Lecture 11 (ETC and ATP synthesis)

1) Draw the mitochondrial inner membrane (just a bilayer) with indicators of the IMS and the matrix sides. Now indicate the proton gradient, and the direction that protons are transported during the action of the ETC.

2) On your map above, Show the direction that protons move when they are used to make ATP. Just direction please.

3) When a weak organic acid is added to mitochondria, what is the effect on the proton gradient? Why does a weak organic acid have this effect?

Weak organic acids break down the gradient by passive diffusion. They do this because the protonated form, A(t), is neutral and hydrophobic, so protons can be carried through the bilayer which is totally impermeant to H+

4) In the “in vitro” isolated mitochondria coupling experiments we discussed in class (and in your book), the first electron source is usually succinate, due to the experimental convenience of using this reagent. Draw a cartoon of the ETC in the inner membrane as it operates when succinate is added to active mitochondria. Include the pumping of H+ to form the proton gradient. Indicate the two sides of the inner membrane.
5) When NADH is used as a proton source, the resulting proton gradient is bigger than when succinate is used. Why is this so? Redraw the little cartoon in 6 to indicate the flow of electrons and the proton pumping that results from NADH being used as an electron source.

Redraw the little cartoon in 6 to indicate the flow of electrons and the proton pumping that results from NADH being used as an electron source.

6) What are the two components of the equation for the free energy stored in the proton gradient called? And what do they physically refer to? What is the significance of each term being a separate entity in the equation?

7) If glycolysis is running forward, you can measure it by looking at the disappearance of glucose, or the appearance of pyruvate. For each situation, describe what happens to glucose disappearance and pyruvate production, both from glycolysis.

a) NAD+ is removed:

b) ADP is removed:

c) free PO4 is removed
8) Now answer the same question for ADP disappearance and ATP production for the following situations during glycolysis:

a) NAD+ is removed:

ADP disappearance stops  
ATP production stops

b) Glucose is removed  
ADP disappearance stops  
ATP production stops

c) free PO4 is removed  
ADP disappearance stops  
ATP production stops

9) Suppose you are observing glycolysis in a yeast cytosol preparation, just like Buchner. It is running along making all the expected products, consuming glucose. Suddenly, you add an inhibitor of pyruvate kinase, and you add enough to completely inhibit that enzyme.

a) You notice that the reaction 2PG → PEP + H2O? rapidly stops, even though the inhibitor is for the next enzyme downstream. Why does this happen?

Since the consumption of PEP is blocked (PEP is substrate of the inhibited enzyme), PEP builds up until the reaction no longer goes forward... ΔG → 0 for PEP generating Rxn.

b) Similarly, you notice that every reaction has stopped. Why?

Eventually, all of the products build up and each reaction stops. Analogies: 1) An accident on the highway will back up traffic for miles. 2) A bend in a hose will stop water flow at every point upstream. 3) Someone conversing with the ticket agent at the theatre will stop everyone from getting a ticket.

c) Now, to the inhibited mix, you add an enzyme that converts PEP into oxaloacetate, and everything needed to make that happen. Soon you observe all the upstream glycolytic steps working again. Why?

Lessening PEP levels by this new alternative will now allow other upstream reactions to flow.

a) Glu → PEP → PEP
b) Glu → PEP → PEP
  PEP blocked, whole pathway stops

c) Glu → PEP → OAA  
  PEP lowered by alternate  
  deleration, upstream rxns resume
10) In a distinct experiment, you are observing isolated mitochondria oxidizing succinate, reducing O$_2$, and making ATP. You have ADP, Pi, and succinate present, and as expected, while succinate is being converted into fumarate, H$_2$O is being formed, and ADP is being converted to ATP.

a) You add an inhibitor of ATP synthase called oligomycin. ALL of these processes stop. Why does blocking ATP production stop succinate from being oxidized? Don’t say, “’cause they’re coupled”. What is the molecular cause of the upstream inhibition?

b) To this inhibited mitochondrial mix, with the oligomycin still present, you add a weak organic acid that carries protons across membranes, from regions of higher concentration to lower concentration. Now what happens to succinate consumption, H$_2$O production, and ATP production?

c) Draw a little cartoon of the three cases: uninhibited, + oligomycin, and +oligomycin + proton carrying drug. Show the proton gradient in each, using the number of H$^+$ you draw to indicate gradient magnitude.

d) This problem is EXACTLY analogous to problem 9. And the proton carrying drug in this problem (that relieves the block to ETC) is exactly analogous to the PEP-consuming enzyme used in 9c to alleviate the block. Why do I say this?
11) In anaerobic cells (those growing in the absence of O2), what would happen if lactate dehydrogenase (LDH) were suddenly inhibited with a drug?

Glycolysis would grind to halt as substrate build up behind the "traffic jam" of pyruvate & and as NAD⁺ is not supplied to GADH

12) Why is pyruvate not available for lactate production when cells are completely oxidizing glucose by Krebs and then ETC?

Pyruvate is efficiently transported into the mitochondrial matrix, leaving not enough for regeneration of NAD⁺

13) If cytoplasmic NADH were not converted back to NAD⁺ during complete oxidation of glucose, what would happen?

Glycolysis would cease because the GADH rxn would stop due to lack of substrates

14) Describe (one sentence each) the two ways that NADH is re-converted to NAD⁺ during complete oxidation of glucose on aerobic cells.

1) Reduces DHAP to glycerol-3-phosphate which is then reoxidized by mitochondrial complex-II like enzyme

2) Cytosolic OPP is reduced to melate, which is transported into matrix and reoxidized by NAD⁺

15) Write the half reaction for the reduction of DHAP to glycerol-3-phosphate. Just the half reaction. Note that this is the same molecule that we see in glycolysis but a new reaction that takes advantage of its presence.

\[
\text{DHAP} + 2H⁺ + 2e⁻ \rightarrow \text{glycerol-3-phosphate}
\]

16) Now write the balanced reaction in which NADH donates the electrons that reduce DHAP, as in 5) above.

\[
\text{CH₂-C-CH₂OH} + \text{NADH} + H⁺ \rightarrow \text{CH₂-C-CH₂} + \text{NAD⁺}
\]

17) Now write the half reaction for the oxidation of glycerol-3-phosphate back to DHAP.

\[
\text{CH₂-C-CH₂} + 2e⁻ + 2H⁺ \rightarrow \text{DHAP}
\]
8) What often-mentioned electron-carrying molecule, present on the surface of the inner membrane, do the electrons generated in 7) end up reducing? Write the balanced reaction for transfer of these electrons to this molecule when glycerol-3P is oxidized back to DHAP.

\[
\begin{align*}
\text{CH}_3-\text{C}-\text{CH}_2 + Q & \quad \rightarrow \quad \text{CH}_2-\text{C}-\text{CH}_2 + \text{QH}_2 \\
\text{OPC}_3 & \quad \text{OH}
\end{align*}
\]

9) In the DHAP-glycerol-3-P cycle, how do the electrons get into the mitochondrial matrix? One sentence.

