NAME: _____ Exam 3

Material for this exam may come from Singh SB, Kang L., Narwrocki AR, ZhouD, Wu M, Previs S, *et al*. (2016) The Fatty Acid Synthase Inhibitor Platensimycin Improves Insulin Resistance without Inducing Liver Steatosis in Mice and Monkeys. *PLoS ONE* **11(10):** e0164133.

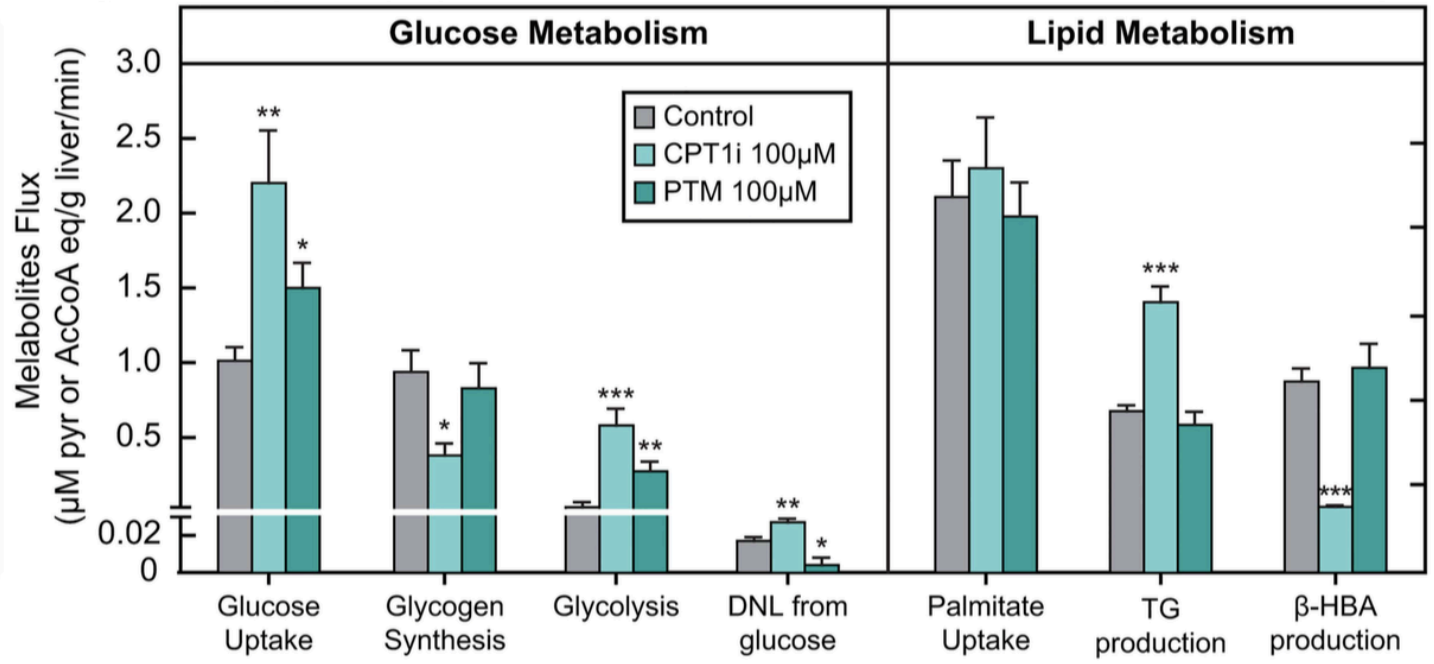
1. In some experiments, the authors administered 30 milligrams of Platensimycin per kilogram of animal mass. The chemical formula for Platensimycin is $C_{24}H_{27}NO_7$.

    a. If the average mouse has a mass of 20 g, how many moles of Platensimycin were administered with each dose?

    b. If the average volume of a mouse liver is 1.67 mL and the authors found the liver concentration of Platensimycin to be 1.445 μM, how many moles of Platensimycin were found in the liver?

    c. The authors find that Platensimycin is present in significantly higher concentrations in the liver than other tissue types. What percent of the original Platensimycin dose seems to make its way to the mouse liver?

