Biochemistry and Metabolism

The content review presented here was derived from a careful consideration of the AAMC MCAT Content Outline as it pertains to Biochemistry and Metabolism. **Challenging assessment questions and passages derived from this content review that emulate the MCAT are available at Med-Pathway.com** With PhD degrees in Biochemistry and decades of MCAT teaching, we are the Biochemistry MCAT experts! Note that this module does not go into detail on enzyme kinetics and thermodynamics. Med-Pathway has a separate testing module on this topic. Further, amino acids and protein structure and function are thoroughly examined in a separate Med-Pathway module.

The following topics are discussed here:

❒ Energy & Metabolism, including free energy, ATP, and acetyl CoA
❒ Hormonal control of glycemia (i.e. insulin vs. glucagon)
❒ The Fed state
❒ Hexokinase and glucokinase, including kinetic comparison
❒ Metabolic fate of glucose 6-phosphate
❒ Glycolysis (including phosphofructokinase I and II, aldolase, glyceraldehyde 3 phosphate dehydrogenase, phosphoglycerate kinase and substrate level phosphorylation, pyruvate kinase)
❒ Fructose metabolism ❒ Pentose Phosphate Shunt
❒ The Warburg effect and cancer ❒ Anaerobic respiration and lactate/fermentation
❒ Pyruvate dehydrogenase and formation of acetyl CoA
❒ Krebs (TCA) cycle
❒ Oxidative phosphorylation and electron transport (including regulation, inhibitors, role of cytochrome c in apoptosis, electron transfer potential)
❒ Glycogen synthesis ❒ Fatty acid and triglyceride synthesis and transport
❒ Biochemistry of lipoprotein particles (chylomicrons, VLDL, HDL, and LDL) ❒ Obesity (leptin and neuropeptide Y)
❒ Fasted state and sources of blood sugar
❒ Glycogenolysis ❒ Gluconeogenesis (including role of fats, protein, lactate, and glycerol, and the Cori cycle)
❒ Ketone bodies (synthesis and utilization)
❒ Protein and amino acid metabolism (including glucogenic and ketogenic amino acids, alanine-glucose cycle, urea cycle)
❒ Diabetes mellitus types 1 and 2

Biochemical Pathways: The Rationale.

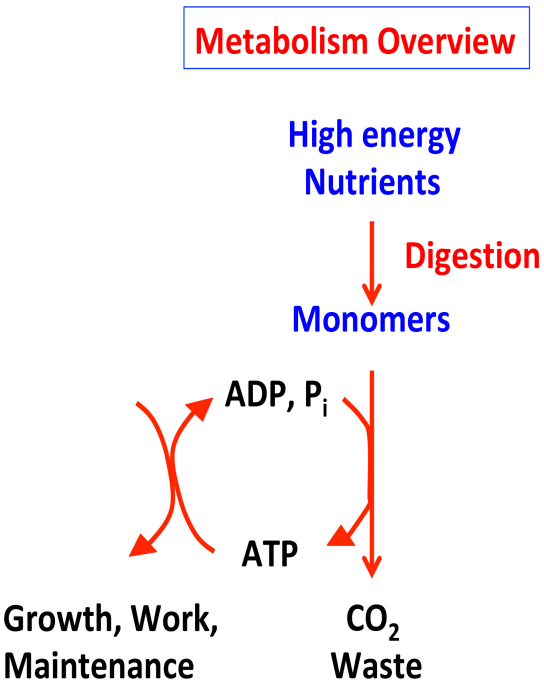
This module discusses major biochemical pathways and how hormones coordinately regulate these pathways to maintain energy balance. Of particular interest is how organisms regulate blood sugar levels (i.e. glycemia), a topic with clear clinical relevance that also reveals how multiple pathways are integrated and regulated by various tissues. Knowledge of this provides the foundation for understanding numerous metabolic diseases such as diabetes, obesity, metabolic aciduria, and glycogen storage disorders.

Although a lot of time has been spent by students memorizing pathway sequences, we will emphasize how pathways are coordinated in both the fed and fasted states. This module assumes that you understand organic chemistry and amino acids/proteins, and of course, you can find content review and assessment at Med-Pathway.com on these topics.

We believe that memorizing pathways per se is largely a waste of time and misses the forest for the trees. Further, there is a strong chance that the MCAT will give you the pathway that is necessary for the passage. You should be able to apply your knowledge of the pathways. Having that been said, it is important to recognize where individual metabolites belong within a given metabolic scheme. You should also be able to determine the relationship between various metabolites within a specific pathway. For example, 3-phosphoglycerate is the oxidized product of glyceraldehyde 3-phosphate and both molecules are glycolytic intermediates.

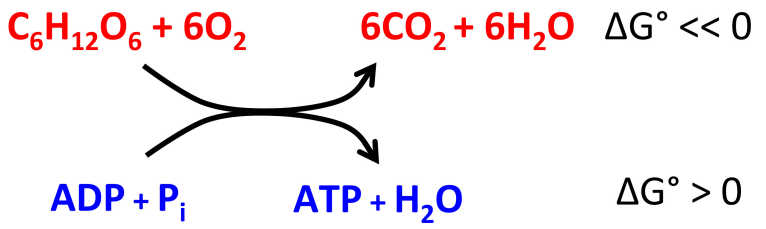
Energy and Metabolism

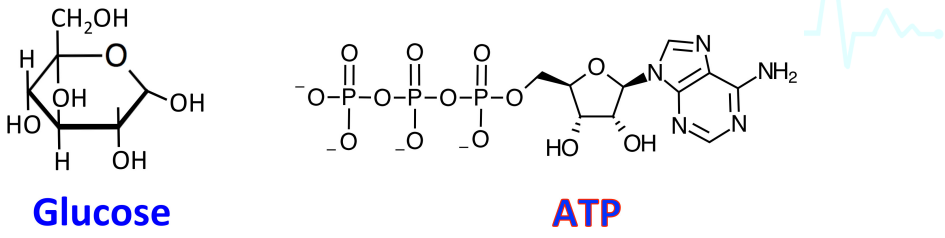
All organisms require energy intake in order to grow and sustain life. The adenylate system is the major source of energy currency in the cell. High energy nutrients including protein, carbohydrates, and nucleic acids derived from food are broken down and absorbed during digestion. The carbon skeletons from these macromolecules are either stored (if in excess) or used to generate energy via oxidation, the loss or transfer of electrons. During oxidation, energy is released as heat and entropy and some of it is converted or "trapped"



(i.e. coupled) in the chemical bonds of high energy carriers such as ATP, NADH, $FADH_2$, and Coenzyme A (CoASH).

All chemical reactions are driven by thermodynamics. This determines if reactions are spontaneous, or whether they can proceed or not. Thermodynamics tells us nothing about reaction kinetics, or the rates of the reactions. We will briefly go over thermodynamics. Med-Pathway has a separate review and assessment module on thermodynamics and kinetics, including enzyme kinetics. According to the first law of thermodynamics, energy can be converted from one form to another and this is seen over and over during





metabolism. The energy available to do work within the cell is referred to as the Gibbs free energy, or ΔG. Spontaneous reactions have negative ΔG values. As discussed below, ΔG values can be manipulated through changing the concentrations of reactants and products.

One of the best examples of the first law is the metabolic process of glucose oxidation that occurs in tissue through pathways such as glycolysis ($C_6H_{12}O_6$) and the Krebs cycle. Oxidation is the transfer of electrons from one molecule to another. Recall that combustion reactions use molecular oxygen (O_2) to completely oxidize carbon atoms residing in chemical bonds (i.e. methylene groups) to carbon dioxide (CO_2), the highest oxidation state for carbon.

The energy released through the oxidation of carbon in the various bonds comprising glucose is converted into the electron transfer potential energy of

the reducing carriers NADH and $FADH_2$. This occurs by the phenomenon of coupling, a key concept in metabolic reactions that create and use energy. During the process of mitochondrial electron transport, the reduction potential of NADH and $FADH_2$ is converted into ATP, the energy currency of the cell. This is discussed below in the context of oxidative phosphorylation, a common application of thermodynamics that you can expect to see on the MCAT.

The meaning of ΔG and ΔG°.

What is the difference between ΔG and ΔG°? ΔG° is the standard free energy change for a reaction that occurs under conditions where the concentrations of reactants and products are 1.0 M each, pH = 0, and any gases are at a pressure of 1.0 atmosphere. Clearly, these conditions are non-physiological. The actual free energy (ΔG) change is what actually occurs in the cell. The relationship between ΔG and ΔG° is related to the equilibrium constant:

$$\Delta G = \Delta G^\circ + (RT)(\ln K_{eq})$$

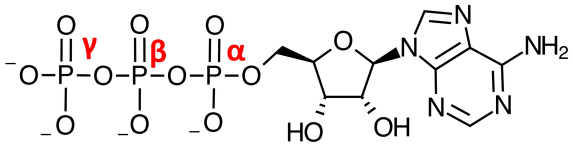
As K_{eq} = [Reactants]/[Products], the value of ΔG, and hence the spontaneity of a reaction can be altered by changing the amounts of reactants and products.

Phosphorylated Metabolite	ΔG° kcal/mol
PEP (phosphoenolpyruvate)	-15.0
Phosphocreatine	-10.0
ATP	-7.0
PP_i (Pyrophosphate)	-7.0
Glucose 6-Phosphate	-3.0

$ATP + H_2O \longleftrightarrow AMP + PP_i$ ΔG° = -7.0 kcal/mol

$PP_i + H_2O \longleftrightarrow 2P_i$ ΔG° = -7.0 kcal/mol

$ATP + 2\,H_2O \longrightarrow AMP + 2P_i$ ΔG° = -14.0 kcal/mol

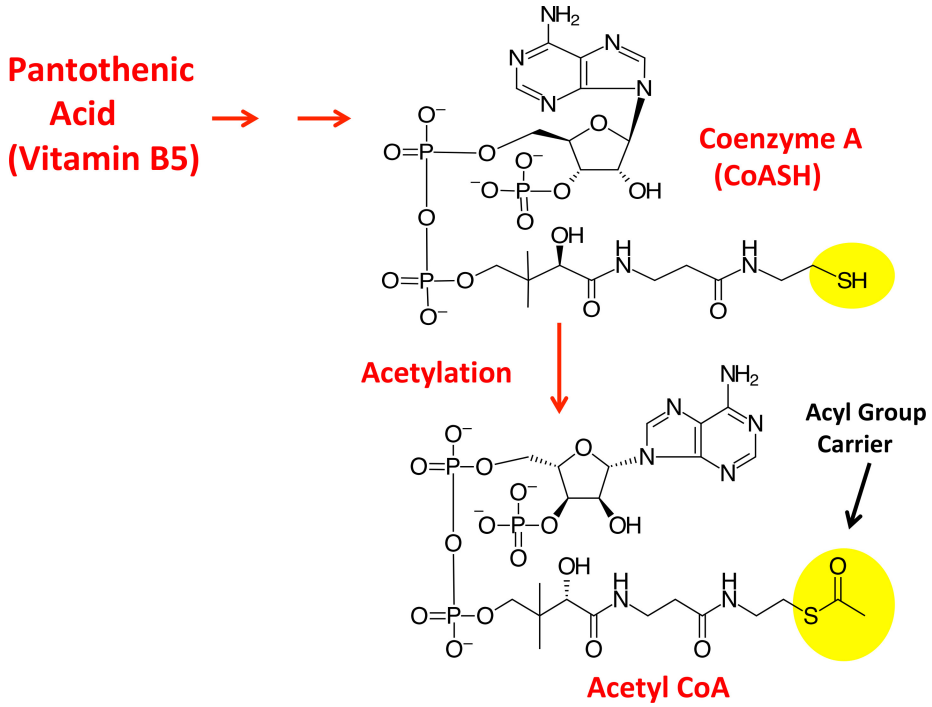


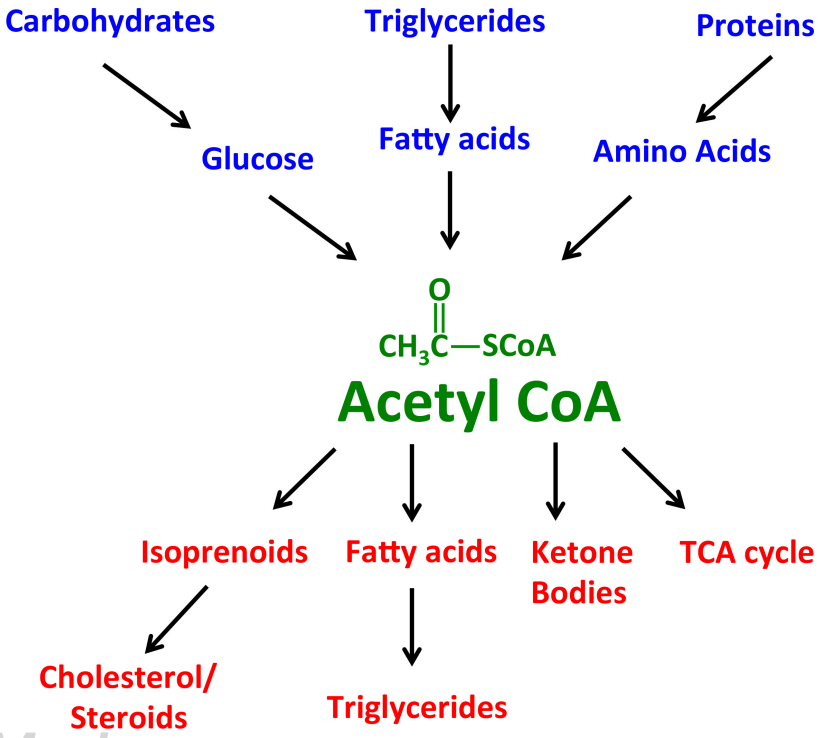
The ΔG° of hydrolysis for phosphorylated metabolites is referred to as the transfer potential or phosphoryl group transfer potential. Approximate values for various metabolites are listed in the Table above.

ATP hydrolysis releases approximately 7 kcal/mol. In some cases, the phosphate proximal to the ribose ring (the α phosphate) is targeted. In this case, AMP is generated and pyrophosphate (PP_i) is released. However, the hydrolysis of pyrophosphate is often coupled to the initial hydrolysis of ATP, generating a reaction that has ΔG = -14 kcal/mol. Such a large release of free energy makes the reaction essentially irreversible.

Acetyl CoA: a key acyl group carrier

Carbon skeletons in macromolecules are often built from and degraded into acetyl CoA (CoA or CoASH stands for coenzyme A). CoA is synthesized from pantothenic acid (Vitamin B_5). Observe that acetyl CoA is a thioester. Upon hydrolysis, these high energy bonds are exergonic and release as much energy as does a phosphoester bond in ATP. Think of acetyl CoA as the activated carrier of two carbon units. As shown below, acetyl CoA is generated during the oxidation of carbohydrates, fats, and proteins and is used in the synthesis of ketone bodies, cholesterol and fatty acids. The two-carbon equivalents of acetyl CoA are oxidized in the TCA cycle. Therefore, knowledge of the biochemistry of acetyl CoA is critical for understanding metabolism.





Glycemia and glucose homeostasis

A central theme of intermediate metabolism concerns glucose homeostasis. The maintenance of glycemia is critical for this as both hyper and hypoglycemic states are deleterious to health. The key hormones involved in regulating blood

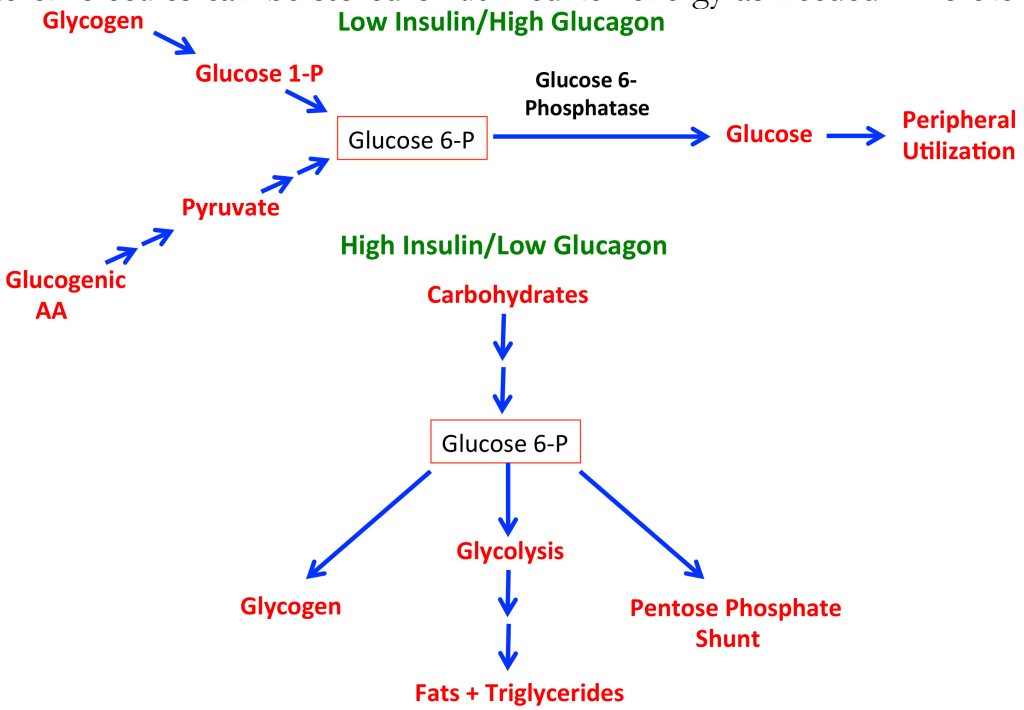
MAJOR HORMONES REGULATING METABOLISM

Hormone	Site of release	Target Tissue(s)	Biochemical Effects
Insulin	Pancreatic β cells	Widespread	↓ Glycemia ↑ Fat, Glycogen, Proteins
Glucagon	Pancreatic α cells	Liver	↑ Glycemia, Gluconeogenesis
Epinephrine	Adrenal medulla	Widespread	↑ Glycemia: Gluconeogenesis, Glycogen degradation, Triglyceride mobilization
Cortisol	Adrenal cortex	Widespread	↑ Glycemia, Protein breakdown

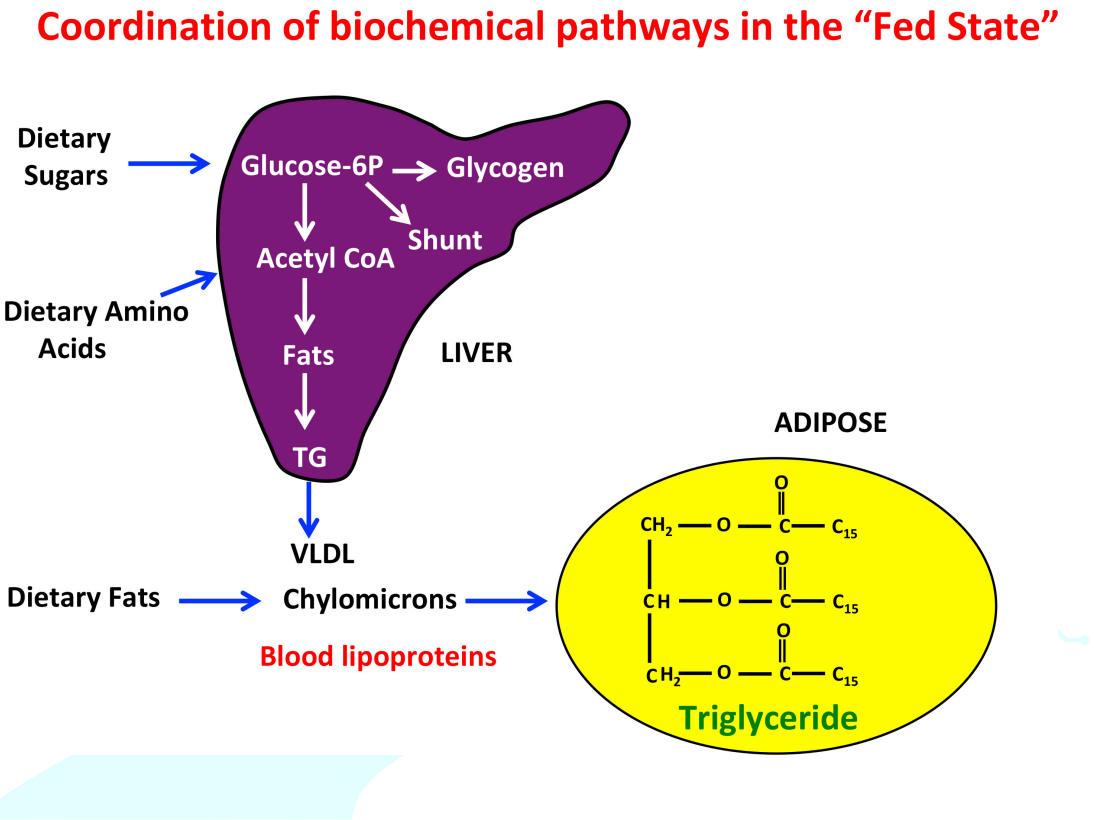
sugar levels are shown in the table. Note that insulin, a 51-amino acid protein, is the key hormone that reduces blood sugar levels. Upon ingesting carbohydrates and even protein and fat, insulin is released by the beta cells of the pancreas.

Insulin binds to its receptor on numerous cell surfaces and activates tyrosine kinase signaling cascades that promote glycogen synthesis, glycolysis, and subsequent fatty acid and triglyceride synthesis, as well as ribosomal protein synthesis. Therefore, insulin is an anabolic hormone that promotes the synthesis and storage of macromolecules though increasing the expression of, amongst other things, glucose transporters, glycolytic enzymes, and enzymes that regulate the synthesis of proteins, glycogen and fatty acids, and triglycerides. Importantly, insulin inhibits the release of glucagon and epinephrine, two hormones that elevate blood glucose levels.

Glucagon, epinephrine, and cortisol act in a counter-regulatory manner to insulin. If insulin is secreted in response to feeding then glucagon, epinephrine, and cortisol are released in the fasted state. Collectively, these hormones increase blood sugar levels through hepatic breakdown of glycogen or hepatic gluconeogenesis. As we will discuss, gluconeogenesis is a hepatic process that is driven through the coordination of multiple biochemical pathways including amino acid (AA) degradation, triglyceride mobilization, and fatty acid metabolism. Think of insulin vs. glucagon et al as a pendulum that swings back and forth as the organism goes from a fed to a fasted state. In this manner, macromolecules can be stored or utilized for energy as needed. Therefore, the



insulin/glucagon et al binary system regulates various pathways to ensure that energy balance (i.e. glycemia) is maintained. This is shown below for the fed state.



The Fed State

Consumption of dietary sugars and amino acids promotes insulin secretion. Many dietary carbohydrates such as glucose, galactose, and lactose (β-D-galactopyranosyl (1→4)-D-glucose are converted into Glucose 6-P. Once formed, glucose 6-P has three major fates that we will examine:

1) Glycolysis and formation of acetyl CoA

2) Glycogen synthesis

3) Pentose Phosphate Pathway (Shunt)

Consumption of fructose does not generate glucose 6-P. All fructose in nature exists in the sucrose disaccharide (Glucose-Fructose). Although fructose is a structural isomer of glucose and can be converted into glucose under basic

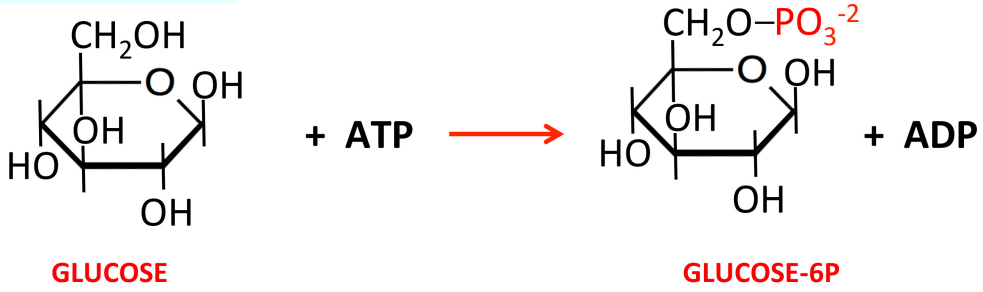
conditions, its metabolism is different than that of glucose. This has important implications for consumption of high fructose corn syrup. Fructose metabolism is specifically discussed below.

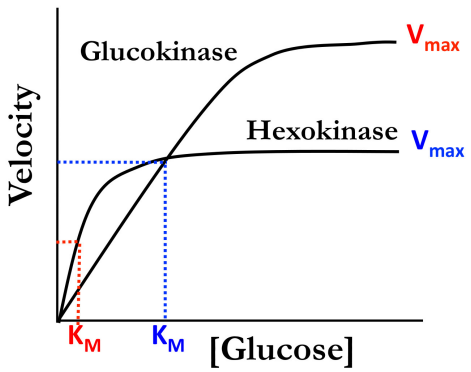
Note that this module does not discuss various aspects of carbohydrate chemistry in detail (stereochemistry, nomenclature, reducing sugars, mutarotation, etc.). This material can be found in the Med-Pathway modules Organic Chemistry as well as in Stereochemistry.

Hexokinase & Glucokinase

Glucose phosphorylation occurs rapidly after glucose enters cells through any of several types of glucose transporters. Insulin increases the surface expression of various glucose transporters. These transporters are tissue specific and operate through facilitated diffusion (GLUT transporters) and through secondary active transport mechanisms (Na^+/Glucose symporters).

The addition of the negatively charged phosphate moiety by the kinase reaction prevents the glucose molecule from leaving the cell. The initial phosphorylation of glucose occurs through muscle hexokinase activity and hepatic glucokinase. Both isozymes catalyze the same reaction: Glucose + ATP → Glucose-6P + ADP, yet have differential modes of expression and regulation.





This differential regulation of isozyme activity has important physiological implications. Importantly, high levels of Glucose-6P (or G-6P) inhibit skeletal muscle hexokinase, but liver glucokinase exhibits no end product inhibition. For example, excess circulating glucose derived from food will fail to be phosphorylated in the skeletal muscle, but rather will end up in the liver where it is rapidly phosphorylated and converted into glucose-6P. The biochemical underpinnings of this lie in the kinetic properties of the two enzymes. Recall that the K_M represents two principles:

1) The substrate concentration at ½ V_{max}
2) An affinity constant where a low K_M value represents high affinity between the enzyme and the substrate.

Observe from the graph that hexokinase has a much lower K_M than glucokinase, but its V_{max} is much lower than that of glucokinase. Interpret this to mean that hexokinase rapidly phosphorylates glucose, but the enzyme saturates at a much lower concentration of glucose than glucokinase. Therefore, when the skeletal muscle is saturated with glucose, excess glucose will not be phosphorylated and sequestered in the skeletal muscle. Rather, glucose will be available for entry into portal circulation where glucokinase will phosphorylate it and sequester it for metabolism.