They pass through the complex II-like enzyme complex that uses glycerol-3P as an electron-donating substrate.

10) For the much fancier malate/aspartate cycle, what is the NET effect of the whole cycle? One sentence.

The net effect is transfer of electrons from cytosolic NADH to matrix NADH.

11) The way we draw the M/A cycle, there is a cyclical set of conversions between 4 carbon molecules, and a separate cyclical set of conversions between 5 carbon molecules.

a) Write the set of conversions for each. Just abbreviations; no structures

\[
\begin{align*}
\text{4C:} & \quad \text{OAA} \rightarrow \text{MAL} \rightarrow \text{MAL} \rightarrow \text{OAA} \\
\text{5C:} & \quad \text{αKG} \rightarrow \text{AKG} \rightarrow \text{Glu} \rightarrow \text{Glu} \rightarrow \text{αKG}
\end{align*}
\]

b) Now, indicate with a T those reactions that are transaminations.

12) A big part of the M/A cycle is the interconversion of alpha-keto acids into their cognate amino acids. This is a very general and very useful idea.

a) Using R groups, keeping it real generic, write the general formula for an alpha keto acid and its cognate amino acid.

\[
\begin{align*}
\text{α-keto acid:} & \quad \text{R} - \text{C} - \text{CO}_2^- \\
\text{amino acid:} & \quad \text{R} - \text{C} - \text{CO}_2^- + \text{NH}_4^+
\end{align*}
\]
b) Now, let’s do the same for pyruvate, oxaloacetate, and alpha-keto glutarate. Write the structure of each, (by now I am assuming you know these structures; they are very useful to know.), and next to each write the cognate amino acids.

23) Using a diagram of the ETC (our usual set of Roman numerated complexes and the inner membrane), indicate where the DHAP-Glyc-3P cycle electrons enter the ETC and where the M/A shuttle electrons enter the ETC.

24) Why do these distinct entry points explain why the DHAP-glyc-3P cycle produces less energy than the M/A shuttle?

Electrons from DHAP cycle only cause complex III and complex IV proton pumping, while those from M/A cause H⁺ pumping.

25) If ADP is low and ATP is high in the mitochondrion, what are the effects of the ongoing respiratory chain?

Lack of ADP, and high ATP slow or stop ATP synthesis. The proton gradient builds up, and the proton-pumping pathway slows and stops. (Think of the H⁺ gradient as a product of the ETC.)

26) When ADP is low and ATP is high in the mitochondrial matrix, the Krebs cycle also slows down to a great extent. Why does this happen? One or two sentences.

The slowed ETC causes a buildup of product NADH, which acts both to slow the rxns that generate it, and acts as an allosteric regulator of several rxns. Similarly, ATP depletion can cause ATP has allosteric effects on several Krebs rxns.
2.7) When ADP is low and ATP is high in the mitochondrion, glycolysis also (!) slows down. Why does this happen? One to two sentences.

Both citrate and ATP that are cytosol when Krebs is slowed by high ATP slow several enzymes. Also, NADH is not delivered to the ETC, when ETC is slowed, and lack of NAD and lack of ADP also contribute to allosteric effects as well as direct effects by substrate availability.

Oops- sorry about the big space ....
Gluconeogenesis is the general term for production of glucose from smaller molecules. It is usually depicted as starting with pyruvate. Two reactions are needed to convert pyruvate into the first glycolytic intermediate. The first produces OAA, and the second produces PEP.

a) What are the two enzymes that catalyze these critical steps in glucose production?

b) Write the reactions for these two key steps that get pyruvate to PEP. Draw the structures of the carbon metabolites, and indicate other cofactors, etc.

1) \[ \text{CH}_3\text{C} = \text{CO}_2^+ + \text{CO}_2^+ + \text{ATP} \rightarrow \text{O}_4\text{C} - \text{CH}_2\text{C} = \text{CO}_2^- + \text{ADP} + \text{P}_i \]

2) \[ \text{O}_4\text{C} - \text{CH}_2\text{C} = \text{CO}_2^- + \text{GTP} \rightarrow \text{CO}_2 + \text{CH}_3\text{C} = \text{C} - \text{CO}_2^- \]

c) So how many energy-rich phosphorylated molecules (ATP or GTP) are needed to convert one pyruvate into one PEP?

\[ 2 \text{ ATP} + 1 \text{ GTP} \]

d) Do all three carbons of PEP come from pyruvate? **Yes**

e) Suppose you have 500 Krebs cycles operating in a single mitochondrion. If PEP-CK catalyzes 250 reactions, how many Krebs cycles can now be supported?

\[ 250 - \text{an OAA is needed for each Krebs cycle initiated} \]

f) In e), how can OAA lost to the PEP-CK be replaced? What are the source molecules for replenishing OAA lost to glucose synthesis? Can AcCoA supply the OAA?

The carbons can come from pyruvate, or from amino acids whose carbon skeletons can be converted into Krebs intermediates with more carbons than acetate. AcCoA can not be used, because there will be no net carbon flow to this point.

2) If AcCoA is abundant, which route for pyruvate utilization is favored, Krebs or gluconeogenesis?

When AcCoA is abundant, PDH tends to be less active and PC tends more active. Thus, \[ \text{AcCoA} \rightarrow \text{more pyruvate} \rightarrow \text{gluconeogenesis} \]
3) The pyruvate kinase reaction has a $\Delta G^\prime 0$ of something like $-23$ kJ/mole. First, review the pyruvate kinase reaction. Write the net reaction of PK, no structures needed. I hope you are starting to appreciate that glycolysis Krebs and pentose phosphate are the language of metabolic biochemistry.

$$\text{PEP} + \text{ADP} \xrightarrow{\text{PK}} \text{Pyr} + \text{ATP}$$

4) So when pyruvate is converted back into PEP during gluconeogenesis, how can this occur with any sort of efficiency? Describe how pyruvate is converted into PEP using words. What is the strategy?

