Lecture 11 Short problems

1) In anaerobic cells (those growing in the absence of O₂), what would happen if lactate dehydrogenase (LDH) were suddenly inhibited with a drug?

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

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

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

5) 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.

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

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

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

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

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
   b) Now, indicate with a T those reactions that are transaminations.
   c) In the M/A cycle, how do the electrons get into the mitochondrial matrix? One sentence.

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.
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.

13) 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.

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

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

16) 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.
17) When ADP is low and ATP is high in the mitochondrion, glycolysis also (!) slows down. Why does this happen? One to two sentences.

18) 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.
   c) So how many energy-rich phosphorylated molecules (ATP or GTP) are needed to convert one pyruvate into one PEP?
   d) Do all three carbons of PEP come from pyruvate?
   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?
   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?

19) If AcCoA is abundant, which route for pyruvate utilization is favored, Krebs or gluconeogenesis?
Lecture 12 Short problems

1) The pyruvate kinase reaction has a $\Delta G^0$ of something like -23kJ/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.

2) 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?

3) 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?

4) 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.

5) Why is this reaction spontaneous? There are two reasons, really.
6) 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.

7) 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?

8) 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).

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

10) 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.
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.

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?

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

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

16) 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:

Glycogen:

Glycogenin:

Glycolysis:

Glycogenesis:

Gluconeogenesis:

Glycogen synthase:
Glycogen phosphorylase:

PFK-1:

PFK-2:

FBP-1:

FBP-2:

17) 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?

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.

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?

18) 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.

19) Now let’s break some glycogen down, people. Why is glycogen phosphorylase called… glycogen phosphorylase?

20) 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.
21) What must occur for the freed glucose monomer to enter glycolysis or to be liberated as free glucose? Write the reaction (no structures).

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

23) Debranching enzyme has two functions to dismantle a four momomer 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?

24) 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 degreased. I will leave a lot of space.
Lecture 13 (lipid catabolism because we are behind)

1) When a triglyceride is consumed, intestinal cells break it down into its parts and then reassemble new triglycerides. Draw a generic triglyceride, showing the fatty acids as hydrocarbon squiggles with a carboxyl group.

2) Now, show the products of complete hydrolysis of this generic triglyceride.

3) The three carbon molecule that results from the complete hydrolysis of triglycerides is called what? Draw it to the right...

4) You are sitting around outside the exam hall before a metabolic final. You overhear a student say “In mammals, fatty acids can not be used to make glucose, but triglycerides can”? Why is this true or partially true?

5) Describe (in words) the route by which glucose can be made from a molecule derived from triglyceride hydrolysis.
6) For 5 above, write the reactions that allow this molecule to become part of glucose.

7) Describe the path by which a free fatty acid is taken up by a cell and oxidized. Say the key steps that happen to this molecule and where they happen. Just words and just a few sentences.

8) What is the new (meaning new to us, rather than CoA-SH) carrier molecule involved in getting fatty acids to the site of their oxidation?

9) During the cyclical oxidation of fatty acids, called beta oxidation, four reactions occur to acyl-CoA. Describe them in words below.

10) Now show the structures that occur during fatty acid oxidation. You only need show up to the beta carbon, since the other just sit there watching in horror…
11) What set of Krebs cycle reactions is this process most like? Would you say they are chemically identical or enzymologically identical?

12) For a 20 carbon fatty acyl-CoA, how many CoA-SH are needed to fully convert this molecule to acetyl-CoA?

13) In the peroxisome, the electrons that are removed in the first oxidation step of beta oxidation produce a potentially dangerous molecule. What is this molecule? Write the reaction that shows its formation?

14) What enzyme breaks down the potentially dangerous molecule produced during beta oxidation in the peroxisome?

15) Why is beta oxidation needed in seedlings to successfully undergo germantion and growth into planthood?

16) Write the net reaction for synthesis of ketone body acetoacetate. Now, how many acetyl CoA actually participate as substrates in the set of reactions?
17) What is the proposed function of ketone bodies produced by the liver?

