**EXAM I**

* **ATP review**
  + ATP is a nucleotide, consisting of a nitrogenous base, adenine, a 5-Carbon sugar, and three phosphate groups. The phosphates are linked via two phosphate anhydride linkages, which when hydrolyzed with water yield –31kJ of work potential per mole of ATP. The reaction from ATP to ADP is as follows:

P-ADP + H2O 🡪 ADP + P-H2O ΔG° = -31 kJ

* + Note that the Pi is just passed from ATP to water, and nothing new is formed.
* **Digestion**
  + Most carbohydrates that we ingest are in the form of starch, and some in the form of glycogen. Monosaccharides are the only carbohydrates that can be absorbed by the intestinal lumen. Carbohydrate digestion begins in the mouth, with the enzyme salivary amylase, which is found in the saliva. This enzyme begins carbohydrate digestion by **hydrolyzing** about every other α 1-4 linkage in glucose. Before this process is able to be completed you swallow your food.
  + In the stomach, the pH is about 1-2, which denatures all proteins, therefore salivary amylase is destroyed, so there is no carbohydrate digestion occurring in the stomach.
  + In the duodenum, the pH is raised so that the pH of chyme is about 7-8. Here, **pancreatic amylase** secreted by the pancreas catalyzes the hydrolysis of alternating α 1-4 linkages between glucose molecules. This produces oligosaccharides of various sizes.
    - One such oligosaccharide that is formed is **maltose**, which consists of 2 glucose molecules connected with an α 1-4 linkage. Also produced are **maltotrioses** (3 glucoses) as well as glc 4—glc 8 sugars. These glucose units are further hydrolyzed by an enzyme called **glucosidase**, which produces single glucose units.
    - Another oligosaccharide that is produces by the two amylase enzymes is **dextrin**. Dextrin is 4-10 glucose units that feature an α 1-6 linkage. Remember that this is the type of linkage that causes branching in glucose and glycogen chains. Dextrins are further hydrolyzed by another enzyme called **dextrinase** to glucose, which enters glycolysis.
    - Dextrinase and glucosidase are **brush border enzymes**, meaning they are secreted by the cells found in the intestinal mucosa. Another BBE is **trehalase**, which can digest **trehalose**, a carbohydrate found in mushrooms and insects.
    - Digestion processes are always hydrolytic, and occur extracellularly, such as in the intestinal lumen, as opposed to catabolic processes, which are oxidative, and occur intracellularly.
  + Other carbohydrates ingested must be digested by other brush border enzymes if they are not in the form of a monosaccharide. **Lactose**, which is glucose + galactose in a β 1-4 linkage is digested by **lactase**. **Sucrose**, glucose + fructose is digested by **sucrase**. Fructose is a monosaccharide so it does not need to be digested. Honey, is a mixture of glucose + fructose, therefore it also does not need to be digested.
  + Carbohydrates that cannot be digested by the body are **dietary fiber**, and pass through the digestive tract without being digested, and offer no nutritional content.
* **Glycolysis** (refer to handout for the steps)
  + **Glycolysis** begins with one 6-carbon sugar and ends with the production of 2 pyruvate/lactate molecules. It is also the entry point for other sugars besides glucose (more to come). Glycolysis is how cells produce ATP anaerobically.
  + Glycolysis has two main stages; the first stage converts glucose to 2 glyceraldehyde-3-phosphate molecules, and costs the cell 2 ATPs. The second stage converts G-3-P ultimately to lactate, and produces a total of 4 ATPs. The net ATP production for glycolysis is 2 ATP per glucose.
  + Some important steps in glycolysis:
    - The final step in glycolysis is a crucial one. Notice that in step 6, G-3-P to 1,3-BPG, NAD+ is reduced to NADH. NAD+ is an important oxidizing agent. The conversion of pyruvate to lactate in the final step of glycolysis regenerates the oxidizing agent. If lactate is not formed, then the oxidizing agent will not be regenerated, and glycolysis will not be able to continue. There are a number of interesting things about lactate. There is no pathway for it to be catabolized, it can only be converted back to pyruvate, but lactate still has a very large work potential left, and it is used to produce more energy. Lactate must leave the cell, otherwise the final step of glycolysis would be able to equilibrate, and the entire pathway would not be able to proceed. Lactate is produced under anaerobic conditions. When the cell is operating aerobically, if it is able to do so, pyruvate moves on to the TCA cycle and another mechanism is necessary to satisfy the “NAD Lust”.
    - The third step in glycolysis is important because it is an important regulation step for the entire pathway. The enzyme for step 3 is Phosphofruktokinase (PFK). PFK has two important functions associated with it, 1) is to catalyze the phosphorylation of fructose-6-phosphate, 2) is to act as an allosteric modulator for glycolysis that is PFK controls how quickly and to what extent glycolysis is able to proceed. Allosteric enzymes have a binding site for the normal substrate, but they also have an additional binding site away from this site that accommodates an allosteric inhibitor. PFK has two allosteric modulators, ATP and AMP. Note that no glycolysis substrate is an allosteric modulator because none of these build up in the cell. When demand for ATP is high, that is when [ATP] is low, or [AMP] is high, the kinetic response curve for PFK moves to the left and vice versa.

The extent of glycolysis depends on [ATP] and the cell’s demand for it. You cannot force glycolysis to run by adding excess glucose, the process is pulled by demand rather than pushed by supply. The following reaction is key in understanding this process:

ATP + H2O 🡪 ADP + Pi ADP + ADP ⇔ AMP + ATP

When ADP goes to AMP you are hydrolyzing a phosphate anhydride linkage, but when ADP goes to ATP you are making another phosphate anhydride linkage, therefore the energetics of this are a wash, ΔG° = 0, so the process is essentially at equilibrium, and it will sit there until you mess with the ATP balance. When the cell is at rest, the ATP/ADP ratio is high because 1) you want to be ready for sudden demand and 2) you don’t want to be making ATP when you need it. When ATP starts to be used, you are making extra ADP (the first equilibrium shifts to the right); when [ADP] increases, the second equilibrium shifts to the right and [AMP] increases. Because AMP has nowhere to go, there is no pathway for it, [AMP] increases and is able to act as a + allosteric modulator for PFK. AMP and ATP compete for the same allosteric sites on PFK, so if [AMP] is high, then it is able to out compete ATP for these allosteric sits and PFK activity increases. How is it that the substrate and the allosteric modulator for PFK can be the same molecule? Because the catalytic and allosteric sites have different levels of affinity for ATP; the catalytic site has a higher affinity. Therefore when [ATP] is low it goes to the catalytic site first, and when [ATP] is high, it goes first to the allosteric site.

* + - **Step 6:** From G-3-P to 1,3-BPG sets us up to make an ATP. Upon oxidizing G-3-P, the intermediate that is formed is the carboxylic acid glycerate-3-phosphate. This is the step that requires the oxidizing agent NAD+. From G-3-P to Glycerate-3-P, the ΔG° is –43.3 kJ. The next half of this reaction is from glycerate-3-phosphate to 1,3-BPG, which has a ΔG° +50kJ. Step 6 has an overall ΔG° of +6.7 kJ. When 1,3-BPG is hydrolyzed, it also yields –50kJ of energy, and this energy is harnessed to move ADP to ATP when 1,3-BPG moves to 3-phosphoglycerate. It takes energy to get from G3P to 1,3-BPG, but this energy comes in the form of oxidizing power from NAD+
    - **Step 10**: from PEP to pyruvate. The ΔG° is –62 kJ, so why are 2 ATPs not made? The reason is that by having a large ΔG° at the end of this process, it allows the process to be very energetically favorable. The last step therefore, is like falling off a cliff energetically, so the whole process is able to move forward.
* **Pentose Pathway/Phosphogluconate Pathway/** **Hexose Monophosphate Shunt**
* Glycolysis is just one direction that the cell can take from G-6-P. The other direction is the Pentose Pathway. This is also known as the Phosphogluconate Pathway, and the **Hexose Monophosphate Shunt**. It is a shunt because it shoots off from glycolysis and comes back. This pathway produces reducing agents that can be used for biosynthesis such as the formation of fatty acids (i.e. NADPH). Energy must be stored as fatty acids instead as carbohydrates because fatty acids have a higher energy content per weight. This process also produces pentoses, which can enter glycolysis at various stages, as well as trioses that enter glycolysis.

1. Normally in glycolysis, G6P would be isomerized to F6P, but in the Pentose pathway, it is oxidized to **6-phosphoglycerate** by NADP+, which becomes the reducing agent NADPH. NADP differs from NAD by having a phosphate attached to it. **(**The intermediate in this step is a cyclic ester).
2. 6-phosphoglycerate is again oxidized by NADP+ to **ribulose-5-phosphate**. Note that this is a 5-Carbon compound, so 1 CO2 is created in this step. CO2 is the most oxidized a carbon atom can be; all of the energy has been extracted from this carbon. Ribulose-5-phosphate is a source of 5-carbon sugars. From here, this pathway has two possible directions. Ribulose-5-phosphate can become ribose-5-phosphate (isomerase), or it can become xyulose-5-phosphate (epimerase). Both of these are 5-carbon molecules
3. R-5-P and X-5-P combine with each other to form a 7-carbon molecule sedoheptulose-7-phosphate and a 3-carbon molecule glyceraldehydes 3-phosphate. To get to this point you need 2 glucose molecules, one to take you out to ribose-5-phosphate, and a second to take you to X-5-P.
4. G3P and S7P combine with each other to form fructose-6-phosphate, which enters glycolysis and produces a net 3 ATP because of the point at which it enters, and a 4 carbon molecule erythrose-4-phosphate.
5. At this point you need another glucose molecule to take you out to X-5-P again, because E-4-P combine with it to give you F-5-P (which nets 3 ATP) and another G3P (which nets 2 ATP), both of which enter glycolysis.

* The pentose pathway sends 15 carbons through the pathway and on to glycolysis, to produce a total of 8 ATP and 2 NADPH. Sending 3 G-6-Ps through glycolysis would produce a total of 9 ATPs, so you sacrifice 1 ATP for 2 reducing agents.
* Note that the Pentose Pathway does not produce any ATP itself.

**The Nefarious concatenation/TCA Cycle/Citric Acid Cycle/Krebs cycle**

* The TCA cycle is the tricarboxylic acid cycle, so named because of one of its substrates **citrate,** which is a triple carboxylic acid. This cycle is also known as the citric acid cycle or the Krebs cycle. The TCA cycle is essentially the reduction/oxidation of citrate; 2 carbons are passed down from ACoA and oxidized to CO2.
* The TCA cycle occurs in the mitochondrial matrix, which is obviously a separate compartment than the cytoplasm, where glycolysis and the Hexose Monophosphate Shunt occur.
* When glycolysis has progressed to the point where it has made 2 pyruvates, there are two possibilities, one of which is important for now. Pyruvate (3 Carbons) is decarboxylated, oxidized, and combined with **Coenzyme A (CoASH)** in what is known as the **pyruvate dehydrogenase system,** so that it is now Acetyl Coenzyme A (ACoA), which has two carbons. This is the substrate that enters the TCA cycle by combining with **oxaloacetate**, which has 4 carbons. This pyruvate dehydrogenase system serves as the link between glycolysis and the TCA cycle, and occurs in the mitochondrial matrix.
  + The **Pyruvate Dehydrogenase System** is a rather complicated process. It consists of 5 cofactors, 4 of which are B-Complex Vitamins, Thiamine (TPP), Pantothenate (CoASH), Niacin (NADH), and Riboflavin (FADH2). Everything keeps going in a circle, and keeps getting reused, except for the carbons that are passed down.
  + In the TCA cycle, citrate, cis-aconitate, and isocitrate are in a **ternary equilibrium**, meaning there are three species in equilibrium. In this equilibrium, citrate exists at 91%, aconitate 3%, and isocitrate 6%. The control point for this equilibrium is **isocitrate dehydrogenase**. If this enzyme is inhibited, then citrate collects because it is the species in the highest concentration.
* Notice that in the TCA cycle, essentially what is happening is that the 3 carbons from pyruvate (2 after pyruvate is converted into ACoA), are being oxidized, by being passed around the TCA cycle substrates. At two points in the TCA cycle, CO2 is released. Remember that CO2 is the most oxidized that a Carbon atom can be, +4. If Carbon atoms are being oxidized, then somewhere, something in the cycle is being reduced: NAD and FAD are reduced to NADH and FADH2. These cofactors, NADH and FADH2 move onto the **electron transport chain**, (ETC). Also in the mitochondrial matrix. to produce ATP. Another important note is that the TCA cycle *only* occurs **aerobically**. The reason for this is that NAD+ is required for the TCA cycle. NAD+ is created in the ETC by reducing complex I. The ultimate electron acceptor in the ETC is O2, which if not present, the ETC cannot proceed, thus NAD+ is not regenerated, and the TCA cycle cannot proceed either.
* **ATP count**
  + Remember that glycolysis nets the cell **2 ATP** for every glucose molecule that is processed. It also makes 2 pyruvates to move onto the TCA cycle.
  + Between glycolysis and the TCA cycle, the pyruvate dehydrogenase system produces 1 NADH. When NADH moves to the ETC, it has enough energy to produce3 ATPs. 2 pyruvates come from glucose, so this makes **6 ATP**
  + For every turn of the TCA cycle, 3 NADH and 1 FADH2 are produced. When FADH2 enters the ETC it has enough energy to produce 2 ATP. (The reason for this is that FADH2 enters the ETC by reducing complex II instead of complex I. Complex II does not have enough energy to make an ATP). The TCA cycle also produces 1 GTP via **substrate level phosphorlyation,** GTP is essentially equal to ATP in energy. Because glucose produces 2 pyruvates and thus causes 2 turns of the TCA cycle per glucose, a total of 6 NADH, 2 FADH2, and 2 GTP, for a total of **24 ATP** per glucose in the TCA cycle.
  + To this point we have 32 ATP, but we’re not done. Remember that the obligatory end product of glycolysis is lactate. Moving from pyruvate to lactate regenerates the oxidizing agent NAD+. If this is not regenerated, glycolysis will choke itself off because the cell will not be able to oxidize glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. HOWEVER, if pyruvate is moving onto the TCA cycle instead of moving to lactate, then the oxidizing agent NAD+ is not being regenerated, which would be problematic for glycolysis. The solution is in the **glycerolphosphate shuttle,** a pathway we have yet to learn, regenerates NAD+. The glycerolphosphate shuttle produces 2 ATP per NADH instead of the normal 3 ATP. Because 2 pyruvates enter the TCA cycle, the glycerolphosphate shuttle, this process occurs twice, therefore a total of 4 more ATP are made. This makes the grand total for catabolism of glucose **36 ATP.**
    - NOTE: The reason that the NAD+ generated from the ETC cannot be used by glycolysis is because they are in two different cellular compartments, the mitochondrial matrix and the cytoplasm.

