G6PDH is activated by elevations of its two substrates $NADP^+$ and Glucose-6-Phosphate. Its activitiy is inhibited by high levels of NADPH.

Nucleotide Biosynthesis and the Pentose Phosphate Pathway

After the removal of one carbon from the six-carbon ring of glucose, the remaining sugar is a pentose, often a ribose. Through a complex series of equilibrium reactions, these sugars can be inter-converted in order to generate nucleotides and nucleic acids (ribose in the case of RNA, deoxyribose in the case of DNA). While non-dividing cells can often recycle their ribose sugars, if a cell is rapidly dividing cells (such as blood cells, skin cells or enterocytes) need substantial ribose to duplicate the six billion bases of DNA in a human cell. Because of these equilibrium reactions, the pentose phosphate pathway can be the source of riboses when needed, NADPH when needed, or a combination of both. The stoichiometry of the pentose phosphate pathway therefore can vary depending on needs. In the case that no ATP is needed, only NADPH this might be the stoichiometry:

$$6G6P + 12NADP^+ + 7H_2O \rightarrow 12NADPH + 6CO_2 + 12H^+ + 12Pi \tag{32}$$

But if a combination of energy and NADPH is needed, the stoichiometry might be the following, which should generate 6.8 ATP equivalents/glucose molecule[154]:

[154] how would this compare with glycolysis to Pyruvate?

$$3G6P + 6NADP^+ + 7H_2O + 5NAD^+ + 8ADP + 5Pi \rightarrow 5Pyruvate + 3CO_2 + 6NADPH + 5NADH + 8ATP + 2H_2O + 8H^+ \tag{33}$$

In reality its always a mixture, depending on the relative demand of riboses, NADPH and energy. Do not bother trying to memorize these reactions, but if you want more details about this see the relevant chapter in [Berg et al., 2013].

The Importance of NADPH

The most important product of this pathway in terms of nutrition is NADPH[155]. While the pentose phosphate pathway is the major source, it is not the only way to generate NADPH, several other mechanisms exist. The first is catalyzed by Malate Dehydrogenase:

$$Malate + NADP^+ \rightarrow NADPH + CO_2 + Pyruvate \quad (34)$$

This pathway removes malate from the TCA cycle to generate NADPH[156]. A third way to generate NADPH is through an isoform of Isocitrate Dehydrogenase:

$$Isocitrate + NADP^+ \rightarrow NADPH + CO_2 + \alpha - Ketoglutarate \quad (35)$$

Finally, recently a fourth pathway has emerged, wherin the reduction of 10-TMF to folate[157] can also generate NADPH in some cells. The details of this, and its potential as chemotherapeutic target due to its importance in rapidly dividing cells is described in Fan et al. [2014]. The relative importance of these three pathways vary based on cell type and metabolic state, but in most cells the pentose phosphate pathway is prominent.

[155] This looks like NADH, but is not, it has an extra phosphate group and is generally not inter-convertable with NADH. However, like NADH it generated largely from Niacin, also known as Vitamin B3.

[156] Think about this, is this cataplerotic, or anaplerotic?

[157] This is part of one-carbon metabolism, which will not be covered in this course, but will be covered in NUTR631.

The Role of NADPH in Anabolism

While glycolysis is a catabolic pathway, the pentose phosphate pathway could be considered an anabolic pathway. This is because anabolic pathways, notably fatty acid and cholesterol biosynthesis requires a large number of NADPH molecules. To make one molecule of palmitate (a 16 carbon fatty acid) you need *14 NADPH molecules*. As described in reaction 32, you would need more than 6 glucose molecules, just to provide the reducing equivalents to make a palmitate (not including all the energy needed for these reactions, which will be described in the lipid synthesis unit). Therefore in cells that make a lot of fatty acids and sterols, such as adipose, liver, the mammary glands, testes and adrenal glands, a substantial fraction of glucose is utilized to make NADPH.

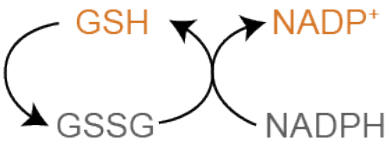

Figure 28: The glutathione:NADPH antioxidant system. The oxidized form of each molecule is shown in orange, the reduced form is in blue.

NADPH is Important for Fighting Oxidative Damage

The other main role of NADPH is to generate reduced glutathione (GSH) from oxidized glutathione (GSSG) by this reaction, catalysed by Glutathione Reductase:

$$NADPH + GSSG \rightarrow NADP^+ + GSH \quad (36)$$

Reduced glutathione is the most important endogenous antioxidant in mammalian cells[158]. When reactive oxygen species, such as free radicals or peroxides are generated, GSH transfers a proton to the oxidative species neutralizing it. This process is shown in Figure 28. Reduced glutathione (GSH) can become oxidized instead of an important protein or lipid in the cell. The oxidized glutathione (now GSSG) can be regenerated by NADPH, and can continue to scavenge for other potentially dangerous oxidants. When cells are exposed to oxidative damage, the pentose phosphate pathway is extremely important for mounting the response and restoring equilibrium.

[158] It also maintains other antioxidants such as Vitamins C and E in their active (reduced) form.

In some cells, such as red blood cells NADPH is the *only* way to reduce glutatione[159]. This makes red blood cells prone to hemolysis[160] and is a common trait in disorders of the pentose phosphate pathway.

[159] We will talk more about how glutathione is generated in the lecture on non-protein compounds generated from amino acids.

[160] The breakage of red blood cells.

Dietary Antioxidants

While GSH is able to scavenge reactive oxygen species on its own, this system is extended by two key vitamins, C and E. This occurs via a series of reactions wherein electrons are passed from Vitamins E and/or C to glutathione and then eventually to NADPH. This is shown in Figure II. Vitamin C is largely soluble, and can scavenge electrons from water soluble areas. Vitamin E is lipophilic and functions to alleviate the oxidative damage to lipids and lipid-bound proteins.

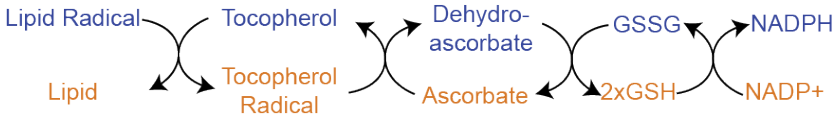

Figure 29: The glutathione:NADPH antioxidant system and Vitamins C and E. The oxidized form of each molecule is shown in orange, the reduced form is in blue. Ascorbate is the oxidized and active molecular form of Vitamin C, while α-tocopherol is the molecular form of Vitamin E

Many foods have high levels of antioxidants, and some of these such as the polyphenols present in blueberries have been suggested to alleviate oxidative damage, and reduce some chronic diseases [Pandey and Rizvi, 2009]. While it is true that these compounds have some *in vitro* anti-oxidant activity in isolation, there is very little evidence that these anti-oxidants have relevant functional antioxidant ability in humans. This is thought to be due to poor bioavailability, potency and cellular levels relative to Vitamins C and E. While Vitamins C and E are both necessary for humans at low quantities, the benefits of extra supplementation are less clear. A recent systematic review of antioxidant supplementation (including Vitamins A,C,E and Selenium[161]) not only demonstrated no beneficial effects on mortality, but found that supplementation led to a 4% increase

[161] An essential cofactor for Glutathione peroxidase, an enzyme which removes peroxide using reduced glutathione.

in mortality [Bjelakovic et al., 2012]. It is possible that there may be some benefits in populations exposed to substantial oxidative stress (people who smoke, live in areas with high levels of air pollution, consume large amounts of alcohol or individuals with non-alcoholic fatty liver disease), but in the general population there seems to be no measurable benefit of supplementation.

NADPH is Required for Innate Immunity

A third major role of NADPH is the oxidative burst. One way in which immune cells can try to destroy invading cells is to use NADPH to generate reactive oxygen species. Phagocytic cells first engulf the bacteria or fungi, then using the enzyme NADPH Oxidase, use NADPH to generate a superoxide molecule. This reacts with proteins, membranes and nucleic acids in the bacteria eventually destroying the invading cell. As you might expect this destructive process must be tightly controlled to avoid oxidative damage to normal tissues.

Disorders of the Pentose Phosphate Pathway

Mutations in G6PDH Mutations in the gene for G6PDH (symbol is *G6PD*) can have varying effects depending on the particular amino acid that is changed[162]. In the most serious cases, where there is effectively no detectable G6PDH activity. This is the most common enzymatic defect, affecting an estimated 400 million people worldwide. These patients are extremely prone to oxidative damage, and can have a buildup of Glucose-6-Phosphate. They are also very sensitive to certain infections and foods, notably fava beans[163] Interestingly, carriers of the mutant G6PD allele also have partial immunity to malaria, which requires host-derived NADPH[164]. This selective benefit may explain why, in contrast to other inborn errors of metabolism, Favism has persisted in human populations.

[162] *G6PD* is on the X-chromosome, so primarily this defect affects males.

[163] This disorder was once known as *Favism*, and has been described since antiquity. For more details see the review by Luzzatto and Arese [2018].

[164] Here is a public health issue to consider (or write a report on if you are really interested). One of the major antimalarials is a drug called primaquine. This drug is effective at reducing NADPH levels and reducing susceptibility to malaria. However, if that person has a deficiency in *G6PD*, they will be probe to primaquine-induced hemolysis, because this can exacerbate inborn defects in NADPH production [Chatterjea et al., 1961]. Since both malaria, and *G6PD* deficiency are both most prevalent in sub-Saharan Africa, it is important that individuals be screened prior to primaquine administration [Howes et al., 2013].

Alcohol Metabolism

Almost three quarters of the population of the United States report drinking alcohol in the past year with a wide range of consumption across the population[165]. Virtually all the alcohol we ingest is absorbed, and the vast majority of it must be metabolized by the liver. There are two major pathways that are engaged in this process. The first involves this sequence, catalyzed by alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and Acetyl-CoA Synthase (ACS). This is diagrammed in Figure IIA:

[165] This is *not* a normal distribution, as the top decile of alcohol consumers average over 10 drinks per day. See the visualization at `https://www.washingtonpost.com/news/wonk/wp/2014/09/25/think-you-drink-a-lot-this-chart-will-tell-you` to see this skewed distribution.

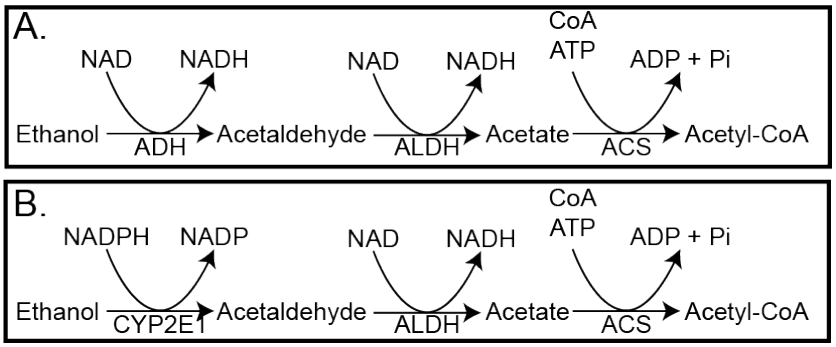

Figure 30: Alcohol metabolism into Acetyl-CoA via A) Alcohol Dehydrogenase or B) *CYP2E1*.

Hopefully by now you can determine that for the ADH-dependent pathway there are 15 ATP equivalents generated, and one ATP used, so the net result is 14 ATP per ethanol molecule. If you recall from the energy balance lecture, the Atwater value of ethanol is 7 kcal/g so more than carbohydrates or protein, but a bit less than lipids. Still, that is a lot of energy to deliver to the liver. The result is that the Acetyl-CoA is often diverted to lipogenesis, which can contribute to MASLD[166], and while cataplerosis may be activated (recall that Acetyl-CoA activates Pyruvate Carboxylase, there is no corresponding increase in amino acids or glycerol from other tissues. This results in depletion of glycogen from the liver and the reduction of TCA intermediates). The situation gets worse in chronic drinkers, where the ADH enzyme cannot process the alcohol effectively enough. This results in the transcriptional activation of Cytochrome P450 enzyme E1 (CYP2E1). This set of reactions (shown in Figure IIB) now *use* NADPH rather than generating NADH. This means that the liver's antioxidant capacity is reduced, and the chances of oxidative stress and chronic liver damage (known as alcoholic fatty liver disease) can occur. While MASLD is increasing in prevalence, most end-stage liver disease is due to chronic alcohol consumption. This is thought to be a combination of lipid accumulation and oxidative damage.

[166] Metabolic Dysfunction-Associated Steatotic Liver Disease

The Cytochrome P450 system detoxifies many other substances. In addition to alcohol, other Cytochrome P450 enzymes cause the enzymatic alteration (generally for purposes of stabilization and excretion) of many other compounds. This is an important process whereby the liver prepares and removes inessential compounds from our bodies. An interesting example is caffeine, which is hydroxylated by *CYP1A2*. There are common variants in *CYP1A2* that result in slower metabolism of caffeine. These individuals have higher sustained spikes in caffeine in their blood, and may be at slightly higher

risk for caffeine-induced hypertension [Palatini et al., 2009].

GENETIC VARIATION IN *ALDH2* IS THE MOST COMMON METABOLIC ENZYME VARIANT WORLDWIDE. Approximately 30-40% of individuals of Asian descent have only one active copy of *ALDH2* the second enzyme in both the alcohol metabolism pathways we have described. This results in elevations in both ethanol and acetaldehyde for longer periods of time, often resulting in flushing, nausea, heart palpitations and worsened hangovers. This differential metabolism is also associated with increased risks of liver disease and alcohol-associated cancers at equivalent alcohol intakes.

Reflection Questions

1. When a person consumes a large amount of alcohol, ADH and ALDH metabolize it to Acetyl-CoA while generating large amounts of hepatic NADH. Analyze how this elevation in hepatic NADH would affect TCA cycle activity, and predict the downstream consequences for: (a) fatty acid oxidation, (b) gluconeogenesis, and (c) the fate of excess Acetyl-CoA in the liver.

2. Compare the redox consequences of moderate alcohol consumption (metabolized primarily via ADH) with chronic heavy alcohol consumption (which induces CYP2E1). Evaluate why CYP2E1 induction shifts the metabolic burden in a way that substantially increases risk of oxidative liver damage, using the NADPH/glutathione antioxidant system in your answer.

3. A supplement company claims their blueberry-derived polyphenol product reduces cellular oxidative stress as effectively as vitamin E supplementation. Based on your knowledge of *in vitro* vs. *in vivo* antioxidant activity, the GSH/NADPH system, and bioavailability, evaluate this claim and describe what evidence would be needed to support it.

Glycogen and Metabolism During Exercise

Glycogen is an important storage macromolecule. While we injest polysaccharides in a variety of forms, glycogen is the major carbohydrate storage form in mammals. This unit will cover the roles of glycogen, and how intracellular and extracellular signals result in glycogen synthesis or release. Finally we will discuss the consequences of abberant glycogen storage. For more details on this topic, we recommend Bollen et al. [1998] and chapter 11 of Lippincott's Illustrated Reviews: Biochemistry[167].

[167] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Evaluate how the structure of glycogen allows for compact but accessible storage of glucose molecules.
- Understand how glycogen synthesis and glycolysis are regulated by intracellular metabolites.
- Explain how protein phosphorylation regulates Glycogen Synthase and Glycogen Phosphorylase activities and how extracellular signals affect glycogen metabolism.
- Assess the tissue-specific roles of insulin, adrenaline and glucagon in glycogen storage.
- Distinguish between the functions of glycogen in liver, adipose and muscle and evaluate how alterations in glycogen metabolism affect the physiological functions of these tissues.
- Explain how glycogen storage diseases can occur and how specific genes result in different pathophysiologies depending on both the gene, and tissue where it is expressed.

Key Concepts and Vocabulary

- Glycogenesis
- Glycogenolysis
- Protein Phosphorylation
- Allosteric Regulation
- Adrenergic Signaling
- Branch Points
- Inborn Errors of Metabolism

Structure and function of glycogen

Glycogen is a homopolymer of glucose units connected to each other by α1-4 or α1-6 glycosidic linkages[168]. This allows for many molecules of glucose to be compactly stored in the cell, and then made available upon energy or glucose demand. A series of α1-4 bonds result in a more or less straight chain of glucose molecules, while a α1-6 linkage results in a branch point. A single glucose monomer can have both an α1-4 and α1-6 linkage, and in glycogen these are typically spaced 8-12 glucose molecules apart (see Figure

[168] This nomenclature refers to a link between the #1 position of one molecule of glucose (the anomeric carbon) and either the #4 or the #6 position on the next glucose.

31). Compare this to the structure of the major dietary polysaccharides we discussed earlier in this unit (Table 26).

Table 26: Structures of some common polysaccharides. Which of these can be digested by human digestive enzymes?

Molecule	Main Linkages	Branches
Glycogen	α1-4	α1-6 (every 8-12)
Amylose	α1-4	none
Amylopectin	α1-4	α1-6 (every 24-30)
Cellulose	β1-4	none

Role of glycogen branching

The branched structure of glycogen means that a single macromolecule can be both very compact, but have many free glucose ends[169]. If glycogen was totally linear then glucose could only be released one at a time from the one free end of a glycogen molecule. By having many branch points, multiple enzymes that liberate glucose from glycogen[170] can release glucose from the many reducing ends at the same time. This allows for rapid mobilization of glucose when needed.

[169] These free ends are known as reducing ends.

[170] Humans only have two enzymes that can do this, one is Glycogen Phosphorylase in human tissues, the other is α-amylase in our digestive tract.

Because branch points need to be removed by a separate enzyme[171], overly branched glycogen is extremely compact, but its digestion may be limited by how well Glycogen Phosphorylase and the glycogen debranching enzyme can work in concert. Defects in debranching enzyme result in a glycogen storage disease characterized by an inability to eliminate these branch points (see the section on Glycogen storage diseases below).

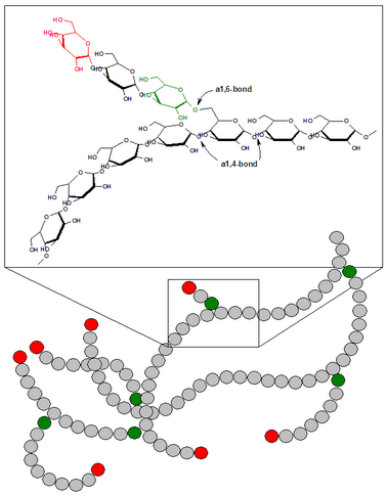

Figure 31: The structure of glycogen. Note the location of branch points (α1-6 glycosidic linkages in green) and reducing ends (red) From https://commons.wikimedia.org/w/index.php?curid=611992.

[171] known as glycogen debranching enzyme or amylo-alpha-1, 6-glucosidase (encoded by the *AGL* gene).

Different tissues use glycogen for different purposes

Glycogen is an easily accessible source of glucose for many tissues. The content of glycogen ranges from 1-3% of total weight in muscle tissue to up to 10% of total mass in a well fed liver. Since glycogen is very hydrophilic, it is estimated that approximately 3g of water are bound for every gram of glycogen [Olsson and Saltin, 1970, Fernández-Elías et al., 2015]. This is one reason why very low carbohydrate diets can result in rapid, initial weight loss, as glycogen is rapidly depleted. Based on what we have already learned about how glucose and phosphorylation differs between cells, different tissues use glycogen for slightly different reasons. In general, muscle cells use glycogen when energy is needed, for example during exercise. Liver cells on the other hand store glycogen to make glucose available for itself and other tissues, rather than for energy.

Glycogen is an important energy source. Muscle tissue can have dramatic and rapid depletions of ATP in response to exercise. The initial resource to replenish ATP is the creatine phosphate system described in Figure 32. Think of creatine phosphate like the battery on your laptop, and can supply (or store) excess ATP[172]. The amount of creatine phosphate can be quickly depleted, so muscle cells next

[172] We will discuss this in the unit on non-protein compounds derived from amino acids later in the semester.

turn to glycogen for glucose. These glucose molecules typically undergo glycolysis (depending on the muscle fiber type) then the TCA cycle and electron transport chain to make ATP[173]. As we will describe below, the primary signals for glycogen metabolism in muscle are energy dependent.

[173] Recall, the absence of glucose-6-phosphatase means that liberated glucose-6-phosphate will not be dephosphoryated for extracellular release.

GLYCOGEN PLAYS AN IMPORTANT ROLE IN ENDURANCE EXERCISE. During prolonged exercise, muscle glycogen can be dramatically depleted. To prevent this, athletes often ingest carbohydrates during exercise in order to continuously provide energy to the contracting muscles. Another approach is to deplete glycogen levels prior to exercise, then eat a large carbohydrate rich meal. This results in *more* glycogen storage than the normal fed states, a condition known as glycogen super-compensation. These extra glycogen stores are thought to fuel a longer sustained effort during exercise[174]. For more information on this concept see Hawley et al. [1997].

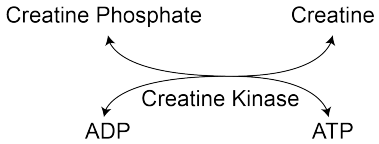

Figure 32: The creatine phosphate system. When ATP levels are depleted, the first reserve is creatine phosphate, which can transfer its high-energy phosphate group to ATP. In times of plenty, creatine phosphate can be regenerated from excess ATP. Muscle has large amounts of the enzyme that catalyzes this reaction, Creatine Kinase

[174] If you are interested in exploring this more, consider the bonus group assignment Glycogen Stores and Sports Nutrition, or doing a research project on this topic.

GLYCOGEN FROM THE LIVER PLAYS AN IMPORTANT ROLE IN GLUCOSE HOMEOSTASIS. The liver on the other hand mobilizes glycogen in order to maintain glucose levels in blood. Whether due to fasting or exercise the body needs to make glucose available for many tissues. This is especially important for the brain, which is very poor at converting fatty acids into ATP. As such, glycogen levels in the liver are generally controlled by indicators of glucose levels, such as glucose-6-phosphate. Unlike the muscle, *once glycogen is catabolized into glucose-6-phosphate it can be dephosporylated into glucose and released from hepatocytes* for transport to other tissues.

Regulation of glycogen synthesis

Glycogen is stored when glucose and energy are plentiful. After a typical meal, in a healthy person glycogen levels increase by about 50% peaking about 4h after a meal [Taylor et al., 1996]. This is due to a combination of increased glucose availability and the postprandial actions of insulin. Glycogen is synthesized starting from Glucose-6-phosphate via the following series of reactions:

$$Glucose-6-phosphate \rightleftharpoons Glucose-1-phosphate \tag{37}$$

$$Glucose-1-phosphate + UTP \rightleftharpoons UDP-Glucose + PPi \tag{38}$$

$$UDP-Glucose + Glycogen_n \rightarrow Glycogen_{n+1} \tag{39}$$

There are two important things to note about this process, one is that the first two reversible steps mean that the levels of G6P are extremely important in this process[175]. The second is that by using UTP to activate glucose, this is an *energy consuming process*. This means that there is an energetic cost (the equivalent of one ATP phosphodiester bond) to storing glycogen[176]. The third reaction is catalyzed by the enzyme Glycogen Synthase[177] and that is the main point of regulatory control in glycogen synthesis.

[175] Recall from the lecture on glycolysis that G6P levels are controlled by the levels of glucose in the cell, the activity of hexokinase/glucokinase and the activity of PFK1, the rate limiting step in glycolysis.

[176] Put another way, this means that to store and then release 1000 molecules of glycogen, you will use up the equivalent of 1000 ATP molecules. In the end, you have used up 1000 ATP molecules but end up where you started, with available glucose. This is another example of metabolic inefficiency, and is measured as part of the energy released during diet-induced thermogenesis.

[177] There are two isoforms of Glycogen Synthase, one expressed in muscle and brain (*GYS1*) and one expressed in the liver and fat, encoded by the *GYS2* gene.

Glycogen Synthase is activated by Glucose-6-phosphate

Both isoforms of Glycogen Synthase are allosterically activated by glucose-6-phosphate as first described in the late 1950s by Leloir et al. [1959]. G6P levels are increased when glycolysis is low, but glucose levels are high. This is generally a situation where nutrient levels are high, but energy demand is low. This is a good time to store extra glucose, so this makes physiological sense.

Kinase	Signal
PKA	Adrenaline/Glucagon
GSK3	Insulin (inactivates)
AMPK	Energy Stress

Table 27: Protein kinases that phosphorylate and inactivate Glycogen Synthase.

Extracellular control and signaling

In addition to this metabolite-level control, Glycogen Synthase is also regulated by reversible protein phosphorylation [Villar-Palasí and Larner, 1960][178]. There are several protein kinases that regulate Glycogen Synthase, and as a general rule they result in the *inactivation* of the enzyme. These kinases are summarized in Table II. Insulin *activates* Glycogen Synthase, and it does so by *dephosphorylating* these sites (see Figure II). Part of this mechanism is by reducing the activity of the kinases (especially GSK3 [179] and PKA) but insulin also functions by activating protein phosphatase activity towards Glycogen Synthase. This is accomplished via a series of proteins that specifically target a protein phosphatase on the glycogen particle. The precise mechanisms by which insulin promotes this dephosphorylation are still unclear. At the same time that Glycogen Synthase is being dephosphorylated (and activated), Glycogen Phosphorylase[180] is also dephosphorylated and inactivated.

[178] This was one of the earliest described examples of metabolic control by protein phosphorylation.

[179] GSK3 is Glycogen Synthase Kinase that phosphorylates Glycogen Synthase and inactivates it thus decreasing the rate at which glycogen is produced. Think of why insulin may deactivate GSK3.

[180] The enzyme that cleaves glucose from an existing glycogen molecule

Separate from the effects of insulin on Glycogen Synthase activity, recall that insulin will also promote glucose uptake (in muscle and fat tissues). This increased glucose flux will result in more Glucose-6-phosphate in the cell and allosteric activation of

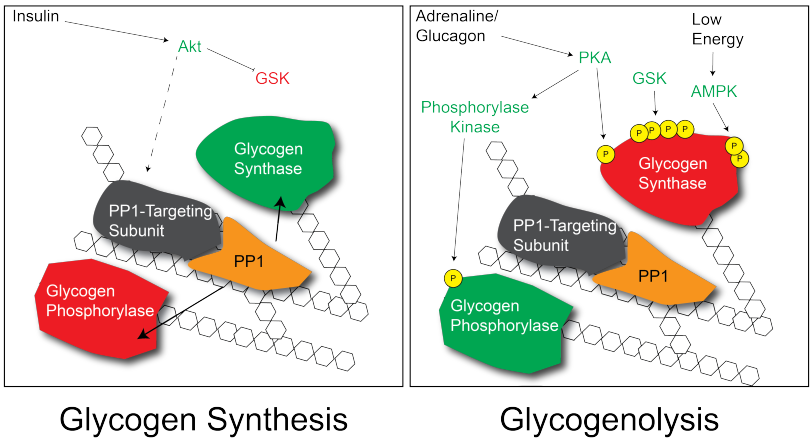

Figure 33: Post-translational regulation of glycogen metabolism. Red indicates inactive enzymes, while green indicates active enzymes. PP1 is a protein phosphatase that removes the phosphate groups from both Glycogen Synthase and Glycogen Phosphorylase. The dashed arrow indicates the mechanisms by which this phosphatase are activated are currently unknown.

Glycogen Synthase. Therefore there are at least two ways by which insulin can promote glycogenesis.

Regulation of glycogen breakdown

Glycogen is broken down to release glucose in two steps[181]:

$$Glycogen_n \rightarrow Glycogen_{n-1} + Glucose - 1 - phosphate \quad (40)$$

$$Glucose - 1 - phosphate \leftrightarrow Glucose - 6 - phosphate \quad (41)$$

The fate of Glucose-6-phosphate depends on the relative activities of PFK1, Glucose-6-phosphate dehydrogenase[182] and, in the case of liver cells Glucose-6-phosphatase. Generally, in the muscle the liberated Glucose-6-phosphate enters glycolysis whereas in the liver it is dephosphorylated and released as glucose when energy is needed in the body.