Catalytic efficiency

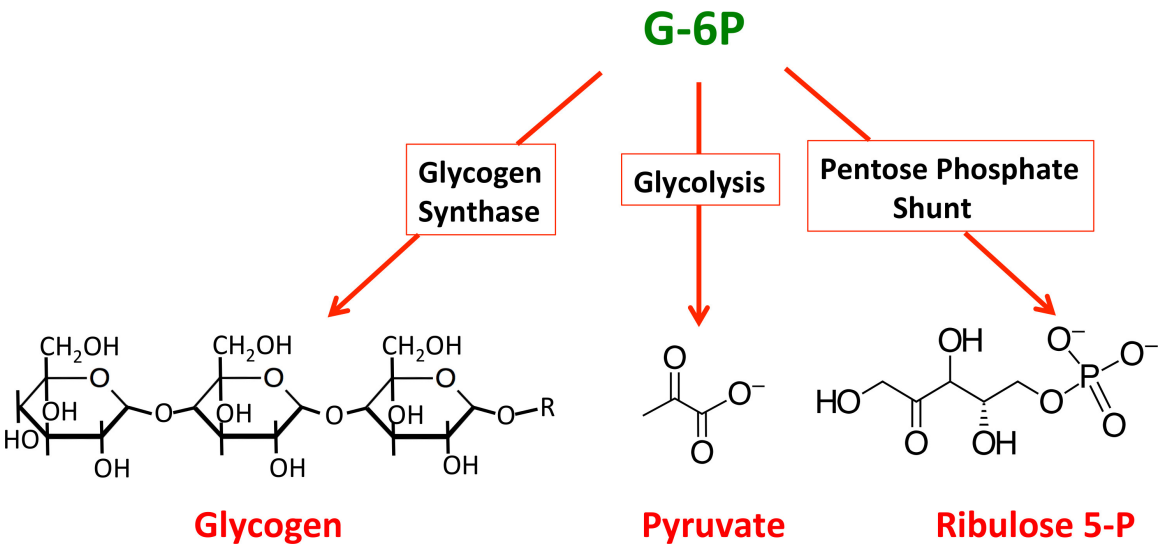
The K_M is also linked to the catalytic efficiency that is defined by the ratio of kcat/K_M. This can often be seen as the rate limiting step of the reaction. Note that the units for this are min^{-1} M^{-1}. This is the rate constant when K_M >> [S] for the reaction E + S = ES. This first order rate constant reflects contributions from both the rate of reaction catalysis (kcat) and the affinity between the enzyme and substrate (K_M).

The kcat is also known as the turnover number and represents the amount of times an enzyme can perform a given reaction per unit time. This is related to the catalytic efficiency, a common MCAT subject. The preference of an enzyme for a given substrate can be determined by comparing the kcat/K_M values for various substrates. Further, in theory the physical limits on enzyme efficiency are determined by the limits of diffusion, which is ~ 10^9 s^{-1} M^{-1}.

Metabolism of G-6P

The phosphorylation of glucose is an exergonic process. Some of the energy that is released from the hydrolysis of the phosphoanhydride bond now exists in the newly created bonds of the activated G-6P molecule. This is the free energy, the energy available to do work (ΔG = -7.0 kcal/mol). Further, some energy has also been released as heat and entropy. Depending on the cell type and metabolic demand, G-6P has three cellular fates as we have mentioned above:

1) Glycolysis
2) Pentose phosphate Shunt
3) Glycogen synthesis



Glycolysis & Bioenergetics

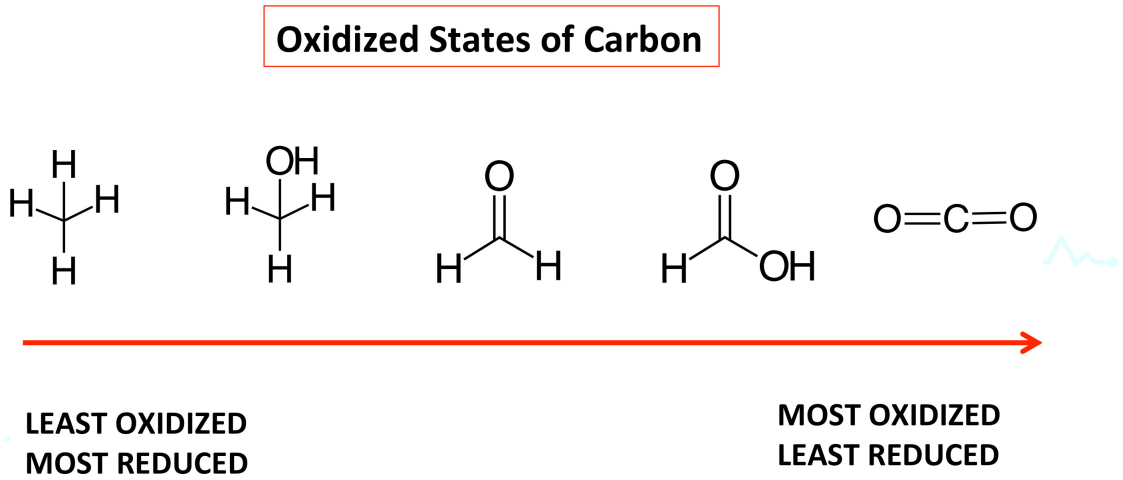
Glycolysis is an anaerobic process promoted by insulin. The glycolytic pathway occurs in the cytoplasm and partially oxidizes glucose to pyruvate through the following net reaction:

Glucose + 2 NAD^+ + ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 ATP

The oxidation of glucose into pyruvate is exergonic (ΔG = -19.0 kcal/mol) and releases heat. This can be performed in the lab or in a cell: the pathway doesn't matter, indicating that enthalpy is a **state function**. However, by capturing the energy in the form of new chemical bonds (pathway intermediates), as opposed

to releasing it directly into the environment as heat and entropy, the energy can be captured and used to do work (i.e. create ATP for muscle contraction). Therefore, some of the energy derived from the oxidation of glucose during glycolysis is coupled to the generation of the electron carrier NADH that is subsequently converted into ATP.

Recall from the **first law of thermodynamics** that energy can neither be created nor destroyed, but can be converted form one form to another. Therefore, during glycolysis the energy in the chemical bonds of glucose (C-C, C-H, C-O, etc.) are oxidized. **Oxidation** is the loss of electrons as often observed through the increase in the number of oxygen atoms bonded to carbon (as shown below). The electrons are transferred to high-energy carriers such as NAD^+, creating NADH. The energy in the chemical bonds of glucose



is released during oxidation and is converted into the chemical energy potential found in the newly created bonds of pyruvate, NADH and ATP. During oxidation, some energy is also released in the form of heat into the atmosphere. However, by converting some of this energy into new chemical bonds (NADH, ATP), the first law of thermodynamics is seen in action. The reducing power (NADH) is ultimately converted into ATP, demonstrating how the energy in the electron bonds of glucose is harvested via thermodynamics.

Pyruvate is the terminal product of glycolysis. Under aerobic conditions, pyruvate enters the mitochondria and is further oxidized by the pyruvate dehydrogenase complex (PDC) into acetyl CoA. The oxidation of the two

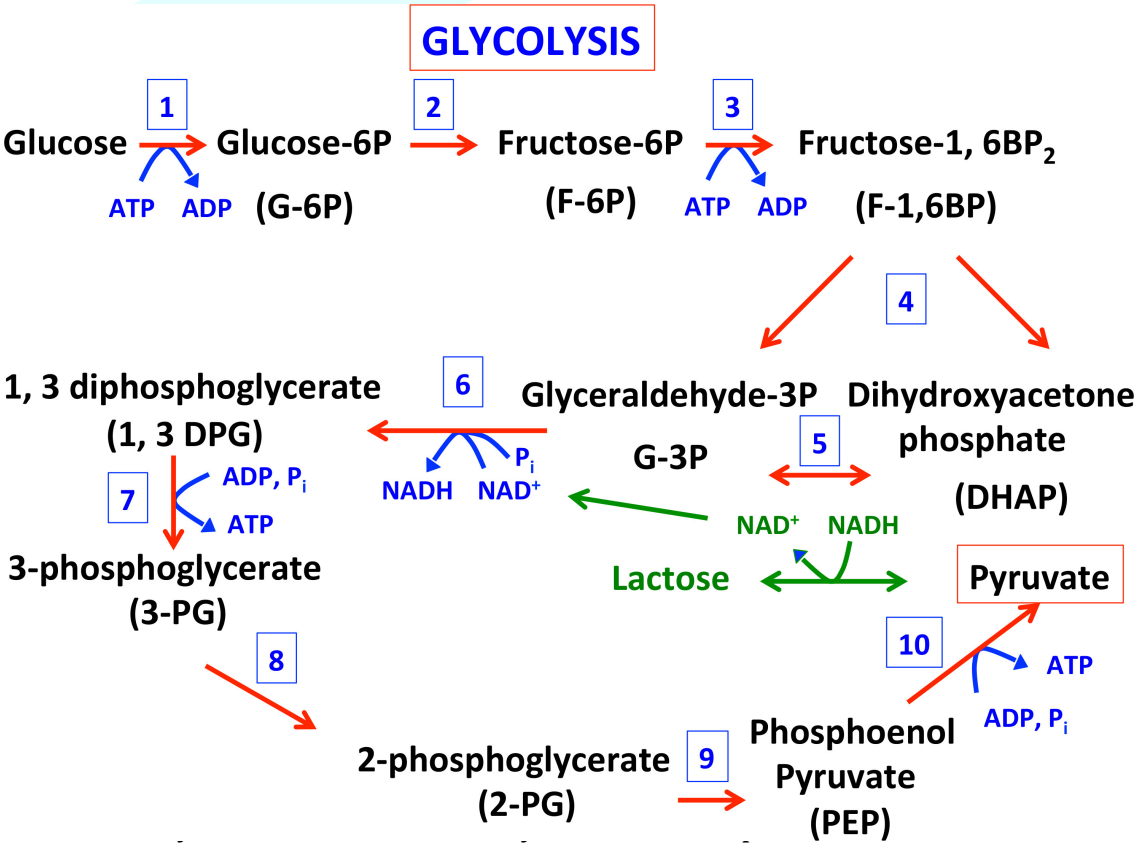
carbon equivalents of acetyl CoA into CO_2 occurs during the Krebs cycle. This will be discussed later.

The entire glycolytic pathway is shown in the image below. We do not recommend rote memorization of the pathway. Rather, we suggest that you understand the biochemical rationale behind each step. Appreciate that in the liver, insulin promotes the flux of carbon through glycolysis through several mechanisms as will be discussed.

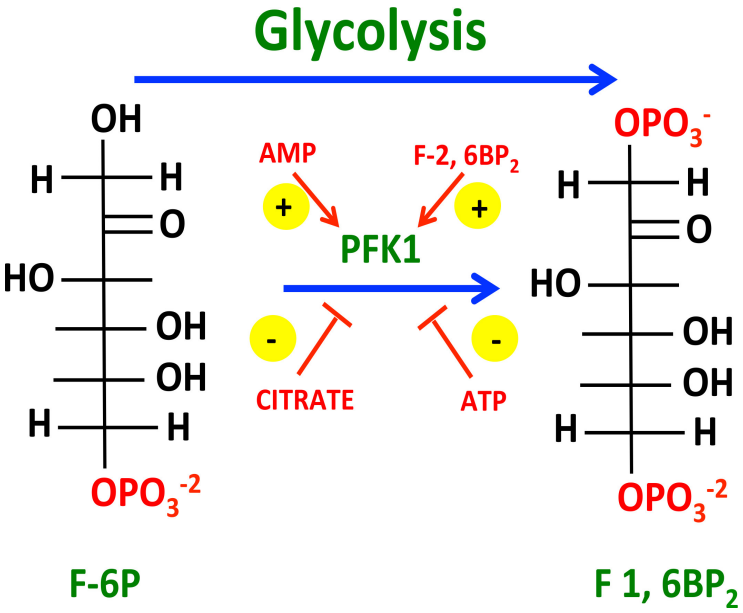
Phosphofructokinase commits G-6P to glycolysis

G-6P is isomerized into fructose-6P (F-6P). Thus, an aldose is converted into a ketose (F-6P) that is a substrate for the enzyme phosphofructokinase 1 (PFK1), a key enzyme in the glycolytic pathway. PFK1 uses ATP to phosphorylate F-6P into fructose 1, 6 bisphosphate (F1, $6BP_2$). The formation of F1, $6BP_2$ commits the carbon skeletons to the glycolytic pathway. Indeed, as a kinase PFK1 hydrolyzes ATP and the ΔG = -5.0 kcal/mol, making the reaction thermodynamically irreversible.

In the liver and skeletal muscle, PFK1 is regulated by multiple allosteric factors, including ATP, AMP, and citrate as shown. AMP, an indicator of low energy status, positively regulates PFK, but both ATP and citrate; indicators of high

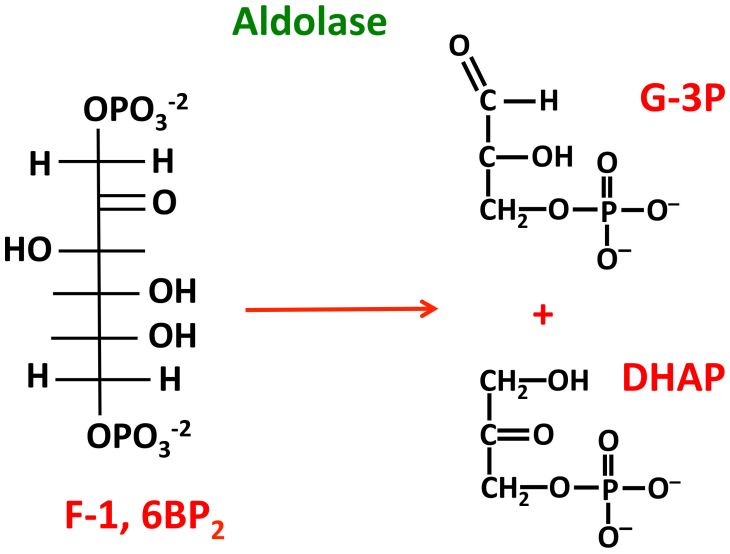


energy inhibit PFK1. However, in the liver, PFK1 is uniquely regulated by the allosteric regulator fructose 2, 6 bisphosphate (F-2, $6BP_2$). Importantly, F-2, $6BP_2$ is synthesized by PFK2, a hepatic enzyme whose expression is induced by insulin. Therefore, insulin promotes glycolysis in the fed state. As we will see, this has important implications for fatty acid and triglyceride synthesis.



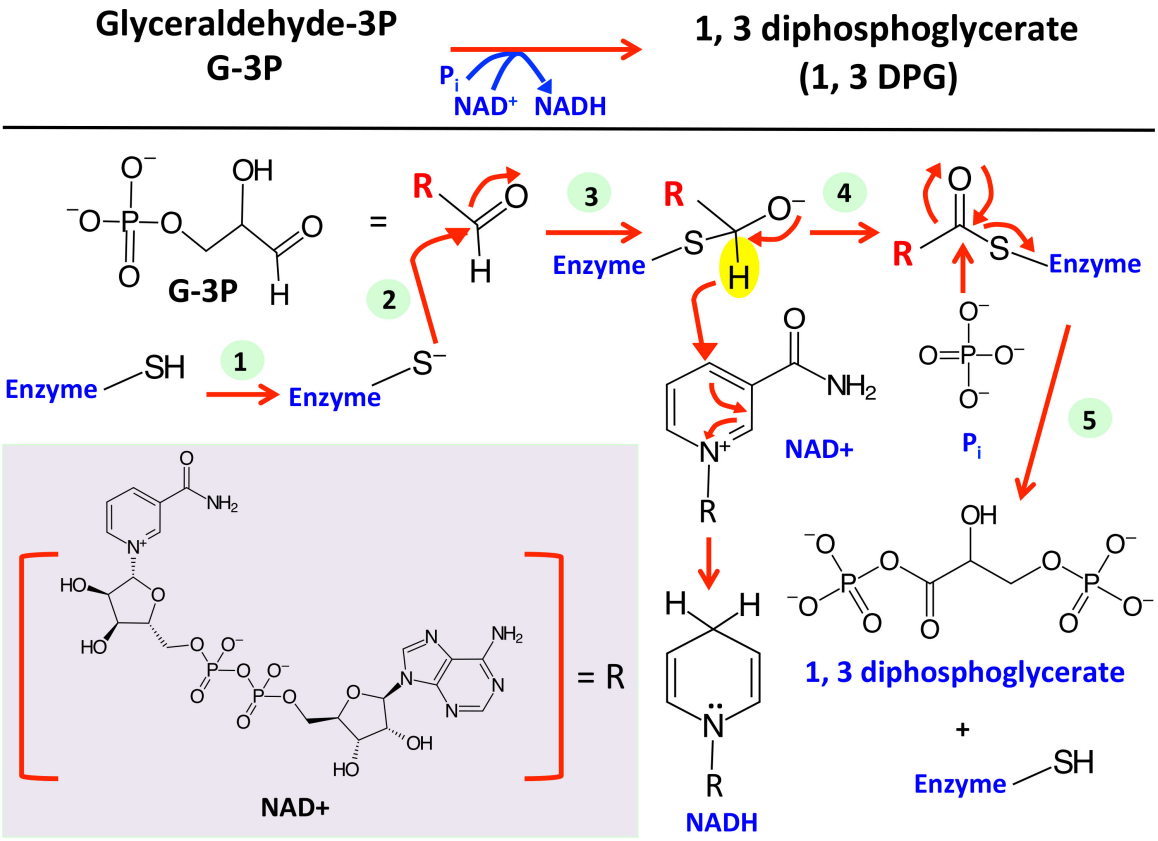
Aldolase generates activated triose-phosphates

Once committed to glycolysis, F-1, $6BP_2$ is used as a substrate by the enzyme aldolase. Aldolase, as its name implies, cleaves C-C single bonds by performing a retro-aldol reaction. Recall that the aldol condensation can be produced from a reaction between an aldehyde and a ketone. The details of this reaction and its mechanism are thoroughly discussed in the Med-Pathway.com O chem module. Note that in the glycolytic pathway aldolase performs the reverse condensation reaction: the retro aldol condensation reaction. This reaction is specifically listed in the AAMC Content Guide (download a free copy from our website); we think that aldolase is what they have in mind. The products of the aldolase reaction are glyceraldehyde 3-phosphate (G-3P) and dihydroxyacetone phosphate (DHAP). These two structural isomers are interchangeable through the enzyme triose phosphate isomerase.



The oxidation of G-3P generates NADH

So far, we have seen that the glycolytic pathway has used two ATP molecules to generate F-1, 6BP$_2$. However, no energy has been harvested from the bonds of glucose. Thus, the beginning stages of glycolysis are used to activate glucose. After converting glucose into two glyceraldehyde 3-phosphate molecules, the oxidative phase of glycolysis begins.



The oxidation of G-3P to 1, 3 DPG converts an aldehyde to the level of a carboxylic acid. This oxidation is coupled to the reduction of NAD^+ into NADH. The requirement for NAD^+ in this reaction will be important for understanding anaerobic metabolism (i.e. fermentation). This is discussed later. The mechanism for oxidizing G-3P into 1, 3 DPG is shown and illustrates that two back to back substitution-elimination reactions are performed to generate the 1, 3 DPG product. The reaction sequence is as follows:

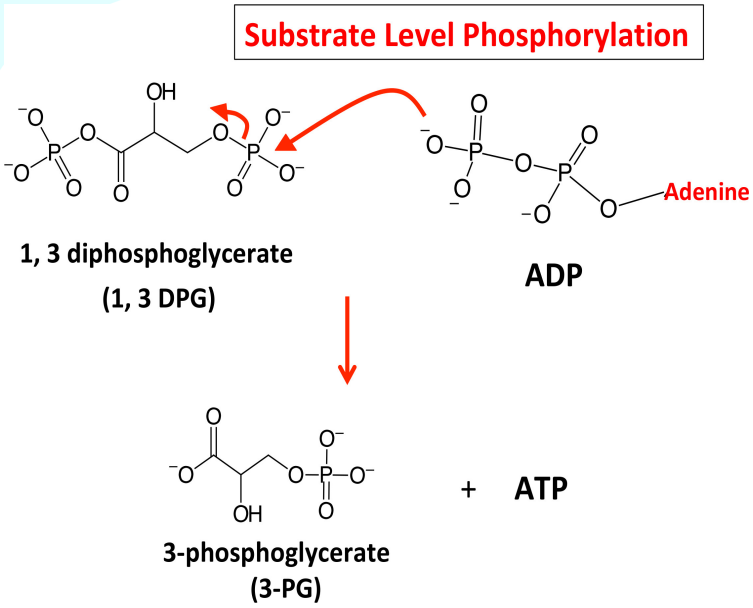
1) An active site cysteine residue (R-SH) in the Enzyme (glyceraldehyde 3-phosphate dehydrogenase) is converted into a stronger nucleophile (R-S$^-$) through general base catalysis.

2) A nucleophilic attack occurs at the electrophilic carbonyl carbon, generating a tetrahedral intermediate.
3) Upon collapse of the intermediate, the hydride anion (H:⁻) is kicked out and transferred to the pyridine ring of NAD^+, creating NADH that dissociates from the enzyme.
4) The high-energy thioester-enzyme intermediate is attacked by a phosphate nucleophile.
5) Upon collapse of the tetrahedral intermediate, a phosphoanhydride bond is formed and released by the enzyme. G-3P has been oxidized to the level of the carboxylic acid (in the form of a phosphoanhydride).

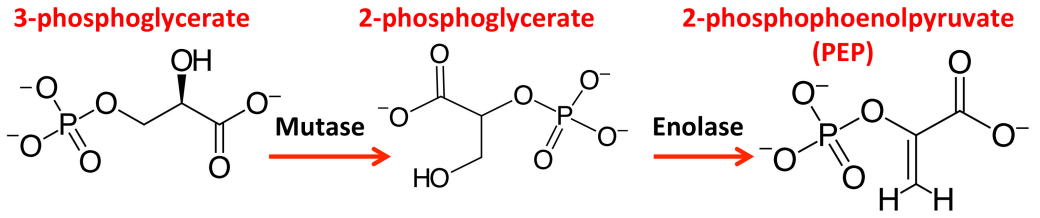
Appreciate that the dehydrogenase enzyme oxidizes the aldehyde group in G-3P. As we know, oxidation releases energy (i.e. $\Delta G < 0$), some of which has been transferred to the electron reduction potential of the newly formed NADH. As NADH holds electrons that were once part of the aldehyde derived from glucose, the chemical energy once present in the bonds of glucose are now being held as potential energy in NADH. Additional energy from this oxidation reaction is also stored in the form of the phosphoanhydride bond of the product 1, 3 DPG. This is the first law of thermodynamics in action.

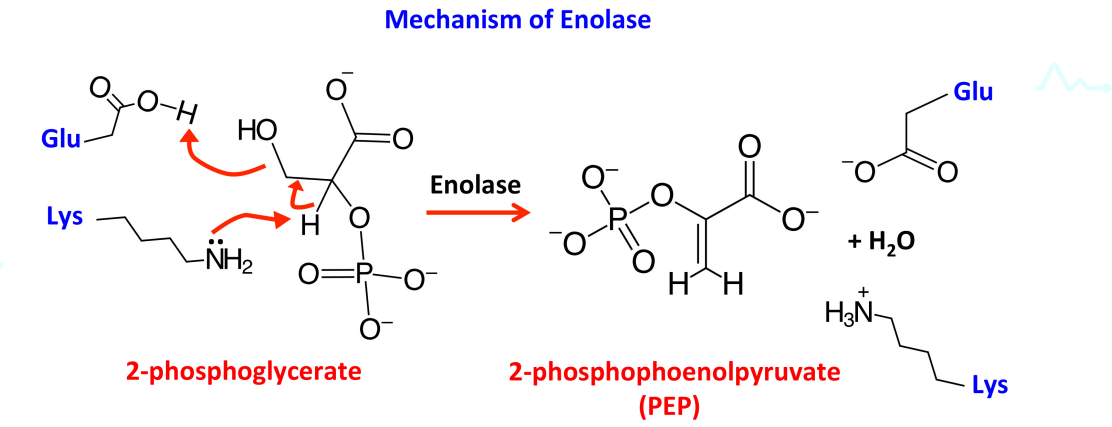
Phosphoglycerate kinase generates ATP

Thus far we have seen the activation of glucose, its conversion into glyceraldehyde-3P, and oxidation to the level of a carboxylic acid. Although this took two ATP molecules to perform, we have yet to harvest any ATP from these reactions. The next step in glycolysis generates ATP. Phosphoglycerate kinase catalyzes the transfer of the phosphate group from 1, 3 DPG to ADP, generating ATP. As the reaction is chemically coupled, the production of ATP from this reaction is called substrate level phosphorylation.



3-phosphoglycerate is converted into 2-phosphoglycerate (2-PG) through a mutase enzyme. 2-PG is a substrate for enolase, an enzyme that generates 2-phosphophoenolpyruvate (PEP). This glycolytic intermediate contains a high energy phosphoenolate bond. The mechanism of PEP formation is shown in the image. This occurs through an E2 elimination reaction. In this reaction, a lysine residue in the active site of enolase abstracts a proton, an example of base catalysis. The electrons collapse, form a double bond, and kick out the alcohol group. As ^{-}OH is a poor nucleophile, a glutamate side chain donates a proton (acid catalysis) to generate a H_2O, a better leaving group than ^{-}OH as it is a weaker base.



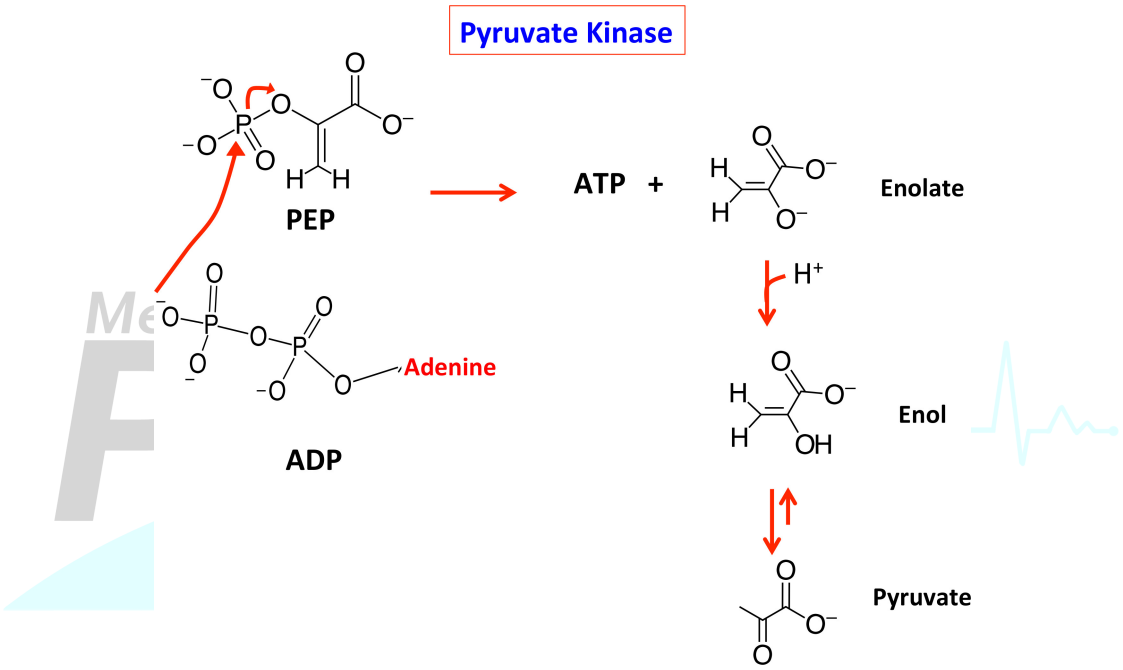


Formation of ATP and Pyruvate: The last step of glycolysis

In a manner analogous to that previously observed for phosphoglycerate kinase, pyruvate kinase uses PEP and ADP as a substrate to generate ATP via substrate level phosphorylation. The hydrolysis of PEP is exergonic (ΔG = - 15.0 kcal/mol) and when coupled to the phosphorylation of ADP (ΔG = + 7.3 kcal/mol), this is sufficient to drive the reaction to generate ATP. The mechanism of this reaction is shown below. Note that the phosphate group in PEP "locks" the molecule in an enol form. As the enol is unstable relative to the keto form, the conversion of the enol into pyruvate (the more stable keto

form) releases energy ($\Delta G < 0$) that is coupled to the synthesis of ATP from ADP and P_i.