What the heck are all of these things?

* FADH2 comes from **riboflavin,** a B-complex vitamin.
* **Coenzyme A** is a mononucleotide, meaning that it contains a nitrogenous base, a 5 Carbon sugar, and a phosphate group. One component of CoA is **Pantothenic Acid**, which is a B-Complex Vitamin. **The role of CoA is to activate Acyl functional groups**; CoA is the active form of Pantothenic Acid.
* **NAD/NADH/NADP/NADPH** is a dinucleotide. This is also an active form of a B-Complex Vitamin, in this case the B-Complex Vitamin is **Niacin or Nicotinic Acid.** Remember that NADP is used in biosynthesis.
* **Thiamin Pyrophosphate** is also an active form of a B-Complex Vitamin, in this case Thiamine. TPP is a carbon carrier.

Carbohydrates in various cells

* In red blood cells, glucose enters the cell, is phosphorylated, and continues on to lactate, which leaves the cell. G-6-P can also continue on to produce pentose phosphates in the Hexose Monophosphate Shunt. Red blood cells are completely anaerobic because they lack mitochondria.
* As opposed to red blood cells, brain tissues are completely aerobic. Glucose entering the cell moves ultimately to the TCA cycle. No lactate is produced.
* In the Liver, glucose has many fates. It can be processed all the way to CO2 in the TCA cycle, it can go to the Hexose Monophosphate Shunt, it can be made into glycogen, it can be made into fat, or it can be made into glucuronides, which can solubolize different substances in the body.

# Glycogen and Types of skeletal muscle

* The liver and skeletal muscle stores most of the glycogen in the body. The liver is about 10% glycogen by mass, and the skeletal muscle is about 2% glycogen by mass, but the majority of the gylcogen in the body is stored in the skeletal muscle because its mass is much greater than the liver.
* White skeletal muscle fibers are located in muscle groups that are used in the short term, such as the flight muscles of a chicken or the muscles of a sprinter. These fibers are largely anaerobic, and they are white because of the lack of mitochondria. Oxygen does not have a chance to get to the muscles to be used before the activity is over. These muscles have a tendency to store more glycogen, because they burn fuel more rapidly.
* Red skeletal muscle fibers are found in the heart, the flight muscles of migratory birds, or the legs of a distance runner. These are largely aerobic because they are used in long-term activities. They are red because they have a lot of mitochondria for aerobic metabolism.

# Glycogen Synthesis

* Glycogen is synthesized from Glucose-6-Phosphate. G-6-P must be isomerized to G-1-P. G-1-P combines with **UTP** (the uracil analog of ATP) to become **UDP-Glucose.** The UTP used to make UDP-Glucose kicks off a Pyrophosphate, PPi, which consists of 2 PO42- stuck together. You’ll notice that in this reaction step, there are 4 inorganic phosphates present. If the reaction were to remain in equilibrium, it would have no incentive to move forward. Therefore the PPi is hydrolyzed to 2 Pi, which destroys the equilibrium and allows this reaction step to proceed. UDP-Glucose is then added onto the glycogen chain in the cell, so that the chain is now one glucose longer. To recover the UTP, ATP is hydrolyzed and its Pi is passed to UDP to regenerate UTP. The enzyme that presides over this glycogen synthesis reaction is g**lycogen synthetase**. You’ll notice that to get from Glucose to Glycogen, the cell must spend 2 ATPs.
* In order to convert glycogen back to glucose, the enzyme **glycogen phosphorylase** adds a Pi onto the glucose to be released, and it leaves the chain. Notice that liberating this glucose requires no ATP input, just a Pi.
* Remember that converting one glucose molecule straight to pyruvate, then onto the TCA cycle produces a **net 36 ATP**. If instead the cell stores this glucose for a while as glycogen, then uses it later for this same process, this glucose will produce a **net 35 ATP**. Notice that even though converting glucose to glycogen costs 2 ATP, one of these would be used anyway in the first stage of glycolysis to convert glucose to glucose-6-phosphate. It costs about 3% of the total energy derived from one glucose molecule to store it as glycogen. BUT…
* The cell stores glucose as the polysaccharide glycogen instead of individual monosaccharides ultimately because this maneuver saves the cell energy in the long run. The normal plasma glucose concentration is about 5 mM. If the cell were to store glucose as glucose, then the intracellular glucose concentration would be about 550 mM. This enormous concentration gradient would cause the intracellular environment to be highly hypertonic, and water would be constantly rushing into the cell. In order to avoid bursting, the cell would have to constantly pump water out, which would require a great deal of energy. The intracellular concentration of glycogen is only around 0.01 mM, which does not contribute much to the tonicity of the cell.

# Fructose Catabolism (occurs mostly in liver)

* Fructose can enter the body either as Sucrose, which is then digested to fructose and glucose, or simply as fructose, which is found in fruits. Similar to glucose, fructose is phosphorylated by ATP (fructokinase), but it ends up as **Fructose-1-Phosphate** instead of Fructose-6-Phosphate. (In a reverse aldol condensation reaction, similar to **Step 4** in glycolysis) F-1-P breaks apart into DHAP and Glyceraldehyde. Because F-1-P only has one Phosphate group on it, it cannot go directly to G-3-P as does glycolysis. Glyceraldehyde is reduced to Glycerol, which is phosphorylated to glycerol phosphate. Glycerol phosphate it oxidized to Glyceraldehyde-3-Phosphate, which can now enter stage 2 of glycolysis. DHAP and G-3-P again are in equilibrium, so as G-3-P is used up, this equilibrium moves toward G-3-P.
* Just as glycolysis, fructose catabolism costs the cell 2 ATP but nets the cell 2 ATP. Carbohydrates always have the same ATP producing potential per gram, regardless of the kind of carbohydrate.
* If an individual has a genetic defect in which they cannot produce the enzyme **fructokinase**, they will not be able to sequester fructose in the liver cells. Fructose will be able to leak in and out of the cells, and it will accumulate in the blood plasma, eventually showing up in the urine. This condition is known as **essential fructosuria**, and is a benign condition.
* Another condition involving the inability to process fructose is known as **Fructose Intolerance**, and unlike the name implies is actually a dangerous condition. Normally Fructose is phosphorylated to F-1-P (fructokinase), then it moves to **glyceraldehyde** in a reverse aldol condensation reaction (aldolase). In fructose intolerance, there is a problem with the aldolase enzyme, so the reaction cannot proceed to glyceraldehyde and beyond. Furthermore, the Pi that is attached to DHAP cannot go on to produce ATP, so eventually the cell runs out of Pi, and suffers from a lack of ATP. Because the fructokinase enzyme is in tact, the cell can phosphorylate fructose, so it is sequestered in the cell and cannot leave, so it builds up in the hepatocytes and causes destruction of the liver.
* The glycemic index of fructose is around 20, whereas that of glucose is 100. It was thought years ago that substituting fructose for glucose in the diets of diabetics would help to solve the problems they have with blood sugar. HOWEVER, even a normal healthy individual has a minimal ability to catabolize fructose, so if fructose now becomes a major dietary component, effects similar to fructose intolerance will be experienced. The fructose that is found in our diets is in relatively low amounts compared to glucose, even in fruits, which even though they contain fructose, they still contain more glucose.

# Regulation of Carbohydrate Metabolism

* The enzyme that is responsible for the phosphorylation of glucose in *most* cells is **hexokinase**, and the enzyme responsible for the phosphorylation of F-6-P is **PFK**. Remember that PFK is inhibited by high ATP concentration in the cell. When PFK is inhibited, the substrate that builds up in the cell is G-6-P, NOT F-6-P. The reason for this is that the K for this step (F-6-P/G-6-P) is less than 1, meaning that the equilibrium favors G-6-P. G-6-P is an inhibitor of hexokinase. What this all means is that when ATP concentration is high in the cell, PFK is inhibited, G-6-P builds up in the cell. If all of the processes that stem from G-6-P have been satisfied (i.e. glycogen synthesis, the shunt etc.) then G-6-P is able to inhibit phosphorylation of glucose. When all of this occurs, instead of being sequestered inside the cell, glucose moves right back out of the cell to go to other places where it may be needed.
* The pyruvate dehydrogenase complex is an important regulation site for the TCA cycle. This enzyme is regulated both allosterically and by covalent modification of the enzyme itself. Allosteric modulation occurs with PDH by ACoA, ATP, and NADH levels in the mitochondria. When these are high, the activity of PDH is decreased. Covalent modification of PDH occurs via two more enzymes in the PDH complex, **PDH kinase**, and **PDH phosphatase**. PDH kinase is responsible for phosphorylating PDH by adding an inorganic phosphate, Pi, to PDH. High concentrations of ATP, NADH, and ACoA signal the PDH kinase to do this. PDH phosphatase is responsible for dephosphorylating PDH, which activates the enzyme. This occurs when concentration of NADH, ATP, and ACoA are low in the mitochondria. **Isocitrate dehydrogenase** is another important allosteric enzyme in the TCA cycle, and is under the influence of NADH (negative modulator). When this enzyme is inhibited, citrate will accumulate in the equilibrium behind it, and citrate is a negative allosteric modulator for its own production, by the enzyme **citrate synthetase**. When citrate accumulates, it moves to the cytoplasm, where it is involved in the production of fatty acids.

EXAM II

* An additional feature involved in the sequestration of glucose in cells is insulin. Insulin works with hexokinase in skeletal muscle cells, and when insulin fails to help glucose move into cells, they don’t get enough carbohydrates. However, in liver cells the enzyme responsible for the phosphorylation of glucose is **glucokinase** instead of hexokinase. Liver cells do not require insulin for glucose to enter them.
* Remember that Km, from the Michalis-Menten equation, is a measure of affinity of an enzyme for its substrate. The lower the Km, the greater the affinity for the substrate. The Km for glucokinase is around 10 mM. In skeletal muscle, the Km for hexokinase is about 0.1 mM. Glucose concentration in the blood plasma is normally around 5 mM. What this means is that at normal glucose concentrations, glucose is more likely to enter into and be sequestered in skeletal muscle cells than liver cells, because at 5 mM, hexokinase is very responsive, whereas glucokinase is not very responsive. So if the plasma glucose concentration is 5 mM, how do liver cells ever get glucose? The answer is that in the Hepatic Portal Vein, glucose concentrations can increase to 12-15 mM after a meal, and these concentrations *are* able to stimulate glucokinase into action.
* Unlike hexokinase, G-6-P concentration has no effect on the activity of glucokinase, so when G-6-P concentration increases, it does not signal the cell to stop sequestering glucose. So how *does* it happen? When all demands for G-6-P have been met (glycolysis, shunt, glycogen etc.) and G-6-P concentration increases, another enzyme in the liver cells is activated. **Glucose-6-Phosphatase**. This enzyme is specific to liver cells and to some extent kidney cells, and it is able to hydrolyze G-6-P back to glucose. The Km for this enzyme is about 3 mM, so when G-6-P concentration reaches this level, glucose is kicked back out of the liver cells.

# The Glycerol Phosphate Shuttle

* Remember that for glycolysis to proceed, the cell must regenerate the oxidizing agent NAD+, which usually happens by the reduction of pyruvate to lactate. However, when pyruvate moves on to the TCA cycle, NAD+ is not regenerated. There are two mechanisms for this to occur, one of which is the Glycerol Phosphate Shuttle. In this shuttle, **DHAP** is used to oxidize NADH, NAD+ is regenerated, and DHAP becomes **Glycerol Phosphate**. Glycerol Phosphate crosses the outer mitochondrial membrane and reduces a protein spanning the inner mitochondrial membrane, **Glycerol Phosphate Dehydrogenase**, thus G-P is turned back into DHAP. FAD reduces this protein to become FADH2. FADH2 reduces Coenzyme Q in the ETC, which makes 2 ATP. The G-P shuttle occurs in skeletal muscle.

# Malate Shuttle (liver and cardiac cells)

* Another mechanism to regenerate NAD+ when the cell is functioning aerobically is the **Malate Shuttle**. The malate shuttle is used to regerate NAD+ in liver and cardiac muscles. This shuttle can work “forward” in order to regenerate NAD+ and make 3 ATP via intramitochondrial NADH, or in “reverse.”
  + Forward—the mechanism to regenerate NAD+ is the reduction of oxaloacetate to malate. Malate is moved into the mitochondrial matrix, where it reduces NAD+ here to NADH. This NADH makes 3 ATP in the ETC. When malate is oxidized, it again becomes oxaloacetate (which itself cannot cross the IMM). Oxaloacetate reacts with the amino acid **glutamate** to form **α keto-glutarate**, and the amino acid aspartate. Both of these leave the matrix, and in the cytosol the react with each other to reform glutamate (which re-enters the matrix to continue the loop) and oxaloacetate, and the loop is completed. (Reactions in which amino acids and α-ketoacids interchange become important when talking about protein catabolism).
  + Reverse—all of the reaction sites of the Malate shuttle are reversible, and even though the “forward” reaction produces ATP, the reverse reaction does not require ATP. The purpose of running this shuttle in reverse is to move reducing power from mitochondrial matrix to the cytoplasm, which can be used for reductive biosynthesis. NADH in the matrix is oxidized to NAD+ by oxaloacetate, which becomes malate. Malate leaves the mitochondrial matrix to be oxidized by NAD+ into NADH, and malate turns back into oxaloacetate. Oxaloacetate reacts with glutamate to make α-keto-glutarate and aspartate. Both of these enter the matrix, and react with each other again to form glutamate (which is able to leave the matrix to continue the loop) and oxaloacetate, which completes the loop.