[181] This mechanism is specific to the α(1-4) linkages, not the branch points, which will be described later.

[182] Leading towards the pentose phosphate pathway.

Intracellular control

The first, and rate limiting step of glycogenolysis is catalyzed by an enzyme named Glycogen Phosphorylase[183]. Glycogen Phosphorylase is allosterically activated by AMP. This activation by AMP is blocked by the presence of ATP or Glucose-6-phosphate. AMP is increased when there is energy demand, so if there is a need for energy, Glycogen Phosphorylase gets activated. This can be over-ridden when ATP is plentiful (indicating a lack of energy stress) or Glucose-6-phosphate is elevated (indicating sufficient glucose levels). While all three isoforms respond similarly in direction, the muscle enzyme is much more sensitive to activation by AMP than the liver enzyme.

[183] There are three isoforms of this gene, *PYGL*, *PYGM* and *PYGB* which are expressed in the liver, muscle and brain, respectively.

This is due to structural differences in the AMP-binding pocket between the muscle and liver isoforms [Rath et al., 2000]. As part of its reaction mechanism, Glycogen Phosphorylase also uses Vitamin B_6-derived Pyridoxal phosphate as a prosthetic group[184].

[184] Vitamin B_6 has many other roles, but has been shown to be effective in treating McArdle's disease, a genetic deficiency in *PYGM*.

Extracellular control and signaling

The activation of Glycogen Phosphorylase by AMP can be overridden by protein phosphorylation by an enzyme named Phosphorylase Kinase (see Figure 34). Once phosphorylated, the enzyme functions as if it is in the AMP-activated state. The phosphorylation of Glycogen Phosphorylase is activated by PKA dependent signaling, induced by either glucagon in the liver or adrenaline in liver, muscle and other tissues. Similarly, PKA-dependent signaling phosphorylates and inactivates Glycogen synthase. This means that adrenergic signaling turns Glycogen Phosphorylase on, and Glycogen Synthase off simultaneously[185]. This makes sense since adrenergic signals would want to increase release of glucose and reduce glycogen storage. A summary of the effects of reversible protein phosphorylation on the enzymes of glycogen metabolism is shown in Table II.

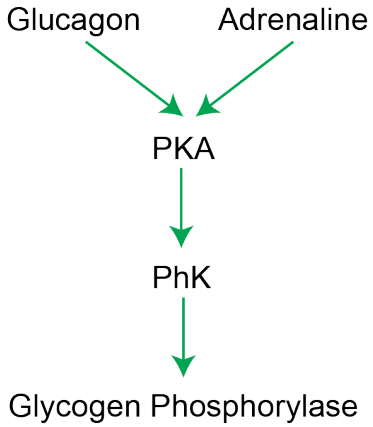

Figure 34: Hormonal regulation of glycogen phosphorylase through PKA and Phosphorylase Kinase (PhK).

[185] Also, insulin turns Glycogen Phosphorylase off, and Glycogen Synthase on.

Regulation of glycogen branching and removal of branch points

The generation and removal of glycogen branch points is an important part of glycogen metabolism. Glycogen Phosphorylase can only cleave at α1-4 bonds and cannot proceed past α1-6 linkages. Whenever an α1-6 linkage is encountered, the Glycogen Debranching Enzyme is recruited, which removes the α1-6 link and allows for Glycogen Phosphorylase to proceed. Currently, there is no strong data suggesting that either Glycogen Branching or Debranching Enzymes are regulated by metabolites, or hormonal signals, but inappropriate activity can result in under- or over-branched glycogen.

Enzyme	Effects of Phosphorylation
Glycogen Synthase	Inactivates - Less Synthesis
Glycogen Phosphorylase	Activates - More Breakdown

Table 28: Summary of how protein phosphorylation regulates Glycogen Synthase and Glycogen Phosphorylase.

Glycogen storage diseases

There are a variety of rare, heritable defects which result in abberant glycogen metabolism (see Table II). Some of these result in an inability to synthesize glycogen, while others prevent glycogenolysis, resulting in pathologically large particles of glycogen resulting in cell death. Some common glycogen storage diseases, and the affected enzymes are below.

Disease	Enzyme	Predicted Glycogen Levels
Fanconi-Bickel syndrome	*SLC4A2*	
von Gierke's disease	*G6PC*	
Tarui's disease	*PFKM*	
GSD Type 0	*GYS2*	
Cori's disease	*AGL*	
Andersen disease	*GBE*	
McArdle disease	*PYGM*	
Hers' disease	*PYGL*	
GSD type IX	*PHKA1/2*	

Table 29: Some examples of glycogen storage diseases and the gene that is mutated. *G6PC*: Glucose-6-phosphatase; *PFKM*: Muscle PFK1; *PHKA1/2*: Phosphorylase kinase; *SLC4A2*: GLUT2. Before class, try to predict what the loss of activity of these proteins would do to glycogen levels.

Reflection Questions

1. A patient with type 1 diabetes consumes a large carbohydrate meal after a prolonged bout of exercise. Analyze what happens to glycogen synthesis in liver and muscle compared to a healthy person, tracing the effects through glucose uptake, glucose-6-phosphate levels, and Glycogen Synthase phosphorylation state. Then explain why inadequate glycogen repletion after exercise creates a risk of fasting hypoglycemia hours later — and why this delayed risk is particularly dangerous overnight.

2. Von Gierke's disease is caused by loss of glucose-6-phosphatase in the liver. Evaluate why this specifically impairs blood glucose homeostasis during a fast even though hepatic glycogen can still be mobilized, and explain why the same enzyme deficiency would have a different metabolic consequence in muscle tissue.

3. An endurance athlete practices glycogen supercompensation before a marathon: first depleting glycogen with hard exercise, then consuming a large carbohydrate meal. Apply your knowledge of both allosteric and hormonal regulation of Glycogen Synthase to explain mechanistically why glycogen stores after this protocol can exceed normal fed-state levels.

Gluconeogenesis and Chronic Fasting

Glucose is not an essential nutrient, and can be generated from a variety of precursors including glycerol, lactate and amino acids. This is important because several tissues, including the brain, are highly dependent on glucose levels. As such, the body maintains blood glucose levels in a very narrow range, and gluconeogensis is essential to ensuring glucose is available in the blood. This unit will describe the function and regulation of gluconeogenesis including its regulation by internal and external signals. For more details on gluconeogenesis, refer to Chapter 17 of Biochemistry: A Short Course[186] *and Chapter 10 of Lippincott's Illustrated Reviews: Biochemistry*[187]*, both on reserve.*

[186] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

[187] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Describe the tissues where gluconeogenesis occurs and the key enzymatic determinants of this specificity.
- Explain the major precursors of gluconeogenesis, including where they are derived from and at what step they integrate.
- Evaluate how the flow of each these precursors is regulated differently, dependent on where they enter the gluconeogenic pathway.
- Analyse the energetic costs of gluconeogenesis, given the precursor substrate.
- Describe how chronic activation of gluconeogenesis results in ketone body production from the liver.
- Understand the importance of the Cori and Cahill cycles in recycling waste products back to glucose.
- Recognize the key steps by which gluconeogenesis is controlled by allosteric control, protein phosphorylation and transcriptional changes, especially in response to insulin, glucagon/adrenaline and Cortisol.
- Evaluate how the cell ensures that gluconeogenesis and glycolysis do not occur simultaneously.

Key Concepts and Vocabulary

- Cori Cycle and Cahill Cycle
- Glucocorticoids, including Cortisol
- Gluconeogenic Substrates
- Direct Regulation (in the liver) vs Indirect Regulation of Gluconeogenesis
- Pyruvate Carboxylase and its regulation
- PEPCK and G6Pase and their regulation
- Alanine Aminotransferase (ALT)

The Importance in Maintaining Blood Glucose Levels

Glucose is not an essential macronutrient. Unlike vitamins, minerals, essential amino acids, and ω-3/6 fatty acids we are able to make glucose even if there is no dietary source. This process is called gluconeogenesis. While many of the tissues that we focus on in this course can use a variety of substrates for fuel, some tissues are highly dependent on glucose. Among these are the brain, and red blood cells. Adding to this problem, neurons are very poor at storing glucose as glycogen, which means they need a constant supply of glucose from the blood to maintain their function. While the brain is generally only 2% of the mass of a human, it consumes around 20% of the glucose in the body [Erbsloh et al., 1958]. Even individuals who consume extremely low carbohydrate diets are able to maintain their blood glucose near the normal range[188] [Bueno et al., 2013]. The ability to maintain blood glucose, with limited dietary carbohydrate ingestion is the primary role of gluconeogenesis. In healthy humans, when gluconeogenesis is active, we produce the carbohydrate equivalent of over 6 cups of cooked rice per day[189].

[188] While individuals on ketogenic diets still use a lot of glucose for brain function, there is evidence that in those individuals the brains can adapt to use ketone bodies as an energy source as well. We will discuss this in the lectures on lipid oxidation later on in the semester.

[189] Calculated from values in Rothman et al. [1991], and the USDA food database.

Gluconeogenesis Primarily Occurs in the Liver

The primary glucose producing tissues are the liver and the kidneys, though in terms of circulating glucose the liver predominates. The molecular determinant of this is the presence of the enzyme Glucose-6-Phosphatase which catalyzes the removal of phosphate from Glucose-6-Phosphate, allowing it to be exported from the cell. While gluconeogenic substrates can become converted to Glucose-6-Phosphate through similar pathways described below, the final phosphate removal and release of glucose only occurs in cells with this enzyme.

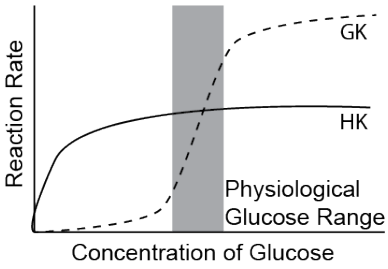

Figure 35: Schematic of the kinetics of glucokinase (GK) and hexokinase (HK). Not the differences in K_m, V_{max} and co-operativity between these enzymes.

Glucokinase plays an important role in this process. If you recall from the lecture on glycolysis, the enzyme kinetics of the Glucokinase is an important site of regulation (see Figure 35. Muscle and adipose tissues have hexokinase, which is very efficient at phosphorylating even low concentrations of Glucose. Glucokinase, which is present in the liver, has very low kinetic efficiency at low glucose concentrations. This means that when glucose levels are low in the blood, Glucokinase will be inactive and Glucose-6-Phosphate dephosphorylation will not be undone.

Gluconeogenic Substrates

There are several substrates that can be converted into glucose as illustrated in Figure 36. The main substrates are glycerol, lactate and alanine. The first thing to note is how similar the overlap of this pathway is to glycolysis. Most of the reactions in glycolysis are reversed in gluconeogenesis, and are catalyzed by the same enzymes, with only a few exceptions. One is that the Pyruvate Kinase step (from PEP to Pyruvate) is not reversible, and the other is that instead of PFK1, a different enzyme known as Fructose bisphosphatase (FBPase1) is used to generate Fructose-6-Phosphate. As you may have guessed, both of these are key sites of regulation. As you read about these reactions, consider that the biochemical steps where glycerol, lactate, or alanine enters the pathway, and see how that dictates what sites of regulation are important to the control its flow to glucose.

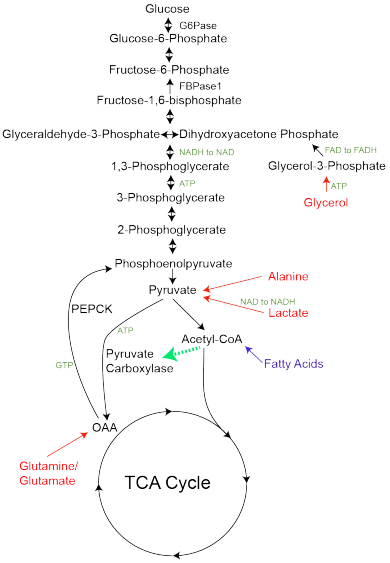

Figure 36: Schematic of gluconeogenesis. Red indicates key gluconeogenic substrates while green indicates energy consuming or generating steps.

Glycerol

Glycerol is derived from the breakdown of triglycerides and is increased during lipolysis. That process releases both glycerol and free fatty acids, both of which traffic to the liver. While fatty acids (shown in blue in Figure 36) are *unable* to be converted to glucose, they play a role in activating Pyruvate Carboxylase via Acetyl-CoA and providing energy to drive gluconeogenesis. Glycerol on the other hand becomes activated by phosphorylation, then is converted into DHAP. DHAP and GA3P are combined by Aldolase into Fructose-1,6-bisphosphate, which is then dephosphorylated by FBPase1. Figure II shows how glycerol flows into glucose.

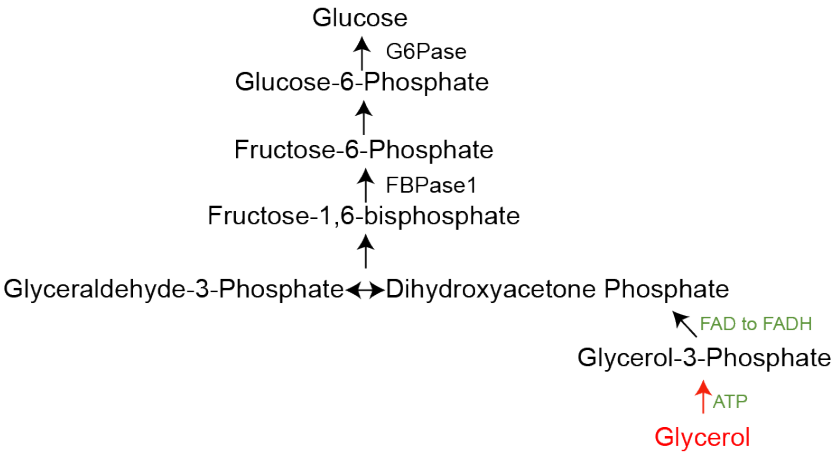

Figure 37: Schematic of gluconeogenesis from glycerol. Red indicates key gluconeogenic substrates while green indicates energy consuming or generating steps. Based on this diagram, you can see that per glycerol 1 ATP is used, and 1 $FADH_2$ is generated. This is a net of +0.5 ATP/glycerol, or since two glycerol molecules are needed to make a glucose, a net ATP increase of 1 ATP/glucose generated.

Lactate and the Cori Cycle

Lactate, the product of anaerobic glycolysis can be inter-converted back to pyruvate via the reversible actions of Lactate Dehydrogenase. This is an important inter-organ cycle called the Cori Cycle[190]. This cycle involves the anaerobic breakdown of glucose to lactate in the muscle, followed by its re-synthesis back to glucose in the liver. This re-formed glucose now travels back to the muscle (see Figure 38). Overall this cycle consumes four ATP molecules, but is an important way to ensure that the muscle has sufficient glucose available for anaerobic metabolism. We discussed this previously, with respect to metabolic efficiency and the basal metabolic rate. If you go through a round of the Cori Cycle, you end up with exactly the same glucose molecule, but you have had to burn four ATP molecules. This is *inefficient* and would increase the basal metabolic rate. The route by which lactate becomes glucose is shown in Figure 39.

[190] Or the Lactic Acid Cycle. This is named after Carl and Gerty Cori who won the Nobel Prize in Medicine or Physiology for this and her work on glycogen metabolism. Gerty Cori was the first woman to win the Nobel Prize in Medicine or Physiology.

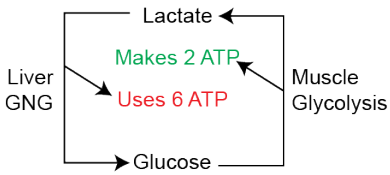

Figure 38: Schematic of the Cori Cycle. Each turn through this cycle uses up 4 ATP (6 ATP used in the liver, 2 generated in the muscle).

Alanine and other Amino Acids

Several amino acids can be converted into glucose. This can be the case either when there is excessive dietary protein, or if proteins (primarily in the muscle) are broken down and sent to the liver. The class of amino acids that can become glucose is known as the *glucogenic* amino acids. The amino acids that cannot be converted to glucose are known as ketogenic amino acids[191]. The catabolic routes of the glucogenic amino acids vary but the most important of them is Alanine. As we discussed regarding the fates of Pyruvate, Alanine and Pyruvate can be inter-converted via the actions of Alanine Aminotransferase (ALT):

$$Alanine + \alpha - Ketoglutarate \rightleftharpoons Glutamate + Pyruvate \tag{42}$$

This enzyme is important for making Alanine (for example when levels are low and Pyruvate levels are high), but also can make pyruvate for gluconeogenesis when alanine levels are high and pyruvate levels are low. As shown in Figure II the conversion of Pyruvate to Phosphoenolpyruvate[192] is not a simple reversible reaction. Instead, for Pyruvate to undergo gluconeogenesis, it first must be carboxylated to oxaloacetic acid (OAA) by Pyruvate Carboxylase and then converted back to Phosphoenolpyruvate by the enzyme Phosphoenolpyruvate carboxykinase (PEPCK). Both of these enzymes are important sites of regulatory control. It is important to note that (absent the balancing influx of Alanine) activation of PEPCK is cataplerotic. It removes TCA cycle intermediates (OAA) and pushes them towards gluconeogenesis. This means that gluconeogenesis oc-

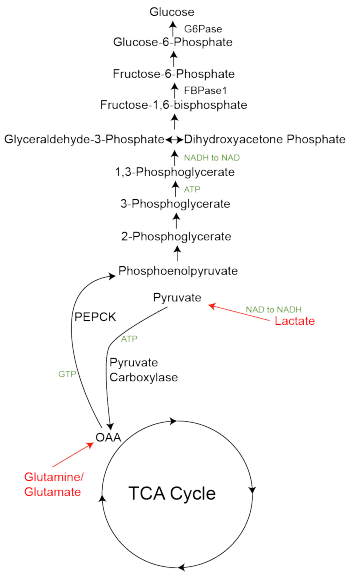

Figure 39: Schematic of gluconeogenesis from lactate. Based on this diagram, you can see that per lactate 2 ATPs and 1 GTP are used, and one NADH is generated and then consumed. This is a net of -3 ATP/lactate, or since two lactate molecules are needed to make a glucose, a net ATP use of 6 ATP/glucose generated.

[191] We will cover this in more detail in the amino acid catabolism lectures.

[192] This is the inverse of the Pyruvate Kinase reaction that was important for glycolysis.

curs at a cost of TCA cycle efficiency, since when OAA is depleted, Acetyl-CoA cannot enter the TCA cycle for energy production. This is the main reason why very low carbohydrate diets result in ketone body production. Gluconeogenesis depletes OAA, and Acetyl-CoA is converted into ketone bodies because it cannot enter the TCA cycle.

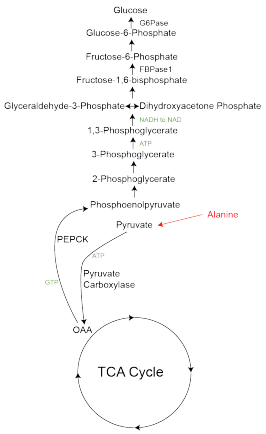

Figure 40: Schematic of gluconeogenesis from alanine. Based on this diagram, you can see that per alanine 2 ATPs and 1 GTP are used, and one NADH is consumed. This is a net of -5.5 ATP/alanine, or since two alanine molecules are needed to make a glucose, a net ATP use of 11 ATP/glucose generated. As we will discuss later in the semester, disposal of the amino group on alanine (which is now on Glutamate) requires the Urea cycle, which comes at a cost of an additional 4 ATP's. Therefore in total gluconeogenesis from alanine costs 15 ATP/alanine.

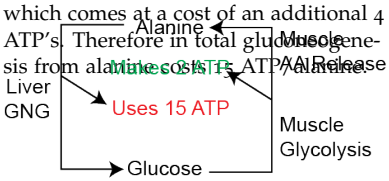

Figure 41: Schematic of the Cahill Cycle. Each turn through this cycle uses up 13 ATP (15 ATP used in the liver, 2 generated in the muscle).

ALANINE RECONVERSION TO GLUCOSE IS KNOWN AS THE CAHILL CYCLE OR GLUCOSE-ALANINE CYCLE. Similar to the Cori cycle, the cycle of Alanine release from muscle, conversion to glucose and then return to blood glucose is an important inter-organ metabolic loop. When muscle is broken down, Alanine is released and can be converted by the liver back into glucose for fuel. This is more costly than the Cori cycle, because Urea must be removed from Glutamate. This cycle uses 4 ATP equivalents for the Urea cycle and 11 ATP molecules for Gluconeogenesis, so requires a lot of energy from the liver. Unlike the Cori cycle, the Cahill cycle is not a closed circuit, as every round depletes amino acids from muscle, a result of muscle protein breakdown.

Energetic Demands of Gluconeogenesis

Gluconeogenesis is typically energetically costly. It requires a variable number of ATP molecules depending on the substrate. The energy consuming steps, are Pyruvate Carboxylase (one ATP), PEPCK, and Phosphoglycerate Kinase (1 ATP). Since at the Aldolase step, two three carbon precursors (GA3P and DHAP) are combined, this means that to make one molecule of glucose you need to follow this pathway twice. That means that to go from Pyruvate to Glucose you need 2 x 3 ATP equivalents plus two NADH equivalents (at the Glyceraldehyde Dehydrogenase step). Since each NADH is equivalent to

2.5 ATP molecules, gluconeogenesis from Pyruvate consumes 11 ATP equivalents per glucose produced. A summary for the main gluconeogenic substrates are shown in Table II.

Substrate	Gluconeogenesis	Urea Cycle	Total
Glycerol	+1	0	+1
Lactate	-6	0	-6
Alanine	-11	-4	-15

Table 30: Gluconeogenic energy use in terms of ATP equivalents.

Key Regulatory Steps in Gluconeogenesis

Since it is counterproductive to have gluconeogenesis and glycolysis operating simulataneously the regulation of these pathways is largely reciprocal. Generally when one pathway is activated, the other is inactivated. In the case of gluconeogenesis, the key points of regulation are Pyruvate Carboxylase, PEPCK, FBPase and Glucose-6-Phosphatase/Glucokinase. These are under a combination of acute (rapid and reversible, mainly allosteric) and chronic (slow and permanent, mainly transcriptional) control mechanisms. Each substrate has its own regulatory path too. For example, as shown in Figure II, Glycerol is not subject to PEPCK or PC-dependent regulation and only Alanine is subject to regulation at the ALT step. All gluconeogenic substrates are sensitive to the activities of FBPase1 and G6Pase.

Gluconeogenesis is regulated both directly and indirectly. Gluconeogenesis requires a supply of its substrates (glycerol, lactate and alanine). Therefore the transport of these molecules from other tissues to the liver is one major regulatory step. As an example, during prolonged fasting, proteins are catabolized and amino acids like Alanine are released. The increased availability of these amino acids results in more glucose production, while also preventing glycolysis (at the PK step) and tipping the ALT equilibrium towards making pyruvate. Another example of indirect regulation is when lipolysis is activated after Cortisol or Adrenaline stimulation. This provides substrates (glycerol), energy (from fatty acid oxidation) and acetyl-CoA ato activate Pyruvate Carboxylase. Gluconeogenesis can also be indirectly inhibited. One example is that insulin suppresses lipolysis. This reduces glycerol and fatty acid delivery to the liver and slows gluconeogenesis (on top of the direct effects of insulin).

Acute Regulation of Gluconeogenesis

As we discussed in the TCA cycle lecture, Pyruvate Carboxylase (PC) is an important anaplerotic enzyme. It is stimulated by Acetyl-CoA levels, to generate OAA from Pyruvate. In terms of gluconeogenesis, a key role of fatty acid-derived Acetyl-CoA is promoting glucose production by activating PC [Perry et al., 2015]. While fatty acids cannot directly be converted into glucose, this is one mechanism by which they induce gluconeogenesis. This has important consequences for understanding the relationships between the regulation of lipolysis on glucose production.

THE SECOND MAJOR SITE OF ACUTE CONTROL IS AT THE FBPASE1 STEP. Similar to PFK1, FBP1 is regulated by AMP, and F26bP though in this case the regulation is in the opposite direction (see Table 31). The levels of F26bP are controlled by the activities of PFK2[193] and FBPase2. In fact, these two activities are the same polypeptide, which comprises a bifunctional enzyme. FBPase2 will remove the phosphate from F26bP, depleting that important allosteric activator of PFK1. F26bP is a potent inhibitor of FBPase1, so its removal activates gluconeogenesis while stopping glycolysis. The same PKA-dependent phosphorylation that that reduced PFK2 activity activates FBPase2 activity resulting in less F26bP and F16bP will be directed towards glucose. In addition to the effects of F26bP, FBPase1 is inhibited by AMP. Since gluconeogenesis is so energy consuming, this regulatory step ensures that ATP is sufficient for gluconeogneesis to occur. A comparison of the regulation of FBPase1 and PFK1 is shown in Table 31 and more details about FBPase regulation can be found in this review: Okar et al. [2001].

[193] As we discussed in detail in the glycolysis lecture

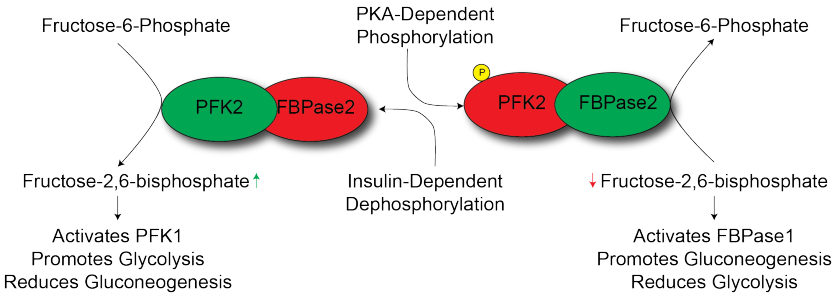

Figure 42: Co-ordinate regulation of PFK2/FBPase2 by protein phosphorylation. This is a bifunctional enzyme, and regulated *in the liver* such that either the phosphatase or kinase activity is activated, while the other activity is suppressed.

PKA, ACTIVATED BY GLUCAGON OR EPINEPHRINE PROMOTES GLUCONEOGENESIS IN THE LIVER. By inactivating PFK2 and activating FBPase2, PKA drives glucose production from precursor molecules in the liver. This, along with promoting the breakdown of glycogen

provides tissues with glucose in times of need. Gluconeogenesis is slower and more costly than glycogenolysis, but is able to use a wider variety of sources to make glucose.

Table 31: PFK1 and FBPase1 Regulation

Regulator	PFK1	FBPase1
F26bP	Activates	Inactivates
AMP	Activates	Inactivates
ATP	Inactivates	-
Citrate	Inactivates	-
PKA	Inactivates	Activates

Transcriptional Regulation of Gluconeogenesis

In contrast to PKA's effects activating gluconeogenesis, insulin potently suppresses the production of glucose. Since insulin is elevated in response to elevations in glucose, this suppression is a major part by which insulin reduces blood glucose[194]. One mechanism by which this is thought to occur is through transcriptional regulation, largely through the transcription factor FOXO. Insulin promotes the Akt-dependent phosphorylation of FOXO, which removes it from the nucleus, rendering it inactive. Several key gluconeogenic genes are FOXO targets including PEPCK and G6Pase. This means that when insulin inhibits FOXO function, the transcription of those genes is markedly reduced (see Figure 43). More information about the role of FOXO can be found in Barthel et al. [2005].