From one mole of glucose, two moles of pyruvate are formed (i.e. 2 moles of ATP). Therefore, the total number of ATP molecules used and generated in glycolysis is -2 + 2 + 2 = 2. Combined with the 2 moles of NADH generated per mole of glucose, we see that the energy in the bonds of glucose have been converted into ATP, the potential energy of NADH, and pyruvate, a partially oxidized molecule.

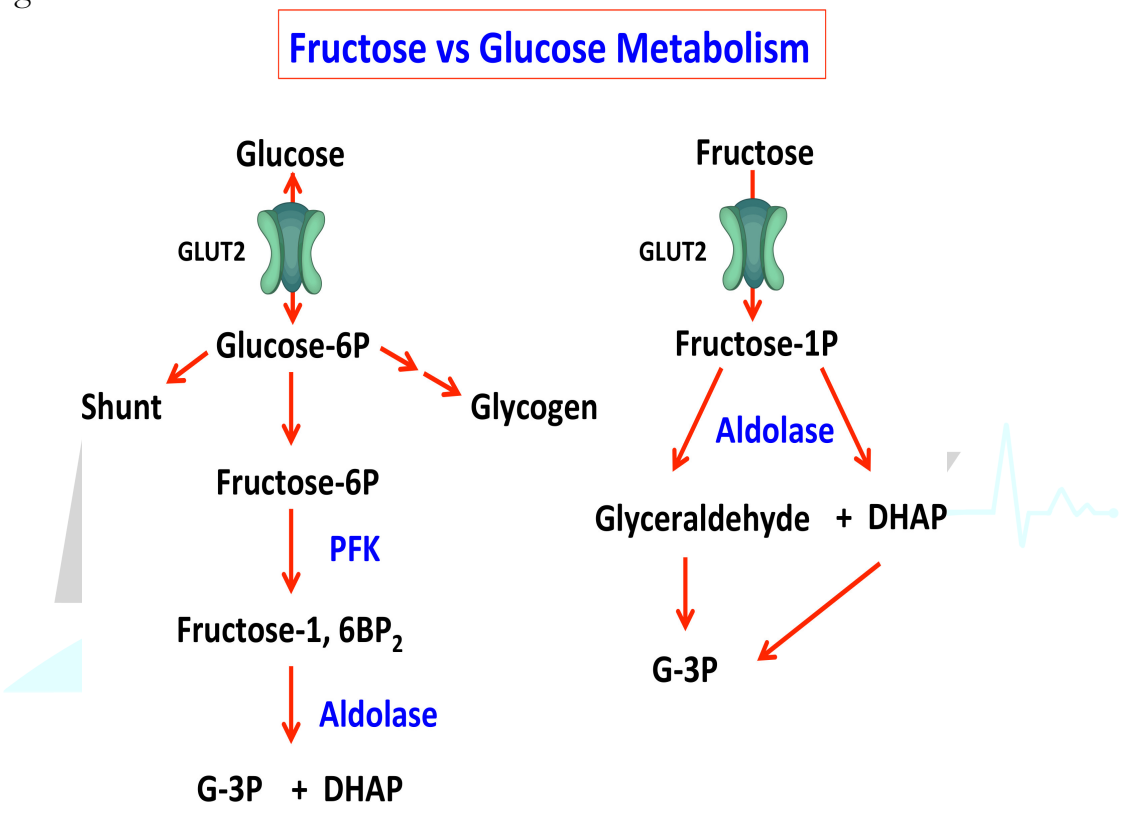


Fructose Metabolism: Altered glycolytic regulation

In nature, fructose does not exist as a monosaccharide, but rather is most commonly found in sucrose, a disaccharide composed of glucose and fructose. In contrast, processed foods often contain fructose in the free, monosaccharide form (i.e. high fructose corn syrup).

The vast majority of fructose is metabolized in the liver. Fructose is transported across the cell membrane via glucose transporters. That is, fructose does not have its own transporters.

The image compares hepatic metabolism of glucose and fructose. First note the fact that glucose and glucose 6-P, but not fructose, is a substrate for the pentose phosphate shunt (discussed below) as well as glycogen synthesis. Importantly, observe that fructose metabolism bypasses phosphofructokinase (PFK) regulation as Fructose-1P is converted into DHAP and glyceraldehyde. This is significant because PFK is regulated by insulin (through PKF2 as discussed above), indicating that fructose metabolism is uncoupled to insulin regulation in the liver.



Glycolysis Summary

Glycolysis is the partial oxidation of glucose into pyruvate:

Glucose + 2 NAD^+ + ADP + 2 P_i → 2 Pyruvate + 2 NADH + 2 ATP

Carbon flux through glycolysis is accelerated by insulin.

The resulting pyruvate can enter into the mitochondria if O_2 is available or is turned into lactate to regenerate NAD^+.

Pentose phosphate pathway (or shunt)

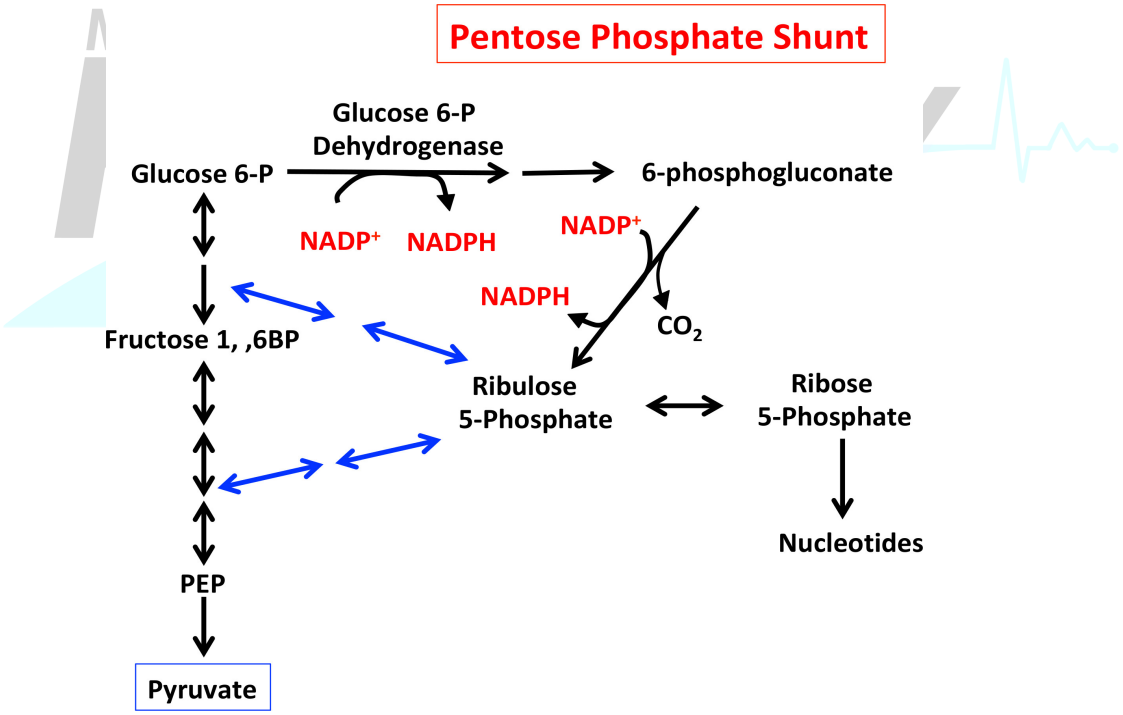
The pentose phosphate pathway, or shunt, oxidizes one of the carbon atoms in glucose. This pathway is divided into two phases:

1) The oxidative phase and generation of Ribulose 5-phophate and;

2) Conversion of Ribulose 5-phophate into glycolytic intermediates.

The first phase oxidizes Glucose-6P and produces reducing power in the form of NADPH. The resulting product (Ribulose 5-phophate) is converted into substrates for glycolysis. The net equation of the first phase is:

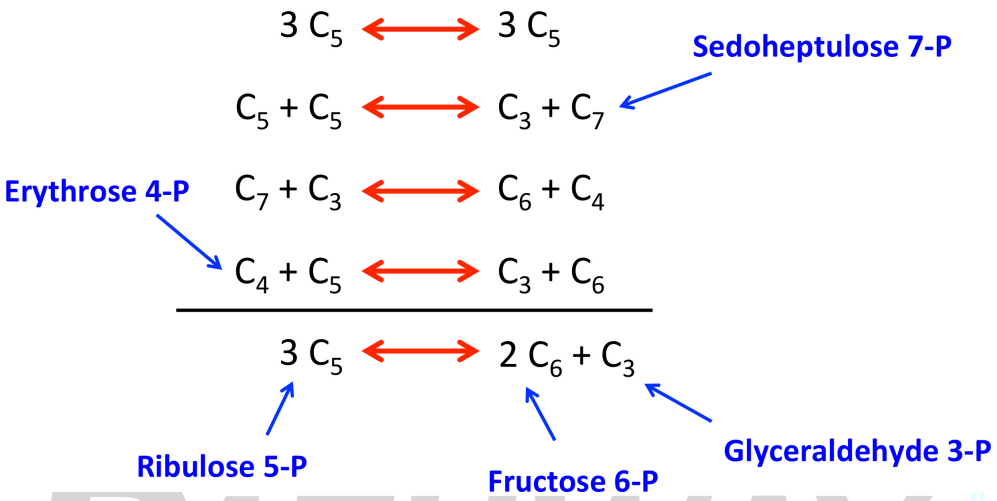
Glucose-6P + 2 $NADP^+$ = Ribulose-5P + CO_2 + 2 NADPH



In the first reaction step, Glucose-6 phosphate dehydrogenase (G-6PDH) oxidizes Glucose 6-P, producing Gluconolactone 6-phosphate. This step generates NADPH. Appreciate that NADPH is involved in various anabolic reactions (i.e. fat and cholesterol synthesis) and does not enter the electron transport chain. CO_2 is generated in the cytoplasm. The first phase of the

shunt generates a 5-carbon sugar, ribulose-5 phosphate, an isomer of the nucleotide precursor ribose 5-phosphate (ribose-5P). Pentose phosphate isomerase converts one isomer into the other.

Intermediate Flow in Pentose Phosphate Shunt



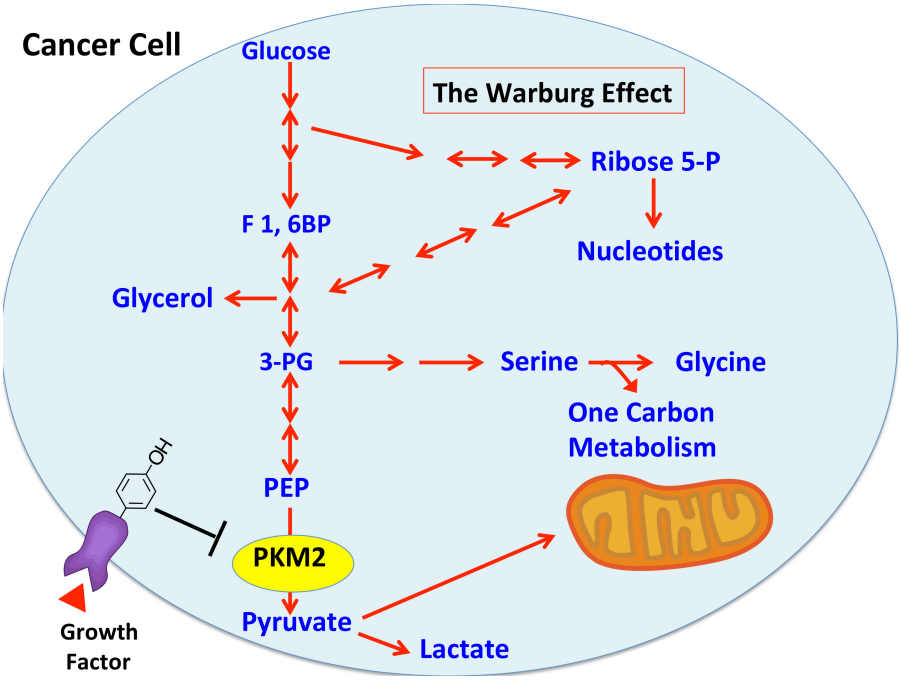
During the second phase of the pentose phosphate shunt, ribulose 5-P is converted into glycolytic intermediates as shown the image above. Through the action of transketolases and transaldolases, the five-carbon sugar produced from the first phase of the pathway is converted into intermediates with varying number of carbons. This allows for the integration of additional sugars (Erythrose 4-P and Sedoheptulose 7-P) into the glycolytic pathway. Ultimately, these products are compatible for entering glycolysis as they be converted into Fructose 6-P and Glyceraldehyde 3-P as shown.

The pentose phosphate shunt generates NADPH in the first phase. In the liver, this is used to generate fats and cholesterol, but in the red blood cell, the reducing power is used in conjunction with the glutathione redox system to combat the oxidative damage that occurs through the handling of oxygen and heme. This occurs through the glutathione redox system. Excessive oxidative damage to RBCs as caused by oxygen free radicals causes cell lysis that leads to hemolytic anemia.

Failure to form NADPH in the red blood cell due to a deficiency in Glucose-6 phosphate dehydrogenase (G6PDH deficiency) is an X-linked disease that affects over 100 million people worldwide. This highly polymorphic disease has hundreds of reported variants. Individuals with G6PDH deficiency are at risk for taking certain medications that are known to create a large oxidative load. This includes antimalarial and sulfa drugs.

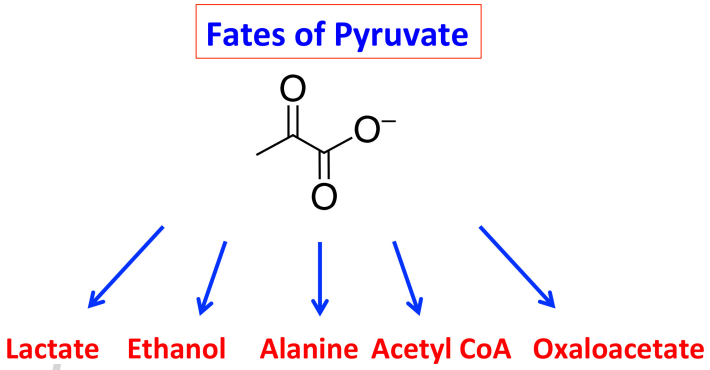
Pyruvate kinase (PK) and cancer

Although the glycolytic pathway produces pyruvate as its end product, many additional metabolites are derived from its intermediates that are used in the synthesis of various macromolecules including amino acids and nucleic acids (see below). In many cancer types, an isoform of pyruvate known as PKM2 is predominantly expressed. This isoform is less efficient at producing pyruvate than the normal pyruvate kinase protein. PKM2 is also negatively regulated through a tyrosine phosphorylation cascade induced by growth factor signaling pathways commonly observed to be unregulated in cancer. By changing the regulation and activity of PK during tumorigenesis, the levels of various key metabolic intermediates change. Consequently, carbon skeletons normally oxidized to CO_2 in the mitochondria (i.e. TCA cycle) are retained as intermediates that are incorporated into newly synthesized macromolecules required for cancer cell proliferation. This is known as the **Warburg effect**, and this is further characterized by converting pyruvate into lactate despite the presence of oxygen. We will examine the additional fates of pyruvate below.



The fates of pyruvate

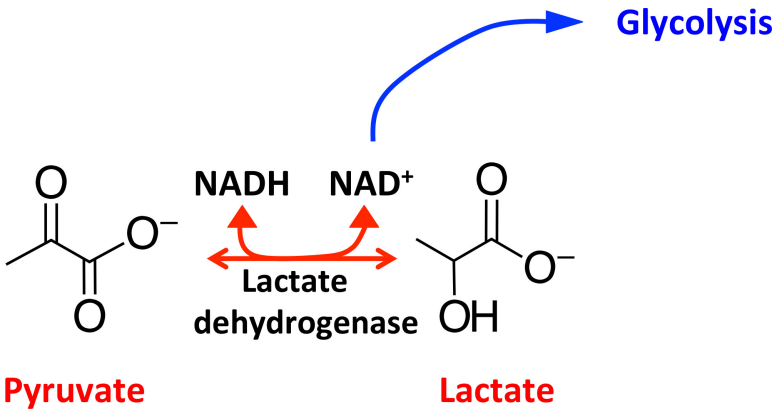
Pyruvate has multiple fates in the cell, depending on the energy status of the cell and the tissue type. We will examine some of these fates below. The role of pyruvate metabolism in the formation of oxaloacetate (OAA) will be discussed in more detail in the context of gluconeogenesis that is discussed later.



Formation of lactate and regeneration of NAD^+

We have just seen how cancer cells can convert pyruvate into lactate, even in the presence of adequate levels of oxygen. However, this is an abnormal scenario. Normal cells will not convert pyruvate into lactate in the presence of oxygen.

Once formed in the cytoplasm under aerobic conditions, pyruvate is transported into the mitochondria where it is further processed during aerobic metabolism. However, under anaerobic conditions (i.e. exercise), pyruvate accumulates in the cytoplasm and is converted into lactate via the action of lactate dehydrogenase. Inadequately oxygenated skeletal muscle produces lactate that is shipped back to the liver for conversion into pyruvate and glucose in the liver. This will be discussed below for gluconeogenesis. During this process in the skeletal muscle, NADH is converted into NAD^+, a substrate in the earlier glycolytic reaction that converted glyceraldehyde-3P into 3-phosphoglycerate. As further oxidation in the mitochondria is inhibited under anaerobic conditions, the conversion of pyruvate into lactate generates NAD^+ that can be used for further rounds of glycolysis. The net reaction for anaerobic glycolysis is: **Glucose + 2 ADP + 2 P_i = 2 Lactate + 2 ATP**.

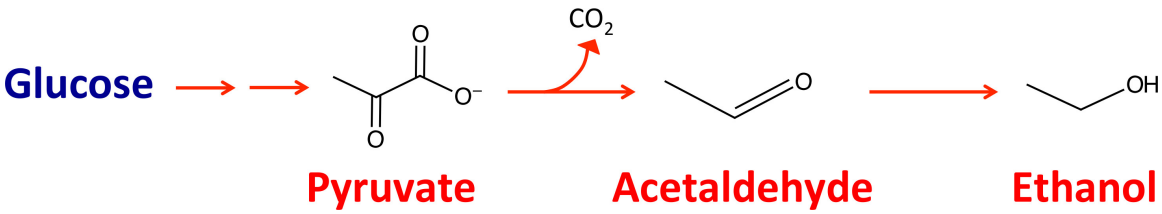


Excess accumulation of lactate is known as lactic acidosis, a serious medical condition. The following is a partial list of metabolic dysfunctional conditions that can lead to the excess production of serum lactate:

1) Warburg effect (cancer)
2) Mitochondrial dysfunction
3) Glycogenolysis defects
4) Defective gluconeogenesis

Fermentation

Fermentation is an anaerobic process that occurs in both bacteria and yeast. Ethanol is the end product of fermentation in yeast and occurs through the partial oxidation of glucose as shown below. One mole of glucose is converted into 2 moles of pyruvate via glycolysis. Pyruvate decarboxylase releases CO_2 to generate acetaldehyde. Alcohol dehydrogenase then reduces the aldehyde to ethanol.



In humans, alcohol is metabolized to acetaldehyde by the action of alcohol dehydrogenase. This is the reverse reaction. The expression of alcohol dehydrogenase allows for the consumption and metabolism of alcohol. Many people of Asian descent have a variant of this enzyme that causes the build up

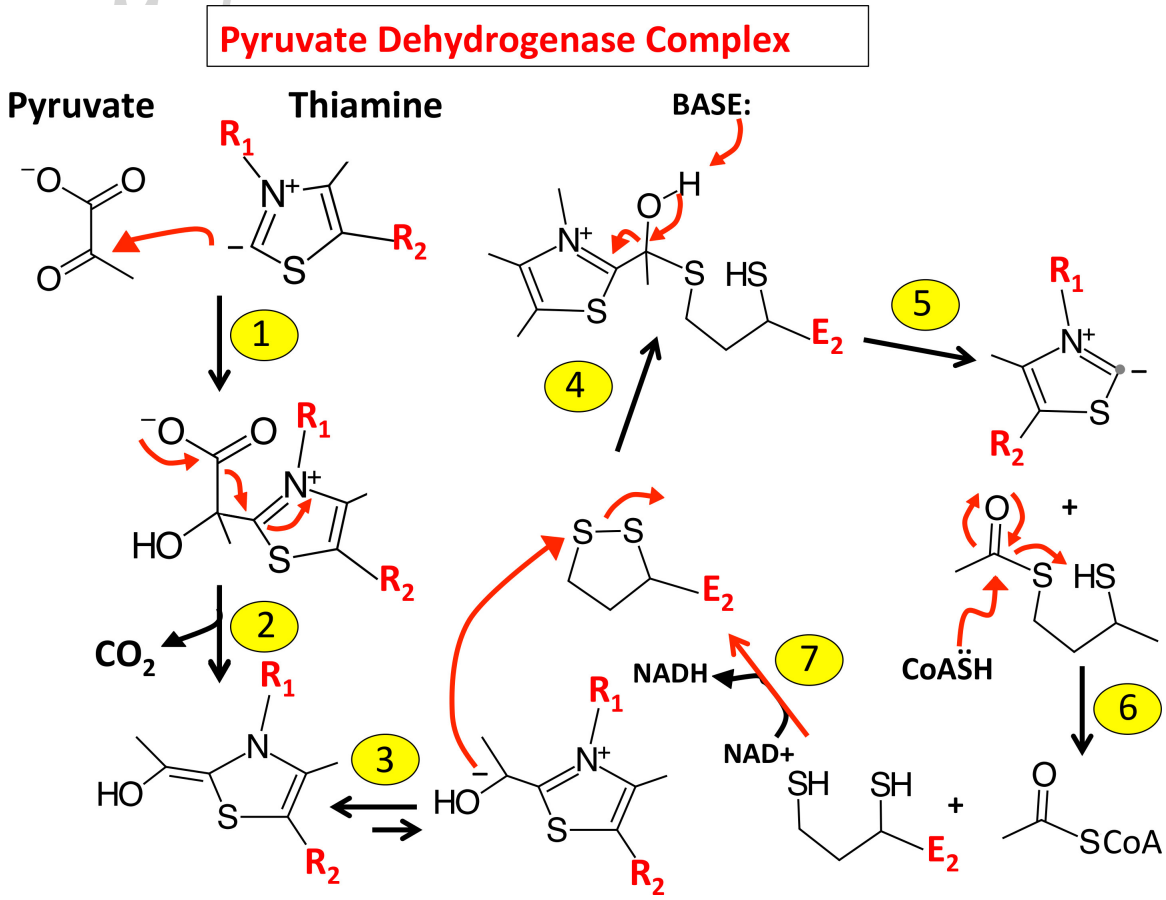
of potentially toxic levels of acetaldehyde that is causal for the "alcohol flush reaction". Therefore, alcohols undergo oxidation reactions. Primary alcohols such as ethanol are metabolically oxidized into aldehydes that can be further oxidized into carboxylic acids.

Acetyl CoA Formation and The Pyruvate Dehydrogenase Complex

Pyruvate that enters the mitochondria under aerobic conditions is a substrate for the pyruvate dehydrogenase complex (PDC), a multi-enzyme complex that converts pyruvate into acetyl coal through oxidation. The net reaction for this is as follows:

Pyruvate + CoA + NAD^+ = Acetyl CoA + CO_2 + NADH

Multiple co-factors are involved in this reaction including coenzyme A, NAD^+, lipoic acid, and thiamine (Vitamin B_1). One major function for thiamine is to break carbon-carbon bonds. The mechanism for this is shown below:



Step 1: The thiamine co-factor is bound to an E1 enzyme. A resonance stabilized-carbanion attacks the alpha keto carbonyl of pyruvate, generating a tetrahedral intermediate.

Step 2: Collapse of the tetrahedral intermediate breaks the C-C bond and releases CO_2. The electrons directionally flow to the heterocyclic nitrogen atom of thiamine, demonstrating that nitrogen serves as an "electron sink" in the reaction.

Step 3: The product is in equilibrium with a resonance-stabilized carbanion that attacks the sulfur atom in the disulfide linkage of lipoic acid. Lipoic acid is linked to an E2 enzyme through an amide bond.

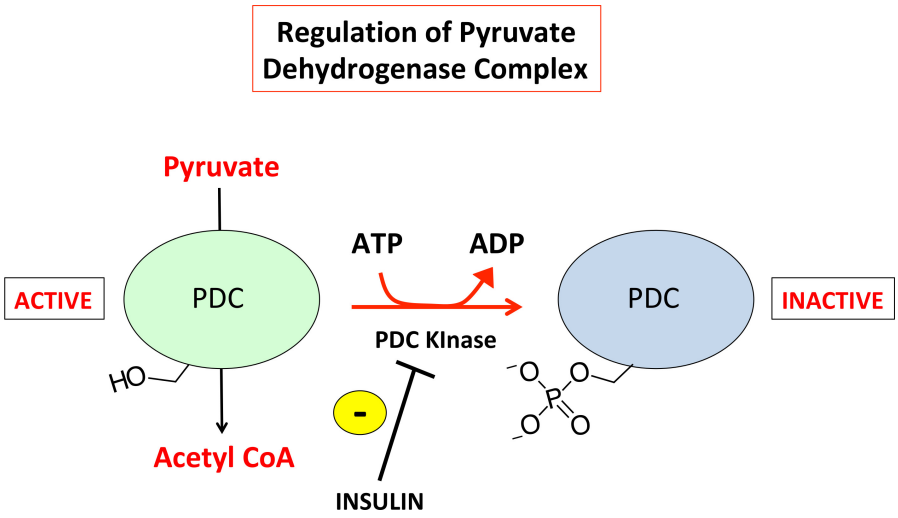
Step 4: A new tetrahedral intermediate is formed. A base draws off a proton and forms the tetrahedral intermediate,

Steps 5, 6: The thiamine moiety is released from the enzyme. Importantly, an acetyl group is covalently linked to lipoic acid. This high-energy thioester intermediate is attacked by Coenzyme A (CoASH), forming a tetrahedral intermediate. Upon collapse, acetyl CoA is released along with reduced lipoic acid.

Step 7: Reduced lipoic acid is converted into the disulfide, oxidized form of the co-factor through a redox reaction coupled to the reduction of NAD^+. This restores the oxidized form of lipoic acid for further reactions.