# Pasteur Effect

The Pasteur Effect describes cells performing under aerobic conditions. When cells are functioning *anaerobically*, and oxygen is applied to the environment, two observations are seen. 1) Lactate concentration stops increasing, because pyruvate goes to the TCA cycle instead of lactate, and 2) the rate of glucose utilization decreases, because cells are getting more ATP per glucose.

### Creatine Phosphate

* **Creatine Phosphate** is skeletal muscle cells’ storage form of ATP. Energy cannot be stored as ATP because high [ATP] inhibits PFK, and stops the further production of ATP. At rest when skeletal muscle is making ATP, it is also passing some of the phosphates from ATP to **creatine** to make **creatine**-**phosphate** (CP) and ADP. ADP can go back and pick up another Pi and the process continues. CP does not inhibit PFK, so ATP can continue to be stored as CP. The reaction goes like this:

Creatine + ATP ⇔ Creatine-Phosphate + ADP ⇔ Creatinine ΔG°1 = +6.3 kJ ΔG°2 = -37.3 kJ

The reaction moves to CP at rest. This step requires an energy input of 6.3 kJ. When the Pi is to be transferred back to ADP to reform ATP, the ΔG° = -6.3 kJ. However, this does not come for free. Anytime you convert from one form of energy to another there is energy lost. The extra energy comes from the reaction moving all of the way to the right. When CP is hydrolyzed instead of passing its Pi back to ADP, it liberates enough energy to fuel the first reaction multiple times. Creatinine has a ΔG° of -37.3 kJ. Creatinine forms to varying extents in skeletal muscle depending on muscle mass and muscle activity. It also appears in the urine as a waste product. Once creatinine is formed, it is of no use to the cell. Urine [creatinine] is an indicator of skeletal muscle activity, because the more the Pi is transferred back to ADP, the more the reaction must leak all the way to the right to fuel the reaction. Note: according to these biochemical conversations, as a supplement, Creatine-Phosphate is useless because phosphorylated things cannot cross the cell membrane. This is why the maneuver to phosphorylate glucose is able to sequester it inside the cell.

CO2

Carbohydrate Catabolism

**FATS**

As an energy source, fats (triacylglycerols) have double the energy content as carbohydrates. The reason for this is that fats are much more reduced than carbohydrates. The more reduced something is, the more work potential it has.

# Fat Digestion

* In the stomach, there is almost zero fat digestion. Instead, the action begins in the small intestine, by the enzyme **pancreatic lipase (steapsin)**. This enzyme is water soluble, therefore because fats are insoluble, it is only able to react with the fats at the surface where the two are in contact. However, as something is broken down into smaller pieces, the surface area increases even though the volume stays the same. More surface area means more area for reaction to occur. **Bile** is what breaks fats down into smaller pieces to increase surface area. Bile functions as an emulsifying agent; it creates a micellular component so that the pancreatic lipase can get at the fats.
* Triacylglycerol digestion is a series of **basic hydrolysis reactions**. OH- is repeatedly added to a triacylglycerol to liberate one fatty acid tail and a diacylglycerol and so on until there are 3 long chain fatty acid tails and one glycerol molecule. These long chain fatty acid tails are amphipathic, and can further act as emulsifying agents. The fatty acids are absorbed by the intestinal mucosal cells, reformed into triacylglycerols, and packaged into **chylomicrons**. The formation of a chylomicron signals the end of the digestion process. The chylomicron leaves the intestinal mucosal cells into a lacteal, and ends up in the lymphatic system. Ultimately, the lymph is dumped into the blood stream.

## Saturated Fat Catabolism

Almost all cells can catabolize fatty acids. Fatty acids are stored in cells in the cytoplasm as fat droplets. Here, hydrolysis occurs with **intracellular lipase**.

* Before fatty acids can be digested, they need to be activated. The **activation** process consists of the fatty acid being made into an ACoA ester, a common maneuver for cells. This reaction requires the input of **1 ATP**, and occurs with a **thiokinase enzyme** (this type of enzyme is important for all activations of fatty acids). The result of the hydrolysis of ATP is AMP and a Pyrophosphate (PPi). The PPi is hydrolyzed to 2 Pi in order to drive the equilibrium forward (a similar action was seen in the formation of glycogen). After the fatty acid is activated it is now long chain **fatty acyl-CoA**, and is ready for catabolism.
* **Carnitine Shuttle:** Fatty acid catabolism occurs in the mitochondrial matrix, so obviously the fatty acyl-CoA must be transported here. It is able to cross the outer mitochondrial membrane without difficulty, but it needs help to cross the inner mitochondrial membrane. This process is the **carnitine shuttle.** The fatty acyl-CoA reacts with an enzyme embedded on the inner surface of the outer mitochondrial membrane known as **carnitine palmidoyltransferase-I (CPT-I).** In this reaction, the fatty acid is liberated from its CoA, and attached to **carnitine**, resulting in a fatty acyl carnitine compound. Carnitine acts as a shuttle to take the fatty acid across the inner mitochondrial membrane via another enzyme (carnitine-acyl carnitine translocase for completion’s sake). Inside the mitochondrial matrix, the fatty acid-carnitine complex now reacts with **CPT-II** to liberate the fatty acid from carnitine and reattach it to CoA. Carnitine is now able to leave the mitochondrial matrix to shuttle another fatty acid across the inner mitochondrial membrane, and the fatty acyl CoA can proceed to β-oxidation.
* **β-oxidation:** Summary: β-oxidation consists of a repetition of oxidation, hydration, oxidation, and thiolytic cleavage of the long chain fatty acid until the last 2 carbon ACoA is produced. After each β-oxidation cycle, the fatty acid will be 2 carbons shorter. Each turn of β-oxidation produces 1 FADH2, 1 NADH, and 1 ACoA. The final thiolytic cleavage produces 2 ACoA. 1) The first step in β-oxidation is to oxidize the bond between the α and β carbons from a single bond to a double bond. This step produces one FADH2 from the oxidizing agent FAD. 2) The second step is to hydrate the double bond. This creates an OH group on the carbon β to the carbonyl (C=O). 3) The third step is to oxidize the OH group on the β carbon to a ketone. This is done by the oxidizing agent NAD, and NADH is produced. 4) The final step is to cleave the fatty acid between the α and β carbons by adding an ACoA to the bond. The result is 1 ACoA and a fatty acid that is identical to the original, only 2 carbons shorter.
* **ATP Count:** β-oxidation produces 1 FADH2, 1 NADH, and 1 ACoA per turn, except for the final one that produces 2 ACoA. All of these products go on to the TCA cycle to produce ATP. Each FADH2 produces 2 ATP, each NADH produces 3 ATP, and each ACoA produces 12 ATP. A 16 carbon fatty acid will yield 7 FADH2, 7 NADH, and 8 ACoA for a total of 131 ATP. Remember that it cost 1 ATP to activate the fatty acid at the beginning, so the net ATP count is 130. Note that fatty acid catabolism *only* occurs aerobically. Therefore, mature RBCs and nervous tissue cannot undergo β-oxidation.

**Unsaturated Fat Catabolism:**

* β-oxidation of unsaturated fatty acids is slightly different at one step, but after that it’s business as usual. Notice that the C=C bond that is formed in step 1 of β-oxidation is **trans** and it is a **Δ2** bond. However, trans fatty acids are not naturally occurring in nature, so they should not appear in our diet. Dietary unsaturated fatty acids are usually **cis**, and can be either Δ odd # or Δ even #. We will concern ourselves with the Δ odd # situation. No matter where the unsaturation(s) occur(s) in the fatty acid, eventually it will come down to be either Δ3 or Δ4. When the unsaturation occurs at the Δ3 location, it is acted upon by an **isomerase enzyme,** which changes it from being Δ3 and cis to being Δ2 and trans. From this point on, the fatty acid can be hydrated and can continue with β-oxidation as usual. HOWEVER, you’ll notice that unsaturated fatty acids must enter β-oxidation one step later than saturated fatty acids. The result is that the unsaturated fatty acid does not undergo the first oxidation step by FAD. The result of this is that for unsaturated fatty acids, there is a **2 ATP shortfall** for every unsaturation. If you’re thinking that substituting all unsaturated fats in your diet for saturated fats will cause you to lose weight, joke’s on you because this amounts to less than a 3% reduction in energy content. There is less energy content because the unsaturation represents a more oxidized state of the fatty acid than a single bond would produce.

**Oxygen utilization:**

* Combustion of a 16-carbon fatty acid would have the following equation:

CH3(CH2)14COOH + 23 O2 🡪 16 CO2 + 16 H2O

Where does the oxygen come from? In the electron transport chain, each NADH or FADH2 requires 1/2 of an O2. This means that each turn of the TCA cycle, which produces 4 reducing agents, 2 O2 are required to oxidize the reducing agents. A 16 carbon fatty acid will make 8 ACoA’s, which will cause 8 turns of the TCA cycle for 16 O2 used. Also, β-oxidation of a 16-carbon fatty acid produces 14 reducing agents, which we said need 7 O2 to oxidize them. This makes the total 23 O2 needed for the combustion of a 16-carbon fatty acid.

**Odd # carbon fatty acid catabolism:**

* Occasionally, we will encounter fatty acids in our diet that have an odd number of carbons. When this occurs, β-oxidation is business as usual until you reach the 3-carbon fragment, known as **Propanyl CoA**. This fragment is carboxylated (CO2 is added)—which doesn’t happen often, but whenever it does, the cofactor **biotin** is used. (Biotin also appears in gluconeogenesis). The product of this reaction is **methyl-malonyl CoA**, which is a 4-carbon molecule. Methly-malonyl CoA undergoes a mutation reaction with the enzyme methyl-malonyl CoA mutase. This reaction requires the cofactor **Cobalamin**. Cobalamin is derived from vitamin B-12. B-12 is typically involved in 1-carbon transfer reaction, which will be discussed more later. When methyl-malonyl CoA is mutated, the product is **succinyl CoA**, which is TCA cycle ready.

# Ketone Bodies

# Ketogenesis

Ketone bodies, which obviously include the functional group C=O, are important metabolic substances. **Acetoacetate** (3-ketobutyrate), **β-hydroxybutyrate** (3-hydroxybutyrate) and **acetone** are the important ketone bodies. The first two a normal products of metabolism, but the third occurs in humans only when there is pathology. Exporting ketone bodies is how the liver moves reducing power to the peripheral sites.

* The liver is the main site for **ketogenesis**. Ketogenesis occurs during fasting or starvation states (12-15 hours after last meal). When the body’s glycogen stores have been depleted, the liver begins to produce glucose from TCA cycle components in a process known as **gluconeogensis,** in order to maintain the blood plasma [glucose] (discusses later). When TCA cycle components are used to produce glucose instead of the TCA cycle reactions, [ACoA] increases, because it is not able to enter the TCA cycle. Remember that ACoA enters the TCA cycle by reacting with oxaloacetate (OAA). However, when OAA is not around because it has gone on to produce glucose in gluconeogenesis, ACoA has nowhere to go and goes on to ketogenesis.
* Remember that β-oxidation of fatty acids results in the production of acetoacetyl-CoA. This is the 4-carbon fragment produced toward the end of β-oxidation. Usually, acetoacetyl-CoA is cleaved into 2 ACoA, but this reaction is reversible. When the liver accumulates a lot of ACoA, the reaction works backward to produce acetoacetyl-CoA again and ketogenesis begins:
  + 1) ACoA is combined with acetoacetyl-CoA by the enzyme **HMG-CoA Synthetase** to form the 6-carbon molecule **Hydroxymethylglutaryl-CoA** (**HMG CoA).** 2) HMG-CoA is liberated from one of the ACoA’s by the enzyme **HMG-CoA Lyase**. The ACoA that comes off is able to react again with Acetoacetyl-CoA to produce another HMG-CoA. The product of this reaction is the ketone body **acetoacetate (a.k.a. 3-ketobutyrate).**
* The liver exports the ketone bodies it makes into the blood stream so that they can be used by peripheral sites. The reason that the liver does not utilize ketone bodies is that it lacks a **short chain thiokinase enzyme.** Recall that this type of enzymes is required to activate fatty acids before they can be catabolized; Thiokinases make fatty acids into Coenzyme A esters (activation). A second, more intuitive reason that the liver can’t catabolize ketone bodies is that it would not make sense for the liver to take something that was already a CoA ester, and make it into a ketone body, just to change it back into a CoA ester.

# Ketone Body Catabolism: peripheral sites such as skeletal and cardiac muscle, CNS tissue, renal cells etc. can utilize ketone bodies

* Ketone bodies can travel in the blood plasma in one of two ways, either as the more reduced **3-hydroxybutyrate**, or as the more oxidized **acetoacetate**. These two ketone bodies are interchangeable, that is 3-hdroxybutyrate is formed by the reduction of acetoacetate by NADH.
  + 1) When 3-hydroxybutyrate reaches a peripheral site, it is oxidized by NAD+back to acetoacetate (or acetoacetate can travel in the plasma itself). 2) Acetoacetate reacts with **succinyl-CoA** and becomes **acetoacetyl-CoA**. This step is equivalent to the acetoacetate being activated, and also recall that the activation step requires an ATP. If you think that we have just completed an activation without sacrificing an ATP then joke’s on you. Remember that succinyl-CoA is a TCA cycle component, and the step after Succinyl-CoA produces 1 **GTP**. If succinyl-CoA passes its CoA onto acetoacetate to become succinate, then this GTP has not been formed. We have used the energy in succinyl-CoA to activate acetoacetate, thus essentially we have still used the ATP. 3) Acetoacetyl-CoA is cleaved by a **thiolase enzyme** (the same type of enzyme used in the last step of β-oxidation) into 2 ACoA’s which go on to enter the TCA cycle.