Two "by pass" reactions are used, both of which are spontaneous. First pyr is carboxylated to OAA, then OAA is converted into PEP.

5) Now write the first reaction of the pair used to convert pyruvate into PEP. Include structures and the name of the enzyme. Where have we encountered the carbon metabolite product before?

$$\text{CH}_3-\text{C}-\text{C}_2^- + \text{CO}_2 + \text{ATP} \xrightarrow{\text{Pyruvate}} \text{OAA}$$

6) Now write the second reaction in the pair needed to convert pyruvate into PEP. Again, include the name of the enzyme and the structures of the carbon metabolites.

$$\text{OAA} + \text{GTP} \xrightarrow{\text{PEPC}} \text{CO}_2 + \text{CH}_3-\text{C}-\text{CO}_2^- + \text{GDP}$$

7) Why is this reaction spontaneous? There are two reasons, really.

The GTP provides ample free energy of hydrolysis and the CO$_2$ is a volatile removable product.
8) The major form of PEP-CK used in liver glyconeogenesis is the cytoplasmic. But OAA is generated in the mitochondrial matrix. How does OAA get out to the cytoplasm? Think malate/aspartate shuttle… Write the set of reactions (just involving OAA) that allow OAA to get out of the mitochondrion. No structures. Indicate the location of the molecules.

\[ \text{OAA} \rightarrow \text{Matrix} \rightarrow \text{Cytoplasm} \]

9) When OAA is transferred to the cytosol, what is the other product generated in the cytosol during the reaction? Why is this not a problem for gluconeogenesis to proceed? What is the enzyme that will catalyze the consumption of this other product?

10) There are two phosphatases used in gluconeogenesis to bypass energetically unfavorable reactions that stand in the way of making glucose from Fr 1,6 bP. What are these two phosphatases called? Name and reaction (no structures).

11) What tissues perform glycolysis, and which ones perform gluconeogenesis and where in the cell do these processes occur?

Glycolysis – essentially all tissues
Gluconeogenesis – Liver, kidneys, (intestine)

Both processes occur in cytosol

12) So the liver, which is really the master metabolic organ, needs to be told whether to do glycolysis or gluconeogenesis. Which two hormones instruct the liver about this decision? Write the names, and write which way these processes are regulated by each hormone.

Glucagon –↑ Glycolysis, ↓ Gluconeogenesis
Insulin – ↓ Glycolysis, ↑ Gluconeogenesis

"Use less, make more"
11) There is a single molecule that mediates glucagon and insulin’s effects on glucose synthesis or breakdown. Write the name and structure of this key regulator. Indicate with an arrow where the phosphate would be in the main pathway metabolite Fr 1,6 bP.

\[ \text{Fr} \text{2,6 bP} \]

Fructose 2,6 bisphosphate

12) Write the reactions (no structures needed) that form and break down Fr 2,6 bP. What are the names of the two enzyme activities that catalyze these opposing processes? What is unique about the actual enzyme that catalyzes these two reactions?

\[ \text{Fr-6P} + \text{ATP} \rightarrow \text{Fr} \text{2,6 bP} + \text{ADP} \text{ phosphofructokinase} \text{-} 2 \text{ (PFK-2)} \]

\[ \text{Fr} \text{2,6 bP} + \text{H}_2\text{O} \rightarrow \text{Fr-6P} + \text{P} \text{ fructose bisphosphatase} \text{-} 2 \text{ (FBP-2)} \]

13) What do insulin and glucagon do to these enzyme activities? How do these two hormones affect this pair of enzyme activities?

Glucagon ↑ PFK2

Insulin ↓ FBP2

15) Finally, describe the actions of Fr 2,6 bP in terms of allosteric regulation.

Fr 2,6 bP is an allosteric activator of PFK 1, promoting glycolysis

and an allosteric inhibitor of FBP 1, inhibiting gluconeogenesis

Importantly, the effects of this regulatory molecule are very strong, serving almost like an on/off switch for these competing steps
17) Using “physiological arrows” describe the process of glucose going up in the blood from pancreatic release of hormones in the blood all the way to alteration of glycolysis and gluconeogenesis. (glucose increases insulin release and inhibits glucagon release from the pancreas).

![Diagram of glucose metabolism]

**Answer**

Extra

- Insulin: due to decreased phosphatase of PEK2/FBP2 protein
- Glucose: due to more phosphatase of PEK2/FBP2 protein
Lecture 13 (lipid anabolism)

1) What is the “starter molecule” that acetyl CoA is converted into to make fatty acids? Why is OMSG useful to understand its structure? Draw it...

\[
\text{malonyl-CoA} \quad \text{Biotin is almost always the case with carboxylating enzymes.}
\]

2) What is the enzyme responsible for activating AcCoA into this starter molecule? What cofactor would you expect it to employ?

\[
\text{acetyl-CoA carboxylase}
\]

3) Write the reaction for the key lipid synthetic enzyme mentioned in 2 above.

\[
\begin{align*}
\text{CH}_3\text{C}-\text{S-CoA} + \text{HCO}_3^- + \text{ATP} & \rightarrow \text{CO}_2 \text{CH}_2\text{C}-\text{S-CoA} + \text{AMP} + \text{P}\; \text{malonyl-CoA}
\end{align*}
\]

4) What is the chemical purpose, if you will allow me this teleological affectation, of the CO\textsubscript{2} in malonyl-CoA produced in fatty acid synthesis?

\[
\text{CO}_2 \text{is an excellent leaving group, facilitating the transfer of the growing acyl chain to the acyl group attached to the CO}_2 \text{in malonyl-CoA...}
\]

5) Just like you did in the lipid catabolism question above, describe the process of fatty acid synthesis. Imagine FAS is empty and take us through one synthetic cycle, words only.

\[
\text{Acetyl-CoA is transferred to the 5th group on the FAS, and malonyl group is transferred to the other (at the “hot peak” in our cartoon). Then the acetyl group is transferred onto the malonyl-derived acetyl group, making a \beta -}
\]

\[
\text{keto acid attached to the } \text{S. Next, NADPH reduces the \beta keto to a \beta OH, then H}_2\text{O is removed to make a double bond, then NADPH reduces that double bond, resulting in an acyl group bound to the malonyl docking site that is 2 carbons longer. This entire acyl group is moved to the \text{“lower” S, making room for the next malonyl group.}
\]

6) Write the key structures that occur when fatty acid synthesis is going on. Is the molecule getting oxidized, or reduced?

\[
\text{two reductions are employed}
\]

\[
\begin{align*}
\text{FAS} \quad \text{FAS} \quad \text{FAS}
\end{align*}
\]
7) What is the main reducing agent for fatty acid synthesis? **NADPH**

8) Where do the acetyl groups for fatty acid synthesis come from. Meaning what metabolite is directly upstream from the AcCoA used to make fatty acids? What enzyme makes AcCoA from this precursor?