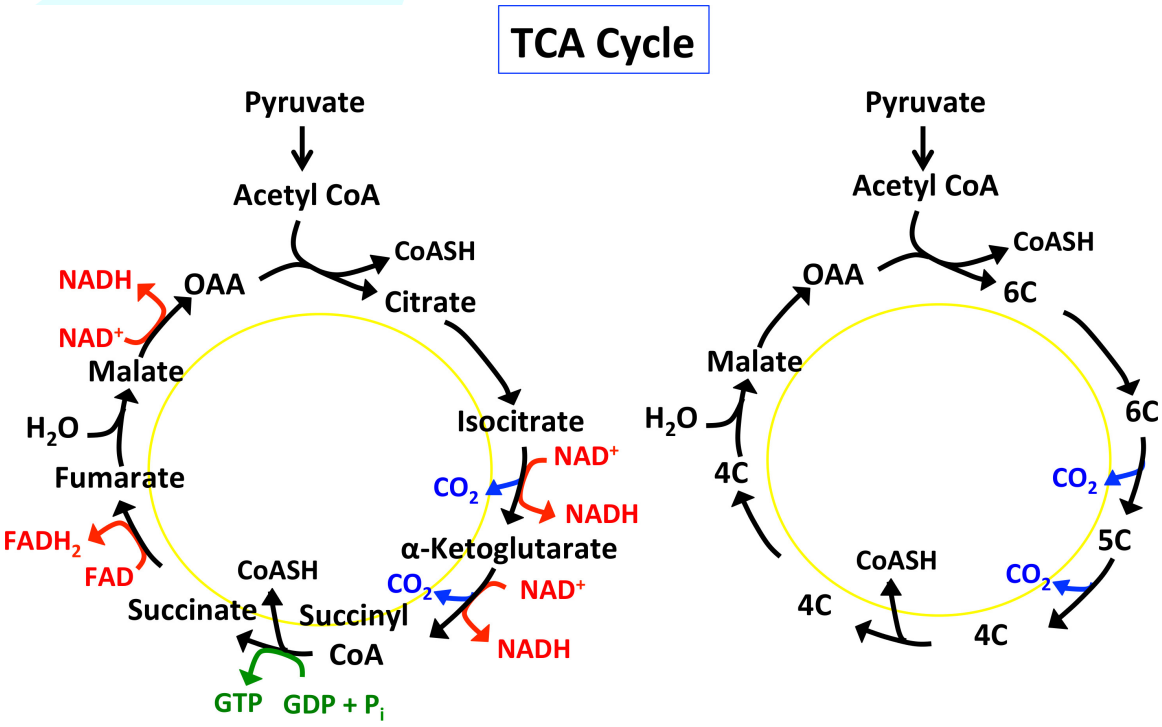
Regulation of the Pyruvate Dehydrogenase Complex

The pyruvate dehydrogenase complex (PDC) regulates the fate of pyruvate. This reaction requires NAD^+ and several other co-factors as shown in the image. In this reaction, pyruvate is oxidized to acetyl CoA, a process positively regulated by insulin (see below). However, pyruvate is also a substrate for hepatic gluconeogenesis, a process supported by glucagon and epinephrine. As a result of the differential fates of pyruvate, there are multiple levels of regulation of the PDC. One important mode of regulation is reversible phosphorylation on various serine residues in a subunit of the complex. PDC kinase inactivates the complex via phosphorylation. In hepatic tissue, this kinase is negatively regulated by insulin, indicating that insulin promotes pyruvate oxidation in the mitochondria.



The oxidation of Acetyl CoA: The Krebs Cycle

Acetyl CoA formed in the mitochondria can be oxidized in the Krebs cycle (i.e. TCA cycle). Upon completion of the TCA cycle, all three-carbon atoms in pyruvate have now been completely oxidized to CO_2. We saw the first CO_2 created from oxidation of pyruvate via the pyruvate dehydrogenase complex. The other two CO_2 molecules created are actually derived from oxaloacetate (OAA), not acetyl CoA. Therefore, the TCA cycle oxidizes the two carbon equivalents of acetyl CoA. However, note that OAA begins and ends the TCA cycle; it is therefore catalytic.



The TCA cycle, by virtue of oxidation, generates a lot of reducing power in the form of NADH and $FADH_2$. Only one high energy molecule (GTP) is produced via substrate level phosphorylation:

Acetyl CoA + 3 NAD^+ + FAD + GDP + P_i = 2 CO_2 + 3 NADH + $FADH_2$

+ GTP + CoASH

Think of the TCA cycle as occurring in three phases:

1) Condensation reaction between Acetyl CoA and OAA to generate citrate which then isomerizes into isocitrate. In this reaction, a two-carbon thioester is added to a four-carbon acid to generate a 6-carbon product (Citrate).

2) Decarboxylation reactions that generate CO_2 and reducing power. During this phase the 6-carbon intermediates are converted to five and then 4-carbon intermediates.

3) Creation of four-carbon compounds and the regeneration of OAA.

TCA cycle intermediates are used in multiple biochemical contexts. For example, citrate is used as a precursor to fatty acid synthesis, malate is used as a substrate in gluconeogenesis, and some intermediates are derived from the breakdown of amino acids. Therefore, the rate of oxidation of acetyl CoA in the TCA cycle changes and will be important when describing fatty acid metabolism as well as gluconeogenesis.

Regulation of the TCA cycle

Several key factors regulate the flux of carbon through the TCA cycle:

1) Substrate availability: various processes including gluconeogenesis and amino acid degradation impact the levels of TCA intermediates. As we will discuss below, gluconeogenesis draws off acids such as OAA and malate and therefore slows down the cycle. On the other hand, the metabolism of some amino acids (fed and fasted states) generates carbon skeletons that are converted into TCA intermediates. Replenishment of TCA intermediates is known as anaplerosis.

2) Enzyme regulation: TCA enzymes such as citrate synthase and isocitrate dehydrogenase are regulated by allosterism. For these enzymes, high levels of

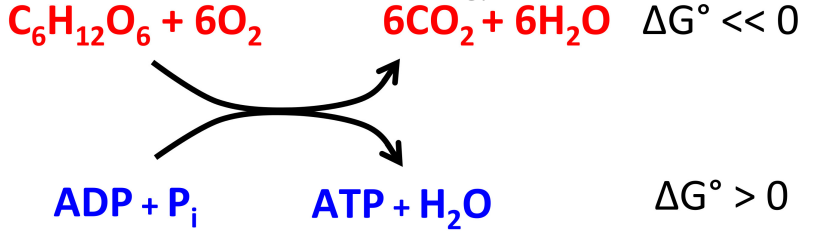
ATP negatively regulate these enzymes and therefore slow the flux of carbon through the cycle.

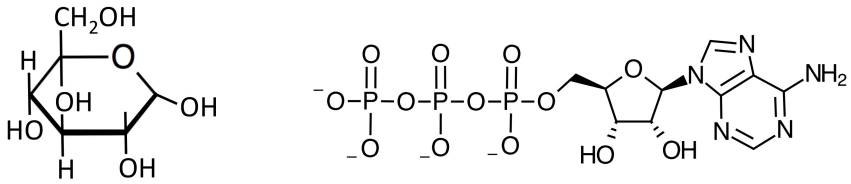
Oxidative phosphorylation

The NADH reducing power created through the oxidation of glucose and acetyl CoA is converted into ATP in the mitochondrial electron transport chain. In contrast to glycolysis that generates ATP from substrate level phosphorylation, oxidative phosphorylation occurs through the mitochondrial coupling of ADP phosphorylation through a proton gradient. This is shown below. The gradient is set up through an inner mitochondrial membrane that is intrinsically impermeable to the free crossing of H^+. The protein complexes of the inner mitochondrial membrane couple the oxidation of reducing equivalents in NADH and $FADH_2$ to the generation of an electrochemical gradient of protons.

Thermodynamics and oxidative phosphorylation. One of the best examples of thermodynamic coupling in biology can be seen in the process of oxidative phosphorylation. Never forget that oxidation is the loss of electrons and reduction is the gain of electrons (OIL RIG). Oxidative phosphorylation is the process by which NADH and $FADH_2$ (i.e. energy derived from the oxidation of glucose $C_6H_{12}O_6$) is coupled to the synthesis o ATP in the mitochondria. This occurs in the electron transport chain (ETC).

Oxidation of the C-C, C-H, and C-O bonds in glucose releases a lot of energy, particularly in the form of heat (enthalpy). As $\Delta G << 0$ for the complete oxidation of glucose into CO_2 and H_2O, energy from this reaction can be used





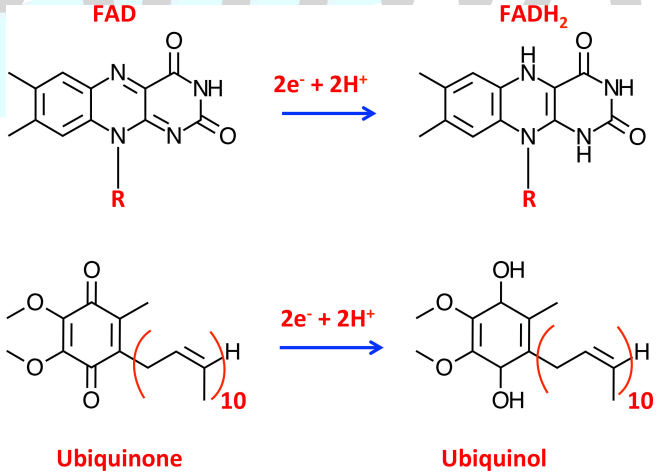
Glucose **ATP**

to couple the phosphorylation of ADP to generate ATP. The conversion of ADP into ATP through the addition of inorganic phosphate is normally an endergonic process ($\Delta G > 7.3$ kcal/mole), but the TOTAL ΔG for the reaction ($C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$) is negative when coupled to the complete oxidation of glucose. Therefore, the electron bonds in glucose are ultimately converted into the high energy potential of the phosphoanhydride bonds of ATP.

Electron transfer potential and synthesis of ATP.

The transfer of energy from glucose oxidation to the chemical bonds in ATP is not necessarily a direct process as much of it is conducted through chemical and electron carrier intermediates. We saw this in glycolysis. The energy derived from the loss of electrons during glucose oxidation is transferred as high energy electrons to the reducing carriers NADH and $FADH_2$. These electrons are deposited into the mitochondrial electron transport chain.

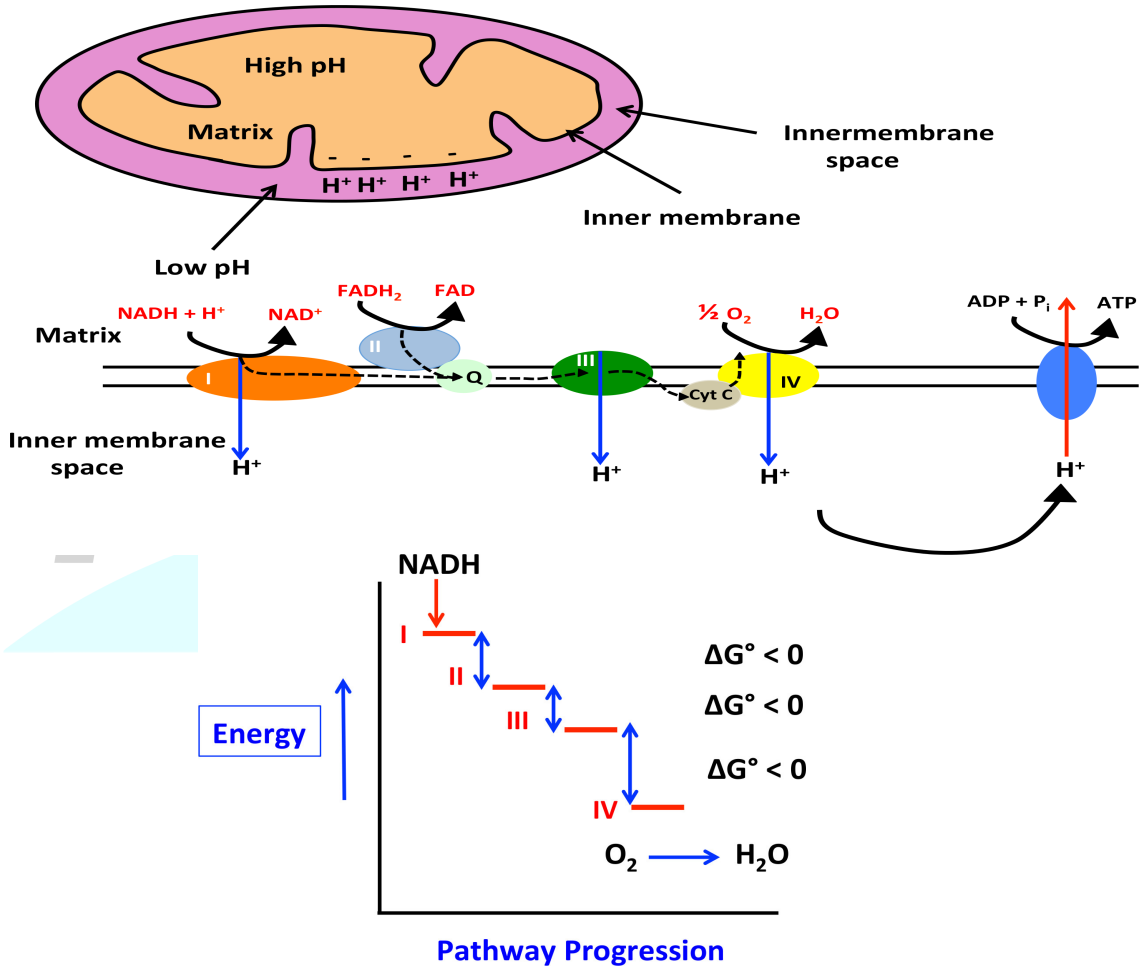
During electron transport, redox events with the hydride anion takes place ($H:^- = H^+ + 2e^-$) with various electron carriers such as quinones and flavins such as $FADH_2$. Electrons flow from more reduced to less reduced carriers. Electron



flow is concomitant with the directional flow of H^+ from the matrix to the inner membrane space. As electrons are transferred from carrier to carrier, protons are released into the inner-membrane space. This is because the inner mitochondrial membrane is impermeable to protons. Therefore, the reduction potential derived from the energy released upon the oxidation of carbon bonds in glucose is converted into a potential energy gradient composed of protons. Electrons are ultimately transferred to O_2, creating water. The energy of the

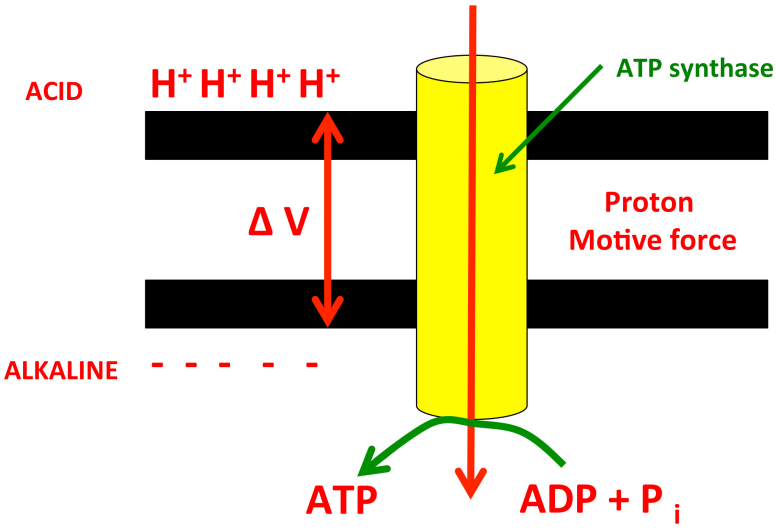
proton gradient is used to synthesize ATP as well as in other processes such as generating heat during the winter for hibernating animals.

Each major step in the transfer of electrons from NADH to O_2 is accompanied by large decreases in free energy as shown. The terminal acceptor in the pathway is oxygen; it is the least reduced acceptor in the entire chain and therefore is at the lowest energy point in the pathway.



Electron transport centers I, III, and IV use various redox factors such as quinones (i.e. ubiquinone and ubiquinol), $FADH_2$, and cytochromes to translocate a pair of protons (proton motive force: ΔP) for the pair of electrons passing through the electron transport chain (see image).

ΔP = chemical gradient ΔpH + charge gradient Δψ.



The transfer of protons generates an electrochemical gradient in the mitochondria that has enough potential to phosphorylate ADP (See figure below). A mitochondrial enzyme known as ATP synthase performs the coupling of the energy in the proton gradient with the phosphorylation of ADP. Under normal circumstances electrons will not flow down the transport chain unless ADP is being phosphorylated into ATP. Thus, the rate of ATP utilization is coupled to the rate of NADH oxidation. ATP synthase couples the conversion of the gradient into the high-energy chemical bonds of ATP. This flow of energy from reactants to products during multi-step biochemical pathways is the essence of bioenergetics and thermodynamics.

Reaction Center	ENZYME	NOTES
1	NADH-Q OXIDOREDUCTASE	Lowest electron affinity in chain; uses NADH
2	SUCCINATE-Q OXIDOREDUCTASE	Enzyme is part of TCA cycle; uses $FADH_2$
3	Q-CYTOCHROME C OXIDOREDUCTASE	Oxidizes coenzyme Q and reduces cytochrome c
4	CYTOCHROME C OXIDASE	Catalyzes the reduction of O_2 to H_2O; Blocked by cyanide, carbon monoxide, azide

Energy yield in the mitochondria

The oxidation of NADH in reaction center I ultimately transfers electrons to oxygen. This electron transfer is accompanied by the translocation of ~10 protons, which allows for the formation of ~3 ATP molecules. In contrast, the $FADH_2$ electron carrier enters the electron transport chain at reaction center 2, a lower energy potential. As a consequence, the transfer of electrons from $FADH_2$ to oxygen is accompanied by the translocation of 6 protons and the formation of ~2 ATP molecules.

The proton gradient derived from the oxidation of NADH and $FADH_2$ is not completely utilized for ATP synthesis. Some of the energy from the gradient is naturally dissipated by thermogenin proteins and produces heat, a great idea for hibernating animals and babies. Further, the ADP/ATP translocase, an enzyme that brings ADP into the mitochondria and sends out newly generated ATP, consumes protons during the process.

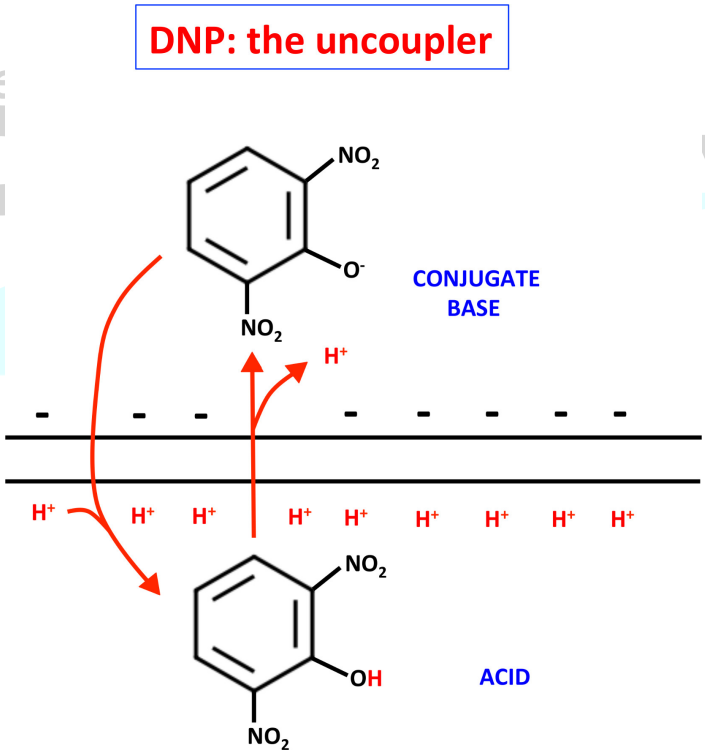
Regulation and inhibition of electron transport

Multiple factors regulate electron transport. Importantly, oxygen is a key regulator. Low oxygen conditions reduce the flow of electrons through electron transport. This leads to a build up of NADH, a soluble electron carrier that enters the electron transport chain in reaction center I. As NADH cannot be converted into NAD^+, a substrate for pyruvate dehydrogenase and TCA enzymes, these pathways are slowed down. In the cytoplasm, pyruvate is converted into lactate in order to regenerate NAD^+ for glycolysis.

Multiple drugs are known to inhibit the electron transport chain at various points in the pathway. For example, amytal and metformin, a drug administered to diabetics to reduce hyperglycemia, inhibit respiratory chain center I. Cyanide and carbon monoxide inhibit reaction center IV. Inhibition of the electron transport chain increases the levels of intermediates upstream of the block. As these intermediates are electron carriers (i.e. quinones and NADH), electrons can be transferred to oxygen in the mitochondria. As a result, oxygen free radicals are notoriously produced through perturbations in the flow of electrons in the respiratory chain.

In addition to chemicals that inhibit the electron transport chain at specific electron transfer reaction centers, some inhibitors act at different points. For example, the high negative charge found on ADP and ATP prevent their free pass through the inner mitochondrial membrane. As a result, the ADP/ATP translocase must bring in ADP and export ATP. Inhibition of the translocase by atractyloside reduces the amount of ADP entering the system and halts electron transport. This is because ATP synthase is devoid of substrate.

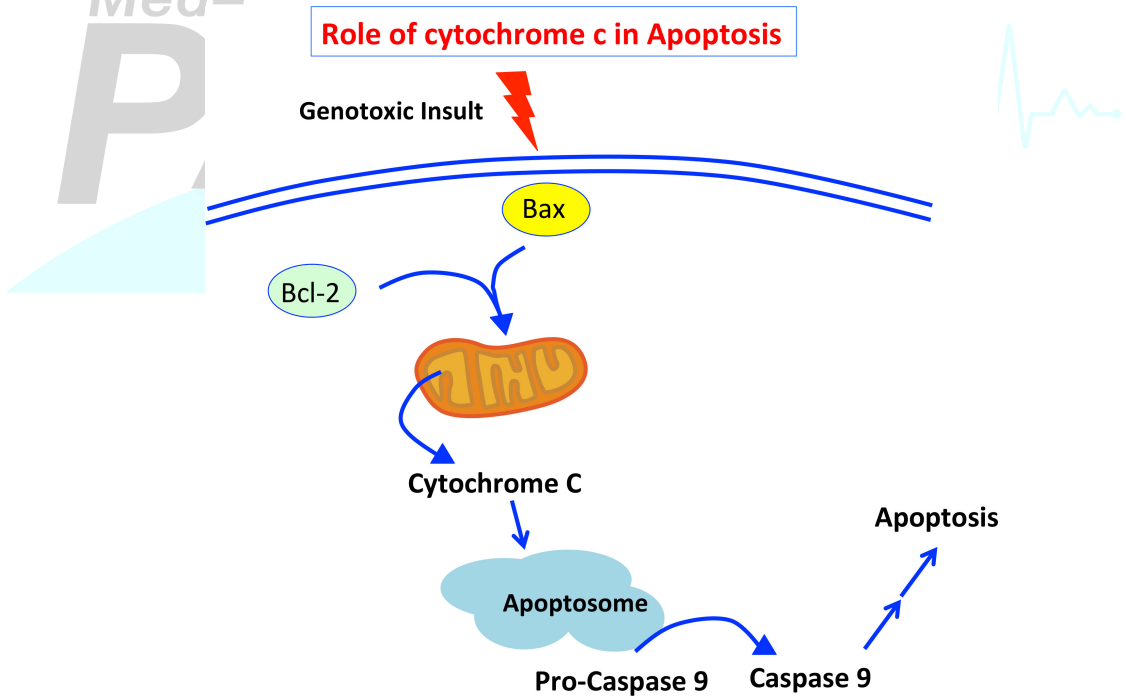
Further, molecules such as dinitrophenol (DNP) "uncouple" the proton gradient from the synthesis of ATP without influencing the flow of electrons down the gradient. The mechanism of DNP is shown below. Although the inner mitochondrial membrane regulates the permeability of protons and therefore the flow of protons during electron transport, uncouplers such as DNP can freely pass through the inner mitochondrial membrane. DNP "uncouples" the proton gradient from ATP synthase activity. This occurs



through its ability to dissipate the proton gradient as shown below. DNP is protonated on one side of the membrane. As DNP diffuses to the other side of the membrane, it releases the proton under basic conditions, generating the conjugate base that is free to cross again and pick up another proton. The net result is the loss of the proton gradient, uncoupling ATP synthase from the gradient. The end result is no ATP synthesis.

The role of cytochrome c in apoptosis

In addition to its role in the electron transport chain, cytochrome c plays a central, intermediary role in the intrinsic pathway of apoptosis, or programmed cell death. In response to genotoxic insults that activate the p53 tumor suppressor (i.e. ionizing radiation), the pro-apoptotic protein Bax is recruited to the mitochondrial membrane where it neutralizes the anti-apoptotic factor Bcl-2. This promotes the release of cytochrome C from the mitochondria. Under normal conditions, cytochrome c binds to the lipid cardiolipin in the inner mitochondrial membrane. The interaction between Bax and Bcl-2 combined with indigenous mitochondrial reactive oxygen species promotes the release of cytochrome c from the mitochondria. Upon its release, cytochrome c becomes part of the apoptosome, a complex that contains pro-caspase 9, a protease zymogen that is activated by cytochrome c. Caspase 9 activates a serine protease cascade that includes additional caspase enzymes that ultimately lead to cell destruction.



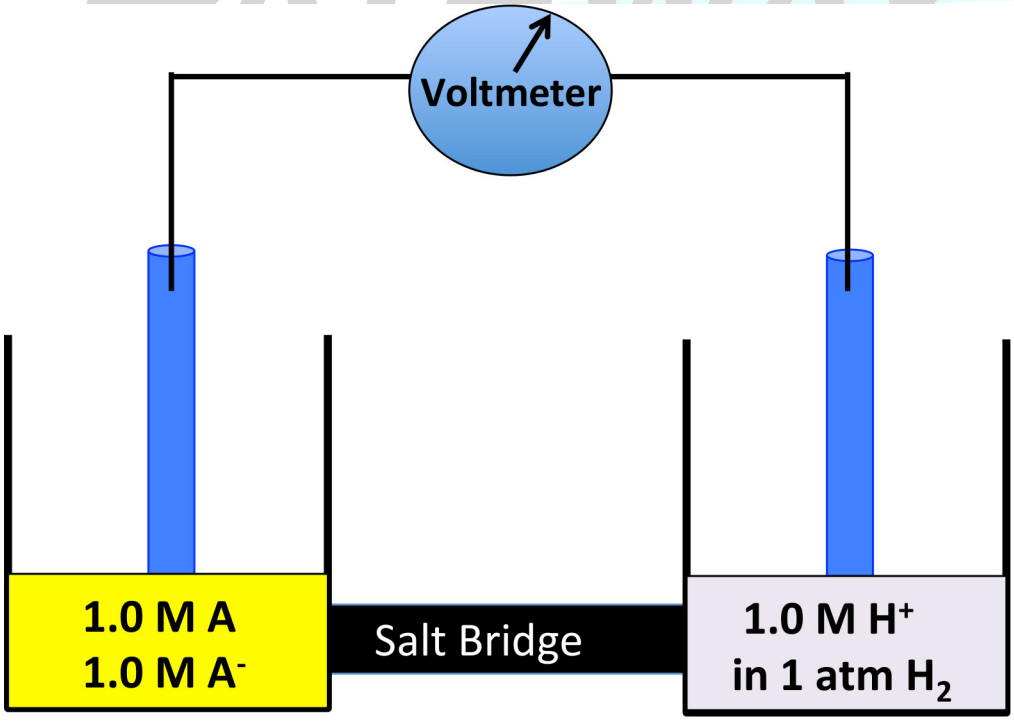
Electron transfer potential

During electron transport the transfer potential of electrons is converted into the phosphoryl chemical transfer potential that resides in the

phosphoanhydride bonds of ATP. Think of the mitochondrial matrix in terms of a battery that stores charge, in this case protons generated from sequential redox reactions in the electron transport chain. The transfer potential of reactions obeys the laws of thermodynamics and can be expressed in terms of the electron reduction potential, designated E_O.

Therefore, electron transport is just an electrical circuit made up of conjugated rings (i.e. quinones and flavins) and ions (i.e. copper) that participate in oxidation/reduction reactions that allows current to flow from NADH to O_2. The proton gradient produced during this electron flow is both electrical and chemical. Stored in this battery is enthalpy.

We know that phosphoryl transfer energy is given by ΔG. However, the standard electron transfer potential, or more commonly referred to as the electron reduction potential, is given by E°. Take for example the redox couple A and A^-. The potential gradient across the membrane (ΔV) can be measured in millivolts (mV). Each unit change in the pH has an effect equivalent to a membrane potential of approximately 60 mV.