2. In Figure 4 (see below), the authors present the metabolic flux in perfused, isolated mouse livers (neither insulin nor glucagon where administered to the cells):



Parallel comparison of Platensimycin (PTM, a Fatty Acid Synthase Inhibitor) and Carnitine-Palmitoyl Transferase Inhibitor (CPT1i) on glucose and lipid metabolism in perfused liver of lean C57BL/6 mice. PTM and CPT1i are used at 100 μM in the perfusion media (n= 3-5 per group). Bars represent means ± SEM. DNL is *de nova* lipogenesis. Asterisks denote statistical significance of treatment groups compared to control: *P≤0.05, **P≤0.01, ***P≤0.001.

a. The units on the y-axis are acetyl-CoA equivalents. For each metabolite, indicate the number of acetyl-CoA molecules that are produced during its breakdown.

   i. Glucose:

   ii. Glycogen monomer:

   iii. Palmitate (16:0):

   iv. Tripalmitoylglycerol (triglyceride, TG)

   v. β-hydroxybutarate

b. For each row in the table indicate whether each process or concentration is significantly increased or decreased or not changed by the presence of Carnitine-Palmitoyl Transferase Inhibitor (CPT1i) or Platensimycin (PTM) compared to the control.

	CPT1i	PTM
Flux through GLUT2		
Flux through glycolysis		
Flux through gluconeogenesis		
Flux through glycogenesis		
Flux through glycogenolysis		
Flux through Pentose Phosphate Pathway		
Transport of acyl-CoA into mitochondria		
Transport of citrate into cytoplasm		
[malonyl-CoA]		
Fatty acid synthesis		
Ketone body synthesis		

c. *De nova* lipogenesis specifically from glucose is significantly increased in the presence of CPT1i compared to the control though the absolute magnitude of the increase is small. Fatty acid uptake by the cell is unchanged by CPT1i, while tripalmitoylglycerol (TG) synthesis is significantly increased. What is the carbon source for the tripalmitoylglycerol (TG)?

d. Why does CPT1i significantly decrease ketone body synthesis?

e. What is the metabolic fate of the increased glucose that is taken up by the hepatocytes in the presence of PTM compared to the control?

3. Imagine that the authors had perfused the mouse livers with isoleucine:
    a. Draw isoleucine.

    b. Convert isoleucine to an α-keto acid.

        i. What enzyme catalyzes this step?

        ii. What cofactor or cofactors are required for this step?

        iii. What glycolysis or TCA cycle metabolites are needed for this step?

    c. Perform the reaction exactly analogous to the reaction catalyzed by α-ketoglutarate dehydrogenase on the isoleucine metabolite from the previous step.

        i. What cofactor or cofactors likely participate in this reaction?

    d. Perform the reaction exactly analogous to the reaction catalyzed by acyl-CoA dehydrogenase on the isoleucine metabolite from the previous step.

        i. What cofactor or cofactors likely participate in this reaction?

e. Perform the reaction exactly analogous to the reaction catalyzed by fumarase or enoly-CoA hydratase on the isoleucine metabolite from the previous step.

f. Perform the reaction exactly analogous to the reaction catalyzed by β-hydroxy-acyl-CoA dehydrogenase on the isoleucine metabolite from the previous step.

    i. What cofactor or cofactors likely participate in this reaction?

g. Perform the reaction exactly analogous to the reaction catalyzed by thiolase on the isoleucine metabolite from the previous step.

h. React the three-carbon acyl-CoA with propionyl-CoA carboxylase to add a carboxylate to carbon two (the α-carbon).

    i. What additional reactants are required for this reaction?

    ii. What cofactor (vitamin) is required for this reaction?

i. A mutase removes the carboxylate on carbon two (the α-carbon) and adds a carboxylate to carbon three (the β-carbon).

    i. Name the product.

j. Is isoleucine glucogenic?

k. Is isoleucine ketogenic?

l. Is isoleucine lipogeneic?