**Citrate is the immediate precursor. ATP-CITRATE lyase, or citrate lyase generates AcCoA (and OAA) from citrate.**

9) In a well-fed person, who has all of their ATP stores full and glucose to spare, fatty acids start being produced in liver cells. Describe the path of glucose, using simple words and arrows. You only need hit the highlights of glucose, pyruvate, citrate, acetyl-CoA, fatty acid and the cellular location(s) of the process. It’s a lot of WORK to make fat!

![Glucose metabolism diagram](http://guweb2.gonzaga.edu/faculty/cronk/biochem/images/anandamide.gif)

10) What are two uses of arachidonic acid that we discussed in class? Both are very important to the functioning of the mammal.

> It is the precursor for a large collection of inflammatory and physiological regulatory molecules known as prostaglandins and leukotrienes. Also, the natural, potent cannabinoid receptor ligand (see we used ligand) called anandamide is made from arachidonic acid.

11) What is the enzyme that aspirin-like anti inflammatory drugs (NSAIDS) inhibits? What is the substrate of that enzyme?

**COX 1,2 - cyclooxygenase - substrate is... arachidonic acid**

12) Why are plant products needed to have reasonable levels of arachidonic acid for the above processes?

> Although we can make arachidonic acid from the appropriate unsaturated fatty acids, we can not generate them from saturated fatty acids, or monoensaturated fat (we need the plant precursor called linoleic acid with radicals $\Delta^6$,$\Delta^9$ from plants)
13) Draw the types of double bonds seen and never seen in natural fatty acids. Why are there trans fatty acids in food?

- cis bonds appear in natural fats
- trans bonds do not - they are products of industrial chemistry to make partially saturated fats

14) Again, we encounter HMG-CoA, but now we see it in lipid synthesis. What lipids are made from HMG-CoA?

- Lipids based on the isoprene structure, including cholesterol and other steroids, rubber, the lipid anchor of Q, and many other molecules.

15) All of the lipids synthesized from HMG-CoA include the “isoprene” structural unit. Using only C-C bonds, draw the basic shape of the isoprene.

```
C - C - C
```

16) Now, draw HMG-CoA 3-OH-3-methy-glutaryl-CoA (using OMSG…). Trace the isoprene structure that is embedded in this molecule.

```
HMG-CoA - C - C - C
          \  |    |    |  \ |
           CO_2  H  CH_3  OH
```

17) How is HMG-CoA assembled in the cytoplasm for lipid synthesis? What pathway is chemically identical to this process?

- 3 acetyl groups are sequentially connected. First 2 AcCoA are combined to make AcAcCoA. Then a 3rd acetyl group is added to make 6 carbon HMG-CoA. EXACTLY the same reactions as the first three of lecture body synth. But different, cytoplasmic enzymes.

18) Where do the building blocks for HMG-CoA synthesis come from in the cell?

- They are immediately derived from citrate via ATP citrate lyase, just like in FA synthesis.

19) What class of widely used drugs inhibit HMG-CoA reductase? What is the product whose production is blocked?

- The statutory inhibitors inhibit HMG-CoA reductase. The block the production of mevalonate, since the enzyme reaction is

```latex
\text{HMG-CoA} + 2\text{NADP}^+ \rightarrow \text{CO}_2 - \text{CH}_2 - \text{C}^\Delta - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 + 2\text{NADP}^+ \text{H}
```
1) Science is FULL of terms that sound the same but mean very different things. Example: lactate and lactose. Describe these different things that sound similar:

Glucagon: Pancreatic hormone released when glucose levels drop “when glucose is gone!”

Glycogen: Animal storage form of glucose monomers, made with α1→4 chains and α1→6 branches

Glycogenin: Protein at core of each glucose granule, has first glucose covalently linked and catalyzes construction of first few linkages

Glycolysis: Common catabolic pathway for glucose. No O2 required! Get 2 ATP per Glu

Glycogenesis: Term for synthesis of glycogen from glucose monomers

Gluconeogenesis: Term for synthesis of glucose from smaller molecules, typically pyruvate or amino acids

Glycogen synthase: Enzyme that catalyzes synthesis (or construction) of α1→4 chains in glycogen.
Glycogen phosphophorylase: enzyme that catalyzes the release of glu-1P monomers from glycogen

PFK-1: glycolytic enz that makes Fr1,6bP from Fr6P

PFK-2: enz that make glucose-regulatory molecule Fr6P from Fr1,6bP

FBP-1: glucoregneric enzynme that catalyzes production of Fr6P from Fr1,6bP

FBP-2: enzyme activity that catalyzes removal of Pi from Fr2,6bP to make Fr6P

2) Glycogen synthase (GS) catalyzes a simple reaction between an activated version of glucose (with a good leaving group) and the growing linear glucose polymer.
a) What is the form of glucose that serves as a substrate for GS?

$$\text{UDP} + \text{Glu} \rightarrow \text{UDP} \text{Glu}$$

(Here again, UDP is a great leaving group!)

b) How is this glucose-based substrate produced? Write the series of reactions from glucose to the substrate of GS ready for addition to the growing chain. No structures needed.

$$\text{Glu} + \text{P} \rightarrow \text{Glu} + \text{P} + \text{P} \rightarrow \text{Glu} + \text{UDP} + \text{P}: \text{P}$$

(\text{UDP-Glu})

c) Write the reaction that GS catalyzes. Do it this once with structures, representing the growing chain with the single non-reducing end monomer and an R group for the rest of the glycogen molecule.
d) What do insulin and glucagon do to the activity of GS? Why do these regulatory effects make sense?

