1. (10 pts, 15 min) Your liver has been actively synthesizing proteins all morning; consequently, its ATP levels are lowered and AMP/ADP levels are high. While crossing the street on your way to your favorite class, a fast moving car comes directly towards you.

   i) What hormone is released by your central nervous system, once you notice the car? (2 pts)

   Epinephrine/adrenalin – the fight or flight hormone.

   ii) What will happen to the phosphorylation state of proteins due to release of this hormone? (2pts)

   Epinephrine, binding to its receptor causes a signal cascade leading to high levels of enzyme phosphorylation. First a G-protein coupled receptor is activated, which activates adenyl cyclase, producing cAMP. cAMP activates protein kinases.

   iii) How will the presence of this hormone affect the levels of F2,6-P in the cell? (Will it rise or fall?) (2 pts)

   The levels will fall. Recall that F26P levels follow glucose levels, epinephrine has the same effect as glucagon, a hormone present when blood glucose levels are low.

   The levels of the regulatory compound F-2,6-P are controlled by the phosphorylation of the two enzymes that make (Phosphofructokinase-2, aka PFK-2) and break it down (Fructose 2,6-bisphosphatase, aka F26-Ptase). When phosphorylated PFK-2 is inactive and F2,6-Ptase is active, resulting in a lowering of the levels of F-2,6-P

   iv) Under these conditions, will the liver produce glucose from glycogen, perform gluconeogenesis or both? Briefly justify your answer with reference to how these pathways are regulated – consider *both* hormonal control, energy sensing, and the availability of ATP for biosynthesis. (4 pts)

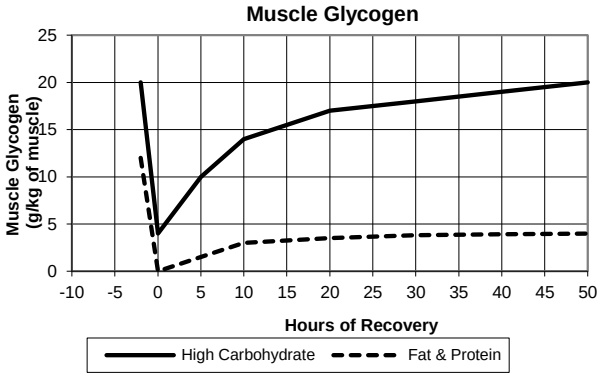
   Glycogen Metabolism: Glycogen phosphorylase, which releases glucose from glycogen, is activated when phosphorylated - it is <u>ON</u>. Therefore, glucose will be released from glycogen. The reverse path, glycogen synthesis will be off.

   Glucose Metabolism:

   Glycolysis will be OFF since F2,6P levels are low, PFK-1 in glycolysis **requires** F-2,6-P to be in the relaxed state. The liver will *not* degrade glucose if glucose is needed in the blood

   Gluconeogenesis is also <u>OFF</u>: The low levels of F2,6P would *normally* activate gluconeogenesis by no longer inhibiting F1-6 phosphatase-1. However, F1-6 phosphatase-1 is inhibited by AMP, which is at high levels in this case because a portion of the ATP has been hydrolyzed to ADP and AMP.

2. (5 pts, 10 min) The diagram to the right shows *actual* data for the recovery of muscle glycogen after exercise for an individual on either high-carbohydrate diet (solid line) or for an individual on a diet that consists of fat and protein (dashed line). The recovery period starts at T=0 after an exercise period of 2 hours (from T = -2 to 0 hours). Explain, using your knowledge of the metabolic pathways discussed in this course, the reason for the slow (almost non-existent) recovery of muscle glycogen for the individual on the fat and protein diet.



   Glucose is required to produce glycogen. Therefore, the person on the high carbohydrate diet can easy convert the ingested glucose to glycogen.

   The person on the high fat, high protein diet cannot generate large amount of glucose. The acetyl-CoA from fatty acid oxidation cannot produce any net glucose because the acetyl-CoA cannot be directly converted to pyruvate (pyr) since this activity is not present in humans. The glycerol backbone on triglycerides can be used to make glucose by entering glycolysis, however this is a relatively small number of carbons.