# Ketoacidosis

* When ketone bodies are in the blood plasma, they exist as an acid, not as the conjugate base; they have a H+to donate. The response of the body to this situation has already been studied, that is the blood buffer system of HCO3-. HCO3- is able to accept the proton from ketone bodies in the following reaction:

Acetoacetate + HCO3- 🡪 CH3C(=O)CH2C(=O)O-+ CO2 + H2O

Small ketone body concentrations in the plasma can be dealt with by the buffer system, but when ketone body concentration is high in the plasma, this maneuver begins to deplete the alkali reserves of the plasma. The result is **1° Alkali Deficit**, which if left uncompensated for can result in a **metabolic acidosis** known as **ketoacidosis**.

* Ketone bodies are formed as you begin to starve, and even though they are used by sites besides the liver for energy, they are cleared by the kidneys and appear in the urine. If a pathology exists such as diabetes, then ketone bodies can form a lot and lead to ketoacidosis.

**PROTEINS**

Proteins are digested into amino acids, which can be used for fuel. Amino acids also have other roles besides an energy source. Amino acids can be used to form the proteins in the body (i.e. structural proteins) and their functional groups (benzene rings, methyl groups, sulfur) can be used as starting materials for hormones etc.

**Protein digestion:** Just as carbohydrate and fat digestion, protein digestion reactions are hydrolytic, and neither require nor produce ATP

* **Stomach:** Protein digestion begins in the stomach, and is aided by the enzyme **pepsin**. Before pepsin can function as an enzyme, it must first be activated from its inactive form **pepsinogen.** Pepsinogen is activated by accepting a H+, which are abundant in the acidic environment of the stomach. Pepsin catalyzes the hydrolysis of only specific peptide linkages. To be hydrolyzed by pepsin, the linkage must be 1) the linkage that involves the carboxylate end of the amino acid 2) provided by an enzyme whose functional group contains a benzene ring. There are only 3 amino acids that have benzene rings in their functional groups, tyrosine, phenylalanine, and tryptophan. These represent about 1/6 to 1/7 of the amino acids. Logic tells us that pepsin will cut proteins into polypeptides that are about 7 amino acids in length.
* **In the Duodenum:** Chyme (partially digested food) now enters the duodenum, and here protein digestion continues. In the intestinal lumen, amino acids are subjected to a number of **pancreatic enzymes** (chymotrypsin, procarboxypeptidase, trypsin, proelastase), which complete their digestion into individual amino acids, and di- and tri-peptides. These are absorbed into intestinal mucosal cells. Eventually, like carbohydrates, amino acids make it into the hepatic portal vein and are taken to the liver.

**Amino acid catabolism**

The majority of amino acid catabolism occurs in the liver. As we know, there are about 20 individual amino acids, and unfortunately for us, they do not all share the same pathway. There is an individual pathway for each amino acid, though some similarities exist between some of them. The characteristic amino acid is **glutamate:**

**-**OOCCH2CH2C(-NH3)HCOO-

* The **first step in amino acid catabolism is to remove the amine group from the amino acid.** Every amino acid has at least one amine group attached to it, and as humans, we must get rid of this before we can begin oxidizing amino acids. The reason for this is that humans are *only* able to oxidize carbon, we cannot oxidize the amine N. There are 2 different mechanisms to remove the N from amino acids.
  + **Oxidative deamination**: Oxidative deamination is most prevalent in the liver, though it can occur in other cells. In this process, the NH3 that was attached to the α-carbon is removed, and NAD+ is used. The enzyme for this particular deamination is L-glutamate dehydrogenase, though other specific enzymes exist for the oxidative deamination of other amino acids. The result is an **α-ketoacid** (see below), NADH, and NH4+ (ammonium must be dealt with by the body in the urea cycle, which is to come).  An α-ketoacid consists of a carboxylic acid group, and a ketone functional group in the α position. Now, the α-carbon of the amino acid has a ketone structure. This pattern, (amino acid to α-ketoacid) is consistent for amino acid catabolism.
  + **Transamination**:In this process, rather than the oxidizing agent NAD being used for the deamination, the amine group is passed onto an **α-ketoacid**. Valine is one amino acid that undergoes transamination. In this case, the amine group from valine is passed onto pyruvate. We have seen pyruvate in other contexts before, but now we are becoming aware of the fact that pyruvate is an α-ketoacid. The products of this reaction are **alanine**, which is the exact same structure as valine but with an amine group at the α carbon instead of a ketone, and a 3-carbon α-ketoacid. Note that α-ketoacids and amino acids are interchangeable, by transferring amine groups back and forth. α-ketoacids consist of only carbon in their structures, and are a major source of fuel (just look at pyruvate, oxaloacetate, and α-ketoglutarate).
    - If you transaminate an amino acid with pyruvate, you get **alanine** and a 3-carbon α-ketoacid.
    - If you transaminate an amino acid with oxaloacetate, you get **aspartate**, and a 4-carbon α-ketoacid
    - If you transaminate an amino acid with α-ketoglutarate, you get **glutamate** and a 5-carbon α-ketoacid.
    - What is the purpose of the transamination maneuver? It seems as though we have just traded one amino acid for another and have gotten ourselves nowhere. The answer is that by reacting the amino acids with pyruvate, oxaloacetate, or α-ketoglutarate, instead of having about 20 possibilities for amino acids, we now only have 3 possibilities.
    - IMPORTANT: the common feature of all transamination enzymes is that they all have **pyridoxal phosphate as a cofactor.** Pyridoxal phosphate (from Vitamin B-6) is the active form of the B-complex vitamin **Pyridoxin,** and it serves as an amine carrier.
* Once the amine group is removed from the amino acid, then it is ready for the TCA cycle. Different amino acids enter the TCA cycle at different points, and these amino acids can be lumped into 2 groups.
  + **Glycogenic amino acids:** These amino acids are the ones whose carbons enter the TCA cycle at Pyruvate, or as TCA cycle components. Malate, a TCA cycle component, is the jumping off point for gluconeogenesis—the production of glucose by the liver. The carbons from glycogenic amino acids produce malate, and furthermore, they go on to produce glucose. Glycogenic amino acids produce glucose and not ketone bodies, even though pyruvate moves on to ACoA anyway, because when these carbons get to ACoA by way of pyruvate, the [ACoA] is not able to build up. Remember from the ketogenesis discussion that it is the build up of ACoA (as in starvation conditions, because of the depletion of TCA cycle components), that causes the production of ketone bodies. Additionally, there is a reaction that takes Pyruvate, combines it with ATP and CO2 to make oxaloacetate. We ignored this reaction in the TCA cycle previously, but it will be discusses later in gluconeogenesis.
  + **Ketogenic amino acids** are amino acids whose carbons enter the TCA cycle as ACoA, Acetoacetate, or Acetoacetyl CoA. Rather than going on to produce glucose, the carbons from these amino acids go on to produce **ketone bodies.** It should be obvious that because the carbons from amino acids enter the TCA cycle, either by way of ACoA or directly as TCA cycle components, that **amino acids have similar calorie content as carbohydrates**, that is **4 calories per gram.**
  + Some of the amino acids donate carbons to both the ketogenic and glycogenic pathways. 19 of the 21 amino acids have at least some glycogenic character, meaning that only 2 of the amino acids are purely ketogenic.
* **Leucine, a ketogenic amino acid:** Leucine is one of the two amino acids that is purely ketogenic. Its catabolism requires a number of steps, but only one of them is a trick reaction that we have not seen before. For the complete process see the diagram below. 1) First, leucine must be transaminated. To do this, α-ketoglutarate is used, which as we know produces glutamate, and a 6-carbon α-ketoacid. 2) The resultant α-ketoacid is oxidized, decarboxylated, and stuck onto CoA, in a step that is identical to the **pyruvate dehydrogenase system** that takes us from pyruvate to ACoA in carbohydrate catabolism (see above). 3) Next, the thioester that we are left with is carboxylated which is the only trick reaction, to give us back the 6-carbon molecule (carboxylations require **biotin**). 4) The 6-carbon molecule is again oxidized, but this time by FAD to produce FADH2 and to insert a double bond in the 6-carbon molecule. You will recognize this reaction from the first step in β-oxidation. 5) Again, similar to β-oxidation, the 6-carbon molecule is hydrated across the double bond, inserting an OH at the β position and a H at the α position. This step is identical to the second step of β-oxidation. The resulting molecule after this step is **HMG-CoA**, which we have seen before in ketogenesis. 6) It should come as no surprise that HMG-CoA is cleaved into ACoA and acetoacetate, because this is the exact same reaction that we see in ketogenesis. ACoA and acetoacetate enter the TCA cycle, making leucine a **ketogenic** amino acid.
* **Phenylalanine, an amino acid that is both ketogenic and glycogenic:** Phenylalanine is an amino acid that has a benzene ring in its R-group. In phenylalanine catabolism, it is reacted with O2, NADPH, and the enzyme **phenylalanine hydroxylase**. This reaction yields a water molecule, NADP+, and the amino acid tyrosine. The only difference between phenylalanine and tyrosine is that tyrosine has a hydroxyl group on its benzene ring and phenylalanine does not. After a number of reactions, tyrosine becomes **fumarate,** a TCA cycle component, and acetoacetate. Both of these enter the TCA cycle. Thus, phenylalanine is an amino acid that has both ketogenic and glycogenic character.
  + **Phenylketonuria (PKU):** About once in every 70,000 births, a child will end up with a genetic condition in which he will not be able to synthesize the enzyme phenylalanine hydroxylase. Instead, phenylalanine will go on to make **phenylpyruvate**, which is a phenylketone. Phenylpyruvate collects in the blood plasma because there is no metabolic pathway for it. If it is not cleared by the kidneys, then it can cause mental and physical retardation, and ultimately death at a young age. This condition is tested for at birth by a blood test that searches for the presence of phenylketones in the child’s blood. An individual with PKU will be treated with a special diet.

The problem with PKU is that phenylalanine cannot take part in metabolic activities, however, the amino acid is still required by the body to synthesize proteins, as phenylalanine is an **essential amino acid** (needed by the body an cannot be synthesized). Additionally, the amino acids tyrosine, phenylalanine, and tryptophan are the body’s sources of benzene rings, which are required for certain hormones and other substances. Therefore, the diet of one with PKU must be *low* in phenylalanine, but NOT lacking phenylalanine because it is still essential. Also, the diet should be high in **Tyrosine** because the individual will not be able to make tyrosine. You should notice that in a “normal” individual, tyrosine is not an essential amino acid because it can be synthesized from phenylalanine. However, in one with PKU, tyrosine becomes essential because the body cannot synthesize it.

# Nitrogen and the Urea Cycle

* Nitrogen occurs in nature as NH3, N2, or NO3-. The only nitrogen that we can use in our bodies is ammonia, NH3. The problems are that we lack the ability to reduce or oxidize Nitrogen, it is needed by the body, and NH3 is toxic. Because we get Nitrogen from amino acids, we need a mechanism to eliminate nitrogen from the body. This mechanism is the **Urea Cycle**. The urea cycle is a hepatic function; if there is liver failure, then there will be failure of the urea cycle, and ammonium will damage body tissues and cause death.

NOTE: Whereas the body cannot stand anymore than a plasma [NH3] exceeding 0.02 mM, the plasma [urea] can be as much as 2 M. It is clear that ammonia must be dealt with by the body. The urea cycle is related to the TCA cycle, and also to the malate shuttle, everything in these processes is recycled and used again except for the N (and CO2 in the TCA cycle) that is thrown away.

* Urea production in the body depends on the amount of Nitrogen intake, that is, how much protein you have in your diet. In a normal diet, urea production will be around **30 grams per day**, and a high diet will cause urea production to exceed **100 grams per day**. If an individual goes on a low protein diet for a number of days, his urea production will decrease, but will eventually bottom out around **2 grams per day**. What this means is that there is a floor to the amount of urea that is produced, and thus to the amount of Nitrogen that is eliminated, and this leads to the idea of the minimum protein intake necessary to maintain a positive Nitrogen balance. One must consume at least **0.8 grams of protein per kg of body weight** to maintain Nitrogen balance.
* **Urea Cycle Summary:** The urea cycle involves 2 new non-peptide amino acids, meaning that they do not take part in peptide bond formation. These are **ornithine and citrulline**. The Urea cycle proper involves the cycle of reactions involving ornithin, citrulline, arginosuccinate, and arginine, but includes input from 2 sources and output to 2 sources. At different points in the cycle 2 NH3 molecules are added, via **carbamoyl-phosphate** and the amino acid **aspartate**, a molecule of fumarate, and one molecule of Urea is released per turn.
* **Steps: 1)** The process begins with any amino acid, which undergoes a transamination reaction with α-ketoglutarate, which as we know produces glutamate and some α-ketoacid. **2a**) Glutamate two possible directions, and to complete this process two glutamate molecules must be involved, one to each direction. The first option is to deaminate glutamate with the oxidizing agent NAD+ to release the ammonium ion, and regenerate the α-ketoglutarate. The α-ketoglutarate goes back to the beginning of the process to react with an amino acid. **2b**) The second option for glutamate is to undergo another transamination reaction with a **oxaloacetate** (a 4-Carbon α-ketoacid) and **aspartate** (aspartate enters the urea cycle by reacting with citrulline to make arginosuccinate). **3)** The ammonium ion formed from the deamination of glutamate reacts with an ATP and a CO2 to form **carbamoyl-phosphate**, which enters the Urea cycle by reacting with **ornithine**, releasing a Pi.The result of this reaction between ornithine and Carbamoyl-P is citrulline, which we have seen combines with aspartate to make arginosuccinate. **4**) Arginosuccinate releases **fumarate**, a TCA cycle component, to become arginine. **5**) Arginine releases urea, and the circle is complete as ornithine is reformed. Urea obviously enters the blood stream, is cleared by the kidneys, and is excreted as urine.