[194] The others being stimulation of glucose uptake and glycogenesis

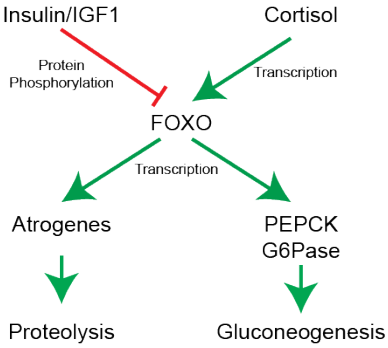

Figure 43: Glucocorticoid- and insulin-dependent regulation of FOXO and its relationship to gluconeogenesis and proteolysis.

Cortisol is another gluconeogenic hormone. By binding to its nuclear hormone receptor, glucocorticoids such as Cortisol can promote the transcription of Pyruvate Carboxylase, PEPCK and G6Pase. You can think of this as the opposite of the effects of insulin. By synthesizing more of these rate limiting enzymes, Cortisol can promote glucose production and make glucose available to the rest of the body during times of stress. Notably, glucocorticoid-induced hyperglycemia is a result of Cushing's disease[195] or prescription glucocorticoids such as predinosone or cortisone. In times of stress, one the main functions of Cortisol is to ensure proper supply of glucose to the brain.

[195] Overproduction of cortisol by the adrenal gland, often associated with pituitary or adrenal tumors.

Consequences of Unrestrained Gluconeogenesis

While gluconeogenesis is essential to maintaining blood glucose levels in times of glucose deprivation, activation of gluconeogenesis is a hallmark of both Type 1 and Type 2 diabetes. Insulin's ability to suppress gluconeogenesis is either absent (in type 1) or impaired (in type 2 diabetes). Diabetics therefore tend to have over-active gluconeogenesis, which results in elevated blood glucose. Insulin resistance promotes both direct (hepatic) and indirect activation of gluconeogenesis, and the rates of gluconeogenesis in diabetics are nearly double than in controls [Magnusson et al., 1992]. Even in the absence of dietary glucose, elevated glucose means that hyperglycemia may be present due to increased gluconeogenesis. Gluconeogenesis is there-

fore an active target for controlling blood glucose in diabetes, which now affects more than 29 million Americans, with another 86 million considered at risk[196].

[196] Centers for Disease Control and Prevention. Diabetes. https://www.cdc.gov/chronicdisease/resources/publicat 2016

Reflection Questions

1. A patient with type 2 diabetes has elevated fasting blood glucose despite not having eaten overnight. Trace the mechanisms responsible, explaining the roles of both the liver and adipose tissue. Include the direct effects of impaired insulin signaling on hepatic FOXO activity and gluconeogenic gene expression, and the indirect effects through dysregulated lipolysis in adipose tissue and substrate delivery to the liver.

2. Cortisol promotes transcription of PEPCK and G6Pase, driving gluconeogenesis. Cortisol is also orexigenic — it increases appetite and food intake. Evaluate how these two effects acting together could create a vicious cycle driving weight gain and worsening hyperglycemia in a patient with Cushing's disease, and predict how each arm of this cycle would reinforce the other.

3. During a prolonged five-day fast, a healthy person maintains near-normal blood glucose. Apply your knowledge of gluconeogenic substrates and their regulation to predict the sequence in which glycogen, lactate, glycerol, and alanine each become progressively more important, explaining the metabolic trade-offs — including the costs to muscle mass and TCA cycle efficiency — as the fast extends.

Carbohydrate Unit Integration Questions

1. A healthy person eats a meal of white rice, steamed broccoli, and a glass of milk. Trace the complete fate of the carbohydrates from digestion through metabolic disposal. Your answer should address: glycosidic bond specificity of digestive enzymes, transporter selection at the enterocyte, the incretin effect and insulin secretion, tissue-specific glycogen responses in liver vs. muscle, and the fate of the indigestible fiber from broccoli in the large intestine.

2. A patient with poorly controlled type 2 diabetes presents with fasting hyperglycemia, elevated triglycerides, and fatigue. Using your knowledge of the entire carbohydrate unit, explain how insulin resistance simultaneously impairs GLUT4-mediated glucose uptake, fails to suppress hepatic gluconeogenesis via FOXO, disrupts intestinal GLUT2 feedback, and promotes lipolysis in

adipose — and how the resulting excess Acetyl-CoA explains the hypertriglyceridemia.

3. During a marathon, an athlete hits the wall when muscle glycogen is severely depleted. Explain why glycogen depletion impairs performance, what gluconeogenic substrates the liver shifts to (including the Cori and Cahill cycles), how PDH regulation changes as lactate accumulates, and why consuming a sports drink containing both glucose and fructose could delay depletion more effectively than glucose alone.

4. A patient is prescribed prednisone — a synthetic glucocorticoid — for three months and develops new-onset hyperglycemia. Connect mechanisms from across the carbohydrate unit to explain this outcome, including glucocorticoid effects on gluconeogenic gene transcription, impaired insulin signaling in muscle and adipose, glycogen metabolism, and the orexigenic effect increasing dietary carbohydrate intake.

Part III

Lipids

Introduction to Lipids

This unit will cover lipid metabolism, with lectures on structure and properties, digestion, synthesis, oxidation and transportation. This particular lecture will cover the general properties of lipids, including fatty acid, steroid and tri- and diglycerides. For more details on general fatty acid properties refer to Chapter 30 in Lippincott's Illustrated Reviews in Biochemistry available in reserve[197].

[197] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Understand the different roles of lipids in our bodies
- Describe the structure and functions of triacylglycerols (triglycerides)
- Recognize that phospholipids are amphipathic and play an important role as structural components within our body
- Identify the structure and functions of cholesterol and other steroids
- Use the common, n- and ω nomenclature systems to describe fatty acids, and be able to draw fatty acids based on these various naming systems
- Describe the structure of fatty acids and analyze how this affects their packing, solubility and physical state
- Explain the roles of the essential fatty acids, including what makes them essential

Key Vocabulary and Concepts

- Neutral Lipid
- Amphipathic Lipid
- Lipid Droplet
- Saturated, Unsaturated, Monounsaturated and Polyunsaturated Fatty Acids
- Essential Fatty Acids
- Cholesterol and Cholesterol Esters
- Phospholipids and Phospholipid Head Groups

Function of Lipids

The defining characteristic of lipids is their poor water solubility. Since these do not mix well in water, they are digested, absorbed and transported very differently from the water soluble molecules we have been discussing up to this point. While solubility presents some challenges in terms of digestion and storage, they also provide several important biological advantages.

Structural Roles of Lipids

Lipids can be considered neutral, or amphipathic. Neutral lipids have little to no charge, this means that they are not soluble at all in water, and generally separate into different layers when mixed. This can be an advantage in terms of storage. For example in adipocytes neutral lipids are packed in water-free organelles called lipid droplets (see Figure 44). This is a very efficient way of storing energy because lipids have a very high energy content. This also sequesters triglycerides and cholesterol esters away from normal cellular machinery. A review on the role of lipid droplets in the storage and release of triglycerides can be found in Walther and Farese [2009]. Neutral lipids include triglycerides and cholesterol esters[198].

[198] Both of these will be described in the next section.

AMPHIPATHIC LIPIDS GENERALLY CONTAIN TWO PARTS, a hydrophilic portion that *is* soluble in water and a hydrophobic part that is not soluble in water. These lipids are useful for generating biological barriers and membranes. Thinking about the lipid droplet example, an amphipathic lipid, such as a phospholipid will orient itself such that the hydrophobic part interacts with the interior triglyceride containing part of the droplet, while the hydrophillic part of the phospholipid orients itself on the outside facing the water of the cellular cytoplasm.

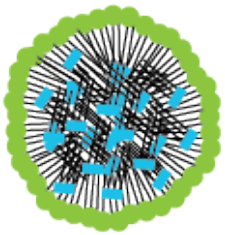

Figure 44: Schematic of a lipid droplet. Amphipathic phospholipids are shown in green, while neutral lipids such as cholesterol esters and triglycerides are shown in blue. The outside of the droplet allows it to be soluble in water, while the inside is hydrophobic.

Roles in Energy Storage

The second major role of lipids is as excess energy storage. Lipids contain much more stored energy both on a per molecule and a per gram basis than either carbohydrates or proteins. In addition we store much more lipid than glycogen on a per gram basis (see Table 32). Finally, in contrast to protein, for the most part storage lipids and glycogen are not part of the normal cellular machinery, so their breakdown does not affect cellular functionality. We will calculate this in the lipid oxidation lecture, but as one example, the oxidation of a triglyceride containing three palmitates conjugated to glycerol generates *330 molecules of ATP* once oxidized completely, compared with 32 for the complete oxidation of one molecule of glucose and 8.5 ATP equivalents for one molecule of Alanine. Dietary lipids or lipids synthesized by our bodies[199] are an excellent long term storage molecule. We will discuss the regulation and importance of both storage and release of lipids in the next few lectures.

Table 32: Total energy stored as glycogen and lipid in a typical person. Mass is the total amount of this storage fuel, converted to calories using Atwater factors. Days indicates how many days worth of TDEE that can be supported by this number of calories. Calculation is based on a 75kg person with 28% body fat and a TDEE of 2000 Calories/day.

Form	Mass	Calories	Days
Glycogen	500g	2000	1
Lipid	21kg	189,000	95

[199] The process by which fatty acids are generated from glucose or amino acids is called *de novo* lipogenesis.

Classes of Lipids

There are two main subclasses of lipids, the sterols and the acylglycerols. These are physically very different and are used in very different ways in the body but are both lipids due to their hydrophobicity.

Cholesterol and other Sterols

Cholesterol is a steroid that can be synthesized by most tissues. It is *not* used for energy but is an essential component of all cellular membranes, aiding in their fluidity. Cholesterol can either be free, or esterified. By esterification we mean that a fatty acid group can be added to cholesterol. This makes it hydrophobic[200], and is important for transportation. Since cholesterol is made endogenously, but is not used for fuel the only way to reduce cholesterol is through bile-mediated secretion, which we will discuss in the digestion lecture. In addition to its membrane-fluidity properties, cholesterol is a precursor for many important steroid hormones including estrogen, testosterone, and cortisol. These molecules are all modifications of the existing cholesterol molecule, and are important for a wide range of biological functions. Associations between circulating cholesterol and cardiovascular events suggested that restrictions of dietary cholesterol may be prudent, but more recent research has shown that dietary cholesterol plays an insignificant role in modulating blood cholesterol levels, and the 2015 Dietary Guidelines published by the Department of Health and Human Services no longer recommends restricting dietary cholesterol[201].

[200] Free cholesterol is amphipathic

[201] United States Department of Health and Human Services, United States Department of Agriculture, and U.S. Department of Health and Human Services and U.S. Department of Agriculture. *2015-2020 Dietary Guidelines for Americans*. 2015. ISBN 978-0-16-093465-0

Glycerolipids

The next group of lipids are those with a glycerol backbone. By that we mean that one, two or three fatty acids are conjugated to a glycerol molecule using via an ester linkage. If three fatty acids are added, the molecule is known as a triglyceride[202]. Its properties are based on which specific fatty acids are added and in which location. Triglycerides are neutral lipids because there is no polar group.

[202] Or triacylglycerol

Phospholipids are a class of diglycerides. These lipids generally contain fatty acids at the first and second positions of the glycerol molecule. *These are known as the sn1 and sn2 positions with the head group at the sn3 position*. At the third position is a phosphate molecule, which has a highly negative charge and then a variable head group. Several head groups are described in Table 33. These

headgroups therefore affect the packing and function of these phospholipids.

Table 33: Common phospholipid head groups. Note that for Phosphatidylglycerol there is a *second* glycerol headgroup in addition to the one conjugated to the fatty acids. For Cardiolipin, there is another entire phosphatidylglycerol molecule, meaning there are two glycerol molecules, and four fatty acids linked via the phosphate group.

Head Group	Lipid
Phosphate Only	Phosphatidic Acid
Ethanolamine	Phosphatidylethanolamine
Choline	Phosphatidylcholine
Serine	Phosphatidylserine
Inositol	Phosphatidylinositol
Glycerol	Phosphatidylglycerol
Phosphatidylglycerol	Cardiolipin

Properties and Structures of Fatty Acids

In addition to properties of lipids that are due to the head group, lipids contain a wide variety of fatty acids. Since each triglyceride can contain three different fatty acids, the number of combinations possible for a triglyceride is very high. The fatty acids are very hydrophobic once esterified to a glycerol backbone, but their length and structure affect their metabolism and functions.

Classes of Fatty Acids

The acyl chains that are conjugated to glycerol[203], a glycerol and phospholipid head group[204] or steroids[205] are defined by two aspects of their structure. The first is their length, or the number of carbon atoms in the fatty acid. Based on this criteria, fatty acids are grouped together as short, medium, long or very long-chain fatty acids (see Table 34). Shorter fatty acids are more soluble, but contain less energy (since energy is released when each bond is broken). Short chain fatty acids are generally derived from fermentation of fiber in the colon, while the other three fatty acid lengths are generally obtained as part of ingested triglycerides and phospholipids.

[203] In the case of triglycerides

[204] In the case of diacylglycerides or phospholipids

[205] In the case of esterified cholesterol, for example

Table 34: Classification of fatty acids by length of the fatty acid tail

Type	Length
Short Chain Fatty Acid	5 or less
Medium Chain Fatty Acid	6-12
Long Chain Fatty Acid	13-21
Very Long Chain Fatty Acid	22 or more

Saturation levels is another criteria for comparing fatty acids. While saturated fatty acids have no double bonds, both monounsaturated[206] and polyunsaturated[207] fatty acids can be made. These double bonds are generated by a class of enzymes known as *desaturases*. For example Stearoyl-CoA desaturase[208] can introduce a double bond at the Δ-9 position[209] of a fatty acid, so could convert a saturated fatty acid into a monounsaturated fatty acid.

[206] containing one double bond

[207] containing more than one bond

[208] also known as Δ-9-desaturase

[209] more about what this means in the nomenclature section. Lots of footnotes today!

The type of double bond is a third criteria. In nature most bonds are in what we refer to as the *cis* position. This means that the hydrogens on either side of a double bond are on the same side. The opposite stereoisomer, where the hydrogens are on opposite sides of the double bond are known as *trans* fatty acids, or more commonly as trans fats. While these are rare in nature, they became abundant in our food supply because of the process of converting unsaturated fats (such as those in corn or canola oil) to saturated fats. This hydrogenation made most double bonds into single bonds, but sometimes also switched the stereoisomer orientation from a *cis* to a *trans* orientation. Nutritional epidemiology studies have associated

trans fatty acid intake with about a 50% increased risk of coronary heart disease [Willett et al., 1993, Mozaffarian et al., 2006]. Since trans fats are dispensable to the human diet, and because of their health risks, they are limited or banned in most countries, including the United States which plans to have them removed from the food supply by January 2020.

Fatty Acid Nomenclature Systems

Based on the above criteria (length, location and types of double bonds) there is a wide variety of potential fatty acids. As such three naming systems have been used, their common names, the Δ notation and the ω notation.

THE COMMON NAME is generally the hardest to remember. Each fatty acid is given a different name, like stearic acid, oleic acid, or α-linoleic acid. Often these common names are offshoots of foods that these fatty acids were found in. Without remembering the name to structure comparison it is very hard to guess anything about the physical properties from a common name.

THE Δ SYSTEM has two parts. The first part refers to the length, so a C16:0 means a fatty acid that is 16 carbons in length, but with *no* double bonds[210]. A fatty acid that is C16:1 has one double bond, C16:2 has two and so forth. This is useful for identifying two of our three criteria; length and saturation level, but it does not tell us about the location and isomer of the double bond. Therefore the Δ system adds another piece of information, how many atoms from the acidic end the double bond is located at. Palmitoleic acid is a C16:1Δ^9-*cis* fatty acid. It can be generated by the generation of a double bond at the 9th carbon, starting from the acid end. This is the product of the enzyme Stearoyl-CoA desaturase acting on palmitic acid, since that enzyme has specificity for generating *cis* bonds at the Δ9 position.

[210] This fatty acid's common name is palmitate, now is it a saturated fatty acid, or an unsaturated fatty acid?

THE ω SYSTEM, also known as the n- system is very similar, but instead counts from the free end, not the acid end. Going back to our example of Palmitoleic acid, while it is a C16:1Δ^9-*cis* fatty acid, it is also a C16:1ω7-*cis* fatty acid. It can also be referred to as a C16:1(n-7) fatty acid, indicating the double bond is 7 carbons from the end. Count the carbons from one end to the other and convince yourself, of the numbering.

Polyunsaturated Fats

We can identify if a fatty acid is polyunsaturated fatty acid (or a PUFA) because the number after the colon is greater than one. For example 18:3ω-6*cis* is also known as Gamma-linolenic acid. By now, hopefully you can appreciate that this fatty acid is 18 carbons long, contains three double bonds and one of them is six carbons from the n-end. But what about the other two double bonds? One way to solve this is to be explicit and indicate that this fatty acid is 18:3, *cis*,*cis*,*cis*-Δ^9,Δ^{12},Δ^{15}. This indicates that the double bonds are at the 9,12 and 15th carbons starting from the acidic end[211]. As a shorthand, double bonds in a PUFA are almost always separated by *three* carbons, so if you know how many double bonds are present, and you know the location of one double bond , you can presume that the other bonds are three carbons away. The convention for this shorthand therefore is to assign the ω or Δ notation to the farthest or closest double bond from the acidic end respectively[212].

[211] For practice, figure out where the double bonds would be from the n-end, or how could you be explicit about this fatty acid's ω naming

[212] If you are looking for more practice in naming or drawing, look up any fatty acid on wikipedia and it should give you the structure, ω and Δ nomenclature

Essential Fatty Acids (ω-3 and ω-6)

A subset of PUFA's are those that have a double bond at the ω-3 or ω-6 position. Fatty acids that have bonds at this location are essential for several physiological functions in humans *but* we cannot synthesize those particular double bonds ourselves. The biochemical reason for this is that human desaturases are all Δ-specific[213]. The specific human isoforms are Δ^9, Δ^6, Δ^5, and Δ^4. This means that if humans synthesize palmitate (C16:0), we could potentially make double bonds at these positions. Switching to the ω nomenclature, the double bond closest to the end is C16:1Δ^9, which is equivalent to C16:1ω7. To make a ω-6 fatty acid humans would either have to start with an odd numbered fatty acid (such as a C15:0, not made by humans) or have a different desaturase. The same logic is true for ω-3 fatty acids. This makes these two fatty acids essential in our diet. Dietarily we can consume these in several forms. For the ω-3 series, some common fatty acid sources include alpha-linoleic acid (ALA; C18:3ω-3), eicosapentaenoic acid (EPA; C20:5ω-3) and docosahexaenoic acid (DHA; C22:6ω-3). These can be inter-converted into each other as long a they are ingested already with the ω-3 bond present. This means that both ω-3 and ω-6 fatty acids are essential in our diet[214]. The recommended daily intakes of lipids are shown in Table 35.

Table 35: Recommended daily intake of lipids. Based on the 2015-2020 USDA Dietary Guidelines [United States Department of Health and Human Services et al., 2015]. Values are for an adult (19-30) year old.

Lipid	Recommended Intake
Total Fat	20-35% of Energy
Saturated Fat	<10% of Energy
Linoleic Acid (ω-6)	17g (M), 12g (F)
α Linolenic Acid (ω-3)	1.6g (M), 1.1g (F)

[213] This means that they bind to a fatty acid from the acidic end, and generate a double bond relative to that position.

[214] Individuals vary quite a lot in how efficiently they can convert, for example ALA into DHA, so it is probably more effective to obtain DHA from dietary sources, rather than rely on our ability to inter-convert these fatty acids. Fish is an excellent source of DHA, this is one of the reasons why the protein package associated with fish is proposed to be so healthy.

These essential fatty acids play several important functions. Due to their structure these fatty acids are less tightly packed, so are important for membrane fluidity in many tissues. Some tis-

sues, such as the brain, have very high levels of ω-3 fatty acids[215]. Another important role of these fatty acids is in the generation of bioactive lipids. These lipids function as hormones to mediate inflammatory responses. In general, the ω-6 derived fatty acids[216] are generally *more* inflammatory than the ω-3 fatty acids. The ω-3 fatty acids generally play a role in the resolution of inflammation, though these are generalizations and not entirely understood. For more details on the role of essential fatty acids on inflammation, see Calder [2013].

[215] Up to 30% of the total mass of the brain is thought to be DHA [Crawford et al., 1976], suggesting a very important role in neural development.

[216] For example arachidonic acid; C22:4ω-6. Some foods with very high ω-6 fatty acids are plant seeds and oils.

ω-6 AND ω-3 FATTY ACIDS CAN COMPETE FOR MANY OF THE SAME ENZYMES. While both these fatty acids are essential, in practice humans generally consume far more ω-6 than ω-3 fatty acids. This can be problematic because since these fatty acids share desaturases and elongases[217]. The result could be an inability to generate enough ω-3-derived fatty acids, and an overproduction of the ω-6-derived fatty acids. This can mean that our dietary shift towards high ω-6:ω-3 ratios may result in higher inflammatory responses.

[217] These are enzymes that extend a fatty acid, say from C16:1 to C18:1.

Reflection Questions

1. Stearoyl-CoA desaturase 1 (SCD1) is the Δ9-desaturase that converts saturated fatty acids such as palmitate (C16:0) and stearate (C18:0) into their monounsaturated counterparts. Two people consume identical diets, but one carries a high-activity SCD1 variant and the other a low-activity variant. Using your knowledge of fatty acid nomenclature and desaturase function, predict how their stored and circulating fatty acid profiles would differ — naming the specific products expected — and explain what downstream consequences this might have on membrane fluidity and lipid packing.

2. Partial hydrogenation of vegetable oils was widely used to create solid fats for cooking and preservation. Evaluate why this process generated trans fatty acids as a byproduct, explain how trans vs. cis double bond geometry affects fatty acid packing and membrane properties, and discuss why trans fats have been associated with greater cardiovascular risk than an equivalent amount of saturated fat.

3. A person adopts a Western diet high in vegetable seed oils (rich in ω-6 linoleic acid) and low in fish consumption. Using your knowledge of essential fatty acid metabolism and competition between ω-3 and ω-6 fatty acids for shared desaturases and elongases, predict the consequences for the balance between pro- and

anti-inflammatory lipid mediators, and suggest a specific dietary modification to address this imbalance.

Digestion and Absorption of Lipids

This unit will cover the digestion and absorption of lipids, and the generation of chylomicrons. Lipid digestion is quite a bit different from the soluble digestion of carbohydrates and lipids, so we will also cover bile acids and their role in absorption. Chapter 15 in Lippincott's Illustrated Reviews in Biochemistry available in reserve[218].

[218] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Identify the roles of Lingual Lipase, Gastric Lipase and Pancreatic Lipase including their locations and specific roles on lipid digestion in the stomach
- Understand the role of bile salts in the formation of micelles, and explain how diseases of bile formation can affect an individual
- Explain the transport mechanisms of lipids across the apical and basolateral membranes, and how they differ between lipid classes
- Describe the re-esterification processes in the enterocyte
- Explain the nature, formation and fate of the chylomicron and its role in lipid transportation

Key Vocabulary and Concepts

- Micelle
- Lipases and Colipase
- CCK and Secretin
- Gallbladder and Bile Synthesis
- Primary, Conjugated and Secondary Bile Salts

Dietary Lipids and Dietary Intake

The three main dietary lipids we ingest are triglycerides, phospholipids and cholesterol. Of these, on average we consume much more in terms of triglycerides (95g for men, 65g/day for women) than phospholipids (1-2g/day) or cholesterol (300 mg/day). For the average person that's about 1/3 of their total caloric intake [National Center for Health Statistics, 2017]. Dietary lipids are also our only source of the essential $\omega 3$ and $\omega 6$ fatty acids. Furthermore several lipid soluble vitamins are carried and absorbed along with lipids. These include vitamins A, D, E and K, so impairments in lipid absorption can affect their absorption as well.

Lipid Digestion in the Upper Digestive Tract

There is substantial debate about whether lipids can be tasted, in the way that we have specific receptors in our tongue that can sense sweet, bitter, sour, salt, and umami flavors. While there are fatty acid

receptors on the tongue, at this stage most lipids are in the triglyceride form. Rather lipid flavor is thought to be a combination of several fatty acid receptors, along with the mechanical sensation of lipids in the oral cavity [DiPatrizio, 2014].

Lipid digestion, relative to carbohydrate digestion is relatively simple from an enzymatic perspective. This process is somewhat complicated by the insoluble nature of fat. Rather than letting food diffuse and break down into aqueous pieces, fat will aggregate in globules and often float as it passes through the digestive tract[219]. Therefore lipid digestion is a combination of enzymatic processing, along with the solubilization needed for absorption.

[219] Think about how oil separates in water.

The first lipid digestive enzymes are secreted in the oral cavity. This enzyme is known as *Lingual Lipase* and is secreted from glands underneath the tongue. While it is present in the mouth, it is only functional in the low pH environment, such as that in the stomach. Lingual Lipase tends to cleave the sn3 fatty acids from triglycerides[220]. This results in a diacylglycerol and a free fatty acid. Lingual Lipase is especially effective in removing medium chain fatty acids from triglyceride molecules [Jensen et al., 1983].

[220] Recall, phospholipids do not have a fatty acid esterified at the sn3 position, that is where the headgroup is located.

Gastric Lipase also functions in the stomach. This enzyme is secreted from chief cells in the stomach, and like Lingual Lipase is most active in the low pH of the stomach[221]. Gastric Lipase prefers to release fatty acids from the sn1 and sn3 positions of a lipid. For both phospholipids and triglycerides this leaves a monoacylglycerol with the fatty acid still in the sn2 position. The removal of the acyl chain make the lipid much more soluble and easier to emulsify into smaller droplets.

[221] Though it is able to continue functioning into the small intestine.

The process of emulsification is essential to lipid absorption. Emulsification is the breaking of lipid droplets in to smaller and smaller particles. A large globule of fat is not going to be able to easily pass through a cellular membrane, so as lipids pass through the digestive tract, both enzymatic digestion and mechanical churning to make the droplets as small as possible.

Absorption and Digestion of Lipids in the Small Intestine

Most lipid digestion and absorption[222] occurs within the small intestine. At this point the semi-digested, emulsified lipid droplets come into contact with bile salts, which further aid in their solubilization into very tiny lipid droplets called micelles. Micelles are generally coated with an amphipathic layer of bile salts, and contain an internal

[222] In most cases greater than 90%.

core of fatty acids, and monoacylglycerols.

Bile Salts and Their Regulation

Bile salts are cholesterol-derived compounds that are generated initially in the liver[223] In the liver through a series of enzymatic steps, cholesterol is converted into *primary bile acids* such as cholic acid and chenodeoxycholic acid. This step is rate-limited by an enzyme known as 7-α-hydroxylase, which in turn is transcriptionally downregulated when liver primary bile acids are high [Ramirez et al., 1994][224].

CONJUGATED BILE ACIDS[225] ARE GENERATED FROM PRIMARY BILE ACIDS. while still in the liver, bile acids have either a glycine or a taurine[226] amino acid group added to them. This yields a conjugated bile acid, of which there are several species. This new bile acid is very amphipathic, with a charged group from the amino acid on one end and the modified cholesterol on the other end. This makes bile acids very effective in interacting with and solubilizing dietary lipids.

BILE SALTS ARE EFFICIENTLY REABSORBED, after their lipid cargo has been absorbed with up to 95% of bile salts being reabsorbed via a sodium co-transporter in the terminal illeum. One approach therefore to remove cholesterol from the blood stream is bile acid sequestrants that impair the uptake of bile salts, and thus (indirectly) the excretion of cholesterol.

Uptake of Lipids in the Small Intestine

After a brief diversion about bile salts and their role in generating lipid micelles lets return to the small intestine where we now have partially hydrolyzed triglycerides and phospholipids. A third Lipase, called *Pancreatic Lipase* is secreted into the small intestine, it again is specific to the sn1/sn3 positions but compared to Lingual Lipase, has stronger activity towards long chain fatty acids [Jensen et al., 1983]. Pancreatic Lipase activity is dependent on a coenzyme called *colipase*. It is secreted as a precursor called procolipase, and is activated by trypsin-mediated cleavage[227]. Recall that at this stage many lipids are solubilized within bile-salt containing micelles. The presence of colipase allows Pancreatic Lipase to be active even on lipids contained within the micelles. More details about how colipase can help Pancreatic Lipase function can be found in Van Tilbeurgh et al. [1999].