The reduction potential of the redox pair A and A^- is determined in a half-cell experiment by measuring the electronmotive force (EMF). Such an experiment is performed with a standard half-cell reference that by convention is 1.0 M H^+ equilibrated in H_2 gas. The direction of the flow of electrons will proceed as per the laws of thermodynamics. If the reactions flow from the sample cell to the standard cell, then the sample cell electrode is arbitrarily said to be negative with respect to the electrode in the standard cell. As a convention, the reduction potential of H^+/H_2 is equal to 0 volts. Negative reduction potentials indicate that the oxidized form of the substance has a lower affinity for electrons than H_2.

The table below shows the standard reduction potentials for various biological molecules, including those involved in electron transport. *E'°* represents the partial reaction: Oxidant + e^- = Reductant. Note that negative reduction potentials indicate that the oxidized form of the substance has a reduced affinity for electrons relative to the standard H_2.

Standard Reduction Potentials

Oxidant	Reductant	# e^- transferred	E° (Volts)
$NAD^+ + H^+$	NADH	2	- 0.32
FAD	$FADH_2$	2	- 0.22
Pyruvate	Lactate	2	- 0.19
Fumarate	Succinate	2	+ 0.03
Fe^{+3}	Fe^{+2}	1	+ 0.77
½ O_2 + $2H^+$	H_2O	2	+ 0.82

The standard free energy change is related to the standard change in reduction potential through the following equation:

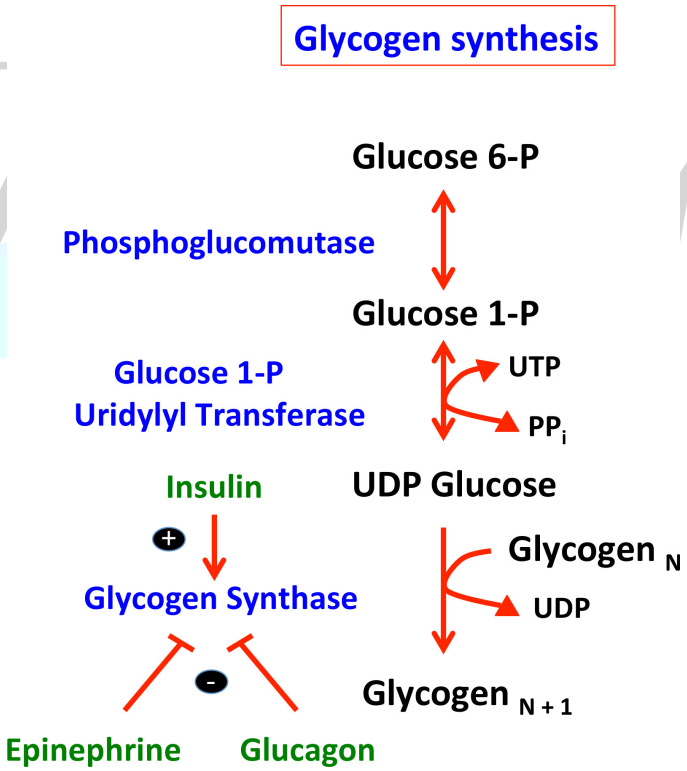
$$\Delta G^\circ = -nF\Delta E^\circ$$

where n = # electrons transferred; F = Faraday (23.0 kcal mol^{-1} V^{-1})

Glycogen synthesis

Thus far, we have seen that in the fed state, glucose is taken up into cells and converted into G-6P. We discussed its conversion into ribulose 5-P via the shunt and into pyruvate via glycolysis. A third fate of glucose, its conversion into glycogen, is discussed here.

Animals store glucose in multiple tissues in the form of glycogen, a polymer composed of α-1, 4 and α-1, 6 glycosidic linkages. Glycogen levels are maintained by the regulation of the two antagonistic enzymes: glycogen synthase and glycogen phosphorylase. Under conditions of excess dietary glucose (high insulin, low glucagon and epinephrine), those tissues that synthesize glycogen (i.e. liver, heart, and skeletal muscle) increase the synthesis and activation of glycogen synthase.



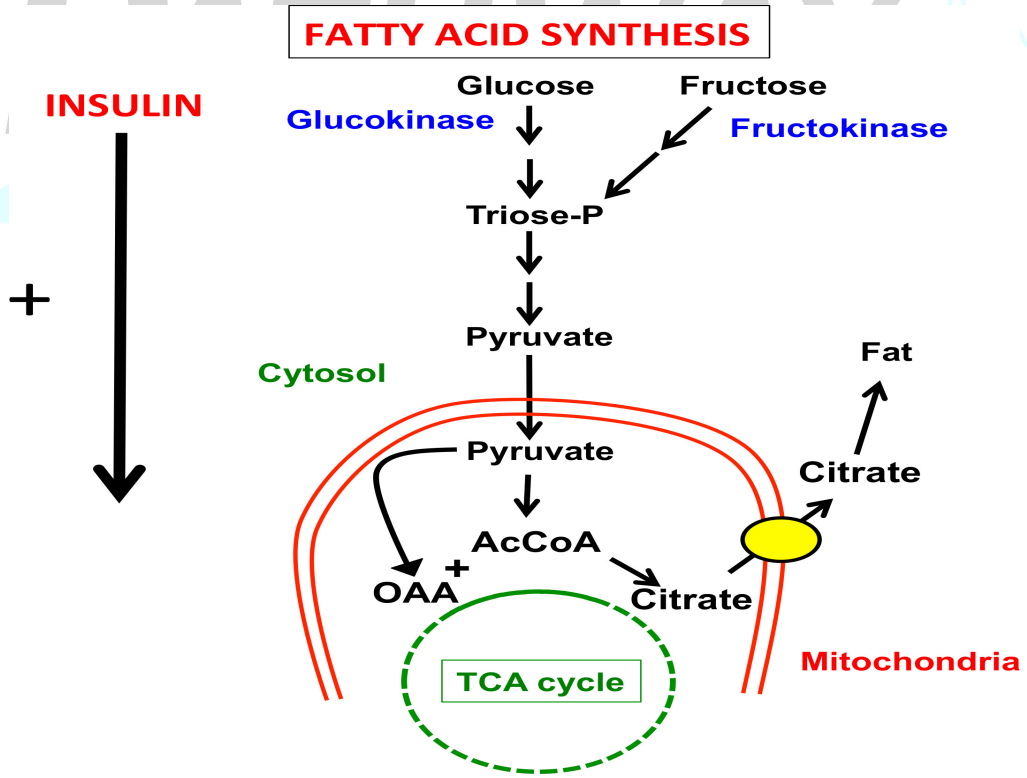
The initial reaction: Glucose 6-P → Glucose 1-P (G1-P) is an isomerization reaction that is driven by mass action, or Le Chatlier's principal. That is, the more G6-P in the system, then more of the structural isomer G-1P is formed. G1-P is activated through a uridylyl transferase enzyme that uses UTP to generate UDP Glucose, an activated sugar that is a substrate for glycogen synthase. This enzyme is the rate limiting step in glycogen synthesis and its

activity is promoted by insulin that keeps it in the de-phosphorylated form. In contrast, epinephrine and glucagon inhibit glycogen synthase by promoting the phosphorylated form of glycogen synthase. Active glycogen synthase catalyzes the addition of a glucose moiety to the glycogen polymer through the creation of a new α-1, 4 glycosidic linkage. Additional branching enzymes create the α-1, 6 linkages which keep the glycogen polymer more soluble.
The net reaction for glycogen synthesis is:

Glycogen $_{N}$ + 2 ATP → Glycogen $_{N+1}$ + 2 ADP + 2 Pi.

Fatty Acid & Triglycerides synthesis

Excess consumption of carbon skeletons in the form of carbohydrate sugars (and amino acids) increases the serum levels of insulin, ultimately leading to fat synthesis in the liver. Glucose transporters induced by insulin import glucose from the serum into tissues. After down regulation of hexokinase in peripheral tissue, excess glucose enters portal circulation and is phosphorylated by glucokinase in hepatic cells. Insulin increases the flux of carbon through



glycolysis as well as the pentose phosphate shunt and into the glycogen synthesis pathway. Glucose is rapidly oxidized via glycolysis and converted into acetyl CoA in the mitochondria. Acetyl CoA is an allosteric activator of

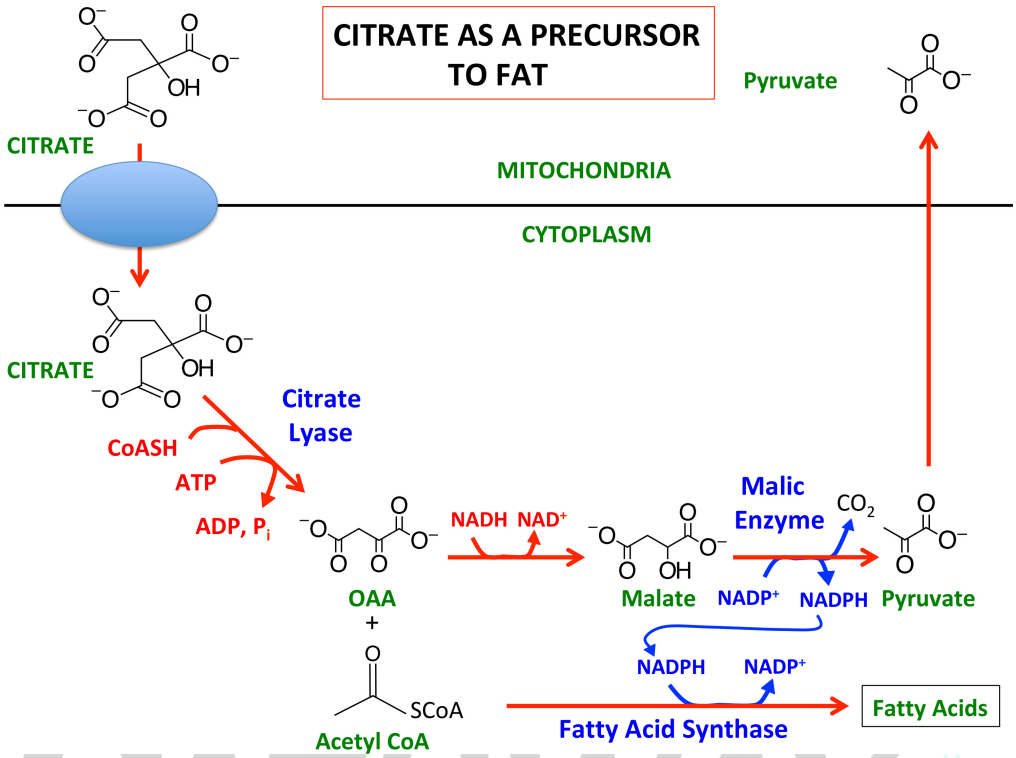
pyruvate carboxylase, a biotin-dependent enzyme that produces OAA in the reaction:

Pyruvate + CO_2 + ATP = OAA + ADP + Pi

Thus, elevated levels of acetyl CoA lead to the production of OAA as shown in the image. When acetyl CoA is produced at levels exceeding the rate of its oxidation in the TCA cycle, citrate accumulates and is transported out of the mitochondria and is re-converted back into acetyl CoA.

Citrate Provides Precursor Carbon for Fat Synthesis

Fat is primarily synthesized in the liver and small intestine. As insulin expedites the flux of carbon down the glycolytic pathway and through the pyruvate dehydrogenase complex, glucose is rapidly converted into acetyl CoA in the liver. In the fed state, the energy charge is high as ATP has been synthesized from glycolysis. The acetyl CoA combines with OAA to form citrate that is in equilibrium with isocitrate. If enough citrate is formed (i.e. high consumption of sugar), then its oxidation in the TCA cycle will slow down because there is too much substrate for the system. If the levels of citrate accumulate to a certain threshold, the citrate transporter takes citrate into the cytoplasm where it is a substrate for citrate lyase. This enzyme converts citrate into acetyl CoA and OAA in the cytoplasm. Observe from the figure that citrate lyase uses ATP and free CoASH in this reaction. Therefore, the energy released from ATP hydrolysis used to generate a high energy thioester, namely acetyl CoA. Think of citrate as a carrier of the two carbon units of acetyl CoA from the mitochondria to the cytoplasm. Indeed, CoASH derivatives are unable to cross the inner mitochondrial membrane.

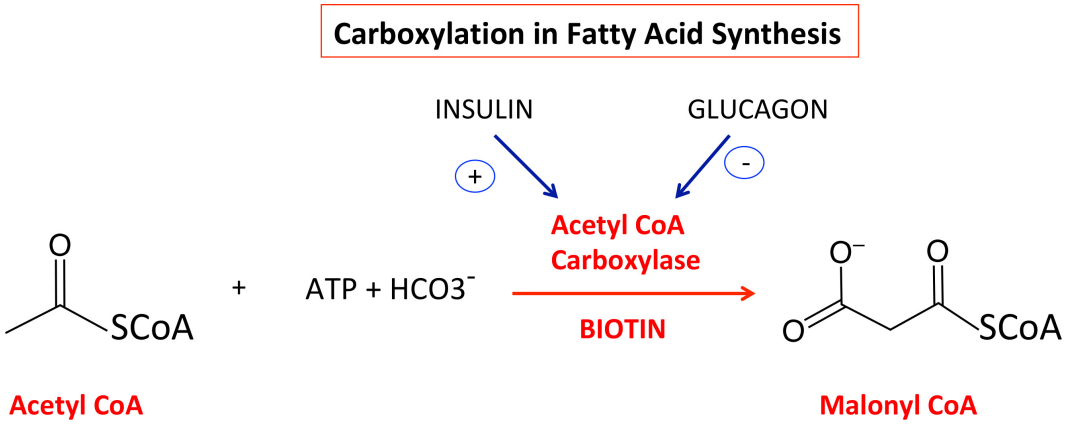


Citrate lyase is a key enzyme that provides precursor carbons for fatty acid synthesis. In fact, its inhibition has been the subject of diet fads as the Asian fruit garcinina cambogia, which contains the citrate lyase inhibitor hydroxycitrate, has been used a weight loss supplement.

In the cytoplasm, OAA derived from citrate lyase is reconverted back into pyruvate through two redox reactions. Note that one of these reactions is performed by the malic enzyme and produces NADPH, a cofactor used by fatty acid synthase.

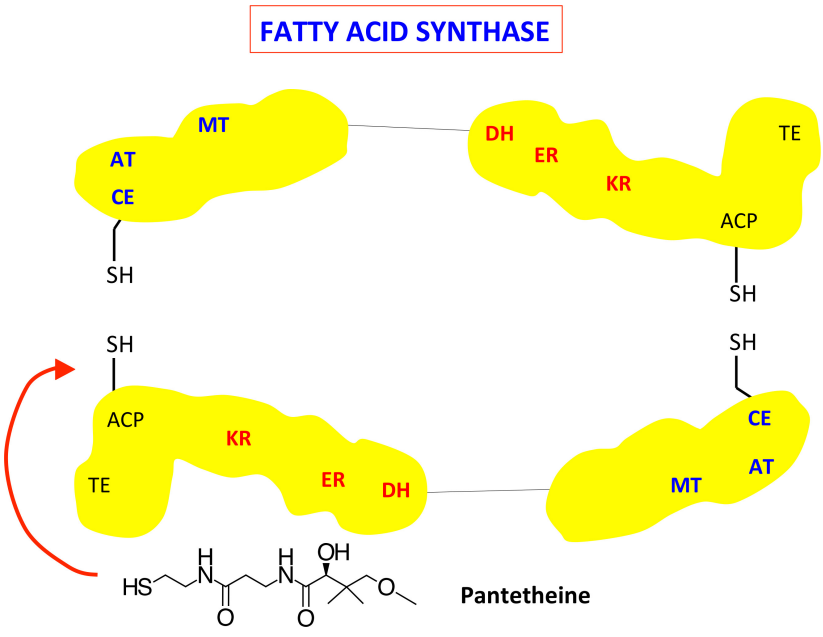
Acetyl CoA carboxylase is the rate limiting step in fatty acid synthesis

Fatty acids are synthesized from acetyl CoA and malonyl CoA. Acetyl CoA is converted into malonyl CoA by the action of acetyl CoA carboxylase, an enzyme that uses ATP and biotin as its co-factor. As acetyl CoA carboxylase is the rate limiting step in fatty acid synthase, it is regulated by insulin and glucagon. Insulin promotes the active, dephosphorylated form of the enzyme. Indeed, acetyl CoA carboxylase is the target of the pharmaceutical and weight loss industries.



Fatty Acid Synthase: A complex of multiple enzymatic activities

The fatty acid synthase (FAS) complex consists of two large dimeric proteins arranged in a head to tail fashion. Each subunit contains multiple enzymatic activities. Acyl carrier protein (ACP) is one biological activity contained within FAS. When covalently linked to pantatheine through a serine residue, apo ACP



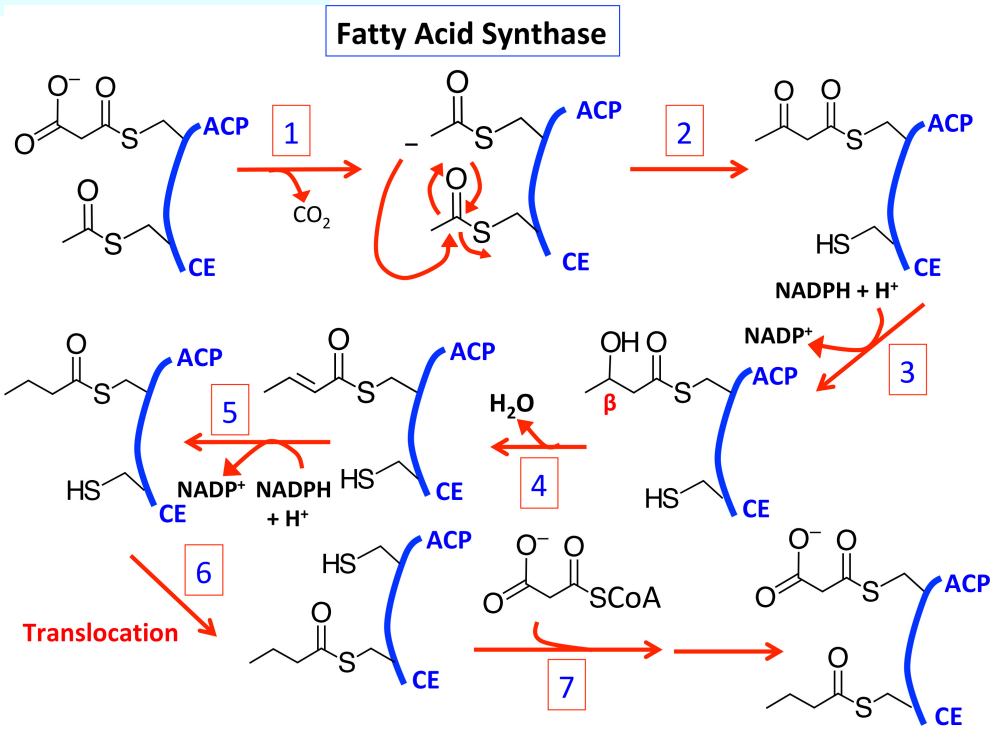
is converted into the holoprotein. This reaction is conducted by 4' phosphopantatheinyl transferase. Note the similarities between the pantatheine prosthetic group and Coenzyme A: they both contain sulfhydryl groups. The principle function of FAS is to generate palmitic acid, a 16-carbon saturated fatty acid ($C_{16:0}$). The net reaction for the synthesis of palmitic acid is:

8 Acetyl CoA + 7 ATP + 14 NADPH + $6H^+$ = $C_{16:0}$ + 14 $NADP^+$ + 8 CoASH + 6 H_2O + 7 ADP + 7 P_i.

Palmitic acid can undergo further oxidation and desaturation on the surface of the ER and the resulting lipids are incorporated into triglycerides along with glycerol.

FAS contains multiple enzymatic activities including acyl carrier protein (**ACP)** and condensing enzyme (**CE**). Both CE and ACP use their respective sulfhydryl groups to form covalent linkages with acetyl CoA and malonyl CoA, respectively. This occurs through the acetyl transferase (**AT**) and malonyl transferase (**MT**) enzymatic activities in FAS. Note that the carbons that will be incorporated into a fatty acid are linked to FAS through high energy thioester bonds.

The assembly line behavior of the complex starts with acetyl CoA and malonyl CoA and through a series of reactions, palmitic acid is generated.



Steps 1, 2: Decarboxylation of malonyl CoA bound to ACP through a thioester linkage generates a resonance stabilized carbanion. Note that the CO_2 released is the same one that was added on to acetyl CoA via acetyl CoA carboxylase. The resonance stabilized carbanion attacks the carbonyl carbon in the thioester linkage bound to condensing enzyme (CE). As a result the free –SH group of CE is restored and a 4-carbon thioester is attached to ACP.

Step 3: The β-ketoacyl-ACP reductase (KR) activity of FAS uses NADPH to reduce the beta carbonyl to the alcohol group. The NADPH is derived from malic enzyme and the pentose phosphate shunt.

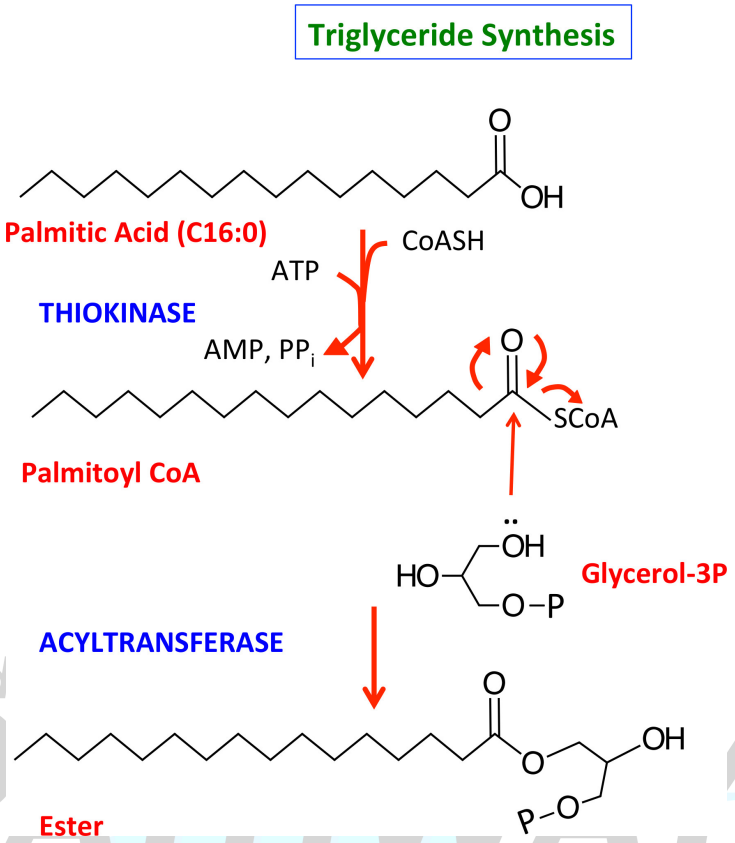
Steps 4, 5: Dehydration of the hydroxybutyryl group attached to ACP is conducted by the hydroxybutyryl-ACP-dehydratase activity of FAS. This generates a double bond that is further reduced to the alkyl state by the action of enoyl-ACP reductase. This completes the β reduction of the carbonyl group.

Step 6: Translocation of the reduced butyryl CoA group to condensing enzyme frees up acyl carrier proteins (ACP). This occurs through a transesterification reaction.

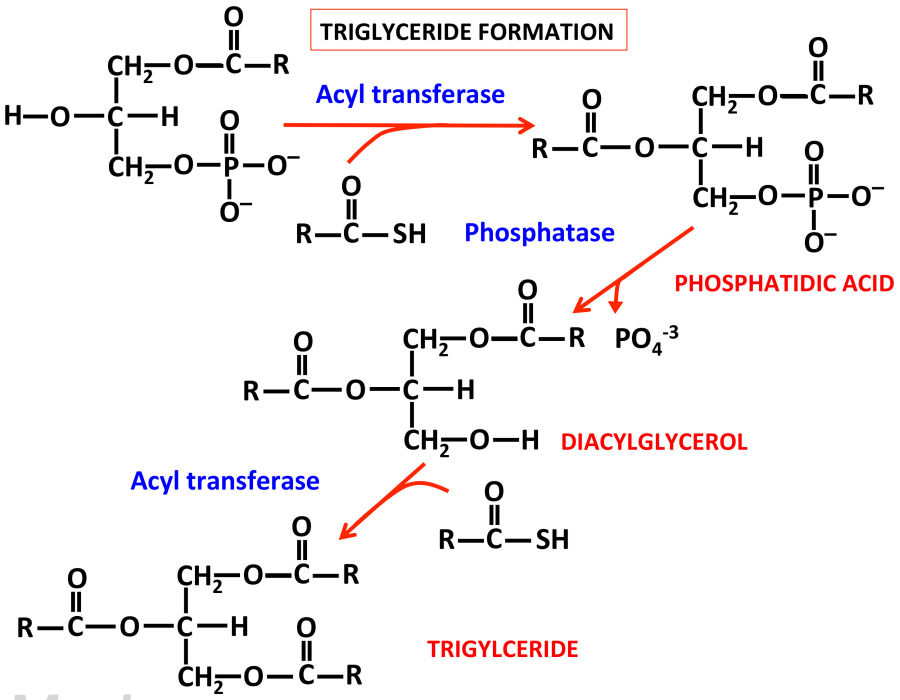
Step 7: A new molecule of malonyl CoA is brought in through malonyl transferase (MT) and loaded onto ACP through the creation of a new thioester bond. After decarboxylation, the 4-carbon fatty acid chain will grow to a 6-carbon chain. This cycle is completed when palmitic acid is generated. At this point, terminating enzyme (TE) hydrolyzes the thioester and releases palmitic acid.

Synthesis of Triglycerides

After release from The FAS machinery, palmitic acid is activated by thiokinase, a cytosolic enzyme that uses ATP and Coenzyme A to generate palimtoyl CoA, a thioester derivative of palmitic acid. This is the activated form of the fatty acid. Next, acyltransferase generates an ester through the combination of an alcohol group on glycerol-3-phosphate and the thioester, a nucleophilic substitution-elimination reaction that releases free Coenzyme A. This is shown below.

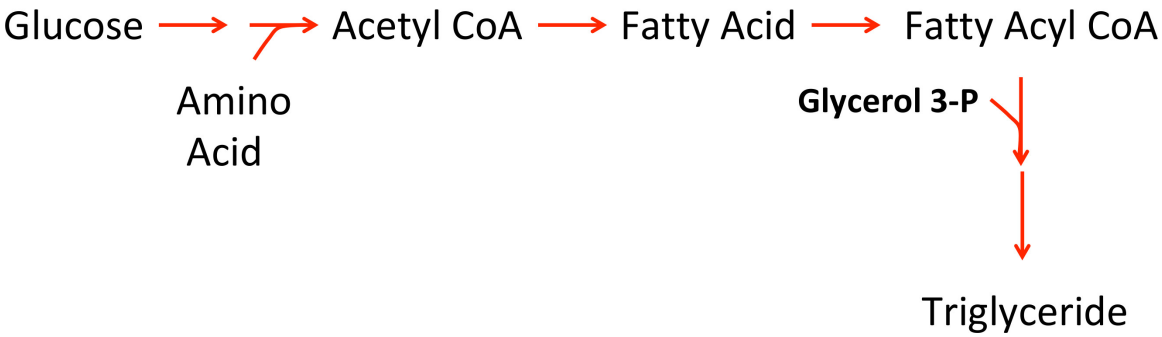


Addition of a second fatty acid via acyltransferase generates phosphatidic acid (PA; see below). For simplicity fatty acid alkyl groups are labeled with "R". PA serves as a substrate for a phosphatase that generates diacylglycerol, a diesterified molecule with one free OH group that is esterified for the third time through the action of acyl transferase. The triglyceride has been generated.