**Glutamine:** Another mechanism of Nitrogen fixation is into the amino acid **glutamine.** Whereas Urea, when formed, is secreted from the body, when glutamine is formed, it is used within the body as a source of Nitrogen. It is especially important for the kidneys, which use the Nitrogen from glutamine to maintain acid/base balance within the body. Nitrogen is also important for central nervous system tissue, as many neurotransmitters require Nitrogen in their structures (Just ask Christy about GABA, γ-**AMINO**butyric acid). Glutamine is formed in the liver by combining glutamate with ammonia, and using an ATP.

**Gluconeogenesis**

Gluconeogenesis is the process of making glucose from non-glucose precursors. This is an important maneuver because many cells in the body (RBCs, nervous tissue) cannot operate anaerobically, thus blood [glucose] must be kept relatively constant so that these tissues will have enough fuel. Until now, everything that we have covered has been catabolic, producing ATP, but gluconeogenesis is a process that is anabolic, therefore it requires the input of ATP. As we will see many of the reactions in gluconeogenesis are just the reverse of glycolysis, but there are a few reactions in glycolysis that are not reversible, thus a different pathway is necessary. Because gluconeogenesis requires the input of ATP, it only occurs when there is a surplus of ATP, or under starvation conditions.

* **Reactions:** For now, consider the starting point for gluconeogenesis to be **Pyruvate**. In glycolysis, pyruvate is formed from PEP, and an ATP is formed. We have learned previously that this reaction has a very negative free energy change, which allows glycolysis to proceed forward to a great extent. For this reason, PEP 🡪 Pyruvate is not reversible as is, and a different path must be taken. Pyruvate is combined with ATP and CO2 to form **oxaloacetate (OAA)**. We have seen in the **Malate** **Shuttle** that OAA cannot leave the mitochondrial matrix, so OAA must be reduced to **malate**, which can cross the inner mitochondrial membrane. Once in the cytosol, malate is re-converted to oxaloacetate, but in this case it is with GTP rather than NAD+, as occurs in the Malate Shuttle. Finally, OAA becomes PEP as a CO2 is released. To this point, this process cost us 2 ATP per Pyruvate, and since Pyruvate is a 3-carbon molecule, we need 2 pyruvates for 4 ATP.

After we reach PEP, the reactions are reversible for a while, but one reaction of note is between 3-phosphoglycerate and 1,3-bisphosphoglycerate. In the forward direction, this reaction makes an ATP, in reverse, it is necessary to utilize ATP, but since these are both 3-carbon molecules and 2 of them are required to make a glucose, we need to use 2 ATP. This brings the ATP count for gluconeogenesis to **6 ATP**. Glycolysis only yields a net 2 ATP, so to go from glucose to pyruvate and back will *cost* 4 ATP.

Aside: When we are moving “forward” in glycolysis, the process is going downhill energetically, some reactions moreso than others. BUT, usually we are concerned with the *standard* free energy change, which means a concentration of 1 M. In the cell, however, we are not under standard conditions. The *actual* free energy change is more along the lines of 0 kJ/mole for many of the reactions in glycolysis. When ΔG = 0, the reaction is at equilibrium, and can go in either direction.

Once we reach Fructose-1,6-Bisphosphate, we must take another path to get to Fructose-6-Phosphate. The forward reaction from F-6-P to F-1,6-P requires the enzyme PFK, and consumes an ATP. Instead of taking this route, a water is added to F-1,6-P, and a phosphate is released. The enzyme required for this reaction is **Fructose-1,6-bisphosphatase.** Fructose-6-phosphate to Glucose-6-phosphate is also reversible, but from G-6-P to Glucose requires another special enzyme (Glucose-6-Phosphatase)and pathway. The forward reaction uses hexokinase in skeletal muscle and glucokinase in the liver to phosphorylate glucose to G-6-P. Recall from previous discussions that the hepatocytes are the only cells that are able to move from G-6-P to glucose, with the enzyme **Glucose-6-Phosphatase**, so that glucose can be exported from the cell. The reason for this is that the liver is the main organ responsible for gluconeogenesis; other cells want to hold on to glucose once they get it. Once glucose is made by these reactions in the liver, it can be exported to by used by other cells in the body. Note that any step

* **Other gluconeogenesis precursors:** Gluconeogenesis can begin from other precursors in the cell besides pyruvate. **Malate** is the main jumping off point for gluconeogenesis. Malate is a 4-carbon molecule, so to proceed from malate, you need 2 molecules, because from OAA to PEP a CO2 is lost. Also, from malate, only 4 ATP are required. Additionally, any molecule that can be made into malate can be a starting point for gluconeogenesis, that is OAA, fumarate, succinate, and succinyl CoA. Note that these are all TCA cycle components. This is why under starvation conditions, ACoA goes on to ketogenesis instead of ATP production, because all of the TCA cycle components are being bled away to form glucose. ACoA IS NOT A GLUCOSE PRECURSOR BECAUSE NO MATTER WHAT, THE REACTION FROM PYRUVATE TO ACoA IS *NOT* REVERSIBLE.
* **A note on starvation:** Once one has not eaten for about 24 hours and gluconeogenesis is in full effect, note that it is amino acids that are being used to maintain blood glucose, *not* stored fats. This is the reason that simply starving oneself is not an effective weight loss technique. Rather than mobilizing your fat stores to maintain blood glucose, your skeletal muscles are used.
* **Where does the liver get its energy?**: During starvation, you will notice that the liver seems to be exporting all of the energy to the other tissues of the body; the liver makes ketones and exports them, the liver makes glucose and exports it, ACoA is not entering the TCA cycle because the cycle is obliterated. All that is left for the liver is β-oxidation. Even though the ACoA from this process is being shipped as ketone bodies to peripheral sites, the liver still gets NADH and FADH2 from β-oxidation, and these enter the electron transport chain to provide the ATP for the liver.

### Lactate

Ignored until now, lactate is a metabolite with a lot of energy still left in it, and it is a very good carbohydrate precursor. First, it must be oxidized to pyruvate, then it is gluconeogenesis as usual, or it can go on to make ACoA and ATP, depending on the conditions.

* **The Cori Cycle:** The Cori Cycle describes how lactate is moved from anaerobically active skeletal muscles where it is produced to the liver to be made into glucose via gluconeogenesis, and back to the skeletal muscles to be converted into glucose. Central to the Cori Cycle is the concept of **Oxygen Debt**, which will be discussed shortly. The Cori Cycle accounts for the movement of carbon from skeletal muscle to the liver.
  + The magic number for the Cori Cycle is 270. That is, a skeletal muscle that is contracting anaerobically, i.e. during a quick sprint, wants to make 270 ATP. To do this, it must utilize 135 glucoses, thus making 270 lactates. The lactate is moved from the skeletal muscle mitochondrial matrix to the blood plasma, (which is required so that glycolysis is able to move forward, as an increase in intramitochondrial [lactate] will choke off glycolysis because the oxidizing agent NAD+ will not be reformed), and then it must be moved to the nearest aerobic cite, a liver cell.

Important points:

1) 1 lactate converted into CO2 is worth 15 ATP.

2) 2 lactate made into 1 glucose will cost 6 ATP.

3) In the Cori Cycle in the liver, the amount of ATP = the amount of ATP consumed

The liver will take the 270 lactates that arrive from skeletal muscle and take them in one of 2 directions. One of these directions is to convert the lactate into CO2 via the TCA cycle, forming 15 ATP per lactate for a total of 675 ATP. This process involves 45 of the lactates. The other 225 lactates that arrive in the liver are converted into glucose, which *consumes* 6 ATP per lactate. These 225 lactates make 112.5 glucoses, which are returned to the skeletal muscle via the blood plasma. These glucoses replace some of the original 135 glucoses that were burned anaerobically. Thus, the skeletal muscle is only out a total of 135-112.5 glucoses, which equals 22.5 glucoses. The ATP per glucose count for the Cori cycle is 270/22.5 = 12 ATP per glucose. This number is between 2 ATP per glucose for glycolysis and 36 ATP per glucose for aerobic metabolism, which should make sense as the Cori Cycle is part aerobic and part anaerobic.

The amount of ATP = the amount of ATP consumed; if you burn x lactate make 15x ATP

15x = ((270-x)/2) \* 6

x = 45 lactates with some algebra.

* **Oxygen Debt:** Go ahead and ask… If the skeletal muscles are working anaerobically during a sprint because the oxygen delivery has not yet increased, how can the liver possibly be working aerobically at the same time in order to undergo gluconeogenesis? The answer is that lactate formation and glucose formation occur at different times. Lactate is formed *during* the anaerobic exertion of the skeletal muscles, glucose is made from lactate *after* the anaerobic activity is complete. This excess usage of oxygen after the strenuous activity is the definition of **Oxygen Debt.**

### Substrate Cycles

A substrate cycle is used by a cell when it must regulate the flow of metabolites in a reaction that runs in both directions, but must use two different pathways. Say for example that one reaction in a long chain of reactions goes in one direction 100 times and goes in reverse 90 times, but 2 different paths are necessary. In this situation, the **flux** or flow is 10, the difference between the forward process and the backward process (or in other words how much the process leaks forward). If the substrate cycle is changed by 20% so that the reaction proceeds forward 120 times, and in reverse 72 times, then the flux is now 48. thus, a small change in the substrate cycle allowed for a much larger change in flux (5 fold greater). Substrate cycles allow for great control over different metabolic processes.

The example that was used was Fructose-6-Phosphate ⇔ Fructose-1,6,-Bisphosphate. The forward reaction uses the enzyme PFK to phosphorylate F-6-P. Traditionally we have known this enzyme as simply PFK, but now it is necessary to refer to the enzyme as PFK-1. The reason for this is that there is another enzyme involved in the substrate cycle, **PFK-2**. PFK-2 catalyzes the reaction of F-6-P to F-2,6-P instead of F-1,6-P. Fructose-2,6-Bisphosphate is the set-up for a substrate cycle. F-2,6-P is a controller for glycolysis and gluconeogenesis. F-2,6-P has positive effects on the forward reaction for glycolysis, and negative effects for the reverse reaction in gluconeogenesis. That is, F-2,6-P facilitates PFK-1, and inhibits Fructose-1,6-Bisphosphatase, and the [F-2,6-P] determines which direction this will go.

#### EXAM III

### Hormones

Hormones are substances that exist in small amounts but can lead to large impacts on an organism. The 3 broad classifications of hormones are polypeptides (insulin, glucagon), steroid hormones (the androgens, estrogens), and catecholamines (epinephrine and norepinephrine). Hormones are usually produced at one site and act at a different site in the body and they often have system-wide effects. For our purposes it is important to keep in mind where the hormone is produced, where it acts, what causes its production, and the effects it has. Another important point for hormones is that they often have a **half-life**. This means that if their production is stopped then the hormone will begin to “decay” and its effects will eventually stop. This is important because you do not want the hormone to linger in the blood when it is not needed anymore. For example, insulin, if allowed to stay in the blood could result in a fatal in plasma glucose concentration. Epinephrine and norepinephrine are trashed by the liver, and steroid hormones enter cells and are trashed there.

* **Hormones and carbohydrate activity:** Glucagon, insulin, and epinephrine are hormones that are important for carbohydrate activity and levels of blood glucose concentration. **Insulin** is a polypeptide hormone secreted by the pancreas in response to rising plasma glucose concentration. Its effect is to increase the movement of glucose into cells, thus lowering plasma glucose concentration. **Glucagon** is another polypeptide hormone secreted by the liver, but as opposed to insulin it is released in response to falling plasma glucose concentration. Its effects are to increase plasma glucose concentration by liberating glucose that is stored in cells as glycogen (see mechanism below). Epinephrine is a catecholamine that is produced by the adrenal medulla. Similar to glucagon, its effects are to increase plasma glucose concentration, *however* different from glucagon, epinephrine is made in response to **fight or flight** situations. The reason for this is to have glucose on hand in the blood plasma to be used by skeletal muscle and other tissues that will be metabolically active in high stress situations like running from a bear. To reiterate, in comparing glucagon and epinephrine, the former responds to an internal stimulus, whereas the latter responds to an external stimulus.
* **Mechanism for glucagon activity:** Note that this process, as opposed to many of the other mechanisms we have seen before is a series of *enzymes* rather than a series of intermediates and metabolites. When glucagon is released from the pancreas, it travels to the liver. It will react with receptors in the plasma membrane of hepatocytes called **adenyl cyclase**. If glucagon stops being secreted by the pancreas, it decays and this process stops. When glucagon reacts with adenyl cyclase on the outside of a hepatocyte, an ATP molecule reacts with it on the inside, and it is split into **cyclic AMP**. cAMP stimulates an enzyme known as **cAMP Stimulated Protein Kinase (cAMP SPK)**. This enzyme has 2 jobs (see below for the second). cAMP SPK stimulates the activation of the enzyme **glycogen phosphorylase kinase** from its inactive form to its active form, and this step involves the phosphorylation of the enzyme. Once glycogen phosphorylase kinase is activated, it can stimulate the activation of the enzyme that clips a glucose molecule off of the glycogen chain. This enzyme is glycogen phosphorylase. It is moved from **glycogen phosphorylase “b” (inactive)** to **glycogen phosphorylase “a” (active).** It is the “a” form that phosphorylates glycogen, thereby clipping of a glucose molecule, which comes off as **Glucose-1-phosphate**. From here it is a simple isomerization to glucose-6-phosphate, which is the phosphorylated form of glucose that we are familiar with. Since glucagon is acting on liver cells that *do* contain the enzyme **Glucose-6-Phosphatase**, G-6-P can be hydrolyzed to glucose, which leaves the hepatocyte to enter the blood stream to be used by peripheral sites. **Note that glucagon never enters the cell**. Once plasma glucose concentration is back to where it should be, the pancreas stops secreting glucagon, which will eventually decay. When glucagon is no longer present outside of the cell, cAMP will decay back to AMP, and the mechanism will stop (see next section).