- Insulin causes GS activity to increase, by favoring dephosphorylation.
- Glucagon causes GS activity to decrease, by stimulating phosphorylation.

These regulatory changes make sense; when glucose is abundant after a meal, it is important to store it for use later. Conversely, when blood glucose is low, it is important to stop storage of glucose but slowing glucogen synthesis.

3) Glycogen has branches, something like one every 8-10 residues along the polymer. What is the name of the glucose-glucose bond that creates a branch? On a glucose structure, indicate with arrows where a regular monomer would be connected, and where a branch monomer would be connected. The bond that defines a branch is an \( \alpha 1 \rightarrow 6 \) glycosidic bond, between 2 glucose molecules.

4) Now let’s break some glycogen down, people. Why is glycogen phosphorylase called… glycogen phosphorylase? Because the \( \alpha 1 \rightarrow 4 \) glucose-glucose bond is broken by adding phosphoric acid, or phosphate, “across it” in direct analogy to hydrolysis where HO is “added across” a bond being hydrolyzed. Hence, GP catalyzes phosphorylase.

5) Write the reaction for removal of a glucose monomer from the glycogen polymer that GP catalyzes. Actually write the structures, including the glucose that will be freed and the penultimate one that will after the reaction be the non-reducing end. Please be comfortable with these terms and structures.
6) What must occur for the freed glucose monomer to enter glycolysis or to be liberated as free glucose? Write the reaction (no structures).

$$\text{Glu-1P} \xrightarrow{\text{phosphogluco-\it{mutase}}} \text{Glu-6P} \rightarrow \text{glycolysis} \rightarrow \text{PP pathway, etc.}$$

7) Glycogen phosphorylase is, not surprisingly, also regulated by glucagon and insulin. What does each do to this enzyme? How is this regulation brought about?

Glucagon increases GP activity by causing its phosphorylation, while insulin decreases GP activity by causing its dephosphorylation.

8) Debranching enzyme has two functions to dismantle a four monomer branch. Using just circles like beads on a string, represent the two things that debranching enzyme does to accomplish this. It involves two separate enzyme activities catalyzed by the same enzyme. What are these enzyme activities called?

(Note: the stippling is simply to help keep track of monomers)

9) Now, to wind up this long problem set (it is short problems, not short problem sets…) write out the entire set of enzyme activity changes that glucagon and insulin bring about, and the consequent effects on pathway enzymes in the cases(es) where an allosteric regulator is being increased or decreased. I will leave a lot of space.
And in the case of the single polypeptide, bi-functional enzyme PKH2/Fpk2, again phosphorylation changes are responsible for the corresponding activity changes:

\[ \text{Frz} \rightarrow \text{Frz} \]

Diagram:

- Gluc
- PKH2
- PKH2:Fpk2
- Frz
- Frz

Text:
- More Frz is made
- Less Frz is made
- Frz is broken down
- Ins

Conclusion:

\[ \text{Frz} \rightarrow \text{Frz} \]
1) Why is urea such a good nitrogen waste-carrying molecule? Compare it to NH$_2$-CH$_2$-NH$_2$ which looks very similar. Why would this not be a good choice?

![Chemical structures]

2) In urea, where does the C=O come from? What is the first reaction of the pathway? Write that reaction. The structures are very simple... so include them. The C in urea comes from bicarbonate, or CO$_2$. The reaction that gets the HCO$_3^-$ carbon into the action is carbamoyl phosphate synthetase (CPS).

$$\text{HCO}_3^- + \text{ATP} + \text{NH}_3 \xrightarrow{\text{CPS}} \text{H}_2\text{O}_2^- + \text{PO}_4^{3-} + \text{carbamoyl phosphate}$$

3) Write the reaction for transfer of a "generic" amino acid's nitrogen to αKG to form the generic α-ketoacid, and a new amino acid. Indicate with an arrow the αKG alpha carbon at the end of the reaction.

![Chemical reaction]

4) What is the name of the amino acid produced in the above reaction that results when the generic amino acid has its NH$_3$ transferred to the αKG?

5) What is the utility of the reaction in 3 and 4 in terms of nitrogen catabolism?

This reaction provides a uniform route for removal of N from amino acid catabolism in the liver. In this way many amino acids have a common nitrogen exit route.

6) The glutamate that is made in 4 is converted back to αKG as part of nitrogen catabolism. What is the name of the enzyme that restores αKG from glutarate. Write out the reaction, no need for structures. What type of reaction is this?

$$\text{Glu} + \text{NAD}^+ + \text{glutamate} \xrightarrow{\text{dehydrogenase}} \alpha\text{KG} + \text{NH}_4^+ + \text{NADH}$$
7) We see glutamate again in transfer of nitrogen from peripheral tissues to the liver. In this case, glutamate is the substrate in a reaction that adds NH₃ so it can be carried safely to the liver. What is the name of the enzyme that adds NH₃ to glutamate, what is the product called? Write the full reaction.

\[
\text{Glutamine synthetase} \quad \text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + \text{P}.
\]

8) What is the cognate α-keto acid of alanine? You may have to look alanine, although most biomedical types know this one.

\[
\text{Alanine} \quad \text{Pyruvate}
\]

9) What is the role of alanine in transfer of nitrogen to the liver from muscle and other tissues? How is this similar to the more general process we discussed in questions 3 and 4. Why is alanine specifically mentioned in this process?

Alanine is synthesized from pyruvate (its cognate α-keto acid) in muscle when there is significant catabolism of muscle protein (and thus excess N) and carried through the blood to the liver, where the N is removed by transamination. The pyruvate is then used to synthesize glucose which can be sent back to tissue, req. glucose. This added “cori cycle” feature is why alanine deamination is mentioned separately.

10) Draw the structure of arginine (you might have to look it up. I would have to; I never get the number of methylenes correct…). Now show the parts that will be urea upon the final step of the urea cycle.

11) In the picture above, complete the reaction to show the liberation of urea and the production of the urea cycles starting molecule. This is called ornithine. Is this an amino acid? Is it used to make proteins? Ornithine is an amino acid (NH₄⁺ - C₆H₄-C₆O⁻) but it is not used to make protein. Oddly enough, since Lys is very similar (with 4 CH₃ groups instead of 3)

12) The structure of aspartame is shown. There are two amino acids in the structure. Which ones are they; indicate by circles? Which one is important for patients with PKU? Why?