   The only way to produce glucose from proteins is by converting amino acids to intermediates that can somehow give pyruvate, which can then be used to synthesize glucose. There are only a small number of amino acids that can be used for this purpose. Alanine is directly converted to pyr by transamination. Cysteine and serine can also be converted to pyruvate. Asp and Asn can produce

oxaloacetate, which can go to phosphoenolpyruvate (PEP) and then to glucose (as part of gluconeogenesis). Glu and Gln can produce keto-glutarate, which can be converted to oxaloacetate and then to PEP.

The key point is that since Pyr to acetylCoA is one way in humans it is relatively inefficient to make large amounts of pyr/PEP from amino acids, consequently it is difficult to make large amounts of glucose and store that glucose as glycogen

3. (5 pts, 10 min) Summarize the various roles of histidine in the mechanism of serine proteases, citrate synthase, and succinate thiokinase.

Histidine can have diverse roles in enzyme mechanisms:

1. Deprotonated His can accept a proton. This is seen in serine proteases, where Ser and water are activated. It is also seen in citrate synthase where the conversion of the alcohol back to the C=O requires His to remove the proton.
2. Protonated His can act as a proton donor. This is seen in serine proteases where the new amino terminal is protonated by $HisH^+$. In citrate synthase the conversion of a C=O to an C-O-H requires proton donation from $HisH^+$.
3. His can also become phosphorylated, which occurs in succinate thiokinase. The phosphoHis is unstable and can donate the phosphate group to GDP to form GTP.

4. (6 pts, 20 min) The reaction catalyzed by Malate dehydrogenase in the TCA cycle has a standard free energy change (ΔG^o) of +29 kJ/mol, but a Gibbs free energy of ~0 kJ/mol during the normal operation of the TCA cycle.

a) Given that the concentration of malate, NAD^+, and NADH are 100 μM in the cell, calculate the concentration of oxaloacetate during the normal operation of the TCA cycle (4 pts). Assume T=300K.
For the reaction A+B→C+D: $\Delta G = \Delta G^0 + RTln[C][D]/[A][B]$

$G = \Delta G^o + RT\ ln[Oxaloacetate][NADH]/[malate][NAD^+]$

$0 = +29\ kJ/mol + 2.5\ ln[Oxaloacetate](.0001)/(.0001)(.0001)$

$-11.6 = ln\ [Oxaloacetic]/(0.0001)$

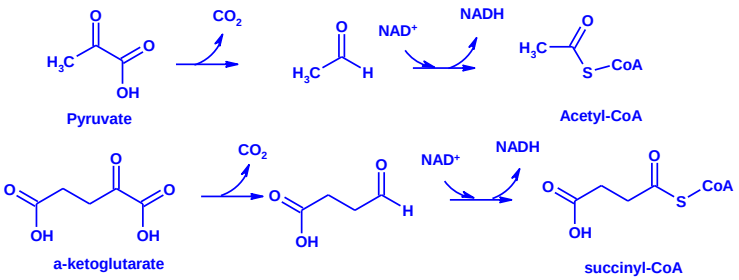
$e^{-11.6} = [Oxaloacetate]/(0.0001)$

$[Oxaloacetate] = e^{-11.6} \times 0.0001 = 9.17 \times 10^{-6} \times 10^{-4} = 9.17 \times 10^{-10}$ M

b) Explain how the Gibbs free energy is reduced to zero for this reaction; is this accomplished by either direct or indirect coupling? (2 pts)

The next step in the TCA cycle is citrate synthase. This must have a large favorable (negative) ΔG^0, which means the equilibrium point of the above reaction is far to the right, i.e. any oxaloacetate that is produced by malate dehydrogenase is immediately converted to citrate, this reduces the concentration of oxaloacetate well below its equilibrium concentration. This is an example of indirect coupling.