18) A boy was found to have a deficiency in mitochondrial HMG-CoA synthase (NEJM 337:1203 (1997)). Lets do a few questions about this young patient.
   a) HMG-CoA stands for 3-hydroxy-3-methyl-glutaryl-CoA. Lets figure out what this looks like, from what you already know. Remember OMSG? What is glutaric acid, or glutarate. Draw that:

   b) Now draw 3-hydroxy-3-methyl-glutarate (this is HMG!)

   c) Finally, draw 3-hydroxy-3-methyl-glutaryl-CoA

   d) Why can’t this patient make ketone bodies even when AcCoA is abundant in the liver?

   e) This patient (at the time of the report he was 11; his first symptoms were seen at age 6 is sensitivity to fasting, becoming severely hypoglycemic. Why does this make sense?
Lecture 14 Short problems (lipid anabolism because we are behind)

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…

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

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

4) What is the chemical purpose, if you will allow me this teleological affectation, of the CO₂ in malonyl-CoA produced in fatty acid synthesis?

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.

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

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?

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!

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

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

12) Why are plant products needed to have reasonable levels of arachidonic acid for the above processes?
13) Draw the types of double bonds seen and never seen in natural fatty acids. Why are there trans fatty acids in food?

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

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

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

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

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

19) What class of widely used drugs inhibit HMG-CoA reductase? What is the product whose production is blocked?
Lecture 15 Nov 26 (amino acid and nitrogen; also purines and pyrimidines)

1) Why is urea such a good nitrogen waste-carrying molecule? Compare it to NH₂-CH₂-NH₂ which looks very similar. Why would this not be a good choice?

2) In urea, where does the C atom come from? What is the first reaction of the pathway? Write that reaction. The structures are very simple… so include them.

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.

4) What is the name of the amino acid produced in the above reaction that results when the generic amino acid has its NH₃ transferred to the αKG?

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

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?
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 NH$_3$ so it can be carried safely to the liver. What is the name of the enzyme that adds NH$_3$ to glutamate, what is the product called? Write the full. No need for mechanism:

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

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?

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?

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?

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:

glutarate:

alpha-keto-glutarate:

glutamine:

alanine:

arginine:

pyruvate:

urea:
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?

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.

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?
21) PRPP is employed differently in the de novo synthesis of purines and pyrimidines. What is this difference?

21) 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:

22) 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….)

23) 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?

24) “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?
Lecture 17 Short problems (Cancer and Aging)

1) HIF-1α 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?

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?

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

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

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

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

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

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…

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

   c) Write the reaction that R132H catalyzes.
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?

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

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

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?

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

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

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

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)

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?

17) Why are people excited about compounds that act like resveratrol, which is found (in extremely small doses) in red wine.
Lecture 18 Short problems (Exercise and Metabolism)

1) Briefly (a few words) describe these four energy-yielding metabolic systems
   a) phosphagen/CP:

   b) glycolysis:

   c) pyruvate oxidation:

   d) lipolysis:

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

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?

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 Giercke’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 Giercke’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:
   b) blood glucose upon exercise:
   c) rate of muscle glycogen breakdown during exercise:

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

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?

10) Endurance training changes the enzyme composition of muscle mitochondria.
   a) Describe in general terms what these changes look like

   b) Which fiber type do endurance trained muscles become more like?

   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?

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.

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?
Lecture 19 Short problems (Obesity and Diabetes)

1) Describe the effect of insulin on these processes or entities:
   a) cellular glucose uptake:
   b) blood glucose levels:
   c) glycogen synthesis:
   d) glycogen breakdown
   e) glycolysis
   f) gluconeogenesis:
   g) fatty acid synthesis:
   h) 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.

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

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

5) Why is the process in 4 useful in normal circumstances of low blood glucose?
6) Write brief descriptions of type I and type II diabetes:

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?

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?

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