CHOLESTEROL AND PHOSPHOLIPIDS ARE ALSO DIGESTED IN THE SMALL INTESTINE. We have been focusing mainly on triglycerides, but two more enzymes in the small intestine are important for the

[223] We will discuss this in the lipid transport and synthesis lectures, but cholesterol is generally made throughout the body, with excess trafficked to the liver. Since it cannot be used for fuel, cholesterol is *only* released in the form of bile acid secretion.

[224] The sensing of bile acids turns out to be an emerging area of research. The actual receptors for bile acids are a transcription factor known as FXR (farnesoid-x-receptor) and a receptor on the cellular surface known as TGR5. For 7-α-hydroxylase, FXR seems to be the more important regulator [Sinal et al., 2000].

[225] A third class of bile acids, known as secondary bile acids are generated in the large intestine by bacterial modification of secreted bile acids. Since these are reabsorbed and sent back the liver, the secondary bile acids, known as deoxycholic acid and lithocholic acid can then also be conjugated in the liver. Secondary bile acids comprise of about 20-40% of the total bile acid pool. Interestingly changes in bile acids are thought to play a role in the metabolic benefits of bariatric surgery [Evers et al., 2017].

[226] Taurine is an amino acid, derived from cysteine that is not used in proteins.

[227] This is common theme that will come up again in the protein digestion unit wherein trypsin is also secreted as an inactive precursor and is activated by cleavage by enteropeptidase.

absorption of phospholipids and cholesterol. For phospholipids, the key enzyme is Pancreatic Phospholipase. This is a class A2 phospholipase[228]. At this stage the phospholipid is known as a lysophospholipid. For cholesterol if the cholesterol is esterified[229] this is removed by an enzyme termed Cholesterol Esterase. Similarly to a lysophospholipid, cholesterol is now in a more amphipathic form allowing for better absorption.

[228] This means that it releases the fatty acid from the sn2 position of a phospholipid, leaving a fatty acid at the sn1 position, and the headgroup at the sn3 position.

[229] Meaning it has a fatty acid conjugated to it.

Cholesterol uptake is mediated by the NPC1L1 transporter. The predominant pathway for cholesterol uptake is via a steroid transporter called NPC1L1[230][Altmann et al., 2004, Iqbal and Hussain, 2005]. Plant sterols, which look very similar to cholesterol are imported along with cholesterol, but then are specifically exported back into the gut lumen via ATP-dependent transporters ABCG5/8[231]. Cholesterol uptake is reduced when enterocyte cholesterol levels are high. For example, bile acid synthesis deficiency[232] results in a dramatic decrease in cholesterol absorption to balance the limited release [Repa et al., 2000, Wang, 2007]. The mechanism for this is via a transcription factor called SREBP2. Normally SREBP2 increases the levels of NPC1L1. When cholesterol levels are high, SREBP2 is inhibited, and cholesterol uptake is reduced (see Figure 45. The upshot of this is that when endogenous cholesterol levels are high, dietary cholesterol absorption is reduced. This is likely one reason why dietary cholesterol intake does not strongly modulate blood cholesterol levels.

[230] Niemann-Pick C1 Like 1, not the most useful name, it is based on homology with the lysosomal cholesterol transporter that causes Niemann-Pick disease, a disorder which involves lipid accumulation within lysosomes of cells.

[231] Why do you think this step requires active transport?

[232] Such as mutations in 7-α-hydroxylase, prevents bile acid production and secretion.

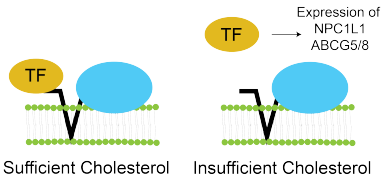

Figure 45: Regulation of SREBP2. When there is insufficient cholesterol, SREBP2 is cleaved releasing the transcription factor (TF) fragment, where it can go to the nucleus and drive expression of cholesterol uptake genes such as *NPC1L1*, *ABCG5/8*.. This means that at sufficient cholesterol levels absorption is largely reduced.

The remaining micelles contain fatty acids, monoacylglycerols[233] and lysophospholipids. These lipids are absorbed across the apical membrane of enterocytes. The precise mechanism seems to be a combination of passive transport and passive diffusion of micelles within the membranes, leaving the bile salts in the gut lumen. Remember that both micelles and phospholipid membranes are very amphipathic, so one theory is that the lipid containing micelles just passively pass through the membrane. Another thought is that they are bound to surface receptors then endocytosed[234]. Either way, the majority of lipids are taken out of the gut lumen into the microvilli in the small intestine.

[233] With a fatty acid still in the sn2 position

[234] This means that they are actively brought into the enterocyte via a biological import process

Short Chain Fatty Acid Absorption

Both short and medium chain fatty acids are more or less soluble in water and therefore do not require micelles for transport into the enterocytes. These fatty acids are thought to be passively absorbed in the small intestine after digestion.

Short chain fatty acids are derived from fiber. On the other hand, short chain fatty acids are more rare in our diet than long chain fatty acids[235]. If ingested directly these can be absorbed by enterocytes, but most SCFA's are generated by our microbiome in the large intestine. These bacteria use undigested fiber and unabsorbed proteins and peptides as fuel. The major products of these reactions are SCFA [Cummings et al., 1987]. Colon epithelial cells can take up these metabolites, and use these SCFA as a fuel source, preferring butyrate. Acetate and propionate typicaly enter the circulation. While this may seem like a minor event, SCFA's provide about 10% of our total caloric requirements and are a major way by which the gut microbiome can affect our energy balance. For more details about SCFA and bacterial physiology refer to a recent review by Koh et al. [2016].

[235] These include things like acetate, butyrate and propionate

Endocrine Control of Lipid Digestion and Absorption

We have mentioned several pancreatic and biliary secretions that aid in digestion of lipids. The major regulators are cholecystokinin and secretin. CCK is released from the lower duodenum and acts at several places including the gallbladder and pancreas. In the gallbladder, CCK promotes contraction of the gallbladder, and relaxation of a sphincter connecting the bile duct to the duodenum[236]. This results in excretion of bile salts into the small intestine. As we have discussed previously CCK[237], is activated by the parasympathetic nervous system and inhibited by the sympathetic nervous system. At the same time, CCK promotes the release of sodium bicarbonate and Pancreatic Lipase and colipase from the pancreas. This process is also aided by secretin which also promotes pancreatic juice release.

[236] This is known as the Sphincter of Oddi.

[237] Secreted from enteroendocrine cells, also known as L-cells.

Transport of Lipids out of the Enterocytes

Lipids are absborbed as free cholesterol, monoacylglycerol, lysophospholipids and free fatty acids into the enterocyte. Each of these can be quite toxic to the cell, so they are very rapidly *reconverted* into storage forms (esterified cholesterol and triglycerides). This re-esterification is critically important for absorption and eventual transport to other tissues.

Within enterocytes, the neutral lipids are packaged into large lipoprotein complexes called Chylomicrons. This is the first lipoprotein complex we will discuss. These particles vary in size from quite large (like chylomicrons) to very small and dense[238] The surface of the chylomicrons contains phospholipids[239], free

[238] The high density lipoproteins or HDL we be described in the lipid transport lecture.

[239] Mostly phosphatidylcholine [Wood et al., 1964].

cholesterol and specific amphipathic proteins called *apolipoproteins*. In the case of chylomicrons, these protein are Apolipoproteins A1 and B48. The chylomicrons are secreted from the enterocytes into the lacteals of the lymphatic system. They then bypass the portal vein at first and travel to peripheral sites for rapid utilization in tissues such as adipose and muscle. Once in circulation, chylomicrons pick up Apolipoproteins CII and E. As we will describe in the lipid transport lecture these allow for the chylomicrons to recognize and activate Lipoprotein Lipase for final delivery of fatty acids and cholesterol to peripheral tissues.

MEDIUM AND SHORT CHAIN FATTY ACIDS ON THE OTHER HAND, are secreted directly into the bloodstream. These more soluble fatty acids are directly transported via the portal vein to the liver. This is one of the reasons why gallbladder and chylomicron-generating diseases can be treated with medium chain fatty acids, and why medium chain fatty acids are rapidly converted into ketones.

Reflection Questions

1. A patient undergoes cholecystectomy (gallbladder removal) and now receives a continuous low-level drip of unconcentrated bile into the duodenum rather than a concentrated bolus release after a meal. Evaluate how this change affects micelle formation, Pancreatic Lipase function, and fat-soluble vitamin absorption, and suggest dietary modifications that could help manage symptoms.

2. Ezetimibe is a drug that blocks NPC1L1-mediated cholesterol uptake in enterocytes. Using your knowledge of SREBP2 regulation and endogenous cholesterol synthesis, analyze why ezetimibe alone often has only a modest effect on circulating cholesterol, and explain the mechanistic basis for why combining ezetimibe with a statin produces a synergistic reduction.

3. A child with cystic fibrosis has severely impaired pancreatic secretion, including near-absent Pancreatic Lipase and colipase. Apply your knowledge of lipid digestion to predict: (a) which classes of dietary lipids would be most severely affected and why, (b) what GI symptoms and nutritional deficiencies would result, and (c) why medium chain triglycerides are used clinically to supplement fat intake in these patients.

4. A patient with elevated LDL cholesterol is advised to increase soluble fiber intake. Using your knowledge of bile acid enterohepatic circulation, fiber's adsorptive properties, and SREBP2-mediated

regulation of cholesterol uptake, explain the chain of events by which co-ingestion of soluble fiber with a cholesterol-containing meal reduces net cholesterol absorption — and predict why this effect requires consistent daily fiber intake rather than occasional high-fiber meals.

Lipid and Cholesterol Synthesis

This unit will cover the synthesis of lipids including cholesterol, fatty acid and triglyceride synthesis. For more details on these topics, refer to Chapters 28 and 29 in Biochemistry: A Short Course available in reserve[240].

[240] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

Learning Objectives

- Describe the conditions and tissues wherein fatty acids are generated, and from what precursors.
- Explain the regulation of fatty acid biosynthesis by metabolite levels and hormones, including how it is ensured that β-oxidation and *de novo lipogenesis* do not occur simultaneously.
- Understand the energy costs of generating fatty acids from glucose, compared with using glucose for fuel. Included in this is the source of carbon units, and reducing equivalents that are necessary for fatty acid biogenesis.
- Analyse the role of citrate in co-ordination of glycolysis, TCA cycle activity and lipogenesis.
- Explain the causes of lipodystrophy, including the metabolic consequences of ectopic fat deposition.
- Understand how transcriptional and post-translational regulation control triglyceride synthesis and cholesterol synthesis.
- Analyse the cellular conditions that lead to cholesterol synthesis and ketogenesis.
- Describe the roles and activation of SREBP1 and SREBP2 in fatty acid/triglyceride and cholesterol biogenesis respectively.

Key Concepts and Vocabulary

- *de novo* lipogenesis and triglyceride synthesis
- glyceroneogenesis and glycerol phosphorylation
- Acetyl-CoA Synthetase, ATP Citrate Lyase and Fatty Acid Synthase
- HMG-CoA Reductase and Statins
- SREBP1 and 2
- Hypercholesterolemia
- Lipodistrophy

Synthesis of Triglycerides from Fatty Acids

Fatty acids are obtained from the diet, or can be made from excess glucose/amino acids[241]. The esterification of these fatty acids within cells is very important because free fatty acids can be toxic to a cell once they build up. Most tissues are able to store excess fatty acids to some degree but the major sites of synthesis are adipose tissue, and liver.

[241] A process known as *de novo* lipogenesis.

Generally cells have to decide between three general fates for the fatty acids that arrive inside the cell[242]:

[242] Though in growing and dividing cells, the fraction of fatty acids that become phospholipids becomes much more relevant.

Without energy demand: esterify with glycerol as triglycerides

With energy demand, but with OAA availability: oxidize fatty acid to Acetyl-CoA and use that for fuel in the TCA cycle to generate ATP.

With energy demand but insufficient OAA: oxidize to Acetyl-CoA and convert that to ketone bodies for release (primarily in the liver[243]).

[243] Two main reasons why this is mainly in the liver, the first is that there is typically less cataplerosis in non-hepatic tissues because there is less gluconeogenesis. The second is that the enzymes of ketogenesis are at higher levels in the liver.

We will discuss the regulation of β-oxidation, which is the main process by which fatty acids become Acetyl-CoA in the next lecture. When fatty acid synthesis is active, the associated elevations in malonyl-CoA[244] will generally suppress β-oxidation via inhibition of Carnitine Palmitoyltransferase I[245]. This ensures that fatty acid synthesis and oxidation do not occur simultaneously.

[244] Described below

[245] Abbreviated CPTI, the rate limiting step of fatty acid breakdown.

TRIGLYCERIDE SYNTHESIS INVOLVES THE SEQUENTIAL ADDITION OF FATTY ACIDS TO A GLYCEROL BACKBONE. This involves enzymatic reactions starting with phosphorylated glycyerol (Glycerol3P) and an activated fatty acid. To prepare fatty acids for esterification, a fatty acid[246] is conjugated to coenzyme A by Acyl-CoA Synthetase:

[246] Also known as an acyl group. Generally this could be any fatty acid, but different Acyl-CoA Synthetases have differing fatty acid length preferences.

$$FattyAcid + CoA + ATP \rightarrow AcylCoA + AMP + PPi \tag{43}$$

Note that this reaction *consumes* two high ATP equivalents for each fatty acid to be added. This fatty acid is now in the *activated* form and is available to be conjugated to phosphorylated glycerol by the following four sequential reactions:

$$AcylCoA + Glycerol3P \rightarrow LysophosphatidicAcid \tag{44}$$

$$AcylCoA + LysophosphatidicAcid \rightarrow PhosphatidicAcid \tag{45}$$

$$PhosphatidicAcid \rightarrow Diacylglycerol + Pi \tag{46}$$

$$AcylCoA + Diacylglycerol \rightarrow Triacylglycerol \tag{47}$$

THE PHOSPHATIDIC ACID GENERATED IN STEP 45 IS THE PRECURSOR FOR MOST PHOSPHOLIPIDS. Enzymes can convert phosphatidic acid to phosphatidylserine, or phosphatidylinositol. Phosphatidylserine is the substrate for the generation of phosphatidylethanolamine, which in turn generates phosphatidylcholine[247]. For more details about phospholipid synthesis see Kent [1995]. These structural lipids are particularly important during development, and are potential drug targets to reduce the growth of cancer cells, which require generation of substantial membranes thus requiring a lot of phospholipid synthesis.

[247] This is the reaction catalyzed by *PEMT*, the enzyme that has variants which confer choline dependence as choline cannot be endogenously synthesized.

FATTY ACIDS ARE ADDED IN A SPECIFIC MANNER. Generally the sn2 position has an unsaturated fatty acid, while there is a saturated fatty acid at the sn1 position. The sn3 position seems to have much less specificity in mammalian triglyceride formation [Brockerhoff, 1971]. This is relevant for phospholipids that form membranes, as these will tend to have one saturated and one unsaturated fatty acid at each of the sn1 and sn2 positions[248].

[248] The sn3 position is where the phosphate group is located.

Glycerol-3-Phosphate Supply for Triglycerides

While each triglyceride molecule needs three fatty acids, they also require a phosphorylated glycerol backbone for reaction 44. The source of this glycerol varies between tissues. In the liver, glycerol-3-phosphate can be generated by phosphorylation of glycerol via Glycerol Kinase at a cost of an ATP:

$$Glycerol + ATP \rightarrow Glycerol3P \tag{48}$$

Adipocytes on the other hand have low levels of glycerol kinase activity. Therefore when fatty acids are presented to adipocytes they *require* phosphorylated glycerol to be made from glucose since they lack the ability to make phosphorylated glycerol from glycerol (since adipocytes lack Glycerol Kinase). The glycolytic intermediate dihydroxyacetone phosphate (DHAP) is generated by Aldolase[249] and can be converted into glycerol.

[249] Two steps *after* PFK1

$$DHAP + NADH \rightleftharpoons Glycerol3P + NAD^{+} \tag{49}$$

This reaction comes at a cost of one NADH molecule, or 2.5 ATP equivalents. The lack of glycerol kinase in adipocytes is thought to be an adaptation to prevent the futile release and then re-esterification of fatty acids post lipolysis, whereby after lipolysis in adipocytes *both* glycerol and fatty acids can go to the liver, as the liver always has sufficient glycerol to prevent fatty acid accumulation. Nonetheless, because of this requirement, a substantial amount of the glucose

used in adipocytes is used to generate glycerol backbones. In the absence of glucose, gluconeogenic precursors such as lactate and alanine can be converted to glycerol in adipocytes, a process known as glyceroneogenesis[250].

[250] This is equivalent to gluconeogenesis, up to the DHAP step, where reaction 49 takes precedence over the Aldolase reaction thus production of Glycerol3P occurs even when Aldolase cannot synthesize DHAP due to glucose insufficiency.

The energy costs of triglyceride synthesis, based on these pathways is substantial. Three acyl chains must be activated at a cost of 2 ATP equivalents each using 6 ATP in total. This is tissue independent. In the liver, one more ATP is needed to activate glycerol to glycerol3P so a total of 7 ATP is required to form one triglyceride. In adipose tissue the cost is more because you use 2 x ATP in the preparation phase of glycolysis (remember that glycerol in adipocytes needs to come from glycolysis due to the lack of Glycerol Kinase) and another NADH to form Glycerol-3-phosphate in reaction 49. This means the cost in adipose tissue is 10.5 ATP (2ATP for glycolysis activation + 2.5ATP/NADH + 6ATP/3FA activation = 10.5), so it is not only more energy demanding, but also consumes some of the glucose that would be used to generate those ATP molecules.

Regulation of Triglyceride Esterification Enzymes

Both in the liver and in adipose, triglyceride synthesis will increase in response to insulin. The mechanisms of this include both short-term and long term regulation. Recall first, that in adipocytes glucose uptake is increased by insulin via stimulation of GLUT4 translocation. Insulin can acutely activate GPAT[251] and Lipin [252]. Together the increased glucose and fatty acid flux postprandial, combined with more activity of these triglyceride synthesizing enzymes will result in efficient triglyceride storage in adipose tissue after a meal. Simultaneous to these effects, as we will discuss in the lipid transport lecture, insulin also suppresses triglyceride breakdown, a process known as *lipolysis*. For more insights into how insulin regulates triglyceride synthesis, see the recent review by Coleman and Mashek [2011].

[251] The enzyme that catalyzes reaction 44.

[252] The enzyme that catalyzes reaction 46, this is mediated by activation of mTORC1 [Harris et al., 2007]

Chronic regulation of triglyceride synthesis is transcriptional and is regulated by the two transcription factors SREBP1c[253] and PPARγ[254]. Both of these nuclear hormone receptors increase the number of the key triglyceride synthesis enzymes, including GPAT, Lipin and AGPAT[255]. The regulation of SREBP1c is quite complicated but involves insulin- and mTORC1-dependent signals (reviewed in Bakan and Laplante [2012]). In this way both insulin and nutritional status can promote the efficiency of triglyceride storage.

[253] Sterol response element binding protein 1c, which has a similar isoform SREBP2, that plays a key role in cholesterol synthesis, more on this in the next section.

[254] This stands for Peroxisome proliferator-activated receptor gamma, a nuclear hormone receptor which promotes both triglyceride synthesis and new adipocyte formation. It is the target of the anti-diabetic drugs of the thiazolidinedione family.

[255] Which catalyzes reaction 44 in adipocytes.

De Novo Fatty Acid Synthesis

Triglycerides can use fatty acids derived from the diet, or that are generated endogenously. Certain fatty acids (the $\omega 3$ and $\omega 6$ derived fatty acids) are entirely dependent on nutritional inputs, but others are generated endogenously. This can vary substantially, with individuals on a high carbohydrate diet generating many of their fatty acids *de novo*, with those on a low carbohydrate diet relying almost entirely on dietary intake. Most tissues can perform this function[256], but the major sites are liver and adipose tissue.

[256] As it may be important for phospholipid synthesis

Fatty Acid Synthesis from Glucose and Ketogenic Amino Acids

Fatty acid biosynthesis occurs in the endoplasmic reticulum, which presents the first problem in terms of substrates. The initial input, Acetyl-CoA is generated from its precursors[257] in the mitochondria, and there are no Acetyl-CoA transporters. Therefore Acetyl-CoA must first be converted to Citrate via Citrate Synthase[258]:

$$AcetylCoA + OAA + H_2O \rightarrow Citrate + CoA \tag{50}$$

[257] Hypothetically from pyruvate via Pyruvate Dehydrogenase, from ketogenic amino acids or as the product of β-oxidation, though the latter is unlikely as the cell will not typically break down fatty acids and then immediately resynthesize them.

[258] This is the same Citrate Synthase that is the first step of the TCA cycle.

Citrate transporters do exist in the mitochondria, so citrate is transported to the cytoplasm and then reconverted to Acetyl-CoA via an enzyme called ATP-Citrate Lyase via the following reaction:

$$Citrate + ATP + CoA \rightarrow AcetylCoA + OAA + ADP + Pi \tag{51}$$

Once the Acetyl-CoA is in the cytoplasm[259], the next and rate-limiting step is catalyzed by an enzyme called *Acetyl-CoA Carboxylase* or ACC. This enzyme catalyzes the following reaction:

[259] As we will discuss later, the Oxaloacetate from ATP-Citrate Lyase (ACL) activity in the cytoplasm is also important for generating reducing agents. This is described in reaction 54 below.

$$AcetylCoA + ATP + HCO_3 \rightarrow MalonylCoA + ADP + Pi + H^+ \tag{52}$$

Malonyl-CoA[260] and Acetyl-CoA are both the substrates for Fatty Acid Synthase, a multifunctional enzyme that catalyzes the next several reactions. Acetyl-CoA serves as both a primer *as in an initial substrate* and a source of carbon units[261]. The first round generates a four carbon fatty acid condensing Acetyl-CoA and Malonyl-CoA and using two NADPH[262] molecules. After that, each round of the reaction consumes two NADPH and another Malonyl-CoA, resulting in the sequential addition of carbons two at a time. This ends when the acyl chain is 16 carbons long, releasing Palmitate, a C16:0 fatty acid. In sum, the overall reaction for a single palmitate is:

[260] When levels of this lipid are high (reflecting high ACL and ACC activity and active lipogenesis), it inhibits the transport of fatty acids back into the mitochondria by inhibiting Carnitine Palmitoyltransferase I. More on this in the lipid oxidation unit.

[261] Through MalonylCoA generated by ACC

[262] Not NADH, remember this from the pentose phosphate shunt?

$$8AcetylCoA + 7ATP + 14NADPH \rightarrow Palmitate + 14NADP^{+} + 7ADP + 8CoA + 7Pi + 6H_2O \tag{53}$$

The NADPH requirements of fatty acid synthesis are high. Each palmitate requires 14 NADPH molecules along with a substantial amount of ATP. The NADPH comes from two sources: the pentose phosphate shunt[263], and the activity of Malic Enzyme. Recall from above, when a Citrate molecule is exported, the other product of ATP-Citrate Lyase, shown in reaction 51, is oxaloacetate. While this is used as a TCA intermediate in the mitochondria, in the cytoplasm OAA can be converted into pyruvate via Malic Dehydrogenase and Malic Enzyme. These reactions are:

[263] Wherein one glucose molecule yields one NADPH molecule.

$$OAA + NADH \rightarrow Malate + NAD^{+} \tag{54}$$

$$Malate + NADP+ \rightarrow NADPH + CO_2 + 2Pyruvate \tag{55}$$

In sum, these reactions use a cytosolic NADH and the released oxaloacetate to generate NADPH and two pyruvate molecules, which can then be re-oxidized in the mitochondria. This means that for each Citrate released and Acetyl-CoA generated by ATP-Citrate Lyase, one NADPH is regenerated (again, at a cost of an ATP from reaction 9). This helps to maintain the NADPH pool. Therefore we can suspect that the 8 Acetyl-CoA's needed in reaction 53 will come with 8 NADPH molecules, so on average we need 6 more from the pentose phosphate shunt to have 14NADPH's needed to syntehsize palmitate. This means we need the equivalent of 6 molecules of glucose to go through the pentose phosphate pathway, and still need to generate 7 ATP, 8 NADH (equivalent to 20 ATP) and 8 Acetyl-CoA molecules (This requires 4 Glucoses, but generates 12 NADH and 4 ATP during partial oxidation) to power the formation of a single palmitic acid. This means a net requirement of 16 glucose molecules to make a single palmitic acid, at a net gain of only 11 ATP equivalents of energy[264]. Recall that if those same glucose molecules were to undergo complete oxidation it would yield 512 ATP[265]. This is a huge diversion of resources for making a single fatty acid.

[264] The exact math here is unimportant, since this is simplified scenario, but the point is that you have to use up a lot of glucose without gaining a lot of energy to make a single fatty acid

[265] 16 Glucose x 32 ATP/glucose.

Desaturation of Fatty Acids

While palmitate is the initial fatty acid made by mammals, this fatty acid (C16:0) can be further modified by both elongases and desaturases. Elongases can extend dietary or *de novo* fatty acids by increments of two by adding another Malonyl-CoA moiety and using two

more NADPH molecules. This results in the synthesis of very long chain fatty acids. There are several elongases in the human genome, but mutations in *ELOVL4* have been linked to macular dystrophy, highlighting the importance of fatty acid elongation in the conversion of ALA to DHA [Zhang et al., 2001]. Adipocytes and liver cells express high levels of ELOVL6, which elongates fatty acids to a maximum of 18 carbons. This is one reason[266] why the primary *de novo* synthesized fatty acids end up as C16:0, C16:1Δ^9, C18:0, and C18:1Δ^9 in these tissues.

[266] The other reason is the activity of SCD1, the Δ^9 desaturase.

Humans have four desaturases that work on the Δ4, Δ5, Δ6 and Δ9 positions of a fatty acid. The enzymes that catalyze these reactions are FADS1 and FADS2, which we discussed in terms of PUFA metabolism and the Stearoyl-CoA Desaturase[267]. The desaturation is a key part of the assembly of triglycerides and phosphpolipids, as a desaturated fatty acid is preferentially attached to the sn2 position. Both desaturases and elongases can alter the structure of both dietary and endogenously produced fatty acids.

[267] Abbreviated SCD.

Regulation of Fatty Acid Synthesis

Two steps of fatty acid biosynthesis are under relatively acute control. ATP-Citrate Lyase is phosphorylated and activated by insulin-dependent signaling through Akt [Berwick et al., 2002], whereas Acetyl-CoA Carboxylase is dephosphorylated and activated, also in an Akt-dependent manner [Witters and Kemp, 1992]. The inactivation of ACC is due to phosphorylation by both PKA[268] or AMPK[269], so the role of insulin is to reverse that inhibition. The precise process by which insulin and Akt mediate this dephosphorylation is not clear at this stage.

[268] In response to glucagon or adrenaline signaling

[269] The AMP-Activated Protein Kinase, In response to energy needs.

Citrate is a feed-forward regulator of ACC. When citrate is exported to the cytoplasm, it serves as an activator of ACC and results in the production of more Malonyl-CoA [Vagelos et al., 1963]. Cytoplasmic citrate is also a potent negative regulator of phosphofructokinase 1[270]. This means that when there is excessive Acetyl-CoA production, and if ATP-Citrate Lyase is active, citrate production will *block* glycolysis while promoting lipogenesis indicating that there is excess glucose in the cell that has to be stored while slowing down glycolysis. In this way, glucose can be saved[271] when there is sufficient substrates to store energy as lipid. This is *not* the case for fructolysis, which bypasses PFK1 regulation by citrate. An implication of this is that even when there is sufficient Acetyl-CoA to make lipids, dietary fructose will continue to be processed into more Acetyl-CoA.