5. (5 pts, 5 min) A reaction that is identical in chemistry to that of pyruvate dehydrogenase occurs in the TCA cycle. Identify the name of the enzyme that catalyzes this reaction and show that the chemical changes are identical to those catalyzed by pyruvate dehydrogenase.



The step is alpha-ketoglutarate dehydrogenase. The reaction steps for both reactions is:

- loss of CO2
- oxidation of aldehyde to thio-ester, with CoA, generating an NADH

6. (10 pts, 10 min) The concentration of chloride in seawater is 0.6 M. An ocean-dwelling bacterium uses chloride transport to generate ATP. What is the minimum number of chloride ions that would have to be moved through its ATP synthase to generate ATP? You can assume the intracellular concentration of chloride is 0.1 M and that the voltage difference across its membrane is 0.06 V, inside positive, and the temperature is 300K.

$$\begin{aligned}\Delta G_{TOTAL} &= \Delta G_{CONC} + \Delta G_{ELEC} \\ &= RT\ln\frac{[Cl]_{IN}}{[Cl]_{OUT}} + ZF\Delta\Psi \\ &= 8.3\times 300\ln\frac{0.1}{0.6} + (-1)96{,}494(0.06) \\ &= -4461 - 5790 \\ &= -10{,}250\ J/mol\end{aligned}$$

The chloride concentration difference, coupled with the voltage difference, will give -10.25 kJ/mol. A total of 3 chlorides would have to be transported to provide sufficient energy to generate on ATP from ADP and P_i (ATP synthesis requires 30 kJ/mol). Note that this is the minimum number, the ATP synthase would have to show near 100% efficiency to synthesize one ATP from three chlorides that are transported.

7. (4 pts, 10 min) View the Jmol page for succinate dehydrogenase and answer the following questions:

i) Is the FAD buried in the enzyme or exposed on its surface? Based on your answer, is it likely that $FADH_2$ will be released after oxidation of succinate, or remain bound to the enzyme?

It is buried in the enzyme, completely surrounded by the protein. Therefore the bound FAD does not leave the enzyme after it is reduced to $FADH_2$, but instead passes its electrons to the iron sulfur centers within the protein.

ii) Describe, or sketch, the electron transfer path from succinate to coenzyme Q What happens to the electrons received from succinate on the way to coenzyme Q?

The two electrons from succinate are passed to FAD and then through the three iron sulfur centers to coenzyme Q. After reduction, the QH_2 carries the electrons to complex III. This regenerates the enzyme, allowing another cycle of reduction of succinate.

8. (6 pts, 10 min) Ethanol can be produced from glucose by yeast (and other organisms) under conditions of low oxygen, a process referred to as fermentation. How efficient is the process of ethanol production, i.e. how much of the original energy content in glucose is retained in the ethanol? Ethanol can be converted to acetyl-CoA by two oxidation steps, generating a total of two moles of NADH.

Glucose produces a net yield of 38 ATP when it is completely oxidized to CO_2. This is calculated as follows:

Glycolysis: 2 ATP + 6 ATP (2 NADH) = 8 ATP
2 Pyr -> 2 acetyl CoA = 6 ATP (2 NADH). Total is 14 ATP to get to 2 x acetylCoA
The acetylCoA enters the TCA cycle and generates:
TCA cycle/acetyl CoA = 3 NADH + FADH2 + GTP = 12 ATP
X 2 for two acetylCoA = 24 ATP
Total for glucose is 14 + 24 = 38.

The conversion of one ethanol to acetyl-CoA will produce 6 ATP (2x3). Therefore, the total ATP/2 ethanol is 12 ATP (to acetyl CoA) + 24 (Acetyl CoA to CO_2) = 36 ATP.

Consequently, the energy difference is 2 ATP. Therefore 36 ATP still remain in the ethanol, 36/38 ~ 95 % of the energy remains in the ethanol.

The actual heats of combustion are -326 Kcal/mol for ethanol and -671 Kcal/mol for glucose, which is close to what you predicted based on the pathways. You need to double the amount for ethanol, since two moles are formed: 652/671 = 97%