Internal regulation within the glucagon mechanism also exists. Remember that the reactions of glycogen to G-1-P and back represents a loop (a substrate cycle if you will). If glycogen is moving “forward” to G-1-P, there is the possibility that the process can leak “backward” to glycogen, which would undo all of the work of glucagon. You could say that G-1-P has a choice in which direction it can go. The enzyme that moves G-1-P back to glycogen is **glycogen synthase,** and this enzyme exists in 2 forms, an “I” form and a “D” form. **Glycogen synthase “I”** is the form that catalyzes this reaction *independent* of the presence of G-6-P, that is, G-6-P is *not* needed as a cofactor. The “D” form is *dependent* on G-6-P being present; it is needed as a cofactor. The second job of cAMP SPK is to move glycogen synthase from the “I” form to the “D” form. This maneuver serves to prevent G-1-P from moving backward to glycogen. The reason this works is that when glucagon is working to increase plasma glucose concentration, G-6-P concentration is not building up in the cell. Instead it is being exported as glucose or used for other processes. Because G-6-P concentration is not increasing it is not present to act as a cofactor for the “D” form, so the maneuver by cAMP SPK serves to turn off glycogen synthase. Another source of internal regulation is G-6-P. G-6-P has 3 jobs:

**1)**, G-6-P stimulates glycogen synthase to move back to its “I” form. The reason for this is to essentially turn the enzyme back on so that G-1-P can again be stored as glycogen. G-6-P is able to build up when it is no longer needed to be made into glucose, that is, when glucagon’s action is no longer needed. Now you may be saying to yourself, “Self, if the ‘D’ form of glycogen synthase *depends* on G-6-P as a cofactor, wouldn’t we want this form to be present to make glycogen?” The answer is no, because even though the “D” form functions well with G-6-P present, the “I” form still works well whether G-6-P is present or not. The maneuver to move from “I” to “D” apparently just serves to turn off glycogen synthesis.

**2)** G-6-P stimulates the movement of glycogen phosphorylase “a” back to “b,” the inactive form.

**3)** Similarly, G-6-P also moves glycogen phosphorylase kinase back to its inactive form. Essentially, when G-6-P concentration increases in the hepatocyte, it undoes everything that glucagon did.

* **WHY?** The reason that all of these steps exist is in order to amplify the effects of glucagon. Remember that a classic characteristic of hormones is that they are made in small amounts but have large effects on the body. If one glucagon molecule causes the formation of 10 cAMP molecules and so on, you quickly have thousands of glucose molecules being exported to the blood stream.
* **Epineprine**: Remember epinephrine is similar in its effects as glucagon, and the mechanism is about identical. There are a few important differences. First, whereas glucagon primarily functions on the liver epinephrine works on the liver *and* skeletal muscle. When it acts on skeletal muscle, the end point is G-6-P, which will go on to produce CO2 and ATP for the cell. This *will not* cause an increase in plasma glucose concentration. However, epinephrine *does* also act on the liver, which *can* release its G-6-P as glucose. This *does* cause an increase in plasma glucose concentration.

### Sources of Carbohydrates in starvation

In the body there are 3 sources of carbohydrate available to maintain plasma glucose concentration. These are exogenous carbohydrates, i.e. from the diet, endogenous carbohydrate, i.e. hydrolysis of glycogen, and carbohydrates made from gluconeogenesis. As you get farther away from your last meal, you use differing amounts of each of these carbohydrates.

**I Absorptive Phase:** in this phase, you are using exclusively exogenous carbohydrate from your last meal to maintain your plasma glucose concentration. This phases lasts about 2-3 hours.

**II Postabsorptive Phase:** As the carbohydrates from your meal are depleted, your body begins to hydrolyze your glycogen stores to maintain plasma glucose concentration. This usage peaks around 5 hours after eating, and declines thereafter. Also during the postabsorptive phase gluconeogenesis is beginning to ramp up slowly. This phase lasts until about 16 hours after eating.

**III Early Starvation** begins around 16 hours after eating, when carbohydrates obtained from gluconeogenesis surpass carbohydrates from the hydrolysis of glycogen. Gluconeogenesis increases until about 30 hours after eating, at which point your glycogen reserves are completely depleted.

**IV Intermediate Starvation** begins at 30 hours after eating and lasts up to 24 *days* after eating. Gluconeogenesis in the liver and kidneys sustains your plasma glucose, which is being used only by the brain, RBCs, renal medulla, and a small amount by skeletal muscle.

**V Prolonged Starvation** lasts up to 40 days after eating, and again it is gluconeogenesis that maintains plasma glucose. However, at this point, only the brain, RBCs, and renal medulla are using glucose.

### Fatty Acid Synthesis

We have already seen how fatty acids can be oxidized in order to produce ATP. The body can also *produce* fatty acids in order to store energy. Remember that fats have a high energy density than carbohydrates, more kJ/gram because fats are more reduced. The production of fatty acids is a reductive process, **reductive biosynthesis** to be precise, and is almost the exact opposite process as β-oxidation. Fatty acid synthesis occurs in the cytoplasm. The steps in fatty acid synthesis are condensation, reduction, dehydration, reduction, condensation etc.

* **16:0 synthesis:** Fatty acid synthesis starts out with a molecule of ACoA (see below for where this comes from and how it gets into the cytoplasm). ACoA is carboxylated (again, biotin is used for all carboxylations) to form the molecule **Malonyl-CoA.** Malonyl CoA and another ACoA are the reacted with **acyl carrier proteins (ACP)** in order to produce 2 ACP esters, Malonyl-ACP and Acetyl-ACP. These 2 ACP esters then react with each other, releasing a CO2 (which can go back to react with ACoA to become Malonyl-CoA to complete the loop) and becoming **β-keto Butaryl-ACP**, a 4-carbon molecule. The step to produce this 4-carbon molecule is a **condensation reaction,** and is the opposite of the thiolytic cleavage reaction seen in β-oxidation. The next step is to reduce this 4-carbon molecule, specifically to reduce one of its ketone functional groups to an alcohol. The reducing agent in this step is **NADPH**, which comes from the hexose-monophosphate **shunt**. The resulting molecule has an OH group at the β carbon, and in the next step this OH group is **dehydrated** to form an alkene functional group. The alkene is again **reduced**, but this time to form an alkane. At this point we have a 4-carbon molecule that is beginning to look like a saturated fatty acid. The final step in this “round” of fatty acid synthesis is to condense the 4-carbon molecule with another molecule of Malonyl-ACP, releasing another CO2, and forming a molecule that is 2 carbons longer. This process continues until the fatty acid is 16 carbons in length.

This process is specific to a 16:0 fatty acid. To form a 16-carbon fatty acid, you need 8 ACoA, 14 reducing agents, and 7 Malonyl-CoA (which are produced from ACoA. You also need some CO2 for the process, but it keeps getting recycled. It is important to note that each time 2 carbons are added to the fatty acid chain, they are added to what will become the carboxylate end of the fatty acid. This point becomes important when we get to the idea of fatty acid elongation.

Because this process only produces the standard 16:0 fatty acid, and the body also requires fatty acids of different size and saturations, it is evident that there must be mechanisms to alter this standard fatty acid. These mechanisms include elongation and desaturation.

* **Elongation** is similar to fatty acid synthesis in that 2 carbons are added at a time, but it differs in that **elongation involves the addition of Malonyl-*CoA* esters and *not* Malonyl-ACP esters.** Remember that malonyl-CoA is produced from the carboxylation of ACoA with biotin as a cofactor. Malonyl-CoA is added to the fatty acid chain, releasing a CO2 and the CoA molecule. After elongation the fatty acid chain will be 2 carbons longer. Again, the 2 carbons that are added end up on the CoA end of the fatty acid (the end that will eventually have a COO- group) not on the methyl end of the fatty acid.
* **Desaturation** involves the insertion of one or more double bonds into the fatty acid chain. Desaturation is important in order to manipulate the melting point of a fatty acid. A 16:0 fatty acid will have a melting point of around 50°C, making is solid at human body temperature of 37°C. In higher primates, desaturation is a very specific process in that the first desaturation that is put into a fatty acid occurs at the Δ9 carbon, that is it is placed between carbons 9 and 10. Remember that the Δ notation represents counting from the carboxylate carbon (COO-) end. Each subsequent desaturation will be placed somewhere between the 9th carbon and the carboxylate carbon. Desaturation involves oxidizing the fatty acid with 1/2 O2, which becomes H2O, and a molecule of NADPH is used to protect the fatty acid molecule from the damaging effects of oxygen. The desaturation that results will be **cis** because trans fats do not occur naturally.

##### Essential Fatty acids: based on this discussion it should be evident that humans can only make insert desaturations into one end of a fatty acid. This leads to the idea of essential fatty acids. These fatty acids are use for things in the body such as prostaglandins, which are hormones used in anti-inflammatory processes. These hormones require fatty acids that have desaturations at the ω (omega) 3 or 6 positions. Remember that the ω designation means that you count from the methyl carbon end of the fatty acid rather than the carboxylate end. These fatty acids are essential because we cannot place desaturations at this end of the fatty acid. Remember that the definition of something that is essential is that it is needed by the body but cannot be synthesized.

* **How would you make a \_\_\_\_\_ fatty acid?** Even though the farthest down from the 1st carbon we can insert a desaturation is the Δ9 position, it is still possible to make fatty acids with desaturations at the Δ11, Δ13 etc. position. To do this we must simply conduct both desaturation and elongation reactions to place the double bond where we want. Δ

**Formation of Lipids (TAGs)**

We already know that a Triacylglycerol (TAG) consists of three fatty acid tails attached to a glycerol backbone. We have already seen how the fatty acid chain is made. In order to make a TAG, we also need to make the glycerol molecule, and stick the fatty acids onto it.

# Glycerol is formed from carbohydrates. DHAP, which comes from the 1st stage of glycolysis, is reduced by NADH to glycerol phosphate. This glycerol-phosphate molecule then reacts with 2 fatty acyl-CoA molecules. Their 2 CoA’s are released, leaving behind the glycerol molecule with 2 fatty acids attached to 2 of its carbons and the inorganic phosphate still attached to the 3rd carbon. This is a phosphatide, which is a polar lipid (because of the charge on the inorganic phosphate), which can go in one of two directions. 1) The phosphatide can remain a polar lipid and go on to be, for example, inserted into a lipid bilayer. Phosphatides are the jumping off point for other polar lipids. 2) The polar lipid can react with another fatty acid tail to become a TAG. Remember that the fatty acids that are being attached to this glycerol molecule come from reductive biosynthesis.

**Carbon Sources (Pyruvate-Citrate Cycle)**

One glaring concept about fatty acid synthesis is how exactly does ACoA get into the cytoplasm. We are used to ACoA, but usually it is located in the mitochondrial matrix, *not* in the cytoplasm. This is explained by the **Pyruvate-Citrate Cycle.**

* For this process, one glucose molecule undergoes its normal reaction steps in glycolysis until it reaches pyruvate. We know that one glucose molecule will produce 2 molecules of pyruvate. These enter the mitochondrial matrix and via the pyruvate dehydrogenase system, one is converted to ACoA and the other is made directly into Oxaloacetate. This is not a reaction step that we are familiar with, but it does occur. In order for pyruvate to be converted to ACoA, it must lose a carbon atom in the form of CO2. This same carbon atom can be thought of to combine with pyruvate in order to form the 4-carbon oxaloacetate. From here, ACoA and OAA undergo their normal reaction to form citrate. Normally, citrate would go on in the TCA cycle to produce ATP, but if there is no demand for ATP, which there wouldn’t be if you had just eaten too many calories and were at rest, citrate concentration would build up, forcing it to leave the mitochondrial matrix. Citrate, unlike ACoA and OAA is able to cross the mitochondrial membranes, and moves into the cytoplasm. Here it separates back into ACoA, which we know goes to form fatty acids. OAA is reduced to malate (a common maneuver we have seen in the malate shuttle). Malate, is decarboxylated and oxidized, to achieve pyruvate again, which moves back into the mitochondrial matrix to complete the loop. This process occurs in adipocytes as a maneuver to store energy as TAGs.
  + Count the carbons: one 6-carbon glucose forms 2 pyruvates. 1 pyruvate loses a CO2, which is picked up by the other pyruvate to form OAA. ACoA and OAA combine to form the 6-carbon citrate. Citrate decays to the 2-carbon ACoA and the 4-carbon OAA again. ACoA is added 2 carbons at a time to form a fatty acid chain. OAA is converted to malate (4-carbons), which is decarboxylated back to the 3-carbon pyruvate. **2/3 of the carbons from glucose end up in the fatty acid chain.** Therefore, for a 16:0 fatty acid, you need 24 glucose molecules.
* At this point it is important to examine where exactly the carbons come from for the formation of fatty acids and TAGs. As far as macromolecules go, it should be obvious that these carbons *do not* come from other fats. It would be a ridiculous waste of time to oxidize fats to ACoA just to take them through reductive biosynthesis to form fatty acids and TAGs again, so fats are out. Amino acids are also out because we know that these amino acids enter the TCA cycle as ACoA or ketone bodies (ketogenic) or as other TCA cycle components (glycogenic). Even though ACoA is where fatty acid reductive biosynthesis begins, when amino acids are being catabolized for energy, the TCA cycle is being obliterated because its components are being bled off to for glucose. Oxaloacetate must be present in order for the Pyruvate-Citrate cycle to occur; OAA must react with ACoA to produce citrate. Therefore, the carbons to be used in fatty acid reductive biosynthesis come from carbohydrates.