[270] Remember this from the glycolysis unit?

[271] For example, stored as glycogen.

This positive feedback loop, ocurring mainly in the liver will result in more lipogenesis. This is proposed to be the biochemical mechanism by which fructose consumption is associated with the development of MASLD[272][Lim et al., 2010].

[272] Metabolic Dysfunction-Associated Steatotic Liver Disease, previously known as Non-Alcoholic Fatty Liver Disease or NAFLD.

At a chronic level, fatty acid biosynthesis is regulated by transcriptional control of several enzymes. SREBP1c, discussed earlier, promotes the synthesis of Fatty Acid Synthase, ACC, ATP-Citrate Lyase, SCD1 and several of the elongases [Horton et al., 2002, Moon et al., 2012]. Another transcriptional regulator is the more recently described ChREBP[273]. This transcription factor is activated by excessive carbohydrates, and especially in the liver, results in the activation of many of the same lipid synthetic proteins[274]. ChREBP is thought to play a key role in fructose's strong lipogenic effects in the liver [Kim et al., 2016].

[273] Carbohydrate Response Element Binding Protein

[274] More information about ChREBP can be found in Baraille et al. [2015].

Lipodystrophy Results from Impaired Triglyceride Storage

We may generally consider fatty acid formation and storage as negative, as increased adiposity is causal of obesity, which now affects over a third of Americans [Flegal et al., 2016]. However fat synthesis and storage is also essential for normal physiological function. Disorders or impairments of either adipocyte generation, or lipid storage result in a disease termed *lipodystrophy*. In this condition, fatty acids are unable to be stored effectively as triglycerides in adipose depots and therefore are ectopically deposited in tissues such as liver and muscle, promoting tissue impairment and inducing insulin resistance. A recent genetic association study showed that among normal-weight seeming individuals with insulin resistance and hyperlipidemia, variants in lipogenic and adipogenic genes were associated with this phenotype [Lotta et al., 2016]. This may also be part of the reason why some ethnic groups, such as those of Chinese descent, have increased diabetes and liver disease risk, even at adiposity levels that pose less risk for those of European descent [Chiu et al., 2011].

Cholesterol Synthesis

All cells are able to produce cholesterol, and in all cases the rate limiting enzyme is *HMG-CoA Reductase*, abbreviated HMGCR[275]. Prior to this step, there are reversible reactions interconverting Acetyl-CoA and Acetoacetyl-CoA and then an irreversible reaction[276] catalysed by HMG-CoA Synthase:

[275] The HMG-CoA here stands for 3-hydroxy-3-methylglutaryl-CoA

[276] This is irreversible only in liver; this reaction is reversible in muscle tissues and other tissues that can utilize ketone bodies

$$Acetoacetyl - CoA + Acetyl - CoA + H_2O \rightarrow HMG - CoA + CoA \tag{56}$$

This produces the intermediate HMG-CoA. At this stage the pathway branches between cholesterol synthesis and Acetoacetate synthesis [277]. HMG-CoA has two potential fates:

[277] ketogenesis

If HMG-CoA Reductase is Active: The HMG-CoA continues along to irreversibly become mevalonate and then cholesterol

If HMG-CoA Lyase is Present and HMGCR is Inactive: HMG-CoA is cleaved to form Acetoacetate, which in turn can be converted into β-Hydroxybutyrate and Acetone the three major ketone bodies. This is thought to occur primarily in the liver.

The reaction catalyzed by HMGCR is:

$$HMG - CoA + 2NADPH \rightarrow Mevalonate + CoA + 2NADP \tag{57}$$

Because the steps other than HMGCR and HMG-CoA Lyase are generally reversible there are some key consequences. First, a build up of Acetyl-CoA will result in increased flux towards cholesterol biogenesis. This will occur when fatty acid oxidation[278] occurs but energy demands are insufficient to pull Acetyl-CoA through the TCA cycle. Second, this means that in most tissues that are not ketogenic, excessive Acetyl-CoA will result in increased cholesterol synthesis. As we will discuss below there is some negative feedback to this system but endogenous cholesterol synthesis is the primary mechanism of hypercholesterolemia. Third, in tissues that are ketogenic, ketone bodies can be produced if there is insufficient oxaloacetate and/or insufficient TCA cycle activity. Efficient ketone body production, export and usage can alleviate this overflow of Acetyl-CoA, but its worth considering that the tissues that the ketone bodies traffic to also need to have efficient energy demands and TCA cycle activity to use those for energy. Otherwise ketone bodies are converted back to HMG-CoA and can become cholesterol there.

[278] This generates a lot of Acetyl-CoA, but this is also true if there is excessive PDH activity or ketogenic amino acid catabolism.

THE ACTIVITY OF HMGCR IS THEREFORE THE MOST IMPORTANT REGULATORY STEP IN CHOLESTEROL SYNTHESIS. This reaction is regulated by allosteric, post-translational and transcriptional mechanisms. There is negative feedback inhibition, via a mevalonate-derived product which reduces the activity of HMGCR, but to date the endogenous inhibitor has not been found. Instead, much like Acetyl-CoA Carboxylase, HMGCR is phosphorylated and inhibited by the AMPK-Activated Protein Kinase (AMPK). This means that

when energy levels are low, Acetyl-CoA is not converted to cholesterol but instead is converted to ATP[279].

[279] Of course, the reverse is also true; that when ATP levels are high, HMGCR activity is also high.

Sensing and regulation of sterol biosynthesis by SREBP2. At the transcriptional level HMGCR is controlled by the transcription factor SREBP2. This transcription factor is normally present in the endoplasmic reticulum where it is inactive. Once it is activated, SREBP2 would drive the transcriptional upregulation of HMGCR, HMGCS and the LDLR[280]. SREBP2 is maintained in the ER, in an inactive form by high cholesterol levels. This mechanism means that when cholesterol levels in the tissue are high, cholesterol synthesis is reduced but when the cholesterol levels in the tissue are low these genes are activated.

[280] The Low-Density Lipoprotein Receptor, which is used to scavenge cholesterol in the form of LDL back into the liver. This will be covered in the lipid transport lecture.

HMG-CoA Reductase is the target of statins. Inhibition of HMGCR by drugs will reduce endogenous cholesterol production, an approach that has proven far more effective than dietary restriction in the reduction of blood cholesterol levels. Currently, one in five Americans above the age of 40 take a statin, which is a HMG-CoA Reductase inhibitor. These drugs are quite effective, reducing the risk of cardiovascular events by approximately 20% [Cholesterol Treatment Trialists' Collaboration, 2010]. There are some serious side effects of statins however, including a small but significant risk of diabetes and an increased risk in muscle breakdown, potentially due to reductions in CoEnzyme Q[281].

[281] This was discussed way back in the TCA cycle lecture, where we discussed how CoEnzyme Q, an electron carrier in the electron transport chain is generated from cholesterol.

The Relationship Between Dietary Fat and Cholesterol Synthesis

There is a strong negative association between MUFA and PUFA intake and both LDL/HDL ratio and cardiovascular risk [Hu et al., 1998, Zong et al., 2016]. Consistent with this, a large randomized controlled trial with a high intake of PUFA and MUFA containing foods reduced cardiovascular events by about 30% [Estruch et al., 2018]. The exact mechanisms by which saturated fats more potently promote cholesterol biogenesis, while MUFA and PUFA do not are still under investigation. Some possibilities include higher energy content, preferential oxidation to acetyl-coA and reduced packing density.

Reflection Questions

1. When the TCA cycle is saturated with Acetyl-CoA, citrate is exported from the mitochondria to the cytoplasm. Analyze how this single event simultaneously coordinates three processes: activa-

tion of de novo lipogenesis via ACC, suppression of glycolysis via PFK1 inhibition, and explain why fructose consumption bypasses this regulatory brake — making fructose particularly lipogenic in the liver even when Acetyl-CoA is already abundant.

2. A lean patient with a loss-of-function mutation in PPARγ presents with severe insulin resistance and MASLD despite having very low adipose tissue mass. Evaluate the mechanistic connection between impaired triglyceride storage capacity in adipose and ectopic fat deposition in liver and muscle, and explain how this leads to insulin resistance in the absence of obesity.

3. In the liver, HMG-CoA sits at a branch point between ketogenesis (via HMG-CoA Lyase) and cholesterol synthesis (via HMG-CoA Reductase). Propose a hypothesis for how impaired hepatic ketogenesis (for example, due to reduced HMG-CoA Lyase activity) would affect cholesterol synthesis and circulating LDL-C levels. Use your knowledge of the reversible reactions upstream of HMG-CoA, SREBP2 regulation, and the fate of excess Acetyl-CoA to support your reasoning.

Lipolysis and Lipid Oxidation

This unit will discuss how lipids are oxidized and converted into energy. Free fatty acids contain substantially more energy per molecule than either carbohydrates or proteins. For more details on this process see Chapter 27 in Biochemistry: A Short Course available in reserve[282].

[282] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

Learning Objectives

- Explain how triglyceride breakdown into glycerol and free fatty acids is controlled in adipocytes by hormonal signals.
- Explain how high carbohydrate diets affect fuel utilization, including effects on lipid fuel utilization. Describe at an endocrine level how this is thought to occur.
- Determine how much energy, in ATP equivalents, is released during the oxidation of a given fatty acid. Be able to relate the energy content of a fatty acid, in general to its physical properties (length and saturation).
- Explain the rate limiting steps of lipid oxidation.
- Explain how ketone bodies are converted to ATP in non-hepatic tissues, and what governs this specificity.
- Demonstrate an understanding of how how *de novo* lipogenesis and β-oxidation are reciprocately controlled.
- Describe how very long chain fatty acids are oxidized differently from long chain fatty acids.
- Explain how odd-numbered fatty acids are catabolized, including the importance of vitamin B12 in this process.
- Evaluate the role of transcriptional regulation and long term adaptations to fatty acid oxidative capacity.

Lipolysis Liberates Fatty Acids from Triglyerides

Fatty acids are generally stored as triglycerides, and those triglycerides are primarily stored in adipose tissue. There are two main sources of fatty acids, the dietary fatty acids liberated from chylomicrons by the activity of lipoprotein lipase[283], and fatty acids liberated from adipose tissue[284]. This step, the conversion of triglycerides to fatty acids is known as lipolysis.

[283] This will be described in more depth in the lipid transport lecture

[284] A quantitatively small amount of lipids may be stored in liver (too much of this is called hepatic steatosis) and muscle (intramuscular triglycerides) tissues as well

In adipocytes the most potent activators of lipolysis are catecholamines such as adrenaline and cortisol. Adrenaline functions to rapidly activate adipocyte triglyceride lipolysis to glycerol and fatty acids via the activation of two enzymes, adipocyte triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). These two enzymes work primarily on triglycerides and diglycerides, respectively[285]. Via PKA-dependent signaling, both HSL and ATGL have

[285] The last step, the conversion of monoacylglycerol to glycerol and a fatty acid, is catalyzed by monoacylglycerol lipase, an enzyme not thought to be regulated by hormones.

increased rates of activity resulting in fatty acid release for use as energy in peripheral tissues. Cortisol also increases lipolysis, potentially through several mechanisms, one of which is increasing the levels of ATGL, in a slower more permanent manner.

Carbohydrate Overfeeding and Lipid Utilization

Insulin potently restricts adipocyte lipolysis. The mechanisms of this are still not clear, but at least part of the actions of insulin are to inactivate the lipases HSL and ATGL by reversing the phosphorylation events caused by adrenaline/PKA. States of insulin resistance such as type 2 diabetes means that insulin is less able to keep lipids in the adipose tissue. Interestingly, studies where *carbohydrates* are given in large quantities as part of overfeeding experiments lead to fat accumulation [Acheson et al., 1988]. This was originally thought to be due to activated *de novo* lipogenesis, but more intricate studies showed that instead, the body was adapting to high levels of carbohydrates by sparing fat [McDevitt et al., 2001] and that the level of lipogenesis was minimal. This lipid sparing effect is the result of increased glucose oxidation and reduced lipolysis and β oxidation. This is a concept we have discussed several times already in this class, when one macromolecule is in relative excess, we tend to alter our metabolic pathways to use this for fuel[286]. Of the multiple potential mechanisms for this lipid sparing effect, one is that carbohydrates induce a strong insulin response, which prevents lipolysis and results in lipids being trapped in adipose tissue. This distinction is key to understanding how high carbohydrate diets relate to obesity.

[286] Some examples we have already discussed include the inhibitory effects of Alanine on Pyruvate Kinase, Citrate on PFK1 and Palmitate on Acetyl-CoA Carboxylase.

Fatty Acid Oxidation

Fatty acid oxidation is important in many contexts, including during endurance exercise, and fasting. It is particularly important in the heart where even under resting or elevated carbohydrate conditions, the majority of ATP is derived from fatty acid oxidation [Neely and Morgan, 1974]. Once fatty acids enter or are made available to the cell, they are quickly converted to "activated" forms known as acyl-CoA molecules[287]. This is also the first step for triglyceride esterification, and consumes two ATP equivalents. The enzymes that catalyze these reactions are known as Acyl-CoA Synthetases. More details about these enzymes can be found in this recent review: [Grevengoed et al., 2014].

[287] The acyl refers to a generic fatty acid molecule, so the specific end product could be oleyl-coA (from oleate) or palmitoyl-CoA (from palmitate).

$$FattyAcid + CoA + ATP \rightarrow AcylCoA + AMP + PPi \tag{58}$$

While there are several Acyl-CoA Synthetases, one in particular,

ACSL1[288] seems particularly important for fatty acid oxidation, as its genetic ablation in mice almost completely prevents fatty acid oxidation [Ellis et al., 2011]. Interestingly, ACSL1 is present on the surface of the mitochondria in a physical complex with CPTI, the most important regulatory protein in fatty acid oxidation [Lee et al., 2011].

[288] Abbreviated from Acyl-CoA Synthetase Long Chain Family Member 1, indicating a preference for long chain fatty acids

MITOCHONDRIA ARE KEY FOR FATTY ACID OXIDATION. Like most amino acid catabolism, fatty acid conversion to energy requires mitochondria. However, unlike both pyruvate and most amino acids, the transport into the mitochondria is the rate-limiting step for fatty acids. This transport[289] is controlled by the carnitine shuttle system.

[289] Which is more difficult than passive diffusion, due to the semi-insoluble nature of fatty acids

The Role of CPTI in Lipid Oxidation

The carnitine shuttle system is a mechanism to get long chain fatty acids[290] through both the outer and inner mitochondrial membranes. It comprises of two reactions and a transporter. The reactions are overall energetically neutral, with the end products identical to the initial reactants. The two reactions are catalyzed by Carnitine Palmitoyltransferase I (CPTI):

[290] Short and medium-chain fatty acids can transport through the membranes freely, and therefore are not subject to this system. This also means that they are not subject to regulation via malonyl-CoA-dependent inhibition.

$$AcylCoA + Carnitine \rightarrow Acyl - Carnitine + CoA \tag{59}$$

and Carnitine Palmitoyltransferase II (CPTII):

$$Acyl - Carnitine + CoA \rightarrow AcylCoA + Carnitine \tag{60}$$

There is a transporter on the inner mitochondrial membrane called Carnitine Acyltransferase (CAT). This transporter *only* works with the carnitine conjugated fatty acids, so this transport system requires carnitine for its activity. As we will discuss in the non-protein compounds made from amino acids unit, carnitine can be made endogenously from the essential amino acid lysine. Carnitine can also be obtained in the diet, with particularly high levels in red meat.

MALONYL-COA INHIBITS CPTI. This is the most important regulatory step in fatty acid oxidation. If Acetyl-CoA Carboxylase (ACC) is activated due to an excess of Acetyl-CoA, Citrate or insulin signaling[291], then there is a buildup of its product Malonyl-CoA in the cytoplasm. This product potently inhibits CPTI [McGarry et al., 1977]. The result of this is to prevent fatty acid import into the mitochondria and therefore fatty acid oxidation. The activated fatty acids are then shuttled towards triglyceride synthesis rather than oxidation. This regulatory process is illustrated in Figure 46. The flip side of this inhibition is that when AMPK or PKA[292] are active, ACC is inhib-

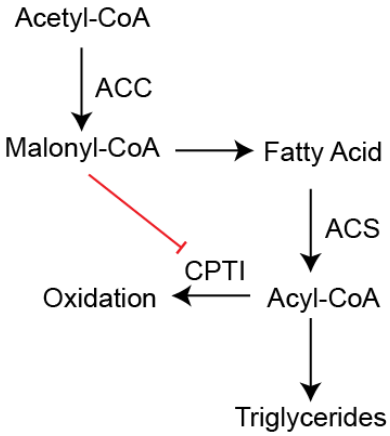

Figure 46: Schematic of the regulation of fatty acid oxidation. ACS indicates Acyl-CoA Synthetase, ACC indicates Acetyl-CoA Carboxylase.

[291] Refer back to the lipid synthesis notes/lecture for more information

[292] Via activation by adrenaline or glucagon for example.

ited, Malonyl-CoA is reduced and CPTI is active. In this way, energy demand or adrenaline can promote fatty acid oxidation.

Lipid Oxidation in the Mitochondria

Once the Acyl-CoA is inside the mitochondria, oxidation occurs through a series of enzymatic reactions. These reactions proceed in a serial manner to extract one Acetyl-CoA at a time, removing two carbons each cycle. The cycles consist of the following:

Desaturation This generates a double bond at the Δ^2 position of the fatty acid[293]. This step *generates* one $FADH_2$ molecule.

[293] The Co-A group is attached at the carboxyl group, or the Δ^1 position.

Isomerization This transfers the electrons from this new double bond to a new ketone group on the Δ^3 carbon. This step *generates* one NADH molecule.

Release The first two carbons are now released as Acetyl-CoA by an enzyme called a thiolase.

After one round, we are left with a fatty acid that is two carbons shorter, and have generated one Acetyl-CoA, one NADH and one $FADH_2$ that can be used as fuel. For a saturated fatty acid such as palmitate (C16:0) this means that this cycle occurs *seven* times[294].

[294] Not eight, because the last reaction ends with two Acetyl-CoA molecules.

Alternative Fatty Acid Catabolism

Long chain, unsaturated fatty acids are catabolized in a very similar manner with one difference. The first desaturation step introduces a double bond, but for unsaturated fatty acids the double bond is already present. That means that during progressive oxidation, if a double bond is already present at the Δ^2 of the now shortened fatty acid the first step is skipped, and $FADH_2$ is not generated.

ODD-NUMBERED FATTY ACIDS after the last thiolase step, you end up with an Acetyl-CoA and a three carbon Propionyl-CoA[295]. Propionyl-CoA is then converted to Succinyl-CoA by a three step reaction that in net consumes one ATP and requires Vitamin B12. Succinyl-CoA is a component of the TCA cycle, and can be further oxidized generating one GTP and one NADH.

[295] Several amino acids, including Isoleucine, Valine, Threonine and Methionine also produce Propionyl-CoA

VERY LONG CHAIN FATTY ACIDS ARE FIRST OXIDIZED IN THE PEROXISOME. The Acyl-CoA Synthetase with activity towards very long chain fatty acids[296] resides on the peroxisomes. Peroxisomes are membrane enclosed organelles that contain similar enzymes to those that perform β-oxidation in the mitochondria. The major difference is

[296] Those with more than 22 carbons.

that while these lipids are catabolized, there is no electron transport chain in peroxisomes, so no ATP is produced. Instead electrons are transferred to peroxide, which is in turn converted to water and oxygen by the enzyme Catalase. This process terminates with short and medium chain fatty acids that are released as acyl-carnitines where they travel to mitochondria for final catabolism and some energy production.

Determining the Energy Content of a Fatty Acid

Based on the series of reactions above, or each two-carbon unit released, one Acetyl-CoA, one NADH and one $FADH_2$ are released. Added together this provides 14 ATP equivalents per two carbon units. Therefore to calculate the ATP in a saturated fatty acid like palmitate (C16:0) we could do the following:

- Consume 2 ATP to activate the fatty acid (see the ACS reaction 58).
- Take the fatty acid length, and subtract 2 then divide by 2. This is the number of fatty acid oxidation cycles. For palmitate that is $\frac{16-2}{2} = 7$. For each cycle we generate 14 ATP units, for palmitate there is $14x7 = 98$ ATP equivalents generated.
- The remaining Acetyl-CoA from the last two carbons left adds another 10 ATP equivalents
- The total ATP is then $98 + 10 - 2$ or 106 ATP equivalents.

For unsaturated fatty acids, you do not need to perform the desaturation step, so for each double bond there is one less $FADH_2$ generated than normal. If we had a fatty acid that was 16:1 it would generate a total of 104.5 ATP equivalents.

Table 36: ATP equivalents of some fatty acids.

Lipid	Net ATP
Butyrate (C4:0)	22
Laurate (C12:0)	78
Palmitate (C16:0)	106
Oleate (C18:0)	120
Linoleate (C18:2)	117

GENERALLY THIS PROCEDURE EXPLAINS TWO BIOENERGETIC PROPERTIES OF FATTY ACIDS. Longer chain fatty acids have more energy than shorter chain fatty acids, and saturated fatty acids have more energy than desaturated fatty acids. I have used the method above to calculate the ATP equivalents of several fatty acids in Table 36 to illustrate this point. Don't try to memorize these, but rather spend some time practicing how to calculate the ATP-equivalents[297]. After you have thought about how to calculate this, take a step back. This is a lot of energy storage. A triglyceride with three C16:0 molecules is catabolized to 318 ATP equivalents. This is close to *ten times* the energy storage of a fully oxidized glucose molecule (32 ATP).

[297] For even more of a challenge, try to calculate the net energy production from an odd-chain fatty acid.

Ketolysis

Ketone bodies are produced when there is excessive acetyl-CoA, but insufficent TCA cycle intermediates in the liver. These ketone bodies[298] can be converted back to Acetyl-CoA in non-hepatic tissues. This specificity is due to the absence of an enzyme called 3-oxoacid CoA-transferase 1[299] in the liver thus not allowing the liver to utilize ketone bodies while allowing other tissues to utilize ketone bodies for energy. This enzyme catalyzes this reaction starting with Acetoacetate[300]:

$$Acetoacetate + Succinyl - CoA \rightarrow Succinate + Acetoacetyl - CoA \quad (61)$$

As a result this generates a single Acetyl-CoA molecule after a thiolase reaction. This reaction is not cataplerotic because both Succinate and Succinyl-CoA are part of the TCA cycle. However bypassing the Succinyl-CoA Synthetase step means that one GTP is not generated during normal TCA cycle progress. Overall, an Acetyl-CoA that is released from the liver as β-hydroxybutyrate costs the liver 3.5 ATP molecules [301]. In the recipient tissue, that β-hydroxybutyrate generates 6.5 ATP in net[302]. Overall, ketone usage means that the original Acetyl-CoA in the liver now only generates 3 ATP equivalents rather than the 10 ATP if it remained in the liver (6.5 ATP generated - 3.5 ATP used in liver to generate the β-hydroxybutyrate = 3 ATP in total). Table 37 summarizes the energy costs of the three major transport processes we have discussed this year. The *inefficiency* of these transport processes comes at a cost to performance, but can cause negative energy balance for weight loss. In terms of low-carbohydrate, high fat diets this may explain the slight increase in energy expenditure (100-200kcal/day) observed in relation to low fat, high carbohydrate diets [Hall et al., 2016, Ebbeling et al., 2018].

[298] Primarily β-hydroxybutyrate then acetoacetate; acetone is a terminal end product and cannot be used for energy.

[299] OXCT1, sometimes called succinyl-CoA-3-oxaloacid CoA transferase (or SCOT).

[300] β-hydroxybutyrate can be interconverted to and from acetoactetate using up one NADH molecule for each acetoacetate generating, and using an NADH for the reverse reaction.

[301] 1 ATP for the ATP-Citrate Lyase step, 2.5 ATP for the NADH used to generate β-hydroxybutyrate

[302] Generate 10 ATP from the TCA cycle oxidation of Acetyl-CoA, but using 1 NADH converting β-hydroxybutyrate to acetoacetate; and 1 GTP skipping the Succinyl-CoA Synthetase step. So 10 - 3.5 =6.5

Table 37: Energy costs of the macromolecule transport processes discussed this year. Refer to the gluconeogenesis notes for details about the Cori and Cahill cycles.

Process	Net ATP Loss
Ketone Body Transport	-7 ATP
Cori Cycle (Lactate)	-6 ATP
Cahill Cycle (Alanine)	-15 ATP

Regulation of Fatty Acid Oxidation

Short-term Regulation

As we described above, short term regulation is accomplished primarily through the ACC/Malonyl-CoA/CPTI system described in Figure 46. Two other key regulatory systems to consider in the short term are the flux of fatty acids, either via the regulation of lipolysis, or the regulation of Lipoprotein Lipase activity[303]. Another regulatory mechanism that might not seem as apparent, is that when there is sufficient non-lipid fuel sources (for example an excess of carbohydrates or amino acids), there will be more Malonyl-CoA and therefore less lipid oxidation. Finally, recall that the products of fatty

[303] Discussed in the lipid transport lecture.

acid oxidation all require TCA cycle and electron transport chain activity. Since the most important driver of the electron transport chain is energy demand, the final oxidation of all that Acetyl-CoA, NADH and $FADH_2$ will only occur if there is some energy demand. Alternately, if there is insufficient TCA cycle intermediates[304] the NADH and $FADH_2$ generated from the partial oxidation of fatty acids might be used for fuel, but the Acetyl-CoA generated from the partial oxidation of fatty acids will undergo ketogenesis for transport to other tissues.

[304] For example due to cataplerosis in a liver undergoing substantial gluconeoegenesis.

Transcriptional Adaptations for Fatty Acid Oxidation

In terms of athletic performance, increasing the ability to oxidize fatty acids is important for endurance athletes. Highly trained athletes tend to have more mitochondria, and higher expression of enzymes such as Lipoprotein Lipase, CPT1, Acetyl-CoA Synthetase and enzymes of the TCA cycle. One major regulator of these adaptations are transcription factors called PPARδ and PPARα. In muscle tissues PPARα and PPARδ activate the transcription of several lipid transport and oxidation genes. In liver tissues, a similar transcription factor, PPARα upregulates many of the same genes[305] along with genes involved in ketogenesis [Kersten et al., 2000, Badman et al., 2007]. Both of these transcription factors are induced by elevated intracellular fatty acid levels [Keller et al., 1993]. The specific natural ligands for the PPAR transcription factors have been difficult to unambiguously identify, but PUFA's seem to play a key role in activating these transcription factors [Forman et al., 1997]. This is potentially one mechanism by which PUFA's alleviate lipid accumulation.

[305] Activation of PPARα is a promising target to alleviate hepatic steatosis and metabolic dysfunction-associated steatotic liver disease (MASLD), though they have not yet reached the clinic.

Interestingly, similar adaptations occur in the context of obesity, likely for the same reasons. The major difference, is that in the absence of energy demand, lipids are only partially oxidized or end up stored as lipids in cells. The buildup of stored, partially oxidized fatty acids is thought to play a role in the pathogenesis of insulin resistance, though the mechansims are not clearly defined.

Reflection Questions

1. Calculate the net ATP yield from complete oxidation of one molecule of oleate ($C18{:}1\Delta^9$) in muscle, accounting for the activation cost and the reduced $FADH_2$ generation due to the double bond. Then compare this to the net ATP a recipient muscle cell would obtain if the liver had instead exported that same Acetyl-

CoA as β-hydroxybutyrate. Use this comparison to evaluate the energetic trade-off of ketone body transport.

2. A patient with a primary carnitine deficiency cannot maintain adequate intracellular carnitine levels. Analyze how this would differentially affect oxidation of short-chain vs. long-chain fatty acids, predict the consequences during prolonged fasting, and explain why the heart would be particularly vulnerable in this condition.