A schematic for the overall synthesis of triglycerides from acetyl CoA is shown below. Note that we have focused on the glucose as a source for fatty acids and triglycerides, but some amino acids that are converted into acetyl CoA (the ketogenic amino acids) are also substrates for fatty acid synthesis. This is covered in the AA/Proteins Testing Module at med-pathway.com.

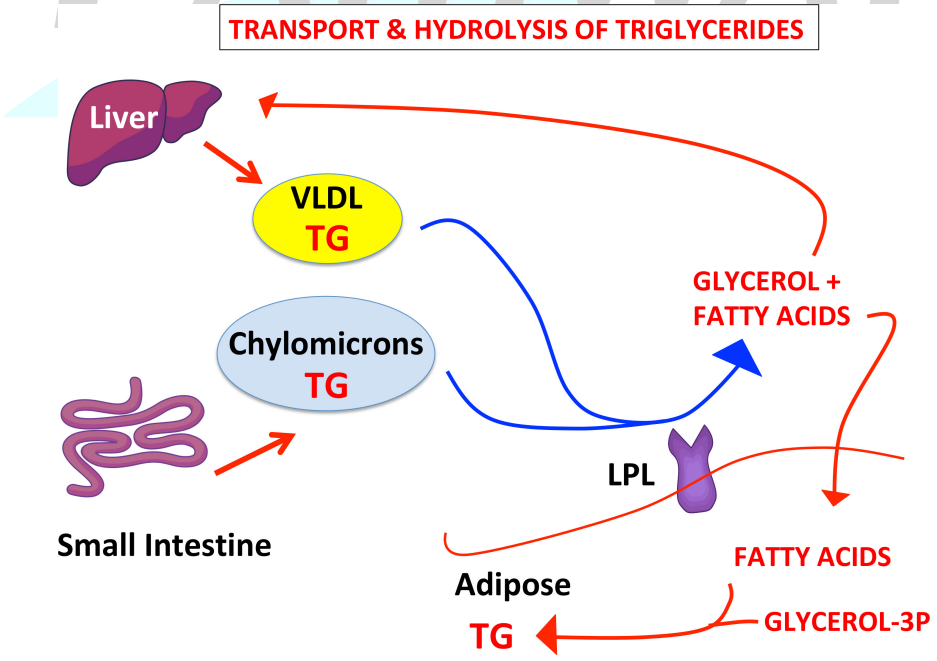
Schematic of Triglyceride Synthesis



Transport of fats and triglycerides

Due to low solubility, hydrophobic triglyceride lipids are packaged into lipoprotein particles that circulate in the serum. Two of these lipoprotein particles (chylomicrons and VLDL) are recognized by lipoprotein lipase (LPL), an adipose serum membrane enzyme that hydrolyzes triglycerides into fatty acids and glycerol, a three-carbon alcohol. LPL expression is stimulated by insulin. Triglycerides do not cross the adipose membrane, but rather are broken down at the adipose surface by LPL, transported across the membrane, and then re-synthesized inside adipose tissue. The glycerol produced from LPL circulates back to the liver where it is phosphorylated by glycerol kinase, a liver-specific enzyme.

The triglyceride is re-synthesized in the adipose tissue. Fatty acids transported into adipose cells are activated by thiokinase, generating a thioester derivative. The activated fatty acid is esterified to glycerol-3-phosphate by acyl transferase. Because glycerol kinase is only expressed in the liver, glycerol-3-phosphate is generated from the reduction of glyceraldehyde-3P, a glycolytic intermediate. Therefore, an active glycolytic pathway in the adipose tissue is required for the re-synthesis of triglycerides.

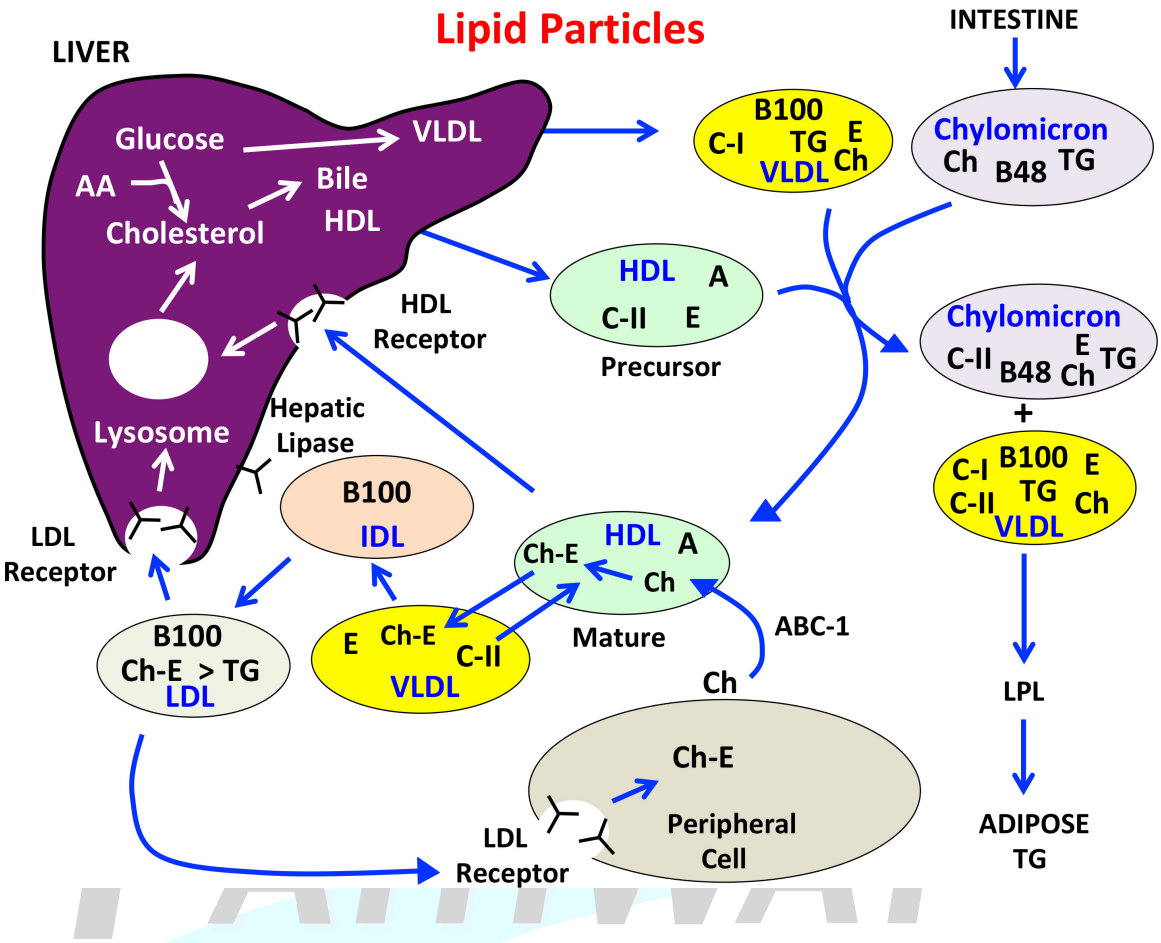


The biochemistry of lipoprotein particles

The biochemistry of lipid particles is a critical topic as its dysfunction is causal for dyslipidemias and cardiovascular disease. Thus, understanding "good" and "bad" cholesterol is a key topic in biochemistry and medicine. Stick with the various apoproteins and nomenclature and you will be an expert.

Triglycerides synthesized from excessively consumed carbon (sugars, fats, proteins) are packaged into very low density lipoprotein particles (VLDLs), but those fats and triglycerides consumed from various products (i.e. dairy) are packaged into chylomicrons in the intestine. In addition to triglycerides, these particles also transport cholesterol and fat soluble vitamins (D, A, K, E). Cholesterol, a lipid, and fat soluble vitamins are more thoroughly discussed in the Med-Pathway Organic Chemistry Module. In brief, cholesterol synthesis begins with acetyl CoA and proceeds through the mevalonate pathway. This 27 carbon molecule is essential for membrane fluidity.

Nascent VLDL particles contain cholesterol (Ch), triglycerides (TG), apoprotein B100 (ApoB100), Apo C-I, and Apo E. The nascent VLDL particle picks up Apo C-II and more Apo E from the high density lipoprotein (HDL) to generate a mature VLDL particle. The mature VLDL can now be



recognized by LPL where its triglycerides are hydrolyzed as discussed above. This is because Apo C-II is a co-factor for LPL. In an analogous fashion to VLDL particles, nascent chylomicrons also pick up apo C-II and Apo E from HDL. Upon hydrolysis of triglycerides by LPL, remnant particles are formed. The remnant donates Apo C-II back to the HDL and is then degraded by liver receptors that recognize Apo B48.

Precursor HDL particles are synthesized in the liver and contain Apo C-II (as discussed above), Apo A and Apo E. In addition to activating VLDL particles and chylomicrons, HDL receives cholesterol from peripheral tissue and takes it back to the liver. Excess cholesterol is removed from the cell membrane by the action of the ATP-dependent ABC-1 transporters. The cholesterol is esterified (Ch-E), generating a mature HDL particle. Once formed, HDL can be endocytosed in the liver and it can transfer cholesterol esters to the VLDL remnant in exchange for triglycerides and phospholipids. After further being subjected to LPL activity and cholesterol ester transfer as well as the enzymatic activity of hepatic lipase, the VLDL is converted into IDL, the intermediate density lipoprotein. IDL is a substrate for hepatic lipase and when the amount

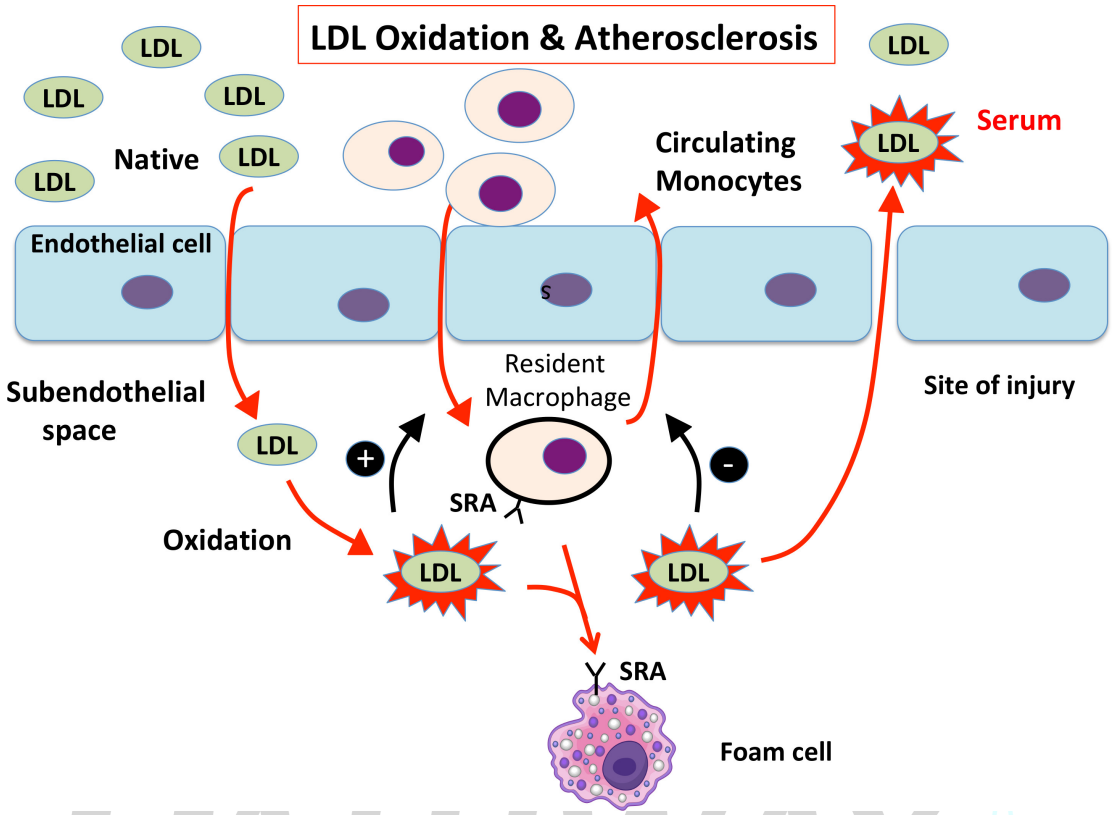
of cholesterol exceeds the amount of triglyceride in the particle, the IDL is converted into an low density lipoprotein (LDL). The primary function of the LDL particle is to deliver cholesterol to peripheral tissue. The LDL receptor recognizes Apo B100 and cholesterol is taken up through receptor-mediated endocytosis.

LIPID PARTICLE SUMMARY

PARTICLE	FUNCTION	APOPROTEINS
Chylomicron	Made in intestine from consumed dietary fats; substrate of LPL; delivers fat to adipose	ApoB48; acquires C-II and E
VLDL	Made in liver from excess dietary carbon; substrate of LPL; delivers fat to adipose	Apo100; acquires C-II and E
HDL	Made in liver; activates VLDL and chylomicrons; reverse cholesterol transport	Apo A, C-II, and E
IDL	Derived from VLDL, substrate of Hepatic lipase	ApoB100
LDL	Derived from IDL when amount of cholesterol exceeds the amount of triglyceride; delivers cholesterol to peripheral tissue; susceptibility to oxidation generates risk for atherosclerosis	ApoB100

Good and Bad cholesterol

In addition to taking excess peripheral cholesterol back to the liver for incorporation into bile and its ultimate deposition in feces, HDL also possesses antioxidant properties. This makes HDL "good cholesterol". In contrast LDL is highly sensitive to oxidation, a property that renders it as a potential risk for atherosclerosis. LDL can be oxidized on its lipid components (i.e. peroxides) as well as its amino acid side chains (i.e. aldehydes).



The basic model for the role of LDL in atherosclerosis is presented and shows that the biochemistry of LDL is intimately associated with the innate immune system. High levels of LDL (and low HDL levels) lead to adherence of circulating monocytes and increased entry of LDL particles into the sub endothelial space (i.e. intima). Entry of native LDL particles into the intima renders it susceptible to oxidation through a variety of mechanisms including the presence of free radicals and metals. Oxidized LDL influences the endothelial cells to release chemo attractants that encourage more monocytes to enter into the intima where they differentiate into resident macrophages. The oxidized LDL and its subsequent physiological effects create endothelial lesions, the site of formation of the eventual arterial plaque observed in cardiovascular disease.

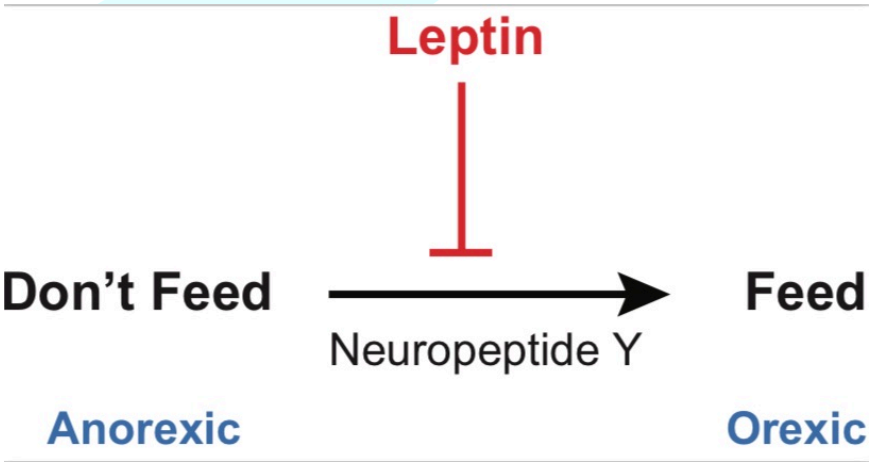
Macrophages have increased expression of the SRA scavenger receptor that can recognize oxidized LDL, but not the native form. As this receptor takes up LDL (i.e. cholesterol) and is not downregulated, macrophages are converted into "foam cells". Eventually the foam cells "burst" and deposit their materials "fatty streaks", the precursor to stenosis and blocked arteries.

Obesity

When excessive fat accumulates to the point of having adverse health effects, one is said to be obese. Clinically, obesity is defined in terms of the body mass index (BMI). The BMI is calculated by dividing the weight in kilograms over the square of the height in meters. Therefore the units are kg/m^2. A BMI value > 30 is considered obese.

Obesity is complex mix of behavior and biochemistry and metabolism. Feeding behavior is regulated by a complex set of hormonal interplay that regulates the hypothalamus. One set of factors involved in this is leptin and neuropeptide Y.

Leptin is a small protein hormone secreted by adipose tissue and is important for regulating body mass. Leptin-deficient mice are famously obese and have very strong appetites, meaning that leptin inhibits feeding behavior. One way this occurs is thought to function by inhibiting Neuropeptide Y (NPY), a factor that promotes feeding. Leptin therefore inhibits feeding behavior, meaning that reduced levels of leptin will increase the duration of hunger in animals. A decrease in leptin will increase the amount of food consumed and would be expected to increase body weight leading to obesity.

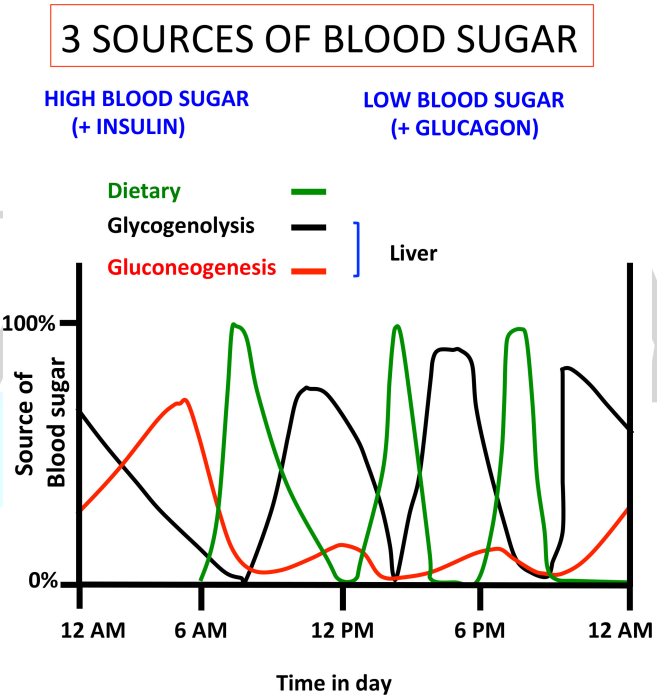


The fasted state

As insulin levels subside, the fasted state begins. Under these conditions, insulin is no longer available to inhibit the release of glucagon and epinephrine. As a consequence, catabolic pathways are promoted in order to maintain glycemia.

There are three sources for maintaining blood sugar levels:

1) food
2) hepatic glycogenolysis
3) hepatic gluconeogenesis



The image shows the relationship between the three methods for maintaining blood sugar through a 24 hour cycle. During sleep, only glycogenolysis and gluconeogenesis contribute to maintaining glycemia and providing glucose to peripheral tissues, especially the brain.

Glycogenolysis

Although multiple tissues can break down glycogen, only the liver releases free glucose for utilization by other tissue. This is because the liver contains an enzyme that de-phosphorylates G-6P, generating free glucose that can exit the cell into circulation.

One key enzyme in degrading glycogen is phosphorylase. This enzyme is regulated via posttranslational modification (phosphorylation) as well as trough allosterism (i.e. activated by AMP and inhibited by ATP). Phosphorylase uses an inorganic molecule of phosphate (P_i) as a nucleophile to break the 1, 4 glycosidic bonds of glycogen.

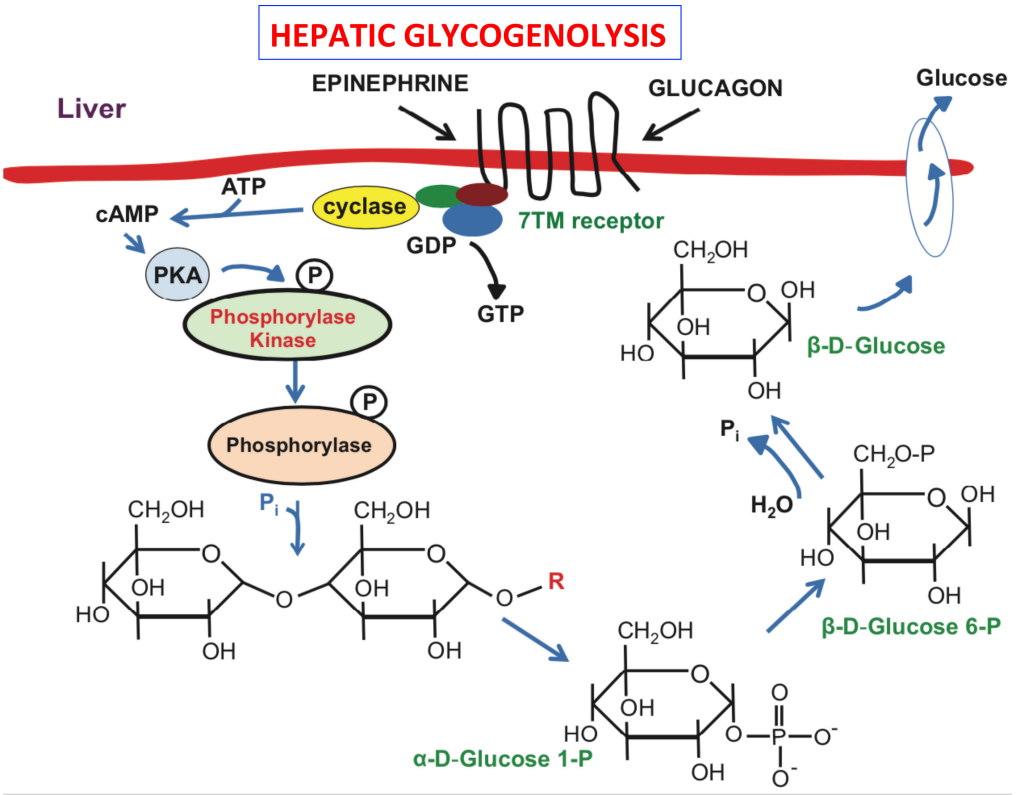
Glycogen also contains branched, 1, 6 glycosidic linkages that are cleaved by debranching enzymes (not shown). By using phosphate instead of water as a nucleophile, phosphorylase performs "phosphorolysis", a reaction that generates glucose 1-P upon cleavage. Thus, the glucose molecule is already activated upon its liberation from the glycogen polymer. That is, no ATP is required to activate glucose. The net equation for glycogenolysis is:

Glycogen $_{(N)}$ + P_i = Glucose 1-P + Glycogen $_{(N-1)}$

The liberated glucose 1-P is in equilibrium with glucose 6-P and this can be used in hepatic glycolysis. However, unlike skeletal muscle, the liver expresses glucose 6-phosphatase. By dephosphorylating glucose 6-P to glucose, the free glucose is now capable of leaving the liver and entering circulation. Here it circulates to needy tissues, such as an exercising muscle.

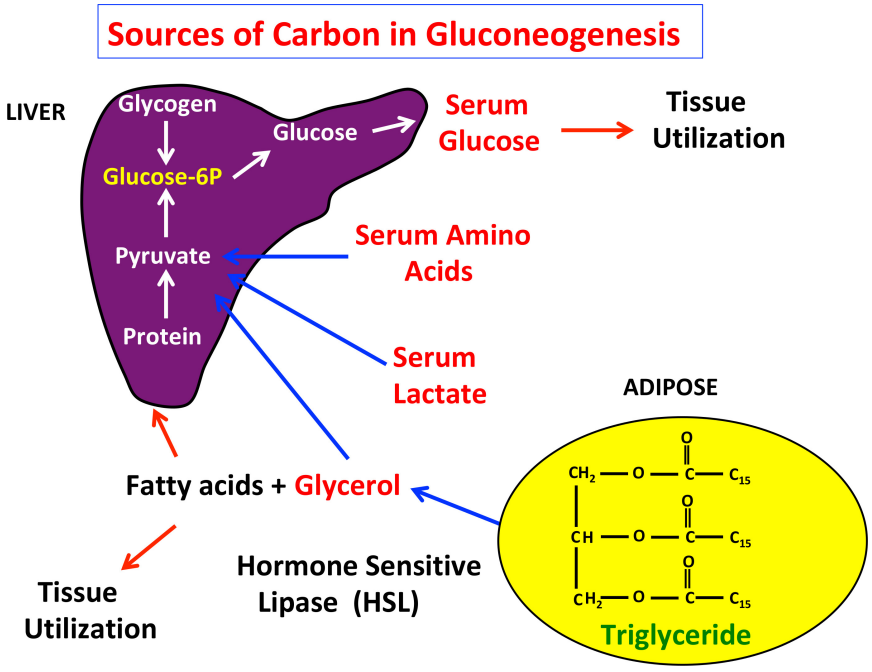
Activation of phosphorylase

In the fasted state, both glucagon and epinephrine are released (cortisol too). Through the use of 7-transmembrane (7TM) spanning receptors linked to heterotrimeric GTP-binding protein signaling, adenylyl cyclase is activated. This enzyme converts ATP into cAMP, a second messenger signaling molecule that allosterically activates protein kinase A (PKA). In this case, the regulatory subunits of PKA dissociate and release the catalytic core of PKA. This initiates a phosphorylation cascade: PKA phosphorylates phosphorylase kinase and this phosphorylates and activates phosphorylase. What a tongue twister!



Gluconeogenesis

As hepatic glycogen levels drop, gluconeogenesis begins. Gluconeogenesis is the process of de novo synthesis of glucose. Glycogen breakdown and gluconeogenesis occur in a staggered temporal fashion as shown above. Two major ingredients are required for gluconeogenesis: carbon skeletons and energy.