### Cholesterol

Cholesterol is a solid, insoluble, amphipathic substance. It is often found in lipid bilayers of cells. Only about 1 gram of sterols are excreted in the feces per day. Most cells make cholesterol, but the majority of it is made in the liver, where it is used as a precursor for bile salts and steroid hormones. **There is no catabolic pathway for cholesterol**.

# Enterohepatic Cycle: Bile made in the liver is sent to the gall bladder for storage. When needed for fat digestion, bile leaves the gall bladder to reach the duodenum of the small intestine. Here it is used for emulsification of fats. Bile is recovered from the small intestine and sent back to the gall bladder via the liver to be used again. A small amount of bile is lost in the feces.

* The gall bladder is able to concentrate bile because its mucosal lining is able to draw out water. Problems can exist with precipitation of bile, leading to gall stones. These are usually washed away and excreted but if they stick in the gall bladder or in the ducts they can cause clogs and lead to problems.
* **Cholesterol Synthesis:** Cholesterol is synthesized from ACoA, which may be surprising considering the size and complexity of the sterol nucleus compared to the simple 2-carbon ACoA molecule. 2 ACoA molecules combine to form acetoacetyl-CoA, and a third combines with this to form HMG-CoA (recall these reactions from ketogenesis). HMG-CoA is reduced via an important enzyme **HMG-CoA reductase** to form **mevalonate**. HMG-CoA reductase is important because it serves as a site of allosteric modulation of the process of cholesterol synthesis. This enzyme is inhibited by all sterols, thus cholesterol inhibits its own formation. Through a series of reactions mevalonate is converted into the 5-carbon molecule (get ready) **3, 3’ dimethylallylpyrophosphate** or **DMAPP** among friends. DMAPP is isomerized to form the 5-carbon isopentenyl pyrophosphate. Three of these molecules combine to form a 15-carbon molecule, and 2 of these combine to form the 30-carbon molecule **squalene**. Squalene undergoes a cyclization reaction in which it begins to form a sterol nucleus, **lanosterol**. Lanosterol then becomes cholesterol. This is a ridiculously complicated process but the take home seems to be that HMG-CoA reductase is inhibited by the presence of sterols, and that cholesterol is formed from ACoA.

### Lipid Transport

It is obvious that lipids have problems with water solubility, therefore there must be some sort of mechanism to help accommodate lipids in aqueous environments so that they can be moved around the body. **Lipoproteins** are the mechanism by which the body transports lipids in the blood**.**

# Lipoproteins are mixed micelles in which the shell is composed of the hydrophilic heads of phospholipids with the hydrophobic heads pointing toward the inside. Inside the micelle is the hydrophobic core, containing agents such as TAGs and cholesterol esters. A cholesterol ester is a variation of a cholesterol molecule in which a fatty acid chain is added where the OH group used to be. Cholesterol esters are a relatively “benign” form of cholesterol. Whereas cholesterol is a slightly amphipathic molecule, a cholesterol ester is completely hydrophobic because the slightly polar OH group is removed. Free cholesterol is found in the amphipathic shell of lipoproteins. Also embedded in the shell are apolipoproteins. There are a number of different apolipoproteins we will encounter, and the purpose of these polypeptide structures is to allow different sites in the body to recognize lipoproteins so that they may utilize their components.

* There are 4 different types of lipoproteins
  + **Chylomicrons:** We have seen chylomicrons before, lipid digestion ends with ingested fats being broken down to individual fatty acids and being packaged into chylomicrons to be dumped into the lymphatic system to make it to the blood. Therefore, chylomicrons are responsible for transporting **exogenous fats**, that is fats in the diet. Chylomicrons are mostly fat (87%) and a little bit cholesterol and cholesterol esters (0.5% and 2% respectively).
  + **VLDL:** Similar to chylomicrons are Very Low Lipoproteins (VLDL). VLDLs are responsible for transporting **endogenous fats**, that is those that are synthesized by the liver (reductive biosynthesis). VLDLs are also mostly fat (55%) with a little bit of cholesterol (1%) and cholesterol esters (5%).
  + **LDL:** Low Density Lipoproteins are different from chylomicrons and VLDLs in that LDLs are responsible for transporting cholesterol and cholesterol esters. LDLs are only a small percentage fat (8%) and cholesterol (8%) but contain quite a bit of cholesterol esters (35%).
  + **HDL:** Among other things, High Density Lipoproteins (HDL) are also responsible for transporting cholesterol and cholesterol esters (5% and 15%). These also contain a very small amount of fat (0.6%).

Lipoprotein metabolism involves 2 different pathways, but there is much overlap between the two. Let’s start with chylomicrons.

* **Chylomicrons** originate from intestinal mucosal cells and make it into the blood stream containing exongenous TAGs from the diet. A chylomicron has 2 important apolipoproteins on its surface, **apolipoprotein C-II** and **apolipoprotein** **E.** E is important for recognition of the chylomicron by the liver, but C-II is the more important apolipoprotein (note that C-II is passed to the chylomicron from HDLs). Apolipoprotein C-II is recognized by specific receptors on adipose cells called **lipoprotein lipase.** This is an enzyme that catabolizes the reaction to remove to fatty acid tails from the TAG, leaving a 2-monoacyl glycerol and 2 fatty acids outside of the cell. These are transported into the adipocyte and re-converted into a TAG for storage. When all of the TAGs have been removed from the chylomicron, what is left is a chylomicron remnant. This remnant still has its C-II receptors on it, which are recycled back to HDLs. Apolipoprotein E is recognized by the liver, which takes up the chylomicron. At this point the chylomicron still contains some dietary cholesterol, cholesterol esters, and fat soluble vitamins, which are deposited into the liver.
* VLDLs originate from the liver and are formed from endogenous TAGs. In addition to carrying TAGs from the liver, VLDLs also serve as the starting point for IDLs and LDLs. VLDLs move into the blood stream, and have attached to their surfaces **apolipoproteins C-II and B-100.**  Since they contain apolipoprotein C-II, which they also obtain from HDLs, they are recognized by the same lipoprotein lipase receptor on the surface of adipocytes. Similar to chylomicrons, this enzyme causes the hydrolysis of TAGs to a 2-monoacyl glycerol and 2 fatty acids, which enter the cell. Apoliproprotein C-II is recycled back to an HDL. Different from chylomicrons, the remnant of a VLDL is an **IDL.** This IDL contains, relatively, a few cholesterol esters, but it receives more cholesterol esters from an HDL, at which point it becomes an **LDL.** Now **apolipoprotein B-100** comes into play. This apolipoprotein allows the LDL to be recognized by a number of peripheral cells (peripheral as in away from the liver), including skeletal muscle cells. The LDL dumps its contents (cholesterol and cholesterol esters) into the skeletal muscle cell. The cholesterol that is dropped off reacts with an enzyme known as **Acyl Coa Cholesterol Transferase (ACAT).** This is an *intracellular enzyme*that catalyses the esterification of cholesterol to a cholesterol ester for storage. The cholesterol esters that are dropped off a simply stored. When the cell needs to produce energy, the cholesterol esters are split back into cholesterol and a **free fatty acid**. The cholesterol is picked up by an HDL.
* **HDLs:** there are a number of important points regarding HDLs. We have already seen how the are a source of apolipoprotein C-II for many of the lipoproteins. HDLs also have an apolipoprotein known as **Lecithin Cholesterol Acyl Transferase.** This enzyme catalyzes the reaction that transfers a fatty acid chain from lecithin to cholesterol to form a cholesterol ester. We also already know that these cholesterol esters are given to IDLs to make LDLs. HDL is also responsible for trafficking cholesterol around. It picks up the cholesterol that is formed in the peripheral cells from the breakdown of a cholesterol ester and takes it back to the liver. HDL is considered the “good” cholesterol because it scours the plasma searching for cholesterol to esterify into a cholesterol ester. Cholesterol that is in the ester form and stored is cells is not available to be roaming around the plasma looking for plaques to form.

**Roles of cholesterol in cell:** When made inside a cell, cholesterol likes to make itself right at home by doing 4 things. 1) Cholesterol can insert itself into the plasma membrane. 2) We already know that cholesterol inhibits its own synthesis at HMG-CoA Reducatase. 3) Cholesterol activates the reaction to convert itself into **cholesterol esters,** the storage form. 4) Finally, cholesterol inhibits the transcription (DNA to RNA) for the protein that is the LDL receptor that brings in more cholesterol.

### Fat Mobilization

# Fats are stored in adipocytes as TAGs, but adipocytes are only able to store fats, they are not able to catabolize them. Therefore, when energy is needed by the body, these fats must be mobilized from the fat cells under your chin and around your waist into free fatty acids in the plasma. These free fatty acids are then available to be taken up by other cells that can act aerobically to be used for energy.

* **Mechanism:** The hormones that start off this process should come as no surprise, glucagon and epinephrine. Both of these act on the same **adenyl cyclase** receptor that we have seen before in the mechanism to mobilize carbohydrate stores. ATP is again converted to cAMP, and cAMP again excites cAMP SPK. A new step is that cAMP SPK then causes the conversion of **intracellular lipase** from its inactive form to its active form. This step requires a bunch of ATP. Intracellular lipase is the enzyme that hydrolyzes TAGs to fatty acids and glycerol. The fatty acids leave the cell into the plasma, where they react with a protein in the plasma called **albumin**. Because fatty acids are amphipathic, this step is necessary to solubolize the fatty acid into a free fatty acid. If this step were not performed then the fatty acids would just clump back together as a micelle and it would be difficult for them to be taken up by a cell for energy. Note that the free fatty acids move on to other cells to be used as energy, but they *do not* go to CNS or RBCs because these cells cannot use them. The purpose of fat mobilization is to spare carbohydrates. If more fats are used for energy, then fewer amino acids need to be broken down via gluconeogenesis into glucose in order to maintain plasma glucose concentration.

The liver is a pretty low metabolical organ. In a starvation state, free fatty acids that go to the liver are partially oxidized into ketone bodies because the TCA cycle has been trashed for gluconeogenesis to occur. We already know that ketone bodies go everywhere except RBCs. **Fat mobilization and ketogenesis go hand in hand.** Free fatty acids and ketone bodies also inhibit carbohydrate catabolism in order to conserve glucose and spare proteins. In order to do this, they literally cause the inhibition of **Pyruvate dehydrogenase**. ACoA is a molecule that inhibits its own formation, and ACoA is what ketone bodies and free fatty acids end up as. Remember it’s dark in there and they all look the same; if ACoA is being formed from these sources, pyruvate dehydrogenase will be inhibited and ACoA will not be made from glycolysis, saving glucose for cells that need it (brain, RBCs).

# The big picture for fats: Fats come from 2 places, exogenous and endogenous. Exogenous fats are broken down from their TAG form by pancreatic lipase into fatty acids and glycerol, and packaged (again as a TAG) into chylomicrons. Chylomicrons end up in the plasma, and via LPL the TAGs are again hydrolyzed to fatty acids and glycerol to be taken up by adipocytes, where they are made *again* into TAGs (what a waste of time). Endogenous fats, those made via reductive biosynthesis are transported from the liver via VLDLs to adipocytes to be stored as TAG. HDLs roam around looking for cholesterol to make into cholesterol esters, which are taken to a number of peripheral cells to be stored as cholesterol esters. When the body needs energy, fats are mobilized into free fatty acids, and cholesterol esters are hydrolyzed back into cholesterol and a free fatty acid.

### Central nervous system fuel usage

As an example of how carbohydrates are spared by fatty acids and ketone bodies, consider metabolism and the central nervous system (the numbers aren’t really important just the changes that occur). In the normal fed state, the difference in the oxygen concentration between the arterial and venous side of the capillaries in the central nervous system is –3.37, whereas in the starvation state this number **falls about 10%** to –2.98. Also, the difference for glucose is about –0.51 in the fed state, and this **falls about 50%** to –0.26. In the starvation state, the central nervous system shows differences of –0.6, –0.34, and –0.02 for acetoacetate, β-hydroxybutyrate (ketone bodies) and free fatty acids (the reason that there is the difference between the two ketone bodies is that they do not appear in the plasma at the same concentration. Remember that β-hydroxybutyrate is more reduced than acetoacetate, and therefore it appears in the plasma more than acetoacetate because it contains more reducing power). These differences are not seen during the fed state. What this example serves to illustrate is that during starvation, the central nervous system begins to use sources of fuel that it would not otherwise use during the fed state. Using these three alternative sources spares carbohydrates for future use, and for tissues that cannot function aerobically at all (RBCs). It is also a protein sparing mechanism.

**Amino acid biosynthesis**.

* Amino acid biosynthesis is a pretty straight-forward process because 1) we have already seen many of the reactions before and 2) about half of the amino acids are essential and can’t be made by the body anyway. We have already seen that:

If you transaminate an amino acid with pyruvate, you get **alanine** and a 3-carbon α-ketoacid.

If you transaminate an amino acid with oxaloacetate, you get **aspartate**, and a 4-carbon α-ketoacid

If you transaminate an amino acid with α-ketoglutarate, you get **glutamate** and a 5-carbon α-ketoacid.

* **The Pyruvate-alanine cycle** is the movement of nitrogen and carbons from the protein stores found in the skeletal muscles to the liver for processing and gluconeogenesis, and the movement of glucose back to the skeletal muscle (and other cells) to be used for energy. Skeletal muscle is made up of amino acids (DUH!) and this serves as an amino acid pool for the body when there is a need for it. As an example, an amino acid that comes from the skeletal muscle is transaminated with pyruvate, which obviously comes from the breakdown of glucose. The result of this transamination reaction is alanine, and an α-keto acid. The α-keto acid can be converted back into glucose-6-phosphate or pyruvate, but the alanine that is made is sent into the blood plasma to make it to the liver. At the liver, the alanine passes its amine group to α-ketoglutarate to produce glutamate and pyruvate. Glutamate enters into the **Urea cycle** by getting rid of its amine group to make carbamoyl-phosphate, and being converted to aspartate (refer to the Urea cycle to see how Carbamoyl-Phosphate and aspartate enter). Pyruvate undergoes gluconeogenesis to make glucose, which is sent back into the blood stream. Notice that the carbons pass to the liver as amino acids, and they pass from the liver as glucose.