The Phe is important for PKU patients, since they have a deficiency in phenylalanine catabolism: they lack the enzyme phenylalanine hydroxylase, that is needed for both breakdown and derivitisation of Phe. Excessive ingestion of this sweetener exacerbates the illness.
13) Here at the boarder of nitrogen catabolism and anabolism we stop for a minute to make sure everyone is on the same page with some very similar sounding terms. Define each of these. Words only.

**glutamate:** 5 carbon, dicarboxylic acid; cognate of αKG. Used in NH₃ catabolism as a common “bottleneck” for delivery of NH₃ from amino acids to the urea cycle.

**glutarate:** The simple 5 carbon dicarboxylic acid from OMSG.

**alpha-keto-glutarate:** The α-keto acid derivative of glutarate, important as a substrate in the transamination reactions that channel amino acid nitrogen into the urea cycle. The cognate α-keto acid of glutamate.

**glutamine:** The amide derivative of glutamate: the R group CO₂⁻ is now amide linked to a NH₂. Thus this amino acid has two amino groups. Used both in N catabolism to carry waste N to liver, but also very important in N anabolism as a substrate of glutamine amidotransferases.

**alanine:** Cognate amino acid of pyruvate. Created from pyruvate in muscle and used to carry NH₃ to liver.

**arginine:** Fancy amino acid (R) with a “guanidino” group that is the source of biosynthetic urea. Also used in proteins as a structural amino acid.

**pyruvate:** Cognate α-keto acid of alanine.

**urea:** Very stable, soluble, unsavory waste storage form of N, used in mammals as a depot for waste nitrogen.
14) Write the simple half reaction for nitrogen fixation using H+ to balance the charges. What is the enzyme that is responsible for this heroic feat of thermodynamic legerdemain?

\[ \text{N}_2 + 6e^- + 6H^+ \rightarrow 2\text{NH}_3 \]

15) Enzymes called glutamine amidotransferases (GAs) are responsible for transferring the amide nitrogen of glutamine into a variety of anabolic pathways. Using “S” as a generic substrate, write the reaction by which GAs produce S-NH₂, the generic product. But include the structure of glutamine and the resulting product.

\[ \text{NH}_3^+ - \text{C} - \text{H}_2 - \text{CH}_2 - \text{O} - + \text{S} \rightarrow \text{GA} \rightarrow \text{NH}_2 - \text{C} - \text{H}_2 - \text{CH}_2 - \text{O} - + \text{S} \]

16) What is PRPP? It is a central starting molecule for both de novo synthesis of nucleotide bases, and for salvage of bases. What is the name, what is the structure of this key molecule.

17) What glucose-consuming pathway is needed for production of PRPP?

18) What is the purpose of the PP in PRPP? What basic general principle of metabolisms that I have mentioned many times is again exemplified in this lovely molecule?

19) What is the difference between de novo synthesis of nucleotides and salvage-based strategies of synthesis?

20) How do we know that purine salvage is critically important to normal human function?

Lesch-Nyhan syndrome is a severe disease caused by the absence of a purine salvage enzyme that adds certain free purine bases to PRPP to generate salvaged purine nucleotides.
21) PRPP is employed differently in the de novo synthesis of purines and pyrimidines. What is this difference? In purine synthesis, the double ring is constructed piece-by-piece on the ribose ring from PRPP. Conversely, in pyrimidine synthesis, the pyrimidine base is synthesized separately and then added as after completion to the PRPP. Purines - made upon the ring; pyrimidines made “over yonder”.

22) What are the two enzymatic steps that are critical for the production of the building blocks for DNA synthesis from those used for RNA synthesis? Name the enzymes and say in one sentence what they do:

- Ribonuclease converts nucleotide ribose into 2-deoxyribose
- Thymidylate synthase methylates the U base to make T used in DNA

23) Draw the generic nucleotide variant (using B for the base and Pi for the phosphate) that is used in DNA synthesis. (hint: it is what the “D” is about….)

24) Which nucleotide base is specifically modified to make a DNA building block? What is the modification? What cofactor is used to accomplish this critical modification?

U is modified to make T for DNA, it is methylated, and the cofactor is 5,10-methylenetetrahydrofolate or 5,10-methylenetetraTHF

25) “Gout is a disease of purine catabolism”. Why is this statement true, and what is the enzyme who is the key player (“play-a”) in this disease?

Gout results from overactive xanthine oxidase (XO) which is along the catabolic pathway for purine bases. Imbalanced XO activity causes a buildup of uric acid, the product of XO. This buildup causes extremely painful joint inflammation. Inhibitors of XO are an effective therapy for this syndrome.

OUT WITH GOUT!
Lecture 17 (Cancer and Aging)

1) HIF-1a transcription factor that is naturally induced when cells are experiencing low oxygen. What are the effects of this factor on glycolysis, and why does that make sense?

HIF-1a induces (increases expression of) the enzymes of glycolysis. This makes physiological sense because glycolysis can provide energy (ATP) in low or even absent oxygen.

2) The same transcription factor causes release of angiogenic factors. What is angiogenesis? Look it up if you need to. Why does this make sense?

Angiogenesis is the formation of new blood vessels, or vasculature. Tissues experiencing low O2 can be oxygenated by better blood flow into the tissue.

3) What did Otto Warburg observe about tumor cells. Why is this called “aerobic fermentation”?

Dr. Warburg noticed that cancerous tissue and cells are much more prone to consume glucose by glycolysis, rather than fully oxidizing the glucose to CO2. This is despite the fact that O2 is available. Hence “aerobic fermentation”

4) What is the current thinking about why tumor cells use so much glucose compared to their normal, socially-obligated neighbors?

It seems that cancer cells channel the glucose into massive anabolism, in much the same way that normal, rapidly growing cells do. The full oxidation of glucose is great if all you need is ATP by for anabolism, carbon must be used, not burnt!

5) What are some of the molecules that glucose can be used to produce if it is not totally oxidized to CO2?

Ribose, glyceral, fatty acids, amino acids, steols, isoprenoids, other sugars, nucleotides, here, etc.
6) Another molecule that is massively consumed by tumor cells is glutamine. For a cell that is invested in rapid growth, why does this make sense?

Glutamine is an important source of amino nitrogen for synthesis of N-containing molecules. It also provides "long" carbon skeletons (e.g., αKG) for use in synthesis of amino acids and other analogues.

7) Why is citrate so important in cancer cell metabolism? Where does citrate come from in the rapidly growing cell?