3. A sedentary person and an endurance-trained athlete consume identical high-fat meals after an overnight fast. Evaluate how their capacity to oxidize dietary fatty acids differs at three levels: (a) hormonal control of lipolysis in adipose, (b) CPTI activity and malonyl-CoA regulation in muscle, and (c) transcriptional adaptations via PPARα/PPARδ. Predict the metabolic fate of dietary fatty acids in each person.

Transport of Lipids and Lipoproteins

Lipid transport is essential to both the efficient storage, and effective use of lipids. Due to their semi-or total insoluble nature, triglycerides, cholesterol and fatty acids present some specific technical problems in terms of transportation. These processes rely on both carrier proteins such as albumin as well as lipoprotein particles to safely move lipids from one tissue to another. Inefficient co-ordination of lipid transport can lead to elevated blood lipids, which are highly associated with cardiovascular disease, a major cause of death in modern society. For more details about lipid transport refer to Chapter 18 in Lippincott's Illustrated Reviews: Biochemistry, available in reserve[306].

[306] Denise Ferrier. *Lippincott Illustrated Reviews: Biochemistry*. LWW, 1496344499, 2017. ISBN 1-4963-4449-9

Learning Objectives

- Explain why lipoproteins are necessary for triglyceride and cholesterol transport.
- Describe the main carriers of cholesterol and triglyceride throughout the body, including how their apolipoproteins affect their endocytosis or catabolism.
- Apply your knowledge of cholesterol transport to explain why someone may have changes in HDL and LDL levels.
- Understand the etiology of high cholesterol and its potential role in atherosclerosis.
- Apply your understanding of cholesterol absorption, synthesis and transport to evaluate the relationships between dietary cholesterol and triglycerides and cardiovascular risk.
- Explain the role of lipoprotein lipase in lipid transport, including how it is regulated.

Key Concepts and Vocabulary

- Lipoprotein particles including:
  - Chylomicrons
  - Very Low Density Lipoproteins (VLDL)
  - Low Density Lipoproteins (LDL)
  - High Density Lipoproteins (HDL)
- Apolipoproteins, especially ApoB48, ApoB100 and ApoCII
- Lipoprotein Lipase and its regulation
- Reverse Cholesterol Transport
- Trans-Intestinal Cholesterol Export (TICE)
- Fatty Acid Transport and Albumin

Triglyceride and Fatty Acid Transport Mechanisms

Transportation of lipids presents some logistical problems. Since they are inherently insoluble, lipids need to be either solubilized prior to transport to other tissues via the blood stream. This is accomplished in two ways. One is the packaging of triglycerides and cholesterol esters into lipoprotein particles, such as the chylomicrons discussed

earlier in this unit. The second mechanism is to break triglycerides down to fatty acids, where they can bind to solubilizing proteins called albumin within the blood.

Lipolysis and Fatty Acid Transport

As we described in the unit about lipid oxidation, the majority of our triglyceride stores are in adipose tissue. The release of free fatty acids and glycerol from adipose tissue is a highly regulated process, activated by adrenaline and inhibited by insulin (for more details see Zechner et al. [2012] for a review). The transport of free fatty acids after release from adipose tissue is mediated by albumin, a very abundant protein produced by the liver. Due to their semi-solubility, fatty acids also require transport systems and fatty acid binding proteins (abbreviated as FABP) to move through membranes and through the cytoplasm.

Lipoprotein Particles in the Body

In terms of moving triglycerides and cholesterol esters, we have a variety of lipoprotein particles that play different roles in the body. These are summarized in Table 38. There are three main transport routes. The first is from the enterocyte to the periphery, mediated by chylomicrons. The second is from the liver to the periphery, mediated by VLDL. The third is from the periphery back to the liver, mediated by HDL and LDL. We will discuss each of these in the next few sections.

The goal of these lipoprotein particles is to move lipids from the source[307] to peripheral tissues which might be better equipped to utilize or store these lipids. As summarized in Table 39, these particles are characterized by distinct lipoproteins.

Table 38: Summary of lipoprotein particles.

Particle	Source	Destination
Chylomicron	Enterocyte	Adipose, Muscle, Liver
VLDL	Liver	Adipose, Muscle
IDL	VLDL	Liver or LDL
HDL	Endothelial	LDL
LDL	IDL/HDL	Liver

[307] The source of chylomicrons transporting dietary lipids is the enterocyte, or the source of VLD is the liver; the function of lipoproteins is to transport the lipids from both these sources, enterocyte and liver

Table 39: Apolipoprotein summary. Some key things to remember, ApoB48 is specifically made in the enterocyte. ApoB100 and ApoE are the ligands for the LDL Receptor allowing for LDL uptake in tissues expressing the LDL receptor (mainly the liver). ApoCII is a coenzyme for LPL thus activating it and allowing for lipid extraction to peripheral tissues.

Particle	ApoA	ApoB	ApoC	ApoE
Chylomicron	AV	B48	CII/CIII	E
VLDL	AV	B100	CI/CII	E
IDL		B100		E
HDL	AI/AII			E
LDL		B100		

The Role of Chylomicrons and VLDL

Both chylomicrons and VLDL function to move lipids to peripheral tissues, either from the gastrointestinal tract or the liver, respectively. These particles transport primarily *neutral lipids* rather than free fatty acids. Their assembly is dependent on the production of the apolipoproteins and the presence of phospholipids, especially phosphatidylcholine that is important for their synthesis. If choline levels are limited, either due to less active variants in *PEMT* or reduced choline dietary intake, the liver will be less able to assemble VLDL. This can result in increased hepatic steatosis, potentially leading to metabolic dysfunction associated fatty liver disease.

The Role and Regulation of Lipoprotein Lipase

Both VLDL and chylomicrons are targeted to peripheral tissues. This specificity is mediated by Apolipoprotein CII. This protein acts as an activator of a triglyceride lipase known as *Lipoprotein Lipase* or LPL. This lipase resides on the lumen of blood vessels, adjacent to muscle and adipose tissues. Once activated by ApoCII binding, LPL breaks down the triglycerides in the particle and releases its free fatty acid content. These free fatty acids enter the cell where they can be stored (if they enter into the adipocytes), or are used as fuel (if they enter into the muscle cells). The levels of LPL are inversely regulated in adipose and muscle tissue. For example, insulin promotes LPL transcription in adipose tissue[308] but decreases LPL transcription in muscle [Spooner et al., 1979]. The inverse is true during fasting, where the muscle LPL transcription is increased while that of the adipose tissue is reduced thus promoting muscle uptake of fat to be used as energy, while limiting adipose tissue ability to store this fat in times of energy need (fasting).

[308] To promote lipid storage. This is accomplished by both transcriptional and post-translational mechanisms, reviewed in Kersten [2014].

LPL IS INACTIVATED BY DIETS HIGH IN SATURATED FATS. This means that when saturated fat levels are increased, LPL activity is reduced. You can imagine how this is no beneficial to the body, as the lipids in the blood cannot be taken up into the muscles or adipose tissue, therefore remaining in the blood and circulation. This is a negative feedback mechanism wherein intracellular lipids can signal to the LPL on the extracellular surface to prevent additional fat uptake (see Figure 47). The molecular underpinnings of this phenomena have recently been determined and involve the transcriptional activation of a protein called ANGPTL4[309]. ANGPTL4 is induced when excess fatty acid levels in the cell activate the transcription factor PPARα. ANGPTL4 is then secreted where it binds to and inhibits the activity of LPL (more details about this can be found in the recent review by Dijk and Kersten [2014]). Mutations in either the *LPL* or *ANGPTL4* genes result in either impaired, or enhanced blood lipid clearance, respectively and as a result either lead to an increased or decreased risk of cardiovascular disease [Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators et al., 2016].

[309] Unhelpfully, an abbreviation for Angiopoietin-like 4.

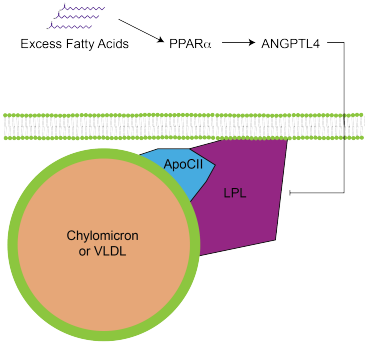

Figure 47: Regulation of Lipoprotein Lipase (LPL) by ANGPTL4.

DEPLETED VLDL ARE KNOWN AS IDL, WHEREAS DEPLETED CHYLOMICRONS ARE KNOWN AS CHYLOMICRON REMNANTS. Once these lipoproteins have delivered their triglyceride content into cells, they are either known as chylomicron remnants or intermediate[310] density lipoproteins (IDL). Due to the presence of ApoE on their surface,

[310] This indicates that this lipoprotein has intermediate density that falls in between VLDL and LDL's density

chylomicron remnants and IDL particles can be absorbed by the liver where the apolipoproteins and phospholipids can be reused.

APOE VARIANTS ARE ASSOCIATED WITH DISEASE RISK. ApoE is present on both chylomicrons and the VLDL that then form IDL/LDL. This gene is the major heritable risk factor for late onset Alzheimer's disease. There are four variants of the *APOE* gene numbered 1–4[311]. Of these isoforms, ApoE2 is thought to be protective of Alzheimer's disease and associated with lower levels of LDL-cholesterol[312] while ApoE4 is a strong risk factor for late-onset Alzheimer's disease, and is associated with higher levels of LDL-cholesterol [Poirier et al., 1993, Corder et al., 1993].

[311] These are variants of the same gene, not different genes

[312] This appears to be specific to the APOE gene, as other genetic factors that have more pronounced effects on LDL-cholesterol do not have as strong an association with AD.

Reverse Cholesterol Transport

Cholesterol is primarily disposed of via bile salt generation and excretion, a process that starts in the liver. Therefore cholesterol, which is made throughout the body, is primarily trafficked to the liver, a process known as *Reverse Cholesterol Transport*. This process is mediated by both HDL and LDL particles. To separate blood lipids between reverse and forward transport processes, sometimes the ratio betwen ApoB and ApoAI is determined[313]. An alternate cholesterol disposal pathway is through the intestine, a process known as transintestinal cholesterol excretion (TICE). In this pathway, cholesterol is transported by either HDL or LDL directly to the intestine for efflux. Estimates vary, but we think that somewhere between 20 and 40% of cholesterol excretion is through TICE, with the remainder being through biliary transport [Temel and Brown, 2015].

[313] Consider based on the data in Table 39 what this ratio is actually measuring. As a hint, a high ApoB100/ApoA1 ratio is indicitave of elevated cardiovascular risk.

Synthesis and Role of HDL

High density lipoprotein particles start off as nascent particles containing ApoAI, ApoAII and very little cholesterol. As they pass through the circulation, they bind cholesterol from the plasma membrane of tissues and become enriched with cholesterol. Since most tissues make but cannot dispose of cholesterol, HDL is important for scavenging excess cholesterol from our cells. The HDL particles may be endocytosed in the liver where cholesterol can be disposed, but most often they transfer their cholesterol to LDL particles using an enzyme known as cholesterylester transfer protein[314]. This ensures that triglycerides are packaged in the LPL-accessible particles for peripheral transport to be used by peripheral tissues, while excess cholesterol is delivered back to the liver or intestine for excretion. Inhibition of CETP results in an increase in the amount of

[314] Abbreviated as CETP

HDL cholesterol in the blood and thus CEPT inhibition was a heavily invested pharmacological area, but these drugs have shown limited cardiovascular benefits. The current thinking in this area is that high HDL cholesterol is a marker of lowered cardiovascular risk but does not cause lowered cardiovascular risk by itself.

LDL-mediated Transport to the Liver

Low density lipoproteins are generated when IDL derived from VLDL remains in the circulation, or when cholesterol is passed from an HDL to an IDL particle. These particles tend to be cholesterol rich, since the triglycerides have been already taken up due to the actions of LPL at peripheral tissues. These particles would normally be endocytosed by the liver where LDL receptors are found. LDLR levels are under control of the SREBP2. Recall that when intrahepatic cholesterol levels are high, SREBP2 is inactive, and thus LDLR would not be produced. This means that when the liver has sufficient cholesterol, LDL particles remain in the circulation[315]. LDL cholesterol levels are correlated with coronary events. If one wants to understand the levels of LDL cholesterol in a person there are two measures to consider. By looking at how much cholesterol is in the LDL fraction (LDL-C, should be less than 100 mg/dL) or how many LDL particles are present in the blood. Since each LDL contains one, and only one ApoB100 particle, and since LDL particles vastly outnumber VLDL particles, then if you determine the concentration of ApoB100 in blood, then that is a measure of LDL particle number. Recent studies have suggested that it is this particle number, more so than the amount of cholesterol in the LDL fraction, that is more predictive of cardiovascular events, though generally both the cholesterol content in LDL and the LDL particle number incresae for most people simultaneously [Cromwell et al., 2007, Mora et al., 2007].

[315] As a thought exercise, consider what would happen if you had a *LDLR* mutation, how would that affect cholesterol retrieval? How do you think it would affect cholesterol synthesis? This is the case for individuals with a disease known as familial hypercholesterolemia.

Cholesterol Export to Bile

Within the liver, bile salt synthesis is controlled by the activity of 7-α-hydroxylase[316] and exported to the gallbladder for release into the digestive system. Separate from the SREBP2-dependent cholesterol regulatory system, the production of bile salts is sensed by the FXR sensing system[317].

[316] We discussed this in the lipid digestion lecture

[317] FXR is a bile acid sensor expressed in entero-hepatic tissues.

Blood Lipids and Cardiovascular Risk

If lipid transport systems (of VLDL and LDL) have exceeded their ability to store lipids, then VLDL and LDL lipids remain in the blood.

Akin to the hyperglycemia associated with impaired glucose disposal, and excessive glucose production, hyperlipidemia is associated with cardiovascular disease due to impaired lipid disposal. Since triglycerides can be metabolized into energy by most tissues, but cholesterol cannot, hypercholesterolemia in particular has been long associated with cardiovascular risk [Keys et al., 1963].

Since cholesterol may exist in several lipoprotein particles, a more prognostic indicator of cardiovascular risk is the amount of cholesterol in HDL particles relative to the amount of cholesterol in LDL particles, with the latter being more pathogenic[318]. Several mechanisms for LDL's specific association with cardiovascular risk have been proposed, but one possibility is that excess LDL is absorbed in blood vessel walls, promoting both atherosclerosis[319] and increasing the risk of thrombosis[320].

[318] HDL cholesterol levels may indicate a surplus of cholesterol transport particles, whereas LDL cholesterol likely indicates a surplus of cholesterol that cannot be absorbed by the liver.

[319] The lipid-based coating of arteries, causing arteries to become narrower and reducing their vascular flexibility.

[320] The release of a blood clot, often by lysis and release of an atherosclerotic plaque. This blood clot could travel to the brain or heart where a stroke or heart attack may occur.

From a dietary standpoint, data from several US-based cohort studies demonstrated that diets lower in saturated fat intake are associated with both lower total and LDL cholesterol, and with reductions in cardiovascular disease [Anderson et al., 1987, Wang et al., 2016]. This has recently been challenged by a large-multi country study[321] which indicated that increased carbohydrate intake plays a key role, maybe more so than saturated fats with respect to cardiovascular disease [Mente et al., 2017]. For this large multi-ethnic study, there is some debate about whether regional dietary differences are fully accounted for, or if this is more reflective of diet-disease risk in a more diverse dataset.

[321] This is known as the PURE study, which evaluated over 135 000 participants in 18 countries.

Reflection Questions

1. A patient carries a loss-of-function mutation in ANGPTL4. Predict the effects on: (a) LPL activity after a high-fat meal, (b) circulating triglyceride levels, and (c) the distribution of dietary fatty acids between adipose and muscle. Then explain why population studies have associated ANGPTL4 loss-of-function variants with reduced cardiovascular disease risk.

2. Patient A has elevated LDL-C but a normal ApoB100 concentration. Patient B has normal LDL-C but elevated ApoB100. Using your knowledge of VLDL-to-LDL progression and what each measurement represents, evaluate which patient is at higher cardiovascular risk and why, and describe what metabolic or genetic conditions could produce each pattern.

3. A patient carries the ApoE4 allele and has elevated LDL cholesterol. They ask whether reducing dietary cholesterol is the best

strategy to lower their LDL. Apply your knowledge of SREBP2 regulation, LDLR expression, and the relationship between dietary and endogenous cholesterol to advise this patient, and explain what interventions targeting different steps in the system might be more effective.

Lipid Unit Integration Questions

1. A person eats a meal containing a grilled salmon fillet (rich in ω-3 PUFA and protein), roasted vegetables (fiber and fat-soluble vitamins), and olive oil dressing (MUFA). Trace the complete fate of the dietary lipids from digestion through absorption, chylomicron assembly, peripheral delivery, and eventual storage or oxidation. Your answer should address bile salt function, LPL regulation, the differential handling of PUFA vs. MUFA, and how the fiber in the meal might modify cholesterol absorption.

2. A patient with type 2 diabetes and MASLD presents with hypertriglyceridemia, low HDL, and elevated LDL particle number despite a normal LDL-C. Using your knowledge of the entire lipid unit, explain how insulin resistance simultaneously drives: excess VLDL secretion from the liver (via unrestrained de novo lipogenesis and impaired insulin suppression), impaired LPL activity in muscle, unrestrained lipolysis in adipose, and how excess hepatic Acetyl-CoA from both fatty acid oxidation and de novo synthesis contributes to the overall picture.

3. An endurance athlete on a very low carbohydrate ketogenic diet completes a four-hour training ride. Trace the metabolic events from adipose lipolysis through fatty acid transport (albumin, LPL, CPTI), β-oxidation, hepatic ketogenesis, and peripheral ketolysis in muscle. Include the role of malonyl-CoA in preventing futile cycling, explain why the brain can shift to using ketone bodies, and calculate (approximately) the energetic cost of using ketone bodies rather than direct glucose oxidation.

4. A patient with familial hypercholesterolemia (loss-of-function LDLR mutation) is started on a statin plus ezetimibe combination. Using your knowledge of cholesterol synthesis (HMGCR, SREBP2), intestinal absorption (NPC1L1, SREBP2 in enterocytes), bile acid cycling (enterohepatic circulation, fiber), and LDL particle clearance (LDLR), evaluate the mechanism of each drug, predict their combined effect on LDL-C, and explain why combination therapy is more effective than either drug alone.

5. Four dietary or lifestyle interventions have been proposed to reduce cardiovascular disease risk: (1) reducing dietary cholesterol intake, (2) replacing saturated fatty acids with monounsaturated fatty acids, (3) increasing soluble fiber intake, and (4) improving insulin sensitivity through exercise or weight loss. Using your knowledge of cholesterol absorption (NPC1L1, SREBP2), synthesis (HMGCR), lipoprotein metabolism, and the role of insulin in lipid homeostasis, compare and contrast the mechanistic basis and expected effectiveness of each intervention on LDL-C and cardiovascular risk. Which intervention(s) would you prioritize for a patient with type 2 diabetes and elevated LDL particle number, and why?

6. Chronic alcohol consumption is associated with hypertriglyceridemia, elevated LDL-C, and increased cardiovascular disease risk. Using your knowledge of alcohol metabolism (ADH vs. CYP2E1 pathways), hepatic NADH generation, effects on TCA cycle activity, de novo lipogenesis, VLDL secretion, and oxidative stress on LDL particles, trace the mechanistic pathway from alcohol consumption to cardiovascular risk. Your answer should distinguish between moderate and heavy drinking, and explain why the shift from ADH to CYP2E1 in heavy drinkers worsens the lipid phenotype.

Part IV

Proteins and Nitrogenous Compounds

Introduction to Proteins and Amino Acids

This lecture is the introduction to the proteins unit. In this unit we will describe some of the important functions of protein and their constituent amino acids including why some amino acids are essential (or conditionally essential), how they are interconverted and how they are used for energy. Amino acids also are the precursors for many other important biological molecules, so we will discuss these non-protein functions of amino acids as well.

Learning Objectives

- Apply your knowledge of amino acid biochemistry to explain why proteins are essential macronutrients
- Understand the basic structure of protein
- Identify the property that makes an amino acid different from another
- Describe different functional roles of protein
- Identify the major proteins present in food
- Differentiate between the different classifications of amino acids
- Understand how nonessential amino acids can become essential
- Explain the nutritional requirements of collagen synthesis

Proteins, Amino Acids and Essentiality

Glucose and other carbohydrates are not strictly essential. As we described in the unit on gluconeogenesis, we can generate glucose even if dietary supplies are low or absent. The same is not true for proteins, which are macromolecules comprised of amino acids. While plants, bacteria and fungi can often synthesize their own amino acids, we have lost the ability to make certain amino acids during evolution (see Table 40). Since most proteins consist of at least multiple of the amino acids, that means that we cannot generate new proteins unless we have a dietary supply of the essential amino acids. In this context, dispensible means that we can make these amino acids from other fuels in our body, such as glucose[322]. Essential means that we must get those amino acids from our diet.

Table 40: Amino Acid Essentiality.

Dispensible	Essential	Conditional
Ala	Phe	Arg
Asp	Trp	Tyr
Asn	Thr	Cys
Glu	Ile	Pro
Gly	Met	Gln
Ser	Val	
	Leu	
	His	
	Lys	

[322] Recall from the glycolysis lecture that pyruvate can be converted to alanine as part of the Cahill cycle via ALT function.

When we say that an amino acid is conditional, that means that we can only make it if we have another amino acid. For example, we can make Tyrosine in the body, but only if we have sufficient amounts of Phenylalanine. If we do not have sufficient amounts of Phenylalanine, then we cannot make Tyrosine, which makes Tyrosine a conditionally essential amino acid. The reaction to convert Phenylalanine to Tyrosine is catalyzed by the enzyme phenylalanine hydroxylase[323]:

[323] BH_4 in this equation indicates tetrahydrobiopterin, a cofactor for this reaction.

$$Phe + BH_4 + O_2 \leftrightarrow Tyr + BH_2 + H_2O \tag{62}$$

Recommended Protein Intake

The USDA recommends increasing protein levels over the lifespan (see Table 41), but suggests a wide range where <35% of calories are from protein. From an essentiality perspective, the amounts of each amino acid needed from the diet depend on the amino acid content of the food. As en example, legumes (beans and nuts) tend to be low in the essential amino acid methionine while grains often contain low levels of the essential amino acid lysine. When thinking about an appropriate protein amount, two things are important to consider: [1] are there sufficient dietary levels of the essential amino acids in a certain food? and [2] if protein levels in that food are low, are the calories from this food coming from lipids or carbohydrates? As an essential nutrient, protein is required, and therefore a RDA was also established at 0.8 g/kg/day for adults [Institute of Medicine, 2005]. This was largely based on nitrogen balance studies which is one way to estimate the typical protein required. There remained (and remains) some controversy about both nitrogen balance as a tool, and the correct DRI. In 2025 the DRI for protein was increased to 1.0–1.2 g/kg/day for adults, which is a significant increase from the previous recommendation [United States Department of Agriculture and U.S. Department of Health and Human Services, 2025]. The new DRI is considered to be more appropriate for the general population[324], but there is still some debate about whether it is sufficient for certain populations such as athletes or older adults. While it may seem like this is a large increase, the typical American diet already contains about 0.9–1.3 g/kg/day, albeit with significant gender differences [United States Department of Agriculture, Food Surveys Research Group, 2021].

[324] Consider evaluating another country's recommendation for protein consumption. Is it in the similar range to those for the United States?

Table 41: Acceptable Macronutrient Distribution Range (AMDR) for protein intake over the lifespan in percent of calories (from [United States Department of Health and Human Services et al., 2015]).

Age (years)	Amount (percent)
1-3	5-20
4-19	10-30
19+	10-35

Diseases of Protein Malnutrition

In the developed world, protein deficiency is rare; however, in some developing nations, protein deficiency is a major public health problem. Protein deficiency can manifest in two main ways, *kwashiorkor* which is a deficiency of protein, but an acceptable total calorie intake; and *marasmus* which is a deficiency of both protein and calories. Protein deficiencies can lead to impaired physical and mental development, fatty liver, hair loss and characteristic distended abdomen.

Protein Storage and the Amino Acid Pool

Unlike glucose[325] and fatty acids[326], there is no storage pool of amino acids for exchange or use. Instead, amino acids are stored as functional proteins in many tissues, but largely in muscle. This

[325] Glycogen stored in the liver, muscle and kidneys.

[326] Triglycerides stored in adipose tissue.

means that protein-deficient diets come at a functional cost, as proteins are broken down from the muscle to enable amino acid liberation for fuel or for synthesis of other proteins.

While the majority of amino acids are part of proteins, there are some free amino acids both in tissues and in the blood. This is known as the *amino acid pool*, which functionally comprises of the available amino acids that can be used for protein synthesis. As proteins are broken down, this pool fills up with amino acids. The available amino acid pool is especially important for the essential amino acids, since once they are depleted they must be obtained from the diet.

Branched-chain amino acids[327] are a special subgroup of essential amino acids. These three amino acids, *Leucine, Isoleucine* and *Valine* are very important as they relate to the amino acid pool for two reasons:

[327] Abbreviated as BCAAs

1. They are extremely abundant in our proteins, comprising of 20% of all amino acids and 35% of indispensible amino acids. Therefore during protein synthesis, these essential amino acids can become limiting.
2. There are very low levels of free BCAAs in tissue. Normally, there are about 3g/kg of amino acids in tissue, but only about 100mg of those amino acids are the three BCAAs.

These two factors combine to mean that BCAAs are nutritionally important, especially during growth and other times of protein synthesis[328]. Some foods that have high levels of BCAAs include red meat, chicken, fish and eggs.

[328] As such, the degradation of BCAAs is under especially tight control, as we will discuss in the amino acid oxidation lecture

Major Proteins in Human Nutrition

There are many thousands of different proteins, each of which have different synthetic requirements and nutritional components. However, some proteins are much more abundant in the food we eat, or in our bodies. Some of the major proteins we will discuss are collagen, actin and myosin. In terms of amino acids, collagen is particularly enriched in glycine, proline, and hydroxyproline.

Actin and Myosin are major proteins in muscle. Foods that are muscle derived, especially meats are high in actin and myosin. Actin and myosin form the contractile units of muscle and are the major components in both meat and in building muscle tissue. These

proteins form long fibers, and therefore are generally denatured by cooking in order to aid digestion of those proteins when they are ingested. Since mammalian-derived meat and human skeletal muscle are similar in composition, these proteins contain high levels of all the essential amino acids needed for muscle growth.

WHEY AND CASEIN ARE ABUNDANT IN MILK PRODUCTS. Casein makes up to 80% of all protein in cow milk. Whey proteins include a variety of soluble globular proteins that are digested quite efficiently. On the other hand, casein tends to be fairly insoluble, and often slow to digest. This results in a faster, but less sustained increase in blood amino acids when digesting whey (which is efficiently and rapidly digested) as opposed to when digesting casein (needs longer time to be digested). Both whey and casein contain high levels of all of our essential amino acids, and just like meat, they are thought of as complete proteins.

THERE ARE SEVERAL VEGETARIAN SOURCES OF AMINO ACIDS. Soy contains high levels of all the essential amino acids and therefor is considered a complete protein sources unlike pea or wheat derived-proteins. The major protein in wheat is gluten, which is low in lysine (an essential amino acid). Legumes on the other hand, are low in methoinine (essential amino acid). Vegetarian diets often combine wheat and legume-derived proteins into the meals to form a complete source of essential amino acids.

COLLAGEN HAS ATYPICAL COMPOSITION AND REQUIREMENTS Collagen is a triple helical protein that makes up much of our connective tissue[329]. Collagen is also a major component in ligaments, tendons and the skin. Collagen is the most abundant protein in mammals, making up 25-35% of the whole body content. Collagen has quite a unique amino acid composition, with extremely high levels of both proline and hydroxyproline. Collagen synthesis is especially important during growth, wound healing and tissue remodeling.