This process is necessary for a number of reasons. First, the liver requires proteins from the skeletal muscle because it is not stupid enough to break itself down in order to make some glucose. You can live without your biceps, you can’t live without a liver. Second, the Urea cycle occurs in the liver. If proteins were broken down in skeletal muscle, the nitrogen would have to be sent to the liver to be made into urea, and you would die before it got there. Third, even if amino acids were to undergo gluconeogenesis in the skeletal muscles, they would only make it as far as glucose-6-phosphate, and would do nothing to help with falling blood sugar.

* **Protein usage in the fed state**: In the fed state, proteins from your diet pass from your gut to the liver, and from here they have a number of possible directions, including storage, synthesis of essential nitrogen containing compounds, catabolism, or export into the blood to be used by other cells. If the amino acids are catabolized, they may end up as glucose, stored as glycogen, or even made into TAGs, and packaged into VLDLs to be sent to adipocytes.
* **In the starvation state,** skeletal muscle proteins are broken down to be used by the body. Alanine and glutamine are 2 of the most prevalent amino acids, and these are both released by the muscles. We already know that destination of alanine. Glutamine can go to the kidneys or to the gut. If it goes to the kidneys, then its job is to get rid of its NH3. Remember from way back that this maneuver is done by the kidneys in order for glutamine to donate the H+ to compensate for a 1° alkali deficit or a 1° CO2 excess. Either way, this is done in order to control acid/base balance. When glutamine exits the kidneys it can do so as serine and alanine, which pass to the liver to be made into glucose or ketone bodies. If glutamine passes to the gut it exits as alanine, which goes to the liver. Notice that in the starvation state, both the carbons and nitrogens from the amino acids are used, the nitrogens are thrown away as urea or as NH4+ and the carbons are oxidized for energy and blown off as CO2.
* Consider the amino acids in the body to be one large pool. This pool is filled by dietary amino acids, and is emptied in a number of directions, energy production (catabolism), acid/base balance (i.e. glutamine), glucose (gluconeogenesis), specialized biomolecules or metabolites (things with benzene rings like certain hormones), or protein synthesis. This idea leads into the idea of essential amino acids and a negative nitrogen balance. Phenylalanine is one essential amino acid, and it is needed for the production of norepinephrine. Consider than phenylalanine is removed from the amino acid pool in order to make this hormone, but the pool is not refilled by the diet. The body returns to the pool in order to produce a protein that requires phenylalanine, but it is not present. For whatever reason (presumably because inserting some other amino acid in phenylalanine’s place when forming the protein would be disastrous, mutations etc. could ensue), the body interprets this situation as not having enough of *any* amino acids, not just phenylalanine. The result is that amino acids will begin to be lost by the body. This is a **negative nitrogen balance**, the body excretes more nitrogen (urea, NH4+, uric acid) than it takes in. Thus, being deficient in even one essential amino acid results in a negative nitrogen balance. If the missing essential amino acid is returned to the diet, the problem is solved.
* Various proteins have various values associated with them. This value is based on the amount of essential amino acids they contained, and (similar to the glycemic index with glucose) this value is based on egg white being 100%. For the most part, animal proteins are better than vegetable proteins. A higher quality protein means that you can consume fewer calories to get the amino acids you need.

### One Carbon Transfers

* There are many times in the body in which a one-carbon transfer must take place (formation of creatine, choline, norepinephrine, and odd # carbon fatty acid catabolism to name a few). The process for one-carbon transfers begins with the amino acid **Methionine**. We can’t just move around one-carbon in the body because our favorite one-carbon molecules are methane (flammable), methyl alcohol (causes blindess and death), formaldehyde (preserving solution), formic acid, and carbon dioxide. Methionine is a sulfur containing amino acid with the R-group S-CH3. It must first be made into its “active form” **S-adenosyl methionine (SAM)**. SAM is a notorious methyl group donor, and is able to undergo one carbon transfers to produce creatine from **guanadoacetate** and norepinephrine from epinephrine. We trafficked in the methyl group in the form of methionine, and when the methyl group is removed from methionine, we are left with a **non-peptide amino acid** known as **homocysteine.** This amino acid is quite dangerous, and can lead to a number of problems. It is taken care of by the B-vitamin **folic acid.**
* **Folic acid** must be consumed in the diet because humans are not able to make it. It is made up of (probably not so important) a methyl pterin group, a para amino benzoic acid (PABA), and glutamate. There are some microorganisms that can make folic acid, and these require PABA to do so. Humans don’t require PABA because we can’t make folic acid. Folic acid must be reduced before it can be used, and this reduction produces H2Folate, and then **H4Folate (tetrahydro folate).** Then, H4Folate receives a methyl group from glycine. **Glycine** is a non-essential amino acid with the minimum possible R-group, H2. This methyl group is stuck onto the H4Folate to produce **5,10 methylene H4Folate.** The byproduct from glycine is an NH4+ (the amine group) and a CO2 (the carboxylate group). 5,10 methylene H4Folate is reduced by NADH to **5 methyl H4Folate**. **5 methyl H4Folate** reacts with homocysteine, passing its methyl group back to it to regenerate methionine, and also regenerating H4Folate, which can again react with glycine to pick up a methyl group. The enzyme that catalyzes the reformation of methionine is **Homocystein H4Folate methyl transferase** (see below).

Folic acid regenerates methionine, and because of this mechanism we do not need to consume so much methionine in our diets. Methionine is still an essential amino acid because we can’t synthesize it. We also can’t make homocysteine; we can only make the two from each other.

The enzyme Homocystein H4Folate methyl transferase requires **cobalamin** as a cofactor. Cobalamin functions as a cofactor for a number of one-carbon transfers, this is one. If there are problems with cobalamin in the diet, then one will not be able to regenerate methionine, *or* recycle folic acid. As a result, the individual will need to consume more methionine and folic acid in the diet. The reason for this is that in addition to this enzyme not working correctly and not being able to reform methionine, the reaction step from 5,10 methylene H4Folate to 5 methyl H4Folate is irreversible. Your folic acid is locked up as 5 methyl H4Folate and not able to be used in other processes.

Cobalamin comes mostly from red meat. When it is ingested, it is conjugated with a glycoprotein known as **Intrinsic Factor** that is generated by the gastric mucosa. Intrinsic factor is able to pull cobalamin out of the diet and combine with it. Cobalamin is then absorbed by the Ileum of the small intestine and moves into the circulation. Lacking Intrinsic Factor leads to a Cobalamin shortage from it not being absorbed. The cascade is a problem with intrinsic factor leads to cobalamin deficiency, then problems with methionine recycling, and folic acid deficiency.

B vitamins are always being used, but they are not used *up,* as in catabolized. Since they are water soluble they can be lost whenever one loses fluids. Cobalamin is lost at a rate of 10-9 moles (a nanomole) per day. The liver stores a micromole of cobalamin. This represents 2000 days worth of cobalamin, so vegetarians only need to eat meat once every 5 years.

**Purines and Pyramidines**

Purines (adenine and guanine) and pyramidines (cytosine, thymine, and uracil) are classes of Nitrogenous bases that are found in nucleotides of all sorts. A nucleotide contains a 5-carbon sugar, a nitrogenous base, and at least one phosphate group. Examples are mononucleotides (ATP, GTP), dinucleotides (FAD and NAD), and polynucleotides (RNA, DNA). Nucleotides are made from simple starting materials (ribose-5-phosphate for purines, which is at least made in the hexose monophosphate **shunt** and aspartate and carbomoyl phosphate for pyramidines).

* **Purine Synthesis** begins with Ribose-5-Phosphate. After a number of reactions, the intermediate **insosinic acid** is made. This contains the purine rings, with R-5-P attached. Inosinic acid is the jumping off point for AMP, and GMP, which in turn become ATP and GMP.
* **Pyramidine Synthesis:** Pyramidines are formed from the combination of **aspartate and carbamoyl phosphate** (found in the urea cycle), which after a number of steps **Uracil Monophosphate (UMP)** is produced. UMP can be made into UTP, which can be made into cytosine triphosphate. UMP can also be made into thymine monophosphate (TMP) and then TTP.
  + In DNA, adenine binds with thymine and cytosine binds with guanine.
  + In RNA, adenine binds with uracil and cytosine binds with guanine. (Just list them alphabetically and the outer two combine and the inner two combine).
* **Uric Acid:** Uric acid is made from the decay of nitrogenous bases. Adenine decays to **hypoxanthine**, which itself becomes **xanthine**. Guanine decays directly to xanthine. Xanthine is then converted into Uric acid. Uric acid is a nitrogenous waste product (similar to NH4+ and urea. However, a build up of uric acid crystals in the blood leads to a condition called **Gout.** These crystals can get into synovial spaces and cause symptoms similar to arthritis. There is a genetic predisposition to gout because the enzyme that produces the molecule **Phosphoribosyl Pyrophosphate** early in the catabolism of ribose, is an allosteric enzyme. This enzyme is inhibited by purines (GTP and ATP). If there is a problem with the genes that code for this enzyme, then the allosteric site can be affected so that the enzyme will not be inhibited. Even though the allosteric site is affected, the main active site is still viable and ATP and GTP will continue to increase in concentration, eventually decaying to uric acid.

**Iron**

* Iron is one mineral that is important for our diets. Iron is water insoluble and when it is ingested, it forms insoluble hydroxides. It is especially important for hemoglobin to carry oxygen, and for cell respiration, (i.e. the electron transport system) as it is a major component in the cytochromes found in mitochondria. There is no mechanism for catabolizing iron, therefore the only ways to remove it are hemorrhage (including, perhaps obviously, menstruation) and as a fecal component.

It is important for the iron that leaves the body to be replaced by iron in the diet. About 1 mg/day is lost via the feces due to the mechanism to recycle RBCs. The majority of the iron is reabsorbed, but about 20% leaves the body. For pre-menopausal, the daily iron requirement should be increased by 0.6 mg/day (because women lose more iron due to menstruation). These numbers represent the **physiological requirement** for iron. The problem is that the iron in foods is not very “bio-available,” so more iron must be taken in so that the body can absorb what it needs. The **nutritional requirement** for iron can be obtained by multiplying the physiological requirement by 10. I’ll spare you Gut’s six-pack joke but for those of you who are bad with numbers, that’s 10 mg/day for men and 16 mg/day for women.

FeSO4, iron sulfate, is the form of iron in food. This is in the 2+ oxidation state. Iron has 2 oxidation states, 2+ and 3+ , Fe(OH)2. Both of these are minimally soluble, but the 2+ form is more soluble. It is useful for iron to be taken with Vitamin C, ascorbic acid, because this is a reducing agent, and encourages iron to stay in its 2+ oxidation state, making it more soluble.

* **Absorption of Iron:** Iron must be in the form of a protein because if it gets free in the plasma it will precipitate. Iron enters the lumen of the small intestine in the 2+ oxidation state. It is absorbed by the intestinal mucosal cells, and combined with the protein **apoferritin** to form the iron-containing protein **ferritin**. Ferritin contains iron in the 3+ state. Iron is then passed to the plasma as Fe2+ where it combines with the protein **apotransferrin (**3+) to become **transferring.** Transferrin is important because it is the transport protein for iron. Transferrin moves iron to one of two places, to liver cells to be stored as ferritin (2+) or to bone marrow cells for RBC formation. Ferritin is used to store iron in intestinal mucosal cells, and the liver. If there is not enough ferritin in the intestinal mucosal cells, then iron will not be able to be absorbed. This condition is known as **mucosal block**.

**The eight B vitamins**

Niacin (NADH) reducing agent

Riboflavin (FADH2) reducing agent

Thiamine (Thiamine Pyrophosphate) carbon carrier used in the pyruvate dehydrogenase system. Deficiency causes **beriberi**

Pantothenate (CoASH) coenzyme A

Cobalamin: Vitamin B-12, involved in **1-carbon transfers**

Pyridoxal phosphate (Vitamin B-6): used as an amine carrier in **transaminations** (amino acid catabolism)

Biotin: used in **carboxylations** (fatty acid oxidation, amino acid catabolism, fatty acid synthesis)

Folic Acid: used in the regeneration of **methionine**

Now I know what you’re saying, “You can sit at your computer and wave your fingers around but if you were really good you could bring *all* of this together.” Well here today, brought to you at great personal expense….

# The big picture

# Carbohydrates

Carbohydrate processing begins with digestion in the mouth and the intestinal lumen by a number of enzymes. Digestion ends with the carbohydrates in the form of monosaccharides. Starch is by far the most prominent carbohydrate and is digested into glucose, which dumped into the blood stream to be taken up by cells and used to produce energy. When glucose is taken up by cells it has a number of destinations. Glucose can be broken down to H2O and CO2 to produce ATP, it can be stored as glycogen, it can enter the hexose monophosphate shunt to produce NADPH (which is important for lipid biosynthesis), or it can be converted into fatty acids and stored as TAGs.

* **Catabolism:** There are a number of important points regarding carbohydrate catabolism. Carbohydrates are broken down by cells for energy via glycolysis, or stored carbohydrates are broken down via glycogenolysis.

Full catabolism of glucose to H2O and CO2 requires oxygen to be present and the TCA cycle. If oxygen is not present then glucose catabolism will end at lactate, which will be exported from the cell into the plasma and transported to the liver where it is made back into glucose and shipped back into the plasma. This of course occurs after the bout of activity because this would be the first time that oxygen is available to the liver.