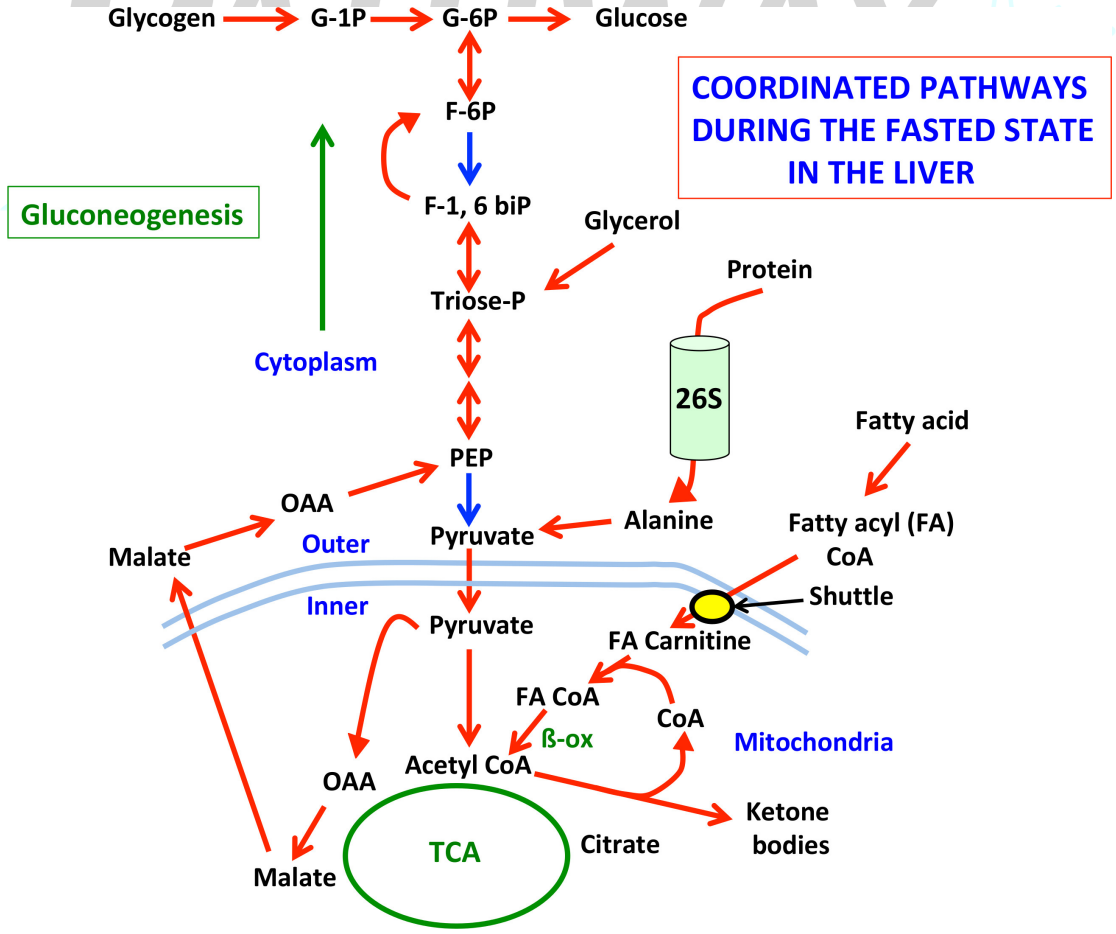
The carbon is derived from several sources as shown. Note that they are ultimately converted into pyruvate. The energy requirement

for gluconeogenesis can readily be seen from the net reaction for gluconeogenesis:

2 Pyruvate + 2 NADH + 6 ATP = Glucose + 2 NAD+ + 6 ADP + 6 P_i

As shown below, multiple pathways are coordinated during gluconeogenesis. The energetic requirement is largely satisfied by the oxidation of fats (β-oxidation), meaning that gluconeogenesis is coupled to fatty acid oxidation. This process is stimulated by epinephrine as discussed below. Further, contrary to what appears from the net reaction, gluconeogenesis is technically not the opposite of glycolysis. This is due to the thermodynamically irreversible steps in glycolysis.

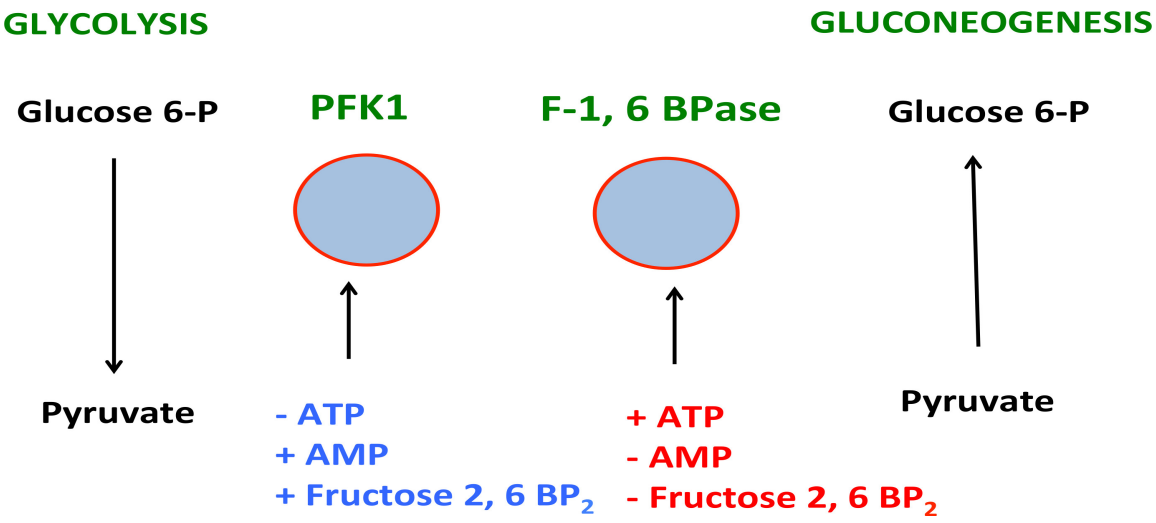
Pyruvate is the major carbon substrate used in hepatic gluconeogenesis, a pathway that synthesizes glucose through the reversal of many, but not all, of the steps in glycolysis. Note the blue arrows show the two irreversible reactions of glycolysis: the conversion of F-6P to F-1, 6biP and PEP to Pyruvate. Recall that glycolysis is an oxidative process that has a net negative free energy. The net reversal of this process (gluconeogenesis) would therefore be expected to



be endergonic. This is why gluconeogenesis requires ATP and NADH: energy is required to to circumvent the irreversible steps of glycolysis.

Hepatic coordination of gluconeogenesis and glycolysis

In hepatic tissue, gluconeogenesis is coordinated with glycolysis such that both processes do not occur at the same time. Such a futile cycle would consume energy and fail to generate any net metabolic products. One major method for preventing futile cycles can be seen through the reciprocal regulation of PFK1 and fructose 1, 6 biphosphatase (F-1, 6 BPase), its gluconeogenic counterpart.

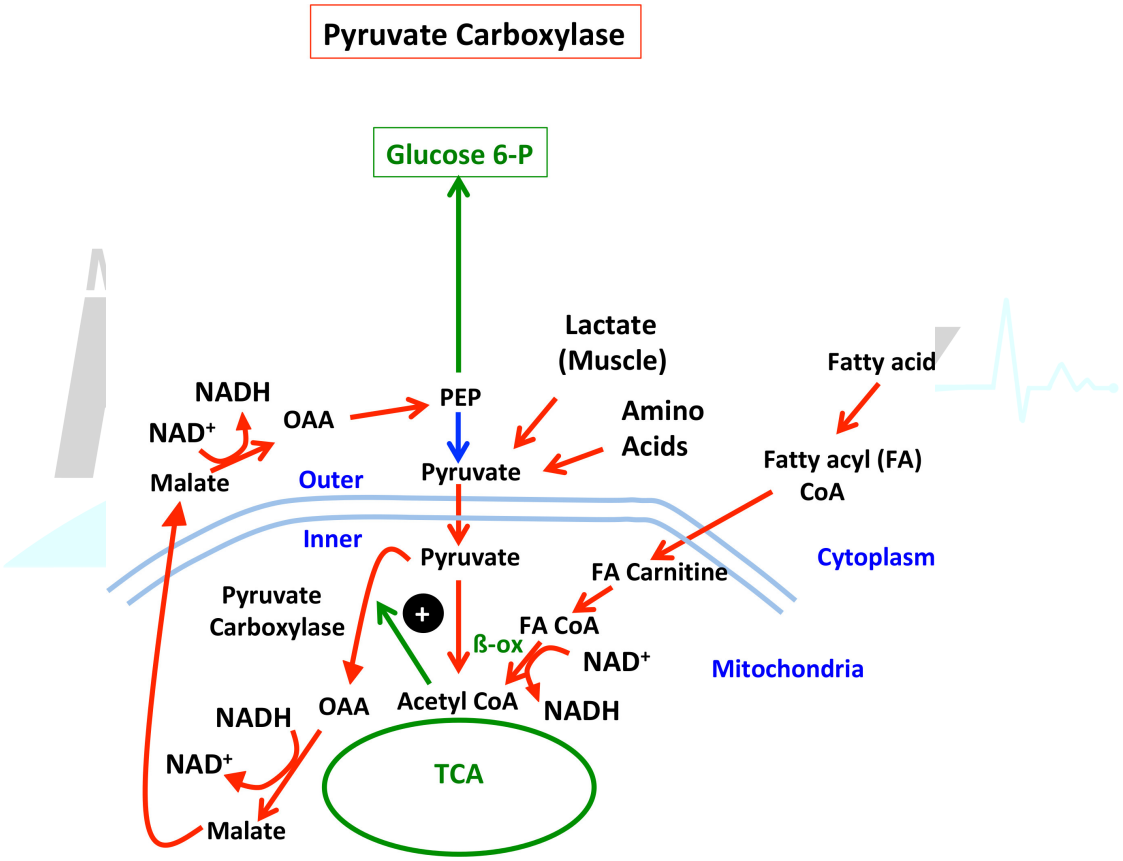


Note that the regulators that activate the gluconeogenic enzyme F-1, 6 BPase inhibit the corresponding the glycolytic enzyme PFK1. Observe that high energy charge (ATP/ADP + AMP) is required for the liver to produce glucose via gluconeogenesis. The bulk of this energy is derived from fatty acid catabolism.

Appreciate that gluconeogenesis occurs in response to high glucagon/low insulin (i.e. fasting, exercise). Therefore, as gluconeogenesis is occurring in the liver under a state of high energy charge, peripheral tissues such as the brain and the muscle might be in a relatively low energy state. Thus, the liver, acting as an altruistic organ, will provide the glucose these tissues need. We will discuss this below with respect to the "Cori cycle", an MCAT essential.

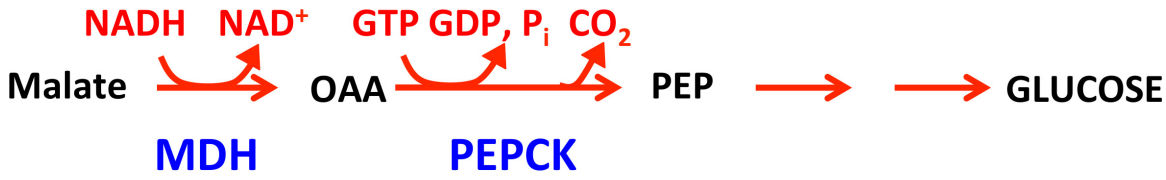
Pyruvate carboxylase: bypassing thermodynamic reversibility

During exercise or the fasted state, amino acids (derived from protein) and lactate are converted into pyruvate in the liver. In parallel, fatty acid oxidation produces acetyl CoA and reducing power (i.e. NADH). Acetyl CoA allosterically activates pyruvate carboxylase and produces oxaloacetate (OAA). In the fasted state, the equilibrium between malate and OAA is in the direction of malate, a precursor carbon source for gluconeogenesis. This is because the high levels of NADH drive the conversion of OAA to malate through Le Chatlier's principal produced via fatty acid oxidation in the mitochondria.



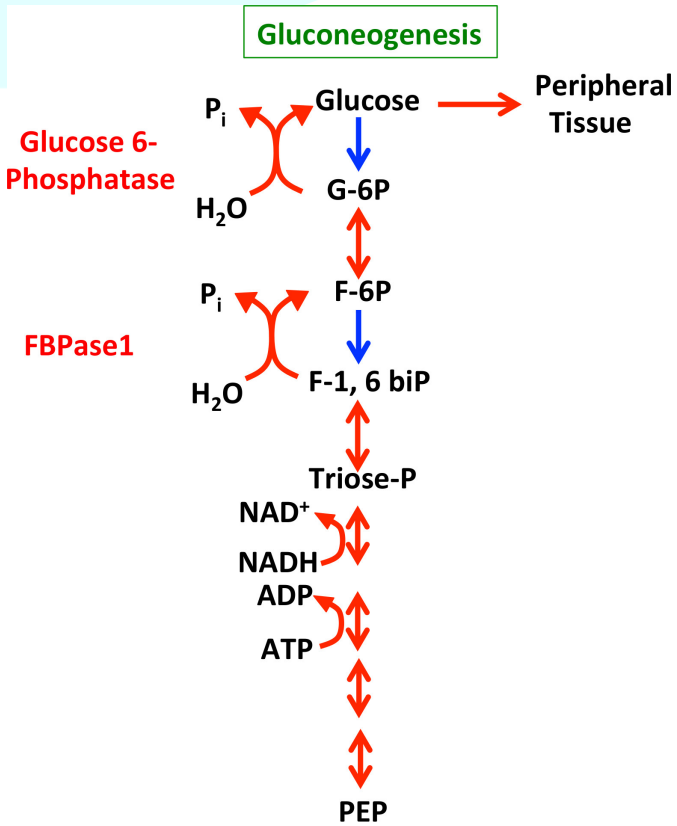
When malate levels malate build up, they are transported out of the mitochondria and are converted back into OAA by cytoplasmic malate dehydrogenase. Although the TCA is slowed down, it is still operative. The OAA is converted into PEP by PEP carboxykinase, an enzyme that hydrolyzes a high energy bond in GTP. As GTP is interconvertible with ATP, GTP is not part of the final equation of gluconeogenesis.

PEP Carboxykinase



What is the fate of the acetyl CoA derived from fatty acid oxidation? In general, it is oxidized through the TCA cycle. However, as we will discuss in detail below, if the levels of acetyl CoA exceed the capacity of the TCA cycle to oxidize it, then ketone bodies will be formed.

Gluconeogenic formation of PEP circumvents the thermodynamically irreversible step of pyruvate kinase. Conversion of PEP into glucose is, in many ways, the reversal of glycolysis. Notably, ATP and NADH are required prior to the generation of the Triose-P sugars: dihydroxyacetone phosphate (DHAP) and glycerol 3-phosphate (G-3P). Two phosphatase enzymes (FBPase 1 and Glucose 6-Phosphatase) specific to gluconeogenesis are responsible for circumventing the other two thermodynamically steps in

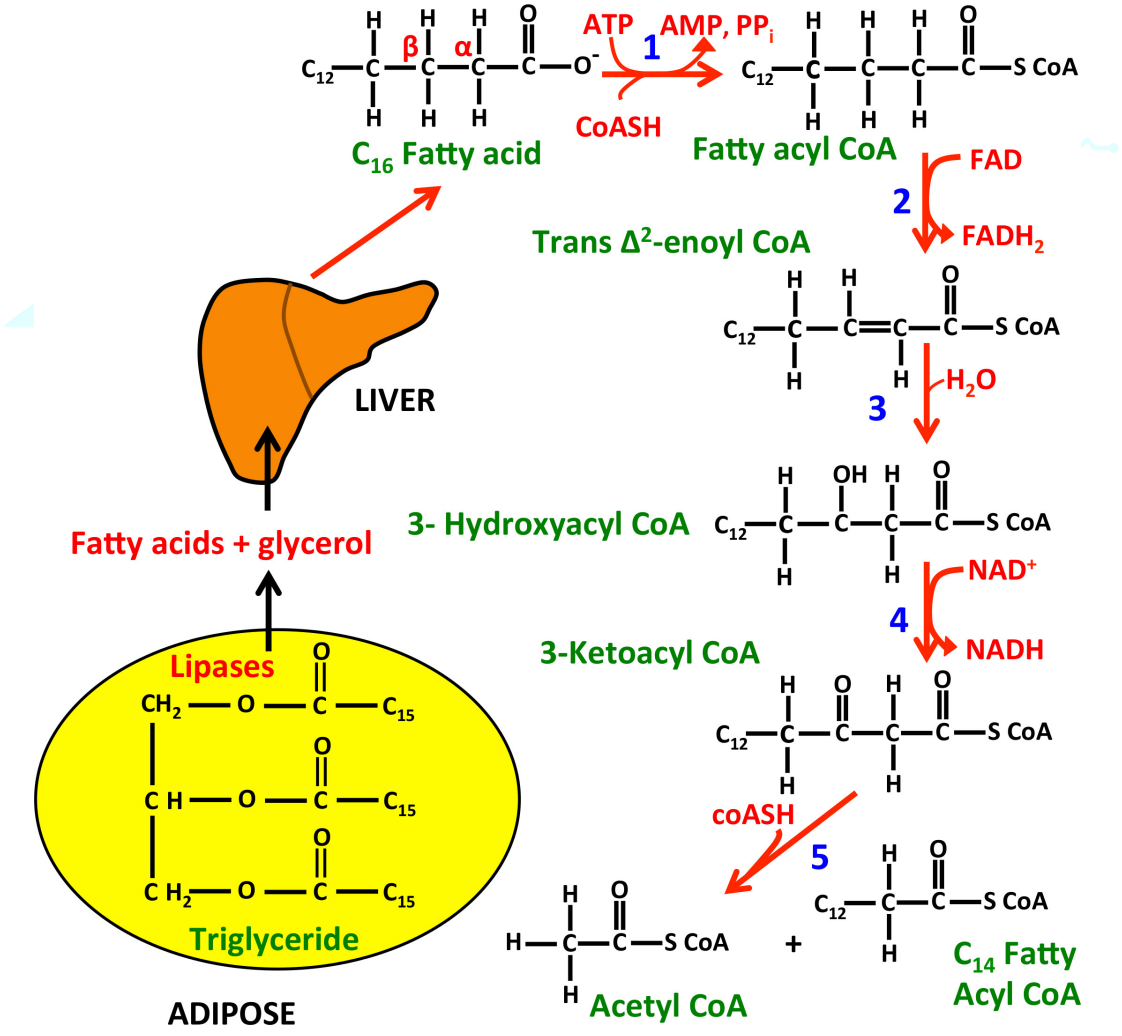


gluconeogenesis (blue arrows in image). This results in the formation of free glucose, the end product of gluconeogenesis.

Degradation of triglycerides and fatty acids during gluconeogenesis.

We have discussed the general role of fatty acids in providing energy during gluconeogenesis. We will examine this in more detail.

Low blood sugar promotes the release of epinephrine from the adrenal glands. Adipose triglycerides are mobilized via hormonal activation of hormone sensitive lipase (HSL) as shown. As is the case for phosphorylase kinase, HSL is also a substrate for protein kinase A. Phosphorylation of HSL activates the enzyme. As a consequence, HSL hydrolyzes triglycerides and releases the previously sequestered fatty acids. The fats circulate (bound to albumin) and are transported to host tissues capable of performing β-oxidation (i.e. those that have oxygenated mitochondria).



Activated fatty acids between 12-16 carbons are imported into the mitochondria through the carnitine shuttle. The shuttle will be discussed below in the context of ketone body formation. Larger fatty acid chains are catabolized in the peroxisomes.

Numerous tissues can oxidize fatty acids for energy. One notable exception is the brain; fatty acids cannot cross the blood-brain barrier and as a consequence, the brain relies on glucose as well as ketone bodies.

Fatty acid metabolism is an important topic on the MCAT as well as in medicine. Fatty acid catabolism occurs in the mitochondria and requires oxygen. Fatty acids derived from adipose tissue are catabolized in various tissues such as the liver. The process is summarized:

Step 1. The fatty acid is initially activated to generate a fatty acyl CoA.

Step 2. The β carbon is oxidized to the level of the alkene.

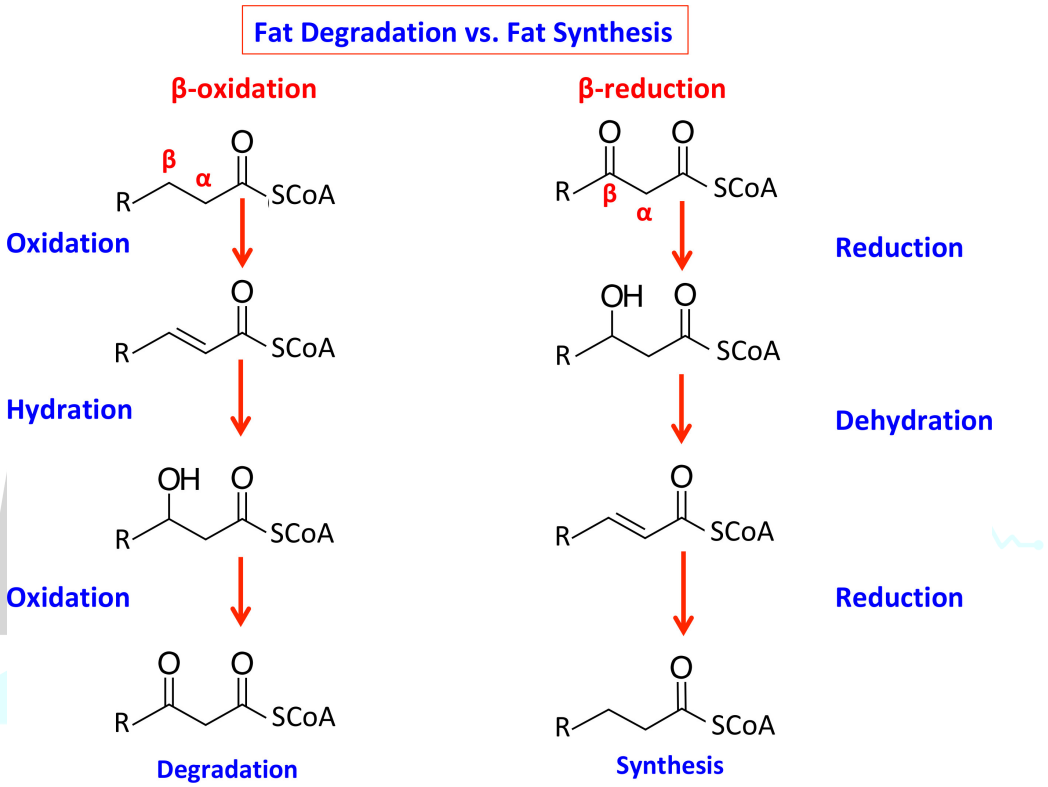
Step 3. Addition of water (hydration) to the alkene creates 3-hydroxyacyl CoA, an alcohol intermediate.

Step 4. Oxidation of the alcohol generates a ketone.

Step 5. A β ketothiolase enzyme uses CoASH to generate Acetyl CoA as well as a fatty acid of N-2 carbons.

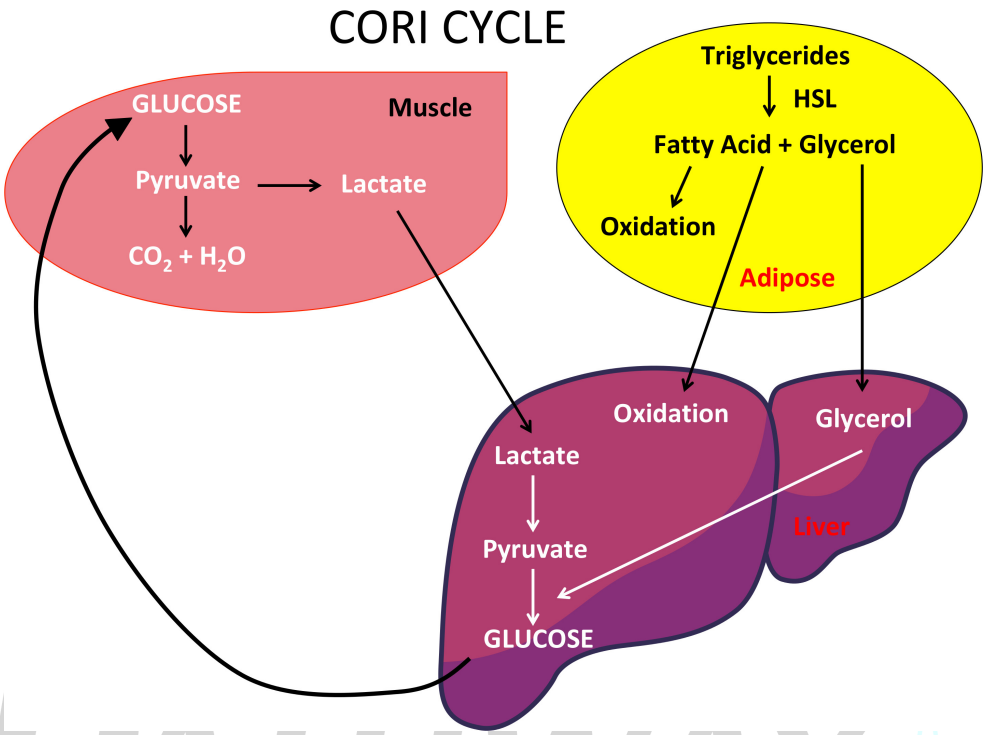
Comparing Fatty acid degradation and synthesis

We have now seen both fatty acid synthesis and fatty acid degradation. Examine the image below and compare the two processes and recognize that they are essentially reciprocal processes. However, fatty acid oxidation occurs in the mitochondria, but fatty acid synthesis occurs in the cytoplasm.



Cori cycle

Lactate and glycerol are significant carbon sources for gluconeogenesis. Lactate is often derived from muscle in the anaerobic state (i.e. exercising muscle), and is shipped to the liver where it is converted back into pyruvate via lactate dehydrogenase. Pyruvate is a substrate for gluconeogenesis. Once formed, hepatic glucose is released into circulation and taken up by muscle. As the muscle oxidizes the glucose into pyruvate and lactate, the Cori cycle is completed. In addition, the adipose tissue is supplying energy for the liver in the form of ATP and NADH derived from fatty acid oxidation. Further, the glycerol derived from triglyceride hydrolysis is shipped back to the liver where it is phosphorylated and incorporated into the gluconeogenic pathway after oxidation into a triose-phosphate.



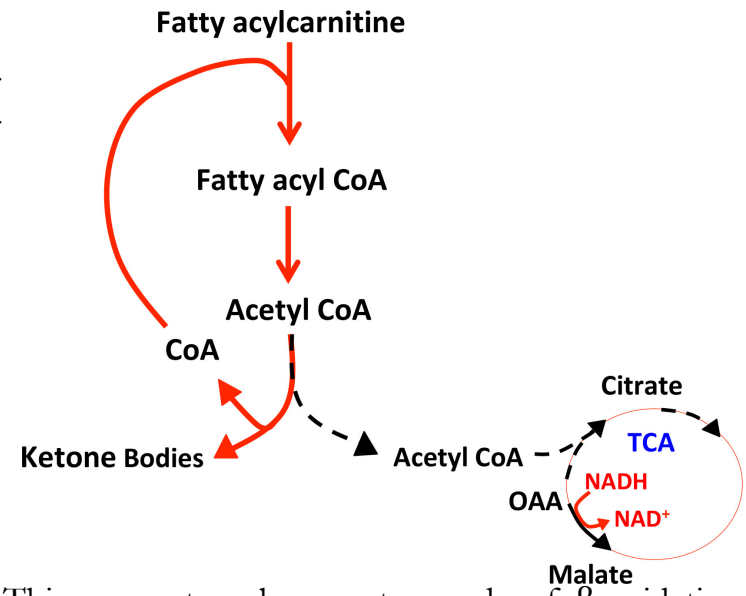
Ketone bodies

Ketone bodies are synthesized as a consequence of excess fatty acid breakdown. This occurs during fasting, exercise as well as in the diabetic state, particularly type I diabetes. Ketone bodies are synthesized from acetyl CoA only in the liver under conditions of low blood sugar.

Large chain fatty acyl CoA molecules (C_{12}-C_{18} carbons) are transported from the cytoplasm into the mitochondria through their conversion into fatty acylcarnitine. Therefore, carnitine is a key player in energy formation. Once inside the mitochondrial matrix, free CoA is used to re-generate fatty acyl CoA molecules that are poised for conversion into acetyl CoA through β-oxidation.