[329] Connective tissue includes the extracellular matrices that hold cells in place

HYDROXYPROLINE IS NOT ONE OF THE STANDARD AMINO ACIDS. It is synthesized from the conditionally essential amino acid Proline[330] via the enzyme *Proline hydroxylase*. Collectively proline and hydroxyproline comprise about a third of the weight of collagen [Bowes and Kenten, 1948]. The conversion of Proline to Hydroxyproline occurs post-translationally, meaning that collagen is translated first, then the reaction occurs on the already assembled protein. Proline hydroxylase requires Vitamin C (also known as ascorbate) to catalyze the reaction. The instability of collagen due to Vitamin C deficiency is the

[330] Proline can be generated from Glutamate, so is therefore conditionally essential and dependent on Glutamate levels.

biochemical basis of scurvy [331].

[331] James Lind. *A Treatise of the Scurvy in Three Parts. Containing an Inquiry into the Nature, Causes and Cure of That Disease, Together with a Critical and Chronological View of What Has Been Published on the Subject*. London, 1753

Other Nutritional Aspects of the Protein Package

While we have focused on the proteins and amino acids contained in particular foods, we appreciate that protein is generally consumed as part of a larger more complex set of foods. As we will discuss in the lecture on non-protein products of amino acids, there are often other key nutrients that should be considered when thinking of a protein-rich food. Some particularly relevant nutrients that may be present or absent in the food depending on the protein source include Vitamin B12, iron, carnitine and creatine. These are important key nutrients to consider in addition to the types of fats and carbohydrates that may also be contained in the protein source.

Reflection Questions

1. A patient with phenylketonuria (PKU) cannot metabolize phenylalanine and must follow a strict low-phenylalanine diet. Using your knowledge of conditional amino acid essentiality and the phenylalanine hydroxylase reaction, explain why PKU patients must also supplement tyrosine, and predict what other downstream consequences a severely phenylalanine-restricted diet might have given tyrosine's broader biological roles.

2. A competitive vegan athlete uses pea protein and wheat protein supplements to meet their daily protein requirements for muscle growth. Evaluate whether this combination constitutes a complete essential amino acid source, identifying the specific limiting essential amino acid in each source. Then explain why adequate supply of BCAAs in particular is critical during periods of intense training, and predict whether this combination would be sufficient to meet both essential amino acid and BCAA requirements for muscle protein synthesis.

3. A patient recovering from extensive burns requires substantial new collagen synthesis for wound healing. Their diet is adequate in total protein and calories, but they are found to have severe vitamin C deficiency. Analyze the specific biochemical step impaired by vitamin C deficiency, explain why this affects collagen stability rather than its initial translation, and predict the clinical consequences for wound healing.

Protein Digestion and Absorption

Learning Objectives

- Describe protein digestion in the organs of the digestive tract.
- Understand the roles of enzymes and compounds in the stomach and small intestine.
- Explain how amino acids and peptides cross the apical membrane of the enterocyte.
- Understand that free amino acids cross the basolateral membrane.
- Explain why not all amino acids are absorbed into the bloodstream.

Digestion

Stomach

Enzymatic digestion of protein begins in the stomach. Hydrochloric acid (HCl), secreted by parietal cells, initiates protein denaturation by lowering the pH. This unfolds the quaternary, tertiary, and secondary structures, disrupting hydrogen and electrostatic bonds but leaving peptide bonds intact.

HCl also activates pepsinogen (from chief cells) into pepsin, which functions as an endopeptidase—hydrolyzing interior peptide bonds, particularly adjacent to hydrophobic or aromatic amino acids.

The result: linear polypeptide chains and oligopeptides.

Small Intestine

As chyme enters the small intestine, hormones secretin and CCK slow gastric digestion and stimulate pancreatic juice release. This juice contains bicarbonate and zymogens including:

- **Trypsinogen** ($\rightarrow$ trypsin)

- **Chymotrypsinogen** (→ chymotrypsin)
- **Procarboxypeptidase** (→ carboxypeptidase)

Enteropeptidase activates trypsinogen to trypsin, which then activates the other zymogens.

Chymotrypsin targets peptide bonds next to tyrosine, phenylalanine, or tryptophan (large neutral amino acids).

Reflection: What are the end products of chymotrypsinogen?

Carboxypeptidase is an exopeptidase that cleaves from the C-terminal, producing free amino acids and shorter peptides.

Brush border enzymes include:

- **Aminopeptidases**: N-terminal cleavage
- **Tripeptidases**: act on tripeptides
- **Dipeptidylaminopeptidases**: act on dipeptides

Absorption

Small Intestine

Digestion products—free amino acids, dipeptides, and tripeptides—must cross:

- Apical membrane (facing lumen)
- Through enterocyte
- Basolateral membrane (facing bloodstream)

Most absorption happens in the lower duodenum and upper jejunum. About 70% of apical absorption occurs as di/tripeptides via the PEPT1 transporter, co-transporting with H^+ ions.

To maintain gradients:

- H^+ exchanged for Na^+
- Na^+ pumped out by Na/K ATPase

Free amino acids use carrier-mediated systems. Their transport is influenced by:

- Side chain structure
- Electrical charge

Reflection: How does amino acid competition affect absorption?

Inside the enterocyte, peptides are hydrolyzed to amino acids. These are then transported across the basolateral membrane into capillaries, ultimately reaching the liver via the portal vein.

Not all amino acids enter circulation—some are retained for:

System	Target Amino Acids
L System	Branched-chain and aromatic AAs
X^- System	Acidic AAs
$B^{0,+}$ System	Neutral and basic AAs
ASC System	Small neutral AAs

Table 42: Selected Free Amino Acid Transport Systems (Apical Membrane)

System	Notes
LAT1	Large neutral AAs
y^+LAT1	Basic AAs
TAT1	Aromatic AAs
SNAT	Sodium-dependent neutral AAs

Table 43: Basolateral Membrane Transport Systems

- Protein synthesis
- Nitrogen-containing compounds
- Oxidation for energy

Large Intestine

About 10–20 g of amino acids escape absorption daily. Gut bacteria use these for growth, and the rest are excreted in feces.

Reflection Questions

1. A patient on long-term proton pump inhibitor (PPI) therapy has virtually no gastric acid secretion. Trace the downstream consequences for protein digestion, starting from the failure to activate pepsinogen and explaining how this affects the subsequent cascade of zymogen activation in the small intestine. Predict which protein structures would be most resistant to incomplete digestion under these conditions.

2. Two athletes consume identical amounts of protein: one as a rapidly digested whey shake (yielding mainly di- and tripeptides), the other as whole egg whites (requiring complete digestion to free amino acids before absorption). Using your knowledge of PEPT1 transport vs. free amino acid carrier systems, predict which source would result in faster peak absorption and why — and explain whether speed of absorption has practical significance for muscle protein synthesis.

3. Large neutral amino acids — including BCAAs, phenylalanine, and tryptophan — compete for the L-system transporter at the apical membrane. A PKU patient on a low-phenylalanine diet begins

taking high-dose BCAA supplements. Evaluate how excess BCAAs in the intestinal lumen could affect absorption of other large neutral amino acids sharing the same transporter, and predict any potential downstream consequences.

Protein and Amino Acid Synthesis

This lecture will cover mechanisms and signals of both protein synthesis and non-essential amino acid biosynthesis. Protein building is important for growth as well as tissue repair. This lecture will also cover in more detail why some amino acids are essential or conditionally essential in our diet.

Learning Objectives

- Understand the mechanistic differences between dispensable and indispensable amino acids.
- Evaluate the roles of insulin, growth hormone, testosterone and cortisol on protein synthesis and degradation.
- Describe the central roles of glutamate and glutamine as a pool of nitrogen.
- Describe the relationships between the glycolytic and TCA cycle intermediates and amino acid biosynthesis.
- Explain why some amino acids are dispensable only if precursors are available.
- Understand how amino acid biosynthetic rates are controlled by utilization and by negative feedback.
- Understand the role that the indispensable amino acids play in controlling protein synthesis.

Key Concepts and Vocabulary

- Essential and Non-Essential Amino Acid
- Negative Feedback
- Protein Synthesis
- Amino Acid Pool and Nitrogen Pool
- Carbon Skeletons
- BCAA, and why they are a special group of amino acids
- mTORC1
- GCN2
- FGF21
- Transaminases
- Insulin, IGF-1, Testosterone and Growth Hormone

Protein Synthesis is a Tightly Regulated Process

As we will discuss throughout this section, protein synthesis involves a complex interplay of detecting the levels of the amino acids[332], integrating a diverse array of hormonal signals and co-ordinating growth with energy demand.

[332] especially the essential amino acids

The Rate of Protein Synthesis Depends on the Levels of Available Amino Acids

In order for most proteins to be made, the cell needs to have an available pool of all the amino acids. Since the non-essential amino acids can be generated when cellular levels are low, a main factor affecting rate is the availability of the essential amino acids. This is particularly important after exercise wherein proteins are degraded for energy but need to be resynthesized [Tipton et al., 1999]. Among the essential amino acids, the branched-chain amino acids[333] are particularly important as they are: used at high levels in human proteins; essential; and often limiting in the amino acid pool. Of the three, Leucine is likely the most important, because it is not only an essential BCAA, but it is also a potent activator of mTORC1, a protein kinase that plays a central role in protein synthesis[334]. In order to induce muscle hypertrophy[335] it is popular to ingest protein, often in the form of a protein shake shortly after a workout. This has been shown to be valuable for post-workout muscle protein synthesis, but due to limitations in digestion, absorption or transport is only beneficial up to about 1.6g/kg/day [Morton et al., 2017][336].

[333] Leucine, Isoleucine,and Valine, abbreviated as BCAA's

[334] It should not be suggested that leucine is the only thing required for protein synthesis, while it is both a potent activator, and a key substrate, protein synthesis cannot occur without sufficient levels of all the amino acid building blocks.

[335] The growth of muscle, often in concert with resistance exercise.

[336] For an average sized woman (75kg) this means that ingesting more than 120g of protein per day has no additional benefit to muscle hypertophy or strength.

Several Endocrine Signals Regulate Protein Biosynthesis

Amino acid levels, particularly essential amino acid levels, are sensed via two systems. One is a slow-acting transcriptional system controlled by GCN2[337]. Short-term regulation is accomplished by the protein kinase mTORC1[338].

[337] This stands for the unhelpful name General Control Non-Derepressable 2 protein.

[338] Mechanistic Target of Rapamycin, again, sorry these names are not exactly easy to remember.

GCN2 REGULATES CHRONIC PROTEIN AND AMINO ACID HOMEOSTASIS. GCN2[339] is a protein kinase that is *activated* by low levels of essential amino acids [Castilho et al., 2014]. One major function it has is to *prevent* protein synthesis when amino acids are low. This is accomlished by phosphorylating and inhibiting the protein synthesis initiating factor eIF2α. In addition to this, GCN2 activates a transcription factor called ATF4. This transcription factor increases the levels of enzymes involved in non-essential amino acid biosynthesis, and amino acid transporters. Together, reduced protein synthesis, increased amino acid biogenesis and increased amino acid transport function to restore amino acid levels.

[339] sometimes referred to as eIF2α-kinase

FGF21 IS A LIVER-DERIVED HORMONE THAT RISES IN RESPONSE TO PROTEIN RESTRICTION. Very recent studies have shown that protein restriction results in the production of FGF21[340], and this has emerged as a signal for restoring amino acid homeostasis [Laeger et al., 2014]. FGF21 production in response to protein restriction is

[340] Fibroblast Growth Factor 21

mediated by GCN2. The mechanisms by which FGF21 might restore protein homeostasis are currently unknown but one hypothesis is that it drives increased appetite[341], as the only way to increase the amount of essential amino acids is to consume them [Solon-Biet et al., 2016]. If you are interested, more details about the relationship between protein and satiety can be found in Morrison and Laeger [2015].

[341] Interestingly this happens in concert with increased energy expenditure, so it may represent an energy balance-neutral adaptation.

Several hormonal signaling and protein sensing systems converge on mTORC1. Growth Hormone/IGF1[342], insulin and testosterone all activate mTORC1 in protein synthetic tissues such as muscle. Catabolic signals such as Cortisol also function in part by reducing mTORC1 activity. In addition to hormonal inputs, mTORC1 can sense the levels of three key amino acids (Leucine, Lysine and Arginine) and energy levels. When these amino acids, energy levels, or the anabolic hormone signaling pathways are elevated, mTORC1 is active. mTORC1 in turn then promotes protein synthesis at several levels, including promoting mRNA translation, ribosome biogenesis and suppressing protein breakdown (both autophagy and proteolysis). mTORC1 has emerged as a master regulator of growth and homeostasis; more details about mTORC1 activity can be found in a recent review by Saxton and Sabatini [2017].

[342] Insulin-like Growth Factor

Protein Synthesis is Energetically Expensive

Protein synthesis is the sequential conjugation of amino acids in a series defined by a messenger RNA molecule. Each addition of an amino acid to an elongating chain requires *four ATP molecules*. These are broken down as follows:

1. First a specific tRNA[343] must have a free amino acid added to it. This costs 2 ATP equivalents.
2. Binding of the charged tRNA to the ribosome costs 1 ATP equivalent.
3. The elongation step requires another ATP equivalent.

[343] Transfer RNA, which is distinct from a mRNA molecule.

Proteins vary widely in their length, but for one example, Actin a very common protein in humans, has 374 amino acids, which is relatively short in length. This means that for to make a molecule of Actin the approximate ATP cost is:

$$374x4 = 1492 \tag{63}$$

That means, to generate a single Actin molecule you would need 46 glucose molecules to undergo aerobic glycolysis through the

TCA/ETC or 748 glucose molecules to go through anaerobic glycolysis[344]. Thats not even accounting for the energy costs needed if any of the amino acids need to be synthesized or transported into the cell. This is one major reason why protein digestion has a very high level of diet-induced thermogenesis, and why energy demands are very high during growth. The flip side of this is that protein breakdown [345] must be only occur under careful control.

[344] Check the math yourself

[345] which we will discuss next lecture

Synthesis of Non-Essential Amino Acids

Amino acids contain both a carbon skeleton and at least one amino group. For the non-essential amino acids, five can be generated under most normal conditions[346]. The other non-essential amino acids require at least one precursor[347]. These relationships are summarized in Table IV.

[346] Mnemonic is ANDES using their single letter abbreviations, meaning Alanine, Asparagine, Aspartate, Glutamate, Serine.

[347] Arginine and Proline require Glutamate; Cysteine and Glycine require Serine, Glutamine requires Glutamate, and as we discussed for PKU, Tyrosine requires Phenylalanine

Humans Have Lost the Ability to Synthesize Several Amino Acids.

Some of the more complex amino acid biosynthetic pathways have been lost during human evolution. A plausible explanation is that these amino acids were easier for us to obtain from the diet, and were too evolutionarily costly to continue to synthesize[348]. There are some remnants of this process where we can generate an amino acid, but not particularly efficiently. For example, Arginine is synthesized from Glutamate in a eight step pathway. This is why Arginine is nutritionally essential during growth and development, because it is so difficult to synthesize.

[348] Plants, on the other hand are not very effective hunter-gatherers and therefore need to make all of their amino acids.

Non-Essential Amino Acids Are Derived from Glycolytic and TCA Cycle Intermediates. As shown in Table IV, Serine, Cysteine and Glycine are all derived from the glycolytic intermediate 3-Phosphoglycerate. Alanine, as we have previously discussed is generated from Pyruvate. Aspartate and Asparagine are eventually generated from Oxaloacetate. Since all amino acids require a nitrogen source, Glutamate and Glutamine are particularly important, not just for Arginine and Proline, but also as a nitrogen source for the remaing amino acids[349].

[349] Except Phenylalanine, which is a special case

The Nitrogen Pool is Key for Amino Acid Synthesis

Glutamate is a part of several *transaminase* reactions[350]. These are near-equillibrium reactions where an amino group is transfered fom glutamate to another amino acid, or vice versa. Some examples are below:

[350] Transaminases require the cofactor pyridoxal phosphate, derived from Vitamin B_6

AA source	Nitrogen Source	Carbon Skeleton	Conditional
Ser	Glutamate	3-Phosphoglycerate	Cys, Gly
Ala	Glutamate	Pyruvate	
Asp	Glutamate	Oxaloacetate	Asn
Gln	Ammonia	Glutamate	Glu
Glu	Glutamine		Arg, Pro
Tyr	Phenylalanine		

Table 44: Summary of biosynthetic pathways of essential amino acids. Amino acids are generally made from a carbon skeleton and a nitrogen source. Conditional indicates that these amino acids are generated by further metabolism of the initial amino acid.

$$\alpha KG + Ala \rightleftharpoons Glu + Pyr \tag{64}$$

$$\alpha KG + Asp \rightleftharpoons Glu + OAA \tag{65}$$

$$\alpha KG + Val \rightleftharpoons Glu + \alpha Ketoisovalerate \tag{66}$$

Since these are easily reversible reactions, the directionality depends on the concentrations of products and substrates on each side. For example in reaction 64, if there are high levels of Glutamate and Pyruvate, then Alanine and α-ketoglutarate will be produced. Because Glutamate and α-ketoglutarate are present on both sides of most transaminase reactions, this is one way in which TCA cycle intermediates (α-ketoglutarate) and amino acids (*i.e.* Glutamate) are kept in balance.

Glutamate and Glutamine are non-toxic carriers of nitrogen. During amino acid breakdown[351], several amino acids can be converted to glutamate via transaminases, then glutamate releases its amino group via the functions of Glutamate Dehydrogenase:

[351] This will be covered in the next lecture

$$Glu + H_2O + NAD^+ \rightarrow \alpha KG + NH_3 + NADH + H^+ \tag{67}$$

In humans this is irreversible, as we cannot re-synthesize glutamate from ammonia. The ammonia released from this reaction is released into the Urea cycle[352].

[352] Also covered in the next lecture

Glutamine is the most abundant amino acid in most cells. Glutamine is another particularly important amino acid, because it contains two nitrogen atoms, and can be quickly be synthesized to or from Glutamate with the following reactions, catalysed by Glutamine Synthetase:

$$Glu + ATP + NH_3 \rightarrow P_i + Gln \tag{68}$$

and Glutaminase:

$$Gln + H_2O \rightarrow Glu + NH_3 \quad (69)$$

Free glutamine is typically present in muscle cells about 4 fold higher than glutamate, and eight-fold higher than the next most abundant amino acid (Alanine). This is our mechanism to store nitrogen and make it available for other amino acid biosynthetic reactions[353]. For example, if Aspartate is required, Glutamine is converted by reaction 69 into Glutamate, which then acts as a nitrogen donor in reaction 65.

[353] Typically the transaminase reactions we described above in Table IV

Regulation of Non-Essential Amino Acid Biogenesis.

There are two main ways that amino acid biogenesis is sensed and controlled, outside of the endocrine signals discussed above. One mechansim is the nature of the transaminase reactions described above. Because these are rapid, near-equillibrium reactions, if an non-essential amino acid such as Alanine has low levels, the equillibrium of this reaction will shift to produced more Alanine[354].

[354] Refer to reaction 64 for example and recall that for a near-equillibrium reaction, the concentration of the products will be nearly equal to the concentration of reactants. In such an example, if Alanine (or α-Ketoglutarate) are low, then Pyruvate and Glutamate will be used to make these reactants.

Negative feedback also plays a role in regulating amino acid biosynthesis. Several amino acids are synthesized via multiple step reactions. For example, Serine is generated from 3-phosphoglycerate via several steps. The first and rate-limiting step is catalyzed by an enzyme called phosphoglycerate dehydrogenase. This enzyme is negatively regulated by Serine. In this way, Serine level controls whether more or less Serine can be generated.

Protein Requirements and Determination Thereof

When amino acids are being oxidized, ammonia is generated[355]. This can be measured by urinary nitrogen levels. If dietary nitrogen and urinary nitrogen are equal, then a person is said to be in *Nitrogen Balance*. During periods of protein catabolism, urinary nitrogen is higher than intake. During periods of protein synthesis, urinary nitrogen is lower. *This is because dietary nitrogen-containing amino acids are not being oxidized.*. This is one way by which dietary requirements are determined, since a lack of any essential amino acid causes proteins to be degraded to release the essential amino acids. An excess of the non-limiting amino acid will then be oxidized and released as urea. Several other methods for determining protein requirements exist, briefly these include:

[355] See reactions 69 and 67 and recall that most amino acids are going to be catabolized via transaminases into Glutamate, which then feeds into reaction 67.

Nitrogen Balance. In this method nitrogen intake is compared to nitrogen release, protein synthesis being associated with positive nitrogen balance.

Direct Amino Acid Oxidation. In this method, stable-isotope labelled Phenylalanine, Lysine, Leucine, Isoleucine of Valine are provided. When catabolized, these indispensible amino acids release the label to the body's bicarbonate pool which is eventually released as $^{13}CO_2$. The oxidation and release of this amino acid will increase if that amino acid is in excess.

Indicator Amino Acid Oxidation. In this method a stable-isotope labelled amino acid is added. If in protein deficiency, that amino acid will be oxidized. As protein intake increases, oxidation will decrease. Therefore the detection of oxidized label (typically $^{13}CO_2$) is inversely proportional to protein levels. More details in this method can be found in Elango et al. [2008].

Reflection Questions

1. A resistance-trained athlete ingests a leucine-enriched protein supplement immediately post-workout. Trace the molecular pathway by which elevated leucine activates mTORC1 and promotes muscle protein synthesis, then explain why leucine supplementation alone (without adequate levels of all other essential amino acids) will ultimately fail to sustain maximal muscle protein synthesis rates.

2. A researcher places subjects on a severely protein-restricted diet for two weeks. Using your knowledge of GCN2 and FGF21, describe the sequential molecular and hormonal responses that occur as essential amino acid levels fall, from the initial phosphorylation of eIF2α through the upregulation of ATF4 target genes and ultimately to the rise in FGF21, and explain how these responses collectively attempt to restore amino acid homeostasis.

3. A patient with chronic liver disease has impaired transaminase activity and low circulating glutamine levels. Using your knowledge of the glutamate/glutamine nitrogen pool and transaminase reactions, predict how impaired nitrogen shuttling would affect the biosynthesis of dispensable amino acids such as alanine and aspartate, and explain why this patient might become functionally deficient in conditionally essential amino acids even on an adequate diet.

Protein Breakdown and Amino Acid Oxidation

In this lecture we discuss protein breakdown and subsequent amino acid catabolism into energy and other products. Amino acids are broken down into carbon skeletons (which often feed into glycolysis or the TCA cycle) and their amino groups (typically transfered to Glutamate/Glutamine for storage, or released via the Urea Cycle). This unit will discuss the functions and the regulation of these processes. For more details on amino acid breakdown and the Urea cycle refer to Chapter 30 in Biochemistry: A Short Course, *available in reserve*[356].

[356] John L Tymockzko, Jeremy M Berg, and Lubert Stryer. *Biochemistry: A Short Course*. W.H. Freeman and Co, New York, NY, 2015

Learning Objectives

- Evaluate the signals that lead to protein degradation and how those signals activate proteolysis and autophagy.
- Explain the circumstances in which amino acids and proteins would be degraded.
- Describe the fates of the carbon skeletons when amino acids are catabolized.
- Consider the importance of branched chain amino acids, and describe the regulation of branched-chain ketoacid dehydrogenase.
- Explain the key role, and the mechanisms of regulation of Glutamate Dehydrogenase in amino acid catabolism.
- Describe the role of the urea cycle and analyse how defects in the urea cycle could be detected and treated.

Protein Breakdown

As we discussed in the last unit, whereas glycogen serves as a store of glucose there is no easily accessible depot for amino acids. Essential amino acids can only be obtained via the diet or by breaking down proteins. Since most proteins play key functional roles breaking down proteins comes at a cost. Furthermore, making proteins is energetically very costly, so breaking down proteins should only be done when absolutely necessary. As such, protein breakdown into amino acids is very tightly controlled in cells.

Mechanisms of Protein Degradation

There are two main organelles in which proteins are broken down: the proteasome or the lysosome. The proteasome is a large multisubunit complex which is the site of protein degradation when the cell requires specific proteins to be catabolized [Finley, 2009]. Individual proteins are tagged, usually by the addition of ubiquitin[357]. Once targeted these proteins move to the proteasome and degraded into individual amino acids. For example a protein that the cell wants to get rid of can be very specifically targeted and removed which leaves other untagged proteins alone. The specificity of this targeting is mediated via a class of enzymes called E3 Ubiquitin Ligase. These proteins recognize a specific protein, and target it for ubiquitinylation. An example of this in muscle tissue is an E3 ligase called MuRF1[358]. During muscle atrophy, MuRF1 activity is increased,

[357] itself a small protein

[358] Muscle ring finger 1

which targets myofibrillar proteins for ubiquitinylation and degradation [Bodine and Baehr, 2014].

THE OTHER MAIN ORGANELLE OF PROTEIN DEGRADATION IS THE LYSOSOME, WHICH CATABOLIZES MACROMOLECULES IN A PROCESS CALLED AUTOPHAGY. In autophagy, instead of targeting individual proteins entire organelles (like a mitochondrion) can be engulfed to be broken down within lysosomes. The lipids (via lipases) and proteins (via proteases) are broken down within the lysosomes[359]. Compared to the proteasome, autophagy is much less specific but has a much higher capacity[360]. Autophagy is often upregulated during times of amino acid starvation. As such, mTORC1 activity[361] is a potent *inhibitor* of autophagy [Noda, 1998].

[359] For more information see https://www.ncbi.nlm.nih.gov/books/NBK9953/

[360] The discovery of which led to the 2016 Nobel Prize in Medicine and Physiology, see https://www.nobelprize.org/nobel_prizes/medicine/laureates/2016/press.html

[361] which is decreased during amino acid or energy deprivation

EXTRACELLULAR PROTEINS SUCH AS COLLAGEN ARE BROKEN DOWN VIA THE SECRETION OF PROTEASES. To digest proteins outside the cell a variety of enzymes known as matrix metalloproteases are secreted by cells. These degrade parts of the extracellular matrix including collagen, elastin and fibronectin.

Endocrine and Metabolic Signals of Protein Breakdown

One protein degradation signal that we have discussed previously is the hormone cortisol. This glucocorticoid signals muscle cells to break down proteins into their constituent amino acids, largely to provide gluconeogenic substrates to the liver. The primary route of action of glucocorticoids is thought to be the FOXO-dependent transcriptional activation of the atrogenes MuRF1 and Atrogin. These ubiquitin ligases then target muscle proteins for degradation and amino acid release. This is one mechanism by which chronic stress or prescription glucocorticoids[362] result in muscle weakness.

[362] such as prednisone, corticosterone or dexamethasone

Another factor in the regulation of proteolysis is the *reduction in anabolic signals*. Insulin and mTORC1 are both potent suppressors of proteasome and autophagosome function, so reductions in these signaling pathways often promotes protein breakdown. This is thought to be especially important when the mTORC1 activators Leucine, Arginine and Lysine are depleted. During insulin resistance[363], insulin signaling in the muscle is reduced and protein breakdown can be accelerated [Wang et al., 2006]. This can increase gluconeogenesis[364] and reduce exercise capacity.

[363] For example, in obese pre-diabetic or diabetic individuals.

[364] By providing more substrates.

Amino Acid Catabolism

For energy to be derived from amino acids, two decisions must be made: first the protein must be broken down into amino acids, then those amino acids must be deaminated and/or oxidized. Proteins are broken down for two major reasons:

- To free up essential amino acids for other protein synthetic requirements.
- To provide energy or generate glucose via amino acid oxidation or gluconeogenesis respectively.