At just around the same time that Warburg noticed the cancer cells’ huge appetite for glucose, Warburg noted that tumor cells derive all the LUBN from de novo synthesis. The acetyl groups used to make lipids de novo came from citrate.

8) Why does citrate synthase inhibition make sense as a cancer cell intervention?

Citrate lyase (also called "AMP citrate lyase (ACL)") generates the acetyl-CoA used in de novo synthesis of both fatty acids and isoprenoids like steroids.

9) We talked about isocitrate dehydrogenase 1 (IDH1), a cytoplasmic version of isocitrate dehydrogenase, as having a very interesting mutation in 80% of glial cancers known as gliomas.

   a) Write the normal reaction of IDH1, including structures and substrates. Remember it is cytoplasmic, so its cofactor choice will be a little bit different from the matrix version...

   \[
   \text{isocitrate} \rightarrow \text{αKG} + \text{NADPH} + \text{H}^+ \]

   b) Now describe the effect of the often-observed R132H mutation on this IDH1, in words.

   The mutation alters the IDH1 enzyme into one that can catalyze the reduction of the αKG in 2-oxoglutarate.

   c) Write the reaction that R132H catalyzes.

   \[
   \text{αKG} + \text{NADPH} \rightarrow \text{2-oxoglutarate} + \text{NADP}^+ \]
d) For all gliomas that have this mutation in the R132 residue, it is always found to be a heterozygous mutation... (fancy!) meaning, it is always found to with the other IDH1 copy being normal. One mutant, one normal. What is this interpreted to mean?

The interpretation is that both the normal enzyme IDH1 and the R132 mutant must be expressed at the same time to benefit the evil needs of the glioma. Simplest interpretation: product of IDH1 is substr. of mutant.

e) Write the combined action of the normal IDH1 and the mutant IDH1-R132H on the normal substrate(s) of IDH1

```
isocitrate + NADP⁺ → IDH1 → α-ketoglutarate + NAD⁺ + NADPH → 2-oxoglutarate + NADP⁺
```

f) What scientific/biomedical question emerges from thinking about the result of e...?

What are the effects of 2-oxoglutarate on glioma cells? Why is this “mini pathway” so frequently and consistently found in gliomas?

10) One model for aging is based on the mitochondrial production of ROS: reactive oxygen species. What are these and where do they come from?

ROS are produced when e⁻ “leak” from the ETC and form highly reactive species. These are typically derived from O₂, on nitrogens.

11) What chemical properties do ROS have that make them good candidates for molecules that cause aging?

They are highly reactive and can damage many macromolecules, like DNA, proteins, lipids and also, they damage the mitus that causes production of even more ROS. Positive feedback!

12) The DAF2 gene in C. elegans was observed to be involved in aging. Describe the effect on worms of missing the DAF2 gene.

When DAF2 is missing (a “null” is the genetic term), the worms have a significantly increased lifespan.

13) Why does the DAF2 gene make some sense when we think about the effects of caloric restriction on aging.

The DAF2 protein appears to be similar to the insulin receptor, so one idea is that it mediates the signal for “abundant nutrition” that consumption of calories would cause. Maybe this is how the worm senses caloric intake!
14) Other aging genes found in C. elegans are involved in the ETC, and others involved in eating behavior. These, combined with the DAF2 gene lead to a simple pathway of effect of calories on aging. Draw that pathway, but remember that their actual role(s) are probably more complex. I don’t mean a metabolic pathway, but rather a process, or a physiological pathway (this causes that which causes that…)

\[
\text{Food} \rightarrow \text{Caloric \ Signal} \rightarrow \text{DAF2 \ Metabolism} \rightarrow \text{Complex I-IV} \rightarrow \text{ETC Activity} \rightarrow \text{Aging}
\]

15) Based on what DAF2 is similar to, what signaling process would you examine for a role in human aging (words; one or two sentences)

\[
\text{Maybe the insulin signaling system, esp in "control" tissues like the brain, or the "insulin-like growth factors (IGF) that are most like the ligands for DAF2.}
\]

16) What is the hypothesis about SIR2 function in aging? How does its role, in very general terms, differ from that proposed for DAF2-like things?

\[
\text{SIR2 is thought to increase lifespan by virtue of its activities. That is, SIR2 action increases lifespan. In contrast, loss of DAF2 increases lifespan. SIR2 \rightarrow \text{Lifespan} \quad \text{DAF2} \rightarrow \text{Lifespan}}
\]

17) Why are people excited about compounds that act like resveratrol, which is found (in extremely small doses) in red wine.

\[
\text{The existence of drugs like molecules (small, easy to synthesize and administer) that can activate SIR2 and related enzymes IMPLIES (but does not prove) that perhaps this approach of developing potent stable specific SIR2 activator is do-able, whether or not such STACs really diminish aging rate remains an open question, but an explorable and exciting one!}
\]
1) Briefly (a few words) describe these four energy-yielding metabolic systems

   a) phosphagen/CP: phosphocreatine (CP) levels are kept high in resting well-fed muscle for high power, low-capacity movements like jumping out of the way of a car gong wild

   b) glycolysis: no oxygen-requiring, rapid, less power than CP but more capacity.

   c) pyruvate oxidation: oxygen-requiring, higher capacity ATP-generating system. Diminish lactate

   d) lipolysis: highest capacity system, used when resting, or in highly trained athlete as an alternative to glucose. Also source of glucose-sparing ketone bodies.

2) Using just “C” for creatine, write the reversible reaction between C and ATP that is used to keep ATP levels buffered during sudden onset of exercise

   \[
   ATP + C \rightleftharpoons ADP + CP
   \]

3) Muscle can store something like 350 grams of glucose as glycogen, the liver something like 80 grams. Why is most of the muscle-stored glucose restricted to only intracellular use, while the liver’s glucose can be released as free glucose as needed?

   Because liver but not muscle possesses Glu-6-phosphatase that is required for the release of free glucose into the blood stream.

4) Write the reaction for the enzyme featured in number 3 answer above. Include structures.
5) When the muscle lactate production exceeds the capacity to oxidize it, what process that involves the liver makes good use of this lactate?