When the rate of β-oxidation is high, free mitochondrial CoA becomes sequestered in the acetyl CoA molecule. High levels of acetyl CoA slow the TCA cycle down, as its enzymes cannot keep up with the substrate levels. Further, as a consequence of increased fatty acid oxidation, the high levels of

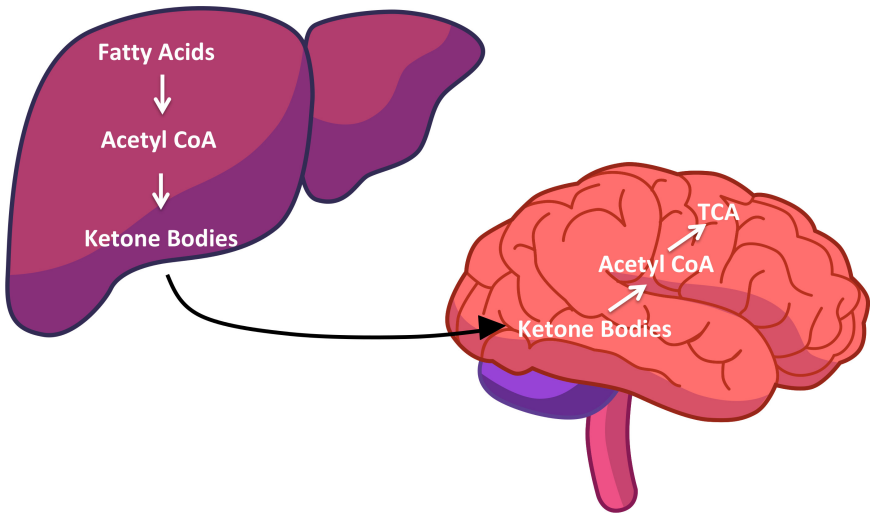
NADH drive OAA into malate for gluconeogenesis: the reverse reaction of TCA. The conversion of OAA to malate is Le Chatlier's principle in action.



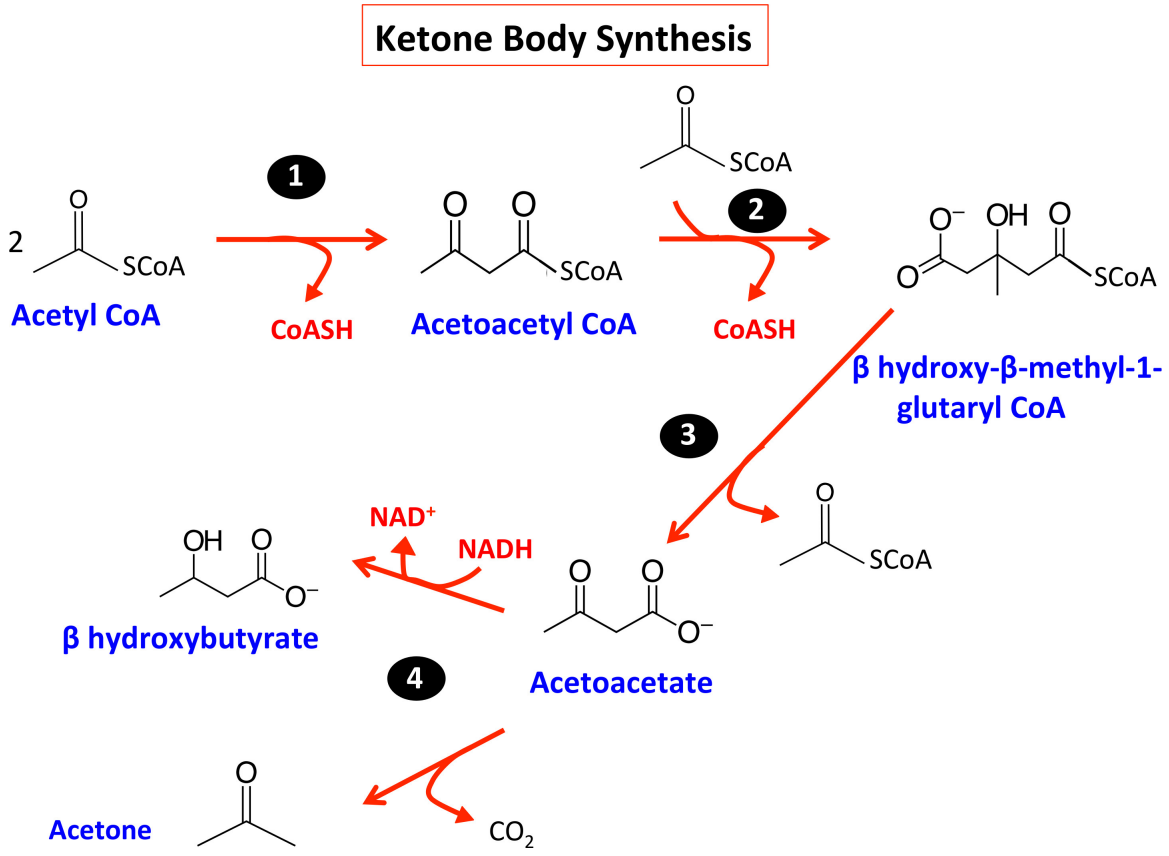
In addition, when the rate of β-oxidation is high, the availability of free Coenzyme A (it cannot cross the mitochondrial membrane) for the carnitine shuttle system is reduced. This prevents subsequent rounds of β-oxidation from occurring. In order to liberate free CoA, acetyl CoA is converted into ketone bodies in the liver. Therefore, the carnitine shuttle is key for understanding why ketone bodies are made: making ketone bodies liberates CoA for the shuttle and the carbon skeletons are used by peripheral tissue for energy.

Ketone body synthesis and utilization

Ketone bodies are synthesized only in the liver (mitochondrial matrix) and are utilized by peripheral tissues such as the brain. Therefore, the liver expresses the enzymes that are used to generate ketone bodies, but the peripheral tissues express the enzymes required for their utilization and subsequent conversion back into acetyl CoA for oxidation into the TCA cycle for energy generation.



The liver begins the synthesis of ketone bodies from 2 molecules of acetyl CoA. As seen in reaction # 1 in the figure, the two acetyl CoA molecules generate acetylacetyl CoA through a Claissen condensation mechanism performed by the β-ketothiolase enzyme. Reaction #2 is the rate limiting step in ketone body synthesis and is catalyzed by HMGCoA synthase, an enzyme that generates the intermediate β hydroxy-β-methyl-1-glutaryl CoA. In step #3, HMGCoA cleavage enzyme releases a molecule of acetyl CoA and forms acetoacetate, the first ketone body. The fate of acetoacetate is seen in step #4. Spontaneous decarboxylation of acetoacetate generates acetone, a volatile substance often detected in the breath of diabetics. Further, given the high reducing atmosphere of the mitochondrial matrix due to fatty acid oxidation and the creation of NADH, acetoacetate is converted into β hydroxybutyrate.



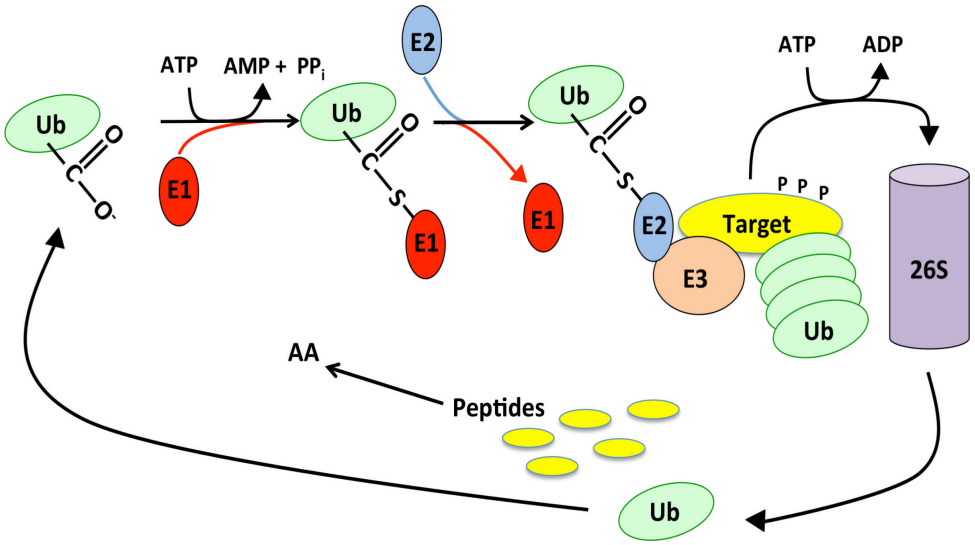
Protein and Amino acid metabolism during the fasted state.

The amino acid pool is derived from a combination of several factors including dietary consumption, in vivo synthesis (non-essential amino acids such as tyrosine, serine, and glycine), as well as protein degradation. Genes required for protein synthesis (i.e. ribosomal proteins) are upregulated by insulin. In contrast, those genes required for protein degradation are often upregulated by cortisol, an adrenal steroid hormone secreted in response to stress (i.e. low blood sugar). Although most commonly associated with muscle, protein degradation occurs in additional tissues (i.e. liver).

A major method of protein degradation is the ubiquitin (Ub) system. Ub, as its name implies, is a ubiquitous protein whose major function is in protein degradation. The Ub system targets proteins for degradation by the cytoplasmic 26S proteasome. Both the expression of Ub and proteosomal components is regulated by cortisol.

Proteins destined for degradation are often phosphorylated on specific residues that constitute a "degron". The Ub degradation machinery recognizes such degrons. For this, the ubiquitin protein is activated at its carboxyl C-terminus through a reaction involving a cysteine residue of an E1 activating enzyme (see image). After transfer to an E2 enzyme, an associated E3 ligase binds to the degron of a selected target where it coordinates ubiquitylation between the carboxyl group of ubiquitin and a substrate lysine residue in the target protein. Multiple ubiquitin moieties on a target are subsequently recognized by the proteasome, a 26S complex of proteins that degrades target proteins into

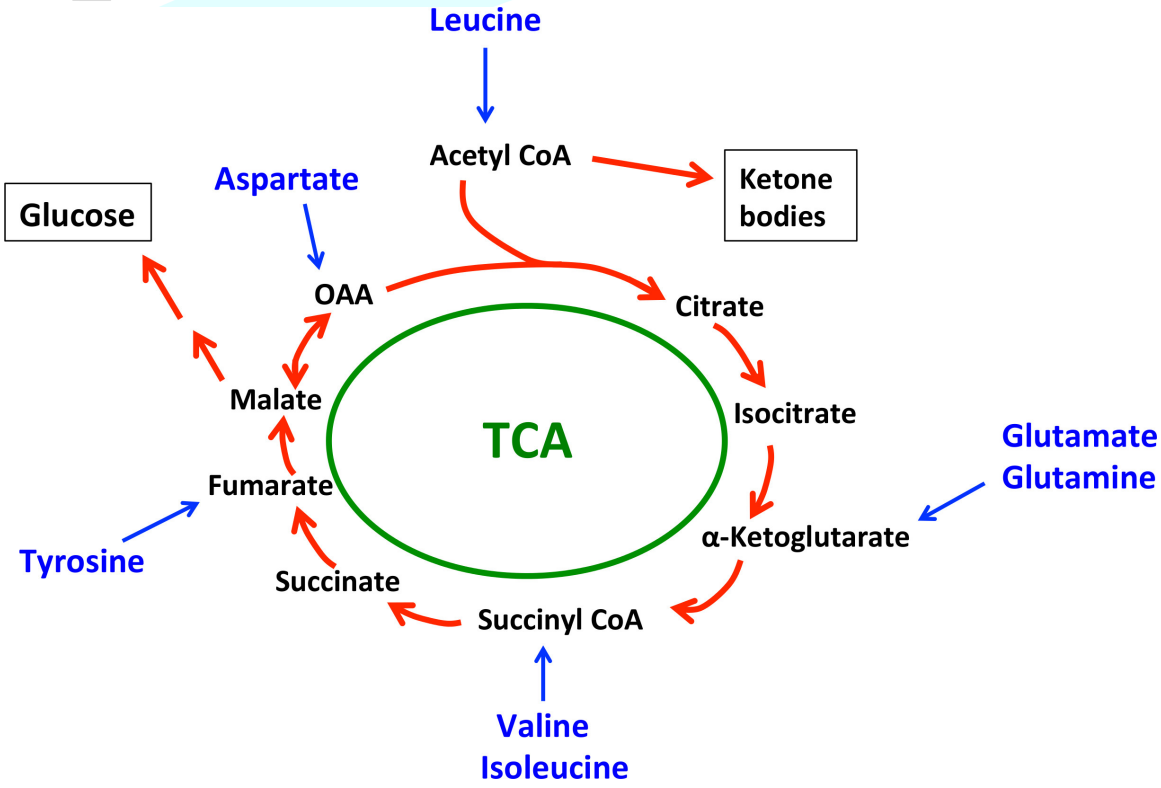
Ubiquitin Degradation of Proteins



constituent peptides and amino acids.

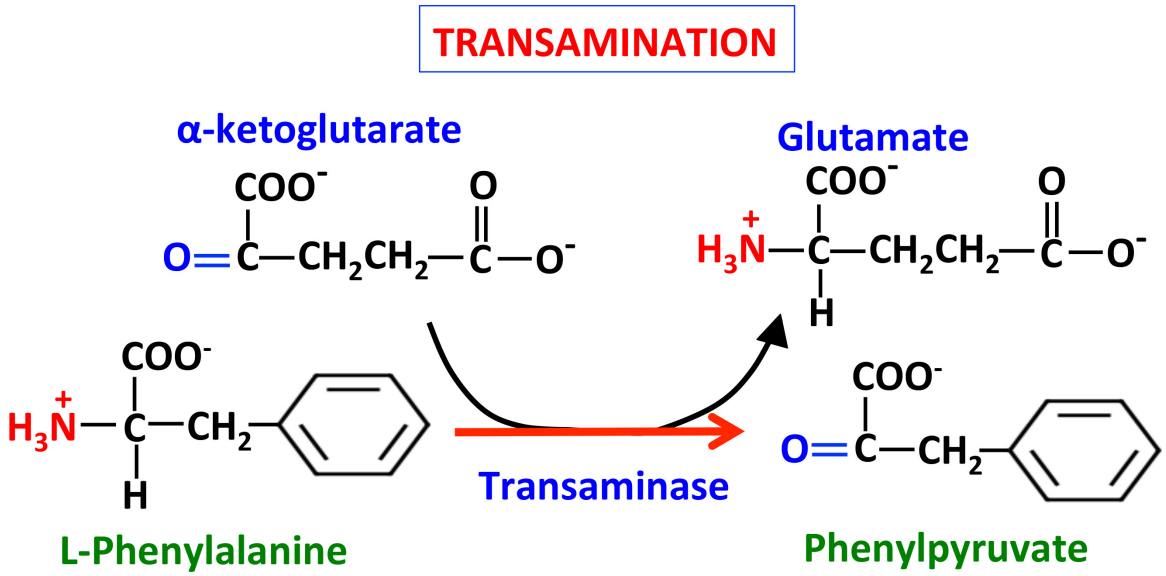
Glucogenic and Ketogenic Amino Acids

What is the fate of amino acids derived from protein breakdown during the low blood sugar state? Depending on their side chain chemistry, amino acids are defined as either glucogenic and/or ketogenic. Some amino acids can be both glucogenic and ketogenic as their catabolism produces multiple products. After processing of the amino function (NH_3^+) of the amino acid through the urea cycle (as discussed below), the carbon skeletons are used as either an energy source in gluconeogenesis (glucogenic) or in ketone bodies (ketogenic). Those amino acids that are catabolized into acetyl CoA (i.e. leucine) are considered ketogenic as they can produce ketone bodies in the liver. In contrast, those amino acids that are catabolized into products that can be converted into malate, a gluconeogenic precursor, are considered glucogenic. This includes aspartate and tyrosine as shown.



Amino acid transamination

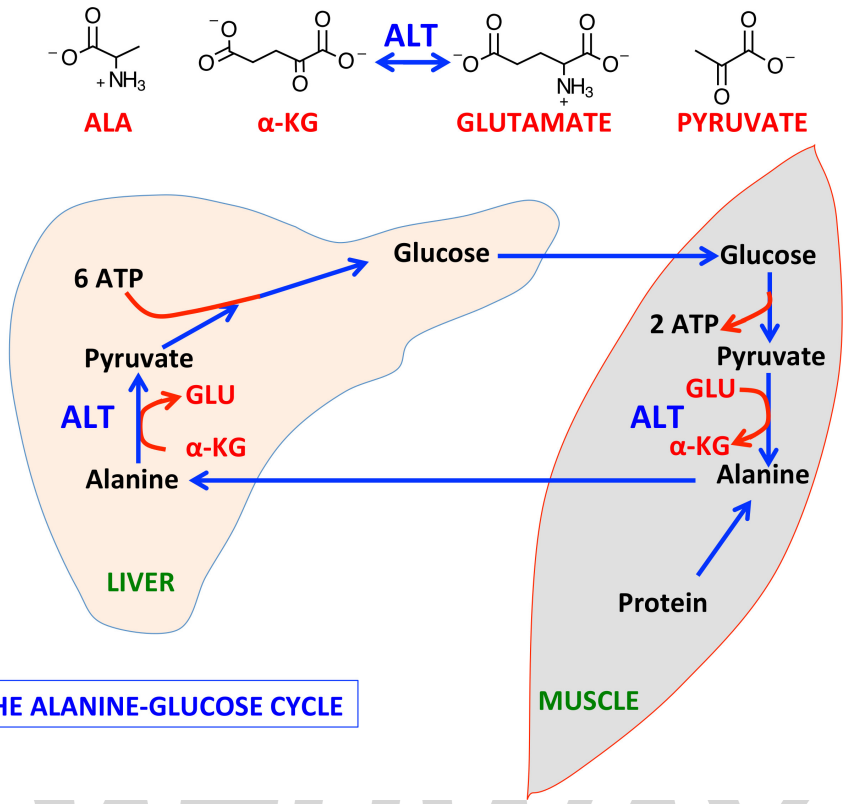
The first step in amino acid catabolism is to remove the α amino group through transamination. These reactions are performed via pyridoxal-dependent transaminase enzymes. The transamination of phenylalanine into phenylpyruvate is shown below. Through the transfer of the amino group in phenylalanine to α-ketoglutarate, glutamate is created along with the cognate α-keto acid of phenylalanine: phenylpyruvate. Therefore, glutamate is a carrier of α amino groups. (Glutamine is also a carrier of amino groups.) The cognate α keto acids derived from transamination are further metabolized (i.e. through gluconeogenic and ketogenic pathways).



The Alanine-Glucose Cycle

The conversion between alanine and pyruvate is an example of amino acid transamination that occurs during the physiological low energy state. This is best illustrated through the alanine-glucose cycle.

In muscle, some of the pyruvate derived from glycolysis can be converted into alanine (lactate too) via a reaction conducted by alanine transaminase (ALT). Further, alanine derived from muscle protein degraded in the fasted, low energy state is shipped to the liver. In the muscle ALT performs the "reverse" transamination reaction. Thus, the carbon skeletons of alanine are used to generate glucose in the liver. By shipping off alanine to the liver, the carbon



skeletons from this amino acid are turned into glucose via the gluconeogenic pathway. In this sense, the muscle can be seen as "storing" proteins and using their carbon atoms for conversion into glucose.

In the liver, ALT removes the amino nitrogen group from alanine, re-generating hepatic pyruvate, a key precursor to glucose in hepatic gluconeogenesis. The amine is transferred onto alpha-ketoglutarate, creating glutamate.

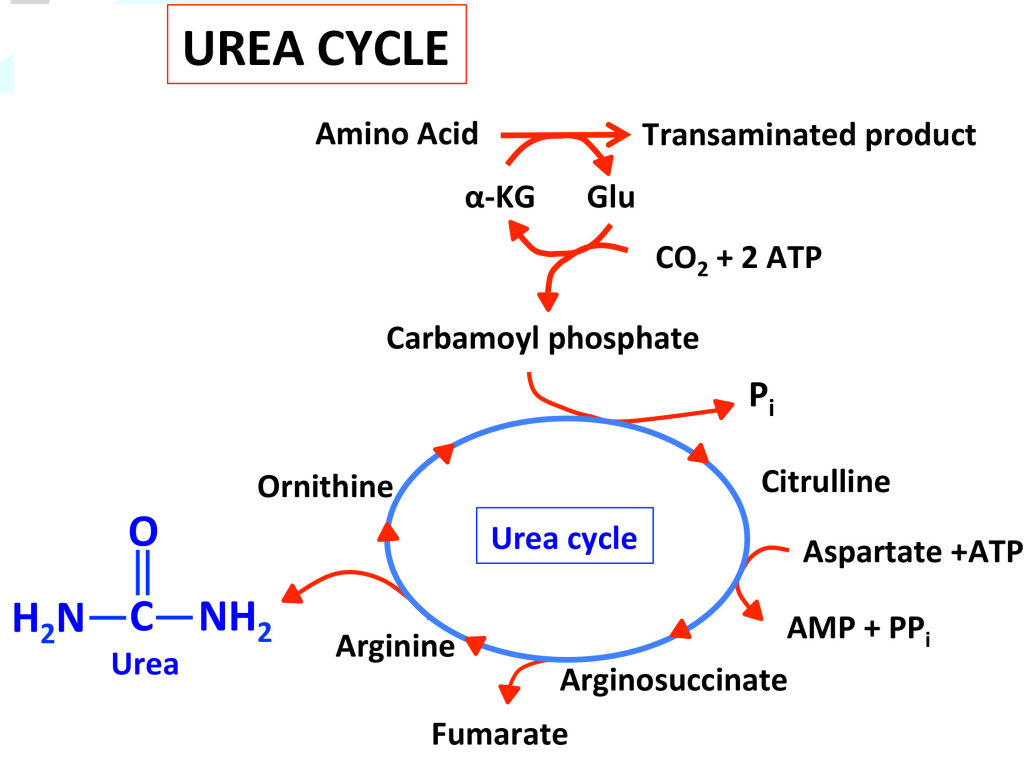
Always think of glutamate (and glutamine) as nitrogen carriers. As such, the ammonia group is released via glutamate dehydrogenase where it is eventually incorporated into the excreted product urea through the urea cycle (discussed below). Note the ALT enzymes catalyze the reciprocal reactions in the liver and muscle.

ALT is an important enzyme that is often measured in blood tests in patients. People with liver disease (i.e. cancer, cirrhosis, hepatitis) have increased levels of ALT in the serum due to its release from damaged hepatocytes. Thus, high ALT levels positively correlate with liver dysfunction.

The Urea Cycle

During transamination of amino acids, the α-amino group from a given amino acid is transferred to α-ketoglutarate, generating the nitrogen carrier glutamate. The nitrogen carried on glutamate is further processed in the urea cycle (see image), a hepatic specific process involved in maintaining nitrogen balance through regulating blood urea nitrogen (BUN) levels.

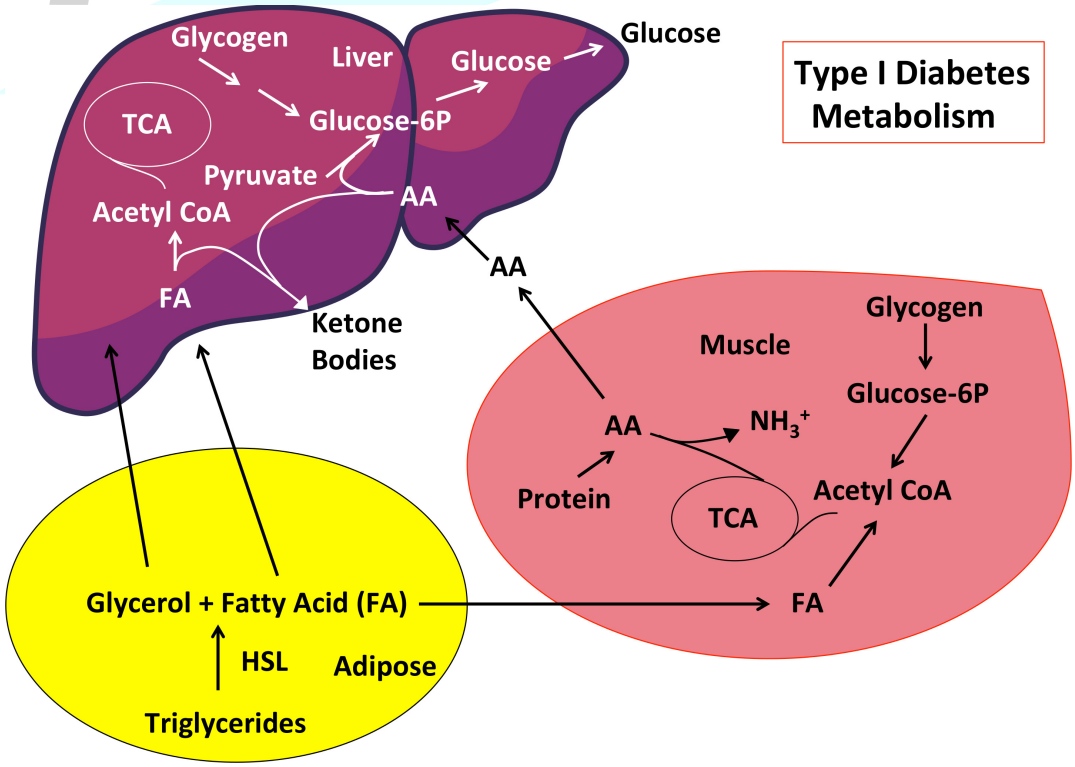
In the mitochondria, glutamate dehydrogenase releases free ammonia from glutamate, regenerating α-ketoglutarate. Carbamoyl phosphate synthetase I uses NH_3 and CO_2 plus ATP to generate carbamoyl phosphate, an activated precursor to urea that combines with ornithine to generate citrulline. In a reaction requiring ATP, citrulline combines with the amino acid aspartate to generate arginosuccinate. Aspartate contributes one of the nitrogen atoms in the urea molecule. After releasing fumarate, arginosuccinate is converted into the amino acid arginine. Therefore, arginine is a non-essential amino acid as it is produced in the urea cycle. Next, the enzyme arginase generates urea and ornithine, thereby completing the cycle.



Diabetes mellitus

Diabetes mellitus (types I and II) is a disease hallmarked by impairment in glucose metabolism. In diabetes mellitus type I (DMI) insulin is not secreted. This is usually due to autoimmune destruction of the β cells of the pancreas. As a consequence, glucose uptake into cells is impaired, generating a hyperglycemic state. Diabetes mellitus type II (DMII) is hallmarked by insulin "resistance", a condition where more insulin is required to achieve the same biological output.

DMI metabolism recapitulates the "starved" state. This is because glucose uptake is impaired from the lack of insulin. Under normal conditions, insulin represses glucagon and epinephrine. In the diabetic state insulin fails to repress glucagon and epinephrine secretion. As a compensatory response, the body increases glycogenolysis and hepatic gluconeogenesis despite the already hyperglycemic state. Therefore, triglyceride mobilization and protein and amino acid catabolism is common in type I diabetics. Further, type I diabetics commonly produce ketone bodies (i.e. acetone) and are notoriously "skinny".



As a result of impaired insulin signaling in DMI, glucose levels in the blood are very high. In the kidney tubules, glucose has an osmotic effect that draws in water. As a consequence, more water is lost relative to normal patients. Indeed, diabetics frequently urinate and are thirsty. The excess glucose is also reactive. Recall that glucose is an aldose sugar, a carbohydrate with a free aldehyde in the open, linear form. Aldehydes are reactive, and when glucose levels are high, the amino groups of various molecules such as hemoglobin (Hb) can react with it to generate glycosylated products through the creation of Schiff bases (imines). For Hb, this is called Hb A1C and high levels are compatible with the diabetic state.

DM2 is far more common than DM1. People with DM2 produce insulin, but have insulin resistance in one or more organs. This means that more insulin is required to achieve the same biological output. The image below shows an example with glycogen synthase, an enzyme that is upregulated by insulin. In the resistant state, observe that more insulin is required to achieve the same levels of enzyme activity relative to the normal state.

There are multiple theories regarding the origins of insulin resistance. In the fat overload model, excess stored fat results in an inflammatory state that interferes with insulin signaling. Because some people with DM2 are not obese, there are other possible scenarios for how DM2 is developed.