In the former example, generally cellular and organismal energy requirements are met, but amino acids are needed for protein synthesis to occur. In the latter example, the cell or organism requires energy or glucose, and protein synthesis is largely inactive. The removal of an amino group[365] from an amino acid is typicaly irreversible. This is an especially important consideration for essential amino acids, since a deficit caused by their catabolism must now be provided by the diet. As you might expect, the regulation and disposal of these amino groups is extremely tightly regulated. We will discuss the regulation of these processes first, then discuss the fates of the remainder of the amino acid[366].

[365] A process called deamination.

[366] Which we refer to as the carbon skeleton.

THE NITROGEN GROUPS FROM AMINO ACIDS ARE OFTEN TRANSFERED TO GLUTAMATE. As we discussed previously, there are a series of transaminase reactions used for both biosynthetic and degradation purposes. These enzymes are particularly important for removing the amino group from an amino acid, leaving a carbon skeleton (also sometimes refered to as an α-ketoacid) and glutamate. We have discussed ALT and AST previously, but a longer list of mammalian transaminases can be found in Table IV .

Substrates		Enzyme	Products	
Alanine	α-Ketoglutarate	ALT	Pyruvate	Glutamate
Aspartate	α-Ketoglutarate	AST	Oxaloacetate	Glutamate
Leucine	α-Ketoglutarate	BCAT	α-Ketoisocaproate	Glutamate
Valine	α-Ketoglutarate	BCAT	α-Ketoisovalerate	Glutamate
Isoleucine	α-Ketoglutarate	BCAT	α-Ketomethylvalerate	Glutamate
Tyrosine	α-Ketoglutarate	TAT	4-hydroxyphenylpyruvate	Glutamate
Tryptophan	α-Ketoglutarate	TTA	(indol-3-yl)pyruvate	Glutamate
Methionine	α-Ketoglutarate	MAT	2-oxo-4-methylthiobutanoate	Glutamate
Cysteine	α-Ketoglutarate	CT	Sulfinylpyruvate	Glutamate

Table 45: Mammalian transaminases

The implications of concentration-mediated regulation of transaminase reactions can be highlighted with the cases of α-Ketoglutarate and glutamate. The non-amino acid substrate in all of these cases is the TCA cycle intermediate α-Ketoglutarate, which makes this step of amino acid degradation highly cataplerotic, since in the absence of α-Ketoglutarate, there is no substrate for the amino group receptor. This also means that amino acid breakdown is driven by a buildup of the free amino acids in the presence of available TCA cycle intermediates. The second implication of all transaminase reactions being rapid, equillibrium reactions is that the concentration of glutamate is important for this first step. If glutamate is building up in the cell, then these amino acids will not be degraded to the same extent.

Several amino acids[367] are not catablolized via transanimase reactions. As we have discussed previously Glutamine is converted to Glutamate via the Glutaminase enzyme, while Asparagine is processed to Aspartate using a similar enzyme, Asparaginase. Phenylalanine is converted first to Tyrosine[368]. Arginine, Histidine, Lysine and Proline have complex paths but end up as Glutamate as well. The only two exceptions to nitrogen flow through Glutamate are Threonine and Serine. These are both catabolized into Glycine, which is broken down into CO_2 and ammonia via the Glycine Cleavage System[369].

[367] the amino acids not shown in Table IV

[368] Via Phenylalanine Hydroxylase, the enzyme deficient among individuals with PKU.

[369] A series of enzymes that completely catabolize glycine in several steps.

Regulation of Glutamate Dehydrogenase

The flow of nitrogen during amino acid catabolism is generally amino acid to Glutamate then Glutamate to ammonia. This second step is controlled by Glutamate Dehydrogenase (GDH), which catalyzes the *irreversible* reaction shown in Reaction 70. The primary location of this enzyme is mitochondrial, so for amino acids to be oxidized they (or the Glutamate derived from them) must be transported into the mitochondria.

$$Glu + H_2O + NAD^+ \rightarrow \alpha KG + NH_4^+ + NADH + H^+ \qquad (70)$$

This reaction replenishes the α-Ketoglutarate consumed in the previous transaminase step, while also releasing Glutamate's amino agroup as ammonia (NH_4^+) and generating a molecule of NADH[370]. As you may have guessed, for an irreversible enzyme of such importance, GDH is under multiple sets of allosteric control. Before you read any further, take a minute to think about the conditions under which this reaction would proceed.

[370] Typically worth 2.5 ATP equivalents

If your thoughts were *energy needs* and *amino acid surplus* you are on the right track! The main positive regulators of GDH are ADP,

GDP, NAD^+ and branched chain amino acids (especially Leucine). The main inhibitors include GTP, NADH and Palmitoyl-CoA[371]. In general that means that Glutamate will be irreversibly broken down only when energy needs are high, essential amino acids are high and fatty acids are low. More details on the allosteric regulation of GDH can be found in Smith and Stanley [2008]'s review article.

[371] This is the first step in the degradation of the fatty acid palmitate, and indicates that there are sufficient fatty acids to use as fuel, rather than breaking down proteins

At a post-translational level, GDH is also under the control of mTORC1 signaling. mTORC1[372] is thought to activate GDH [Csibi et al., 2013]. One hypothesis is that this could be a pathway by which nutrient excess leads to the oxidation of un-needed amino acids, especially Glutamine and Glutamate.

[372] Which as you may recall is activated by some amino acids, insulin and high energy levels.

The Urea Cycle

The final nitrogen products of Glutamate Dehydrogenase, Glutaminase, Arginase and the Glycine Cleavage System[373] is ammonia. Ammonia is very toxic to cells and organs, the accumulation of which is a condition known as hyperammonia. As such, ammonia needs to be efficiently converted to the less damaging, and more easily excreted molecule Urea. The urea cycle begins in the mitochondria, typically in the liver via the synthesis of free ammonia and bicarbonate to form a molecule called Carbamoyl Phosphate (CPS), the first committed step of this cycle. Somewhat similarly to the TCA cycle, CPS is attached to Ornithine, which goes through several conversion steps, releasing Urea and regenerating Ornithine. This step, diagramed in Reaction 71, is the key regulator of the Urea cycle.

[373] Which ends the catabolism of Serine, Threonine and Glycine.

$$NH_4^+ + CO_2 + 2ATP \rightarrow CPS \tag{71}$$

The activity of the Urea Cycle is controlled by the levels of N-Acetylglutamate. The activity of the Urea cycle should be coupled to the amounts of amino acid breakdown products, namely Glutamate. As such, a Glutamate-derived molecule called N-Acetylglutamate (NAG) is a potent allosteric activator of Carbamoyl Phosphate Synthetase, the enzyme which catalyzes reaction 71. NAG synthase is itself regulated by Arginine, so that when amino acids[374] and Acetyl-CoA[375] are elevated, NAG increases, which in turn activates the Urea cycle[376]. Urea cycle enzymes are also upregulated by gluconeogenic hormones including glucagon and cortisol. This ensures that the deamination products of gluconeogenesis are able to be removed.

[374] Specifically Arginine and Glutamate

[375] Generated either via fatty acid β-oxidation, Ketogenic Amino Acid catabolism or from Pyruvate Dehydrogenase.

[376] It might help to sketch this out in the margins.

Branched-Chain Amino Acid Catabolism

The branched-chain amino acids[377] are under a another level of

[377] Leucine, Isoleucine and Valine

metabolic control. Because they are high-demand, low-availability essential amino acids, it is especially important that their levels remain protected. The first step in BCAA catabolism is their transamination (see the Branched-Chain Aminotransferase, BCAT in Table IV). This reversible reaction equillibrates the pool of BCAA's with their respective α-ketoacids[378]. The rate-limiting step of their catabolism is the next step, mediated by an enzyme called Branched Chain Ketoacid Dehydrogenase (BCKDH). This enzyme catalyzes the following *irreversible* steps of BCAA catabolism, starting with the transamination products of Leucine, Valine and Isoleucine respectively:

[378] Think about what could cause a build-up of these α-ketoacids.

$$\alpha Ketoisocaproate + CoA + NAD^+ \rightarrow IsovalerylCoA + NADH + CO_2 \tag{72}$$

$$\alpha Ketoisovalerate + CoA + NAD^+ \rightarrow IsobutyrylCoA + NADH + CO_2 \tag{73}$$

$$\alpha Ketomethylvalerate + CoA + NAD^+ \rightarrow \alpha MethylbutyrylCoA + NADH + CO_2 \tag{74}$$

These reactions are all fairly similar in that they take an α-ketoacid and generate an activated form[379], release CO_2 and produce NADH. These activated products are then catabolized further as described in the next section. Since BCKDH is the rate limiting step for all three of these reactions, and is the main control point by which BCAA's are released, it is unsurprising that it is controlled by both internal and external signals.

[379] The CoA version; the activation of a product prior to complete oxidation will come up again when we discuss lipid oxidation.

BCKDH IS INHIBITED BY PROTEIN PHOSPHORYLATION. Similar to PFK2 and Pyruvate Kinase, BCKDH is *inactivated* by protein phosphorylation. The kinase that is responsible for BCKDH phosphorylation is *inactivated* by a build-up of the branched chain ketoacids, especially the Leucine catabolite α Ketoisocaproate (see reaction 72). In this way, a buildup of the ketoacids turns off the inhibitory protein kinase and allows for BCAA catabolism. It has been reported that BCKDH expression is induced by glucocorticoids, and reduced by insulin, suggesting that chronic gluconeogenic signals can modify the activity of this process.

The Fates of Amino Acid-Derived Carbon Skeletons

We have focused on the amino groups of the amino acids and how they often end up in the Urea cycle, but what about the rest of the amino acid carbon skeleton? Generally, in steps that are thought to

Amino Acids	Carbon Skeleton Fate	Notes
Leucine, Lysine	Acetyl-CoA	Ketogenic
Tyrosine and Phenylalanine Fumarate	Acetyl-CoA and Partially Ketogenic	
Isoleucine	Acetyl-CoA, Succinyl-CoA	Partially Ketogenic
Threonine and Tryptophan	Acetyl-CoA, Pyruvate	Partially Ketogenic
Asparagine and Aspartate	Oxaloacetate	via AST
Arginine, Proline, Histidine, Glutamine, Glutamate	α Ketoglutarate	via GDH
Methionine	Succinyl-CoA	
Cysteine, Alanine	Pyruvate	

Table 46: Carbon skeleton fates. These often involve other side products being generated, but note that most of the amino acids end up as Acetyl-CoA, TCA cycle intermediates (like Fumarate, Succinyl-CoA and α Ketoglutarate or Pyruvate.)

be in near-equillibrium, the carbon skeletons are catabolized into molecules you are probably already familiar with. These endpoints are summarized in Table IV

The ketogenic amino acids, Lysine and Leucine, are converted into Acetyl-CoA, while the partially ketogenic amino acids[380] are broken down into both Acetyl-CoA and another potentially glucogenic molecule. This is an important difference because it means that the products of Leucine and Lysine catabolism *can not* enter gluconeogenesis. That is because Acetyl-CoA cannot become glucose. Rather it can be catabolized in the TCA cycle, enter *de novo* lipogenesis, or be released as a ketone body.

[380] Phenylalanine, Isoleucine, Threonine and Tyrosine

The gluconeogenic amino acids on the other hand are either anaplerotic or they can become Pyruvate. Anaplerotic gluconeogenic amino acids[381] can either function in the TCA cycle or be converted via PEPCK into phoshpoenolpyruvate. Gluconeogenic amino acids destined to become Pyruvate[382] can then undergo gluconeogenesis via the activities of Pyruvate Carboxylase and PEPCK. To conceptualize whether amino acids will be catabolized to energy or enter gluconeogenesis, consider the metabolites and hormones that govern the rates of gluconeogeneic flux and oxidative phosphorylation.

[381] Tyrosine, Phenylalanine, Asparagine, Aspartate, Arginine, Proline, Histidine, Glutamine, Glutamate and Methionine

[382] Alanine, Threonine, Tryptophan, Cystine

Reflection Questions

1. A critically ill patient on high-dose corticosteroids for two weeks develops significant muscle weakness and elevated urinary nitrogen. Using your knowledge of FOXO-dependent atrogene activa-

tion, MuRF1/Atrogin ubiquitin ligases, and BCKDH regulation by glucocorticoids, trace the pathway from cortisol signaling to muscle protein breakdown and predict how the elevated urinary nitrogen arises from both the amino group and carbon skeleton fates of the catabolized amino acids.

2. A patient presents with a urea cycle defect (carbamoyl phosphate synthetase deficiency) and develops hyperammonemia after a high-protein meal. Using your knowledge of N-Acetylglutamate regulation of CPS and the role of Glutamate Dehydrogenase in releasing ammonia, explain why protein intake is particularly dangerous in this patient and predict how manipulating dietary protein composition (specifically the ratio of glucogenic to ketogenic amino acids) might reduce ammonia load.

3. A lean, well-trained endurance athlete and an obese insulin-resistant individual both undergo a 24-hour fast. Compare and contrast the signals governing protein breakdown in each person, focusing on the roles of mTORC1 suppression, insulin signaling, and amino acid availability. Predict which individual will show greater net protein catabolism and why this difference has implications for preserving lean mass during weight loss interventions.

Non-Protein Nitrogen Containing Compounds

An amino acid contains nitrogen through its amino group. Beyond an amino acid's role as the backbone structure for a peptide, they can also be utilized to make nitrogen-containing non-protein compounds that have nutritionally relevant roles. We will discuss four of these compounds plus their role in neurotransmitter synthesis.

Learning Objectives

- Understand the metabolism of nitrogen-containing compounds from amino acids
- Explain the role of these compounds in our body
- Explain the role of amino acids in neurotransmitter biosynthesis
- Understand the role of these compounds in our body
- Describe MAOIs influence in serotonin production and on tyramine

Review of Amino Acid Structure and Role in the Body

An amino acid consists of four distinct functional groups, a carboxyl, a hydrogen, an amino, and a side (R) group. The amino group makes the amino acid a nitrogen-containing structure within our body. Amino acids are the building blocks of proteins but they can also be used for the synthesis of other compounds within our body. Nitrogen-containing non-protein compounds are found in dietary sources but can also be biosynthesized. Once synthesized, they have varying roles in the body such as an antioxidant, a methyl donor, a transporter, or a neurotransmitter. In the following sections, we will discuss the constituents, biosynthesis, and main roles of glutathione, carnitine, choline, creatine, and serotonin, all nitrogen-containing compounds.

Glutathione

Glutathione is synthesized in the liver from glutamate, cysteine and glycine. It plays an important role as an antioxidant, thus it is found in the majority of cells in our body. The cysteine of glutathione provides the structure with a thiol (-SH) group. The majority of glutathione present in our cells is in the reduced form, but it can be oxidized in the presence of free radicals and lipid peroxides. The glutathione donates its hydrogen group to stabilize free radicals and lipid peroxides. Following oxidation, NADPH, from the pentose phosphate shunt, quickly reduces glutathione again. The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is commonly used as a biomarker for disease and inflammation. For example, if the hepatocyte cells have undergone stress or become damaged, the tissue GSH:GSSG would be low [Zitka et al., 2012, Sentellas et al., 2014]. Depending on the tissue and purposes of measuring GSH:GSSG, varying analytical techniques are utilized which include

high performance liquid chromatography, ultraviolet absorbance and fluorescence detection, mass spectrometry or electrochemical detection.

Carnitine

Carnitine is made in the liver and to some extent the kidneys. Three methyl groups are added to lysine by the universal methyl donor S-adenosyl methionine (SAM) via one-carbon metabolism. Methylated lysine is hydroxylated to form carnitine. Once synthesized, carnitine is stored in muscle. Because muscle is the reservoir of carnitine, we can also obtain carnitine through dietary sources, specifically animal sources such as meats and fish. Carnitine is also made in supplemental form. About 60–80% of carnitine is absorbed with intake levels of 0.5–0.6 grams [Rebouche, 2004]. One of the primary roles of carnitine is to transport long chain fatty acids through the inner mitochondrial membrane. The fatty acid is covalently joined to carnitine through a reaction catalyzed by carnitine palmitoyltransferase 1 (CPT 1)[383]. Once bound, the fatty acid-carnitine complex moves across the inner membrane and is released by carnitine pamitoyltransferase II (CAT II). The long chain fatty acid can then undergo oxidation. This process was discussed in detail in the Lipid Unit.

[383] Sometimes you may find that this is refered to as CAT (carnitine acyltransferase).

Choline

Choline is synthesized in the liver and to some extent the kidneys. Serine is decarboxylated until it forms phosphatidylethanolamine (PE). Three methyl groups are donated by SAM to PE via one-carbon metabolism to form phosphatidylcholine, which is the body's internal source of choline. Choline is also abundant in dietary sources like eggs, wheat germ, legumes, salmon and liver. In can be in the free form or bound to lecithin.

Role in One-carbon Metabolism

Choline is formed from the addition of methyl groups from SAM, but it can also serve as a methyl donor (Figure 3B) by feeding methyl groups into one-carbon metabolism. Thus, adequate levels of choline are required for one-carbon metabolism to run efficiently, a concept that has been studied and manipulated extensively. Altered functioning of one-carbon metabolism can result in homocysteine build-up and altered nucleic acid synthesis and cell division. Homocystinuria is a condition of high blood homocysteine levels. Build up of homocysteine in the blood can cause vascular wall damage leading

to atherosclerosis associated with stroke and heart disease [Millard et al., 2018]. Altered cell division can increase risk of neural tube defects like spina bifida [Zeisel, 2006].

Role in Neurotransmission

Choline is necessary for the synthesis of a neurotransmitter called acetylcholine. Choline crosses the blood brain barrier and acetylcholine is formed by the transfer of acetyl (from acetylCoA) to choline – this reaction is catalyzed by choline acetyltransferase. Acetylcholine is important in long-term memory, muscle control and motor skill development. Choline deficiency during pregnancy and the effects of offspring has been studied extensively. Mothers deficient in choline result in offspring with neural tube defects and structural abnormalities in brain resulting in impaired long-term memory [Craciunescu et al., 2010].

Role in Fatty Acid Transport

Choline is structurally part of very low-density lipoprotein (VLDL). Thus, a deficiency of choline directly affects the transport of fat and cholesterol out of the liver by VLDL. This results in an accumulation of fat in the liver leading to a fatty liver, a topic also heavily discussed in the Lipid Unit.

Creatine

In the kidney, glycine and arginine react to form guanidoacetate. Guanidoacetate is then methylated via SAM from one-carbon metabolism mainly in the liver. Following methylation, creatine is transported out of the liver to muscle where about 95% of resides within the body. Creatine can also be taken in through diet. It is found in meat, fish products and is commonly used as a performance enhancement supplement. The main role of creatine in our body is that it acts as a storehouse for high-energy phosphate because it can exist in a phosphorylated form, phosphocreatine. More than half of the creatine in resting muscles is in the form of phosphocreatine. When the muscle contracts the phosphocreatine can phosphorylate ADP producing a quick source of ATP for the muscle. The ATP then phosphorylates creatine making it a coupled reaction. This cyclic reaction is catalyzed by creatine kinase (Figure 4). This process acts as a buffer of ATP during contractions from anywhere to 8-30 seconds resulting in the delay of the breakdown of muscle glycogen. Over time creatine and phosphocreatine do get eliminated from the muscle in the form of creatinine. Creatinine is transferred from the muscle to the kidney

where it is excreted from the body through urine. Urinary creatinine levels are often used as a biomarker of muscle mass and kidney function. Because creatine plays a critical role in energy availability in the muscle, it has been used as a supplement for performance enhancement especially in short-duration maximal exercise like sprinting and weight lifting [Kreider et al., 2017].

Neurotransmitter Synthesis

There are active transport systems for amino acids present at the blood brain barrier. Thus amino acids can enter the brain and biosynthesis of neurotransmitters can occur locally. Tryptophan is needed for the synthesis of serotonin. Serotonin is a neurotransmitter that can affect mood and appetite. Serotonin levels have been widely studied with the association of depressive disorders. Nitrogen groups are removed from serotonin by monoamine oxidase (MAO). MAO also plays a role in breaking down tyramine, which is a compound found in cheeses, alcohol, meats and fish. If not broken down properly, tyramine can build up in the brain causing neural dysfunction. Monoamine oxidase inhibitors are a common class of antidepressants that block the action of MAO to increase levels of serotonin in people with mild to moderate depressive disorders. Thus individuals taking MAOs must take dietary precautions by eliminating tyramine-containing foods.

Part V

Abbreviations, References and Index

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List of Abbreviations

ABCG5/8	ATP-Binding Cassette Transporters G5 and G8
ACC	Acetyl-CoA Carboxylase,
ACL	ATP-Citrate Lyase
ACS	Acetyl-CoA Synthase
ACSL1	Acyl-CoA Synthetase Long Chain Family Member 1
ADH	Alcohol Dehydrogenase
ADP	Adenosine Diphosphate,
ALA	Alpha-Linolenic Acid
ALDH	Aldehyde Dehydrogenase
ALT	Alanine Aminotransferase, , ,
AMDR	Acceptable Macronutrient Distribution Range,
AMP	Adenosine Monophosphate, , , , ,
AMP	antimicrobial peptide
AMPK	AMP-Activated Protein Kinase, , ,
ANGPTL4	Angiopoietin-like 4
Apo	Apolipoprotein (e.g. ApoB, ApoE, ApoAI),
AST	Aspartate Aminotransferase
ASV	amplicon sequence variant
ATGL	Adipocyte Triglyceride Lipase,
ATP	Adenosine Triphosphate, , , , , , ,
BCAA	Branched-Chain Amino Acids, ,
BCAT	Branched-Chain Aminotransferase
BCKDH	Branched-Chain Ketoacid Dehydrogenase
BH_4	Tetrahydrobiopterin
BMR	Basal Metabolic Rate
cAMP	Cyclic Adenosine Monophosphate
CAT	Carnitine Acyltransferase
CCK	Cholecystokinin, ,
CETP	Cholesterol Ester Transfer Protein
CFU	colony-forming unit
CHO	Carbohydrate
ChREBP	Carbohydrate Response Element Binding Protein

CoA	Coenzyme A (e.g. Acetyl-CoA), , ,
CoQ	Coenzyme Q (Ubiquinone)
CPS	Carbamoyl Phosphate Synthetase
CPTI	Carnitine Palmitoyltransferase I,
CPTII	Carnitine Palmitoyltransferase II
CRC	colorectal cancer
CREB	cAMP-Response Element Binding Protein
CYP	Cytochrome P450 (e.g. CYP7A1, CYP2E1)
DHA	Docosahexaenoic Acid
DHAP	Dihydroxyacetone Phosphate, ,
DIT	Diet-Induced Thermogenesis
DPP-4	Dipeptidyl Peptidase 4
EAT	Exercise Activity Thermogenesis
EPA	Eicosapentaenoic Acid
ETC	Electron Transport Chain,
F-2,6-BP	Fructose-2,6-bisphosphate
FABP	Fatty Acid Binding Protein
FAD	Flavin Adenine Dinucleotide (e.g. FAD or $FADH_2$)
$FADH_2$	Flavin Adenine Dinucleotide (reduced form)
FADS	Fatty Acid Desaturase
FAS	Fatty Acid Synthase
FBPase	Fructose-1,6-bisphosphatase
FGF21	Fibroblast Growth Factor 21
FODMAP	Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols
FOS	fructooligosaccharides
FOXO	Forkhead Box O transcription factor (e.g. FOXO1, FOXO3),
FXR	Farnesoid X Receptor,
G6P	Glucose-6-Phosphate
G6Pase	Glucose-6-Phosphatase
GALT	Galactose-1-Phosphate Uridylyltransferase (or Gut Associated Lymphoid Tissue)
GCN2	General Control Non-Derepressible 2
GDH	Glutamate Dehydrogenase
GH	Growth Hormone,
GHIH	Growth Hormone Inhibiting Hormone
GHRH	Growth Hormone Releasing Hormone
GIP	Gastric Inhibitory Peptide
GLP-1	Glucagon-like Peptide 1, , ,
GLUT	Glucose Transporter (e.g. GLUT1, GLUT2, GLUT4, GLUT5),
GLUT2	Glucose Transporter Type 2

GLUT4	Glucose Transporter Type 4
GLUT5	Glucose Transporter Type 5
GOS	galactooligosaccharides
GSD	Glycogen Storage Disease
GSH	Glutathione (Reduced),
GSK3	Glycogen Synthase Kinase 3,
GSSG	Glutathione (Oxidized),
GSV	GLUT4 Storage Vesicle
GTP	Guanosine Triphosphate, ,
HCl	Hydrochloric Acid
HDAC	histone deacetylase
HDL	High-Density Lipoprotein, ,
HFCS	High Fructose Corn Syrup
HIF	Hypoxia-Inducible Factor
HIF1α	Hypoxia Inducible Factor 1α
HMG-CoA	3-Hydroxy-3-Methylglutaryl-CoA
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
HMP	Human Microbiome Project
HSL	Hormone-Sensitive Lipase,
IBD	inflammatory bowel disease
IBS	Irritable Bowel Syndrome
IDL	Intermediate Density Lipoprotein
IGF-1	Insulin-like Growth Factor 1, ,
LDL	Low-Density Lipoprotein,
LDL-C	LDL Cholesterol
LDLR	LDL Receptor,
LPL	Lipoprotein Lipase,
LPS	lipopolysaccharide
MAO	Monoamine oxidase
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease, , , ,
mRNA	Messenger RNA,
mTORC1	Mechanistic Target of Rapamycin Complex 1,
MUFA	Monounsaturated Fatty Acid
NAD	Nicotinamide Adenine Dinucleotide (e.g. NAD^+ or NADH),
NADH	Nicotinamide Adenine Dinucleotide (Reduced), ,
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (Reduced)
NAG	N-Acetylglutamate
NEAT	Non-Exercise Activity Thermogenesis
NHANES	National Health and Nutrition Examination Survey,

NPC1L1	Niemann-Pick C1 Like 1
OAA	Oxaloacetate, ,
OTU	operational taxonomic unit
OXCT1	3-Oxoacid CoA-Transferase 1 (also SCOT)
PC	Pyruvate Carboxylase,
PDH	Pyruvate Dehydrogenase,
PDK	Pyruvate Dehydrogenase Kinase
PE	Phosphatidylethanolamine
PEMT	Phosphatidylethanolamine methyltransferase
PEP	Phosphoenolpyruvate,
PEPCK	Phosphoenolpyruvate Carboxykinase, ,
PEPT1	Peptide Transporter 1
PFK-1	Phosphofructokinase-1, , ,
PFK-2	Phosphofructokinase-2, ,
Pi	Inorganic Phosphate
PKA	Protein Kinase A, , , ,
PPAR	Peroxisome Proliferator-Activated Receptor, ,
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
PRR	pattern recognition receptor
PUFA	Polyunsaturated Fatty Acid, ,
rRNA	ribosomal RNA
SAM	S-Adenosyl Methionine
SCD	Stearoyl-CoA Desaturase
SCFA	Short Chain Fatty Acids
SCFA	short-chain fatty acid
SCOT	Succinyl-CoA-3-Oxaloacid CoA Transferase (also OXCT1)
SGLT1	Sodium-Glucose Linked Transporter 1,
SGLT2	Sodium-Glucose Linked Transporter 2
SOD	Superoxide Dismutase
SREBP	Sterol Response Element Binding Protein,
SREBP1c	Sterol Response Element Binding Protein 1c
TCA	Tricarboxylic Acid Cycle, , , , ,
TDEE	Total Daily Energy Expenditure, ,
THF	Tetrahydrofolate
TICE	Trans-Intestinal Cholesterol Excretion
TLR	Toll-like receptor
TMAO	trimethylamine N-oxide
TPP	Thiamine Pyrophosphate
Treg	regulatory T cell
tRNA	Transfer RNA
UDP	Uridine Diphosphate,
USDA	United States Department of Agriculture

UTP	Uridine Triphosphate
VLDL	Very Low-Density Lipoprotein,
WGS	whole genome shotgun sequencing
WWEIA	What We Eat in America,

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