6) von Gierke’s disease is the most famous of a group of maladies called “glycogen storage diseases” often referred to as GSDs. It is also called GSD I, but von Gierke’s disease sounds more dramatic, like some terrifying dirigible from the WWII era. von Gierke’s disease is a deficiency in glucose 6-phosphatase. What would you expect the effects of this disease to be on the following entities:

    a) liver glycogen levels:

        Liver glycogen levels would be abnormally high, and stay high even during exercise

    b) blood glucose upon exercise:

        It would drop more rapidly than normal since it won’t be replenished by liver glycogen breakdown or gluconeogenesis

    c) rate of muscle glycogen breakdown during exercise:

        This will be normal, or perhaps a bit faster because no liver help...

7) There is another GSD, called McArdle’s disease or GSD V, is due to a deficiency in levels of glycogen phosphorylase in muscle. When people with GSD V start exercising, they get severe muscle cramps due to an abnormal buildup in ADP.

    a) What is the function of glycogen phosphorylase:

        It releases usable glucose in the form of Glu-1P that can readily be used for ATP generation

    b) Why do patients with GSD V not have symptoms when they are sedentary (not moving much):

        During rest or very low level activity, muscle glycogen is not needed. This is why doctors advise sedentary lifestyles to GSD V’s

    c) It is often observed that GSD V patients gain noticeable relief from their muscle cramps by continuing exercise after 10 or 15 min. Why might this make sense? (hint blood flow):

        Because as blood flow and heart rate increase in that time frame, other sources of glucose and other fuels like FA and lactate bodies become available.
8) Two people are measured in a metabolic room (these exist). One has an RER of .75, and the other has an RER of .95. Which is metabolizing more glucose as fuel? Why do you say this?

Theoretical RER for glucose oxidation is 1.0, RER for FA ox is 0.7. So .75 is almost totally lipolytic by this measure, while .95 is almost totally “glycolytic.”

9) It is thought that a person with a high proportion of type I muscle fibers would be expected to be a better endurance athlete than one who has a higher proportion of type II fibers. Why do people think this?

Because type I fibers are good at sustained oxidation and especially use of the abundant fatty acid fuel needed for successful endurance exercise, like biking 100 miles, or running 26.2, or both...

10) Endurance training changes the enzyme composition of muscle mitochondria.
   a) Describe in general terms what these changes look like
   One sees an increase in the levels of oxidative enzymes of the Krebs cycle, and FA-oxidizing enzymes.

   b) Which fiber type do endurance trained muscles become more like?
   They become more like “Type I” fibers, in terms of enzyme composition. The actual ratios don’t seem to change.

   c) So what would you predict high intensity training (like power lifting and sprinting) changes to look like in terms of enzyme activities and fiber type resemblances?
   One would expect lowering of oxidative enzyme levels, fewer fibers, and increased glycolytic capacity.

11) What are the changes that strong expression of PPAR-δ in mouse muscles cause? Describe a few of the phenotypes (traits) associated with this genetic modification of mice.

   Increases in Type I fiber enzyme levels, increased capacity for lipolysis, increased exercise endurance, less fear of cats...

12) Why do we think that the PPAR-δ transcription factor normally plays a role in setting the exercise endurance capacity of (at least) a mouse?

Because mice who are lacking the PPAR-δ gene have LESS than normal lipolysis, type I fibers and endurance than their wild-type siblings.
Lecture 19  
(Obesity and Diabetes)

1) Describe the effect of insulin on these processes or entities:

   a) cellular glucose uptake: increases uptake of glucose into cytoplasm
   b) blood glucose levels: decreases blood glucose levels
   c) glycogen synthesis: increases glycogen synthesis
   d) glycogen breakdown: decreases glycogen breakdown
   e) glycolysis: increases glycolysis
   f) gluconeogenesis: decreases gluconeogenesis
   g) fatty acid synthesis: increases fatty acid synthesis
   h) fat cell lipid storage: increase adipocyte (fat cell) lipid storage

2) Why is increasing the activity of liver hexokinase useful for lowering blood sugar?  
What kind of regulator was developed to accomplish this, as described in class.

   Once glucose is phosphorylated it can not get out of the cell through the transporter that takes it up. So the activity of hexokinase is one important determinant of glucose levels in blood. They devised an allosteric activator.

3) Why is there a high abundance of acetyl-CoA when insulin signaling is low or absent in the liver cell?

   The liver responds as though blood glucose is low, since insulin signal is how it knows otherwise. So there is a large increase in gluconeogenesis to make glucose to supply the perceived (but not true) shortage. The Krebs cycle is very low in this circumstance because OAA is being tipped for glucose synthesis. AcCoA produced by FA oxidation thus builds up.

4) When acetyl-CoA is abundant in the liver, and there is low Krebs cycle activity, what happens to that acetyl-CoA?

   The high levels of AcCoA promote ketone body synthesis like we discuss in lipid catabolism. These molecules (AcAc & beta-butyrate) are released into the bloodstream.

5) Why is the process in 4 useful in normal circumstances of low blood glucose?

   The released ketone bodies can be used by tissues as an alternative fuel to glucose. When glucose is actually low, this is a good thing...
6) Write brief descriptions of type I and type II diabetes:

*Type I - loss of insulin production due to autoimmune destruction of pancreatic insulin-producing cells*

*Type II - deficiency in insulin responsiveness*

7) When a diabetic injects insulin, sometimes they can show very severe sudden effects like disorientation and wooziness. These effects are almost instantly alleviated by a glass of orange juice or similar beverage. What do you think happened?

*If a bit too much insulin is injected (this happens now and then), why one of you needs to invent a synthetic pancreas, blood glucose will drop enough to cause central nervous effects, and fast. The glucose in the juice does the trick.*

8) What does the adipocyte-released hormone leptin do in the mammal? If a human had a leptin deficiency what would you expect to observe in this patient?

9) What was the rational behind blocking the endocannabinoid receptor (CB1) as an appetite-suppressing drug?

10) Why are the Pima people of great interest to understanding obesity and its connection to type II diabetes?

The Pima people have a profound response to Western, industrialized diet. ~50% of them get type II diabetes when consuming a processed food “modern” diet. ~95% of these people are obese. Their genetic makeup is a Rosetta stone for understanding these prevalent and increasing maladies.

11) What is James Levine’s NEAT hypothesis, which he tested in his Science paper we discussed in class?

He is exploring the idea that natural variations in NEAT, that vary between people, are determinants of BMI and energy balance. He and others advocate that standing while at work can have significant beneficial effects on calorie balance and body mass. “Get up”