NAME: _____ Exam 2

1. Imagine that a heated sample of small intestine was incubated with an olive oil emulsion for 30 minutes and required 2.34 mL of 0.018 M NaOH to reach the end point in a titration with thymolphthalein as an indicator. An unheated sample of small intestine was treated similarly but required 5.67 mL of 0.018 M NaOH to reach the end point in a titration. One unit of lipase will hydrolyze 1.0 μmole of fatty acid from triglycerides in one hour. How many units of lipase activity were observed in the small intestine?

The following questions pertain to concepts and data presented in:

Aubert *et al.* (2016). The Failing Heart Relies on Ketone Bodies as a Fuel *Circulation* **133:** 698-705.

Bedi *et al.* (2016). Evidence for Intramyocardial Disruption of Lipid Metabolism and Increased Myocardial Ketone Utilization in Advanced Human Heart Failure. *Circulation* **133:** 706-716.

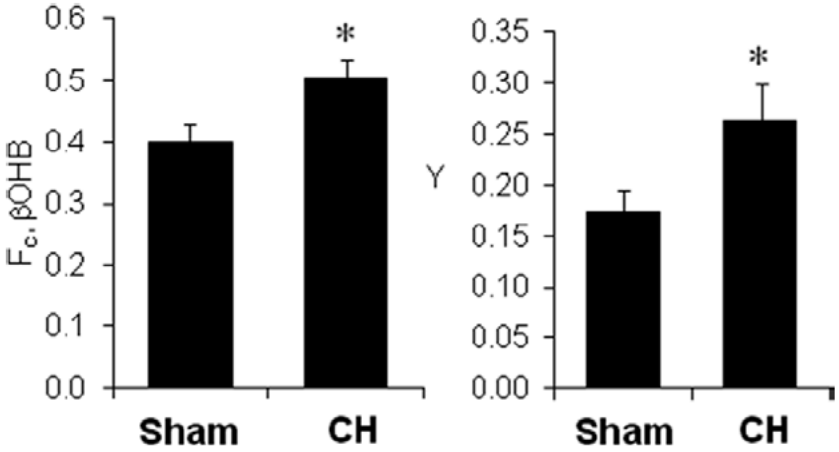


**Figure 3.** Increased βOHB oxidation in the hypertrophied heart. **Left,** The fraction of $^{13}$C-enriched acetyl-CoA entering the TCA cycle from $^{13}$C-labeled βOHB ($F_c$, βOHB) is shown. **Right,** The fraction of carbon entering the TCA cycle via anaplerosis relative to that entering via citrate synthase (Y) is shown for CH and sham-operated controls. Data are shown as mean±SEM (n=10, sham; and n=11, CH). *$P$<0.05. CH indicates compensated hypertrophy; CoA, coenzyme A; βOHB, β-hydroxybutyrate; SEM, standard error of the mean; and TCA, tricarboxylic acid.

2. The authors find that the compensated hypertrophic state results in an increase in the amount of carbon entering the TCA cycle from reactions that generate intermediates (anaplerosis) compared to citrate (right panel in Figure 3). The authors suspect that the increase in carbon entry into the TCA cycle other than by acetyl-CoA with citrate synthase is through succinate.

    a. Use a chemical reaction (draw chemical structures and include enzyme names) to explain why increased ketone utilization by cardiomyoctyes might result in increased levels of mitochondrial succinate.

For this process to occur, the cell requires additional succinyl-CoA. Succinyl-CoA results from the anaplerotic reactions after the oxidation of fatty acids with an odd number of carbons.

b. Draw the fatty acyl-CoA (7:0).

c. How many total C-C and C-H bonds are present in the acyl-CoA (7:0)?

d. How many redox reactions will you find in the complete oxidation of the acyl-CoA (7:0)?

e. Acyl-CoA (7:0) can undergo 2 rounds of β-oxidation, ending with propionyl-CoA (3:0)

   i. How many redox reactions does this account for?

   ii. How many NADH are produced?

   iii. How many $FADH_2$ are produced?

   iv. How many acetyl-CoA are produced?

f. Propionyl-CoA is acted upon by propionyl carboxylase, which adds a carboxylate function group to C2.
   i. Draw the product of this reaction.

   ii. What other reactants are required for a carboxylase catalyzed reaction?

   iii. What other carboxylase enzymes have we encountered?

g. In the final step, the carboxylate functional group is moved from C2 to C3 to form succinyl-CoA.

   i. What is the relationship between the molecule that you drew for 2.f.i and succinyl-CoA?

   ii. If the absolute identity of the atoms in the carboxylate on C2 <u>are not</u> the atoms found in the carboxylate at C3 in the product succinyl-CoA, what class of enzyme catalyzes this reaction?

3. Consider the indicated acyl-CoA molecules quantified in Table 2 [Dodecyl = 12].

    a. Indicate a non-anaplerotic reaction that produces each entry by drawing the reactant and naming the enzyme that catalyzes the reaction. Exclude CoASH.

**Table 2. Total Short-Chain and Medium-Chain* Acyl-CoA Profile of Nonfailing Versus Failing Hearts Quantified by SID-LC-MS/MS**

| | Nonfailing | | Failing | |
|---|---|---|---|---|
| Acyl-CoA | pmol/mg | SEM | pmol/mg | SEM |
| CoASH | 0.32 | 0.03 | 0.19 | 0.04 |
| Acetyl | 7.74 | 1.45 | 14.98 | 5.53 |
| Propionoyl | 1.76 | 0.20 | 0.89 | 0.18 |
| Succinyl | 17.74 | 2.35 | 10.54 | 2.22 |
| Butanoyl | 3.04 | 0.92 | 2.28 | 0.32 |
| Malonyl | 1.83 | 0.19 | 1.95 | 0.20 |
| 3-Hydroxybutanoyl | 0.29 | 0.05 | 0.57 | 0.13 |
| Hexanoyl* | 0.16 | 0.04 | 0.16 | 0.04 |
| Octanoyl* | 0.11 | 0.03 | 0.09 | 0.02 |
| Decanoyl* | 0.13 | 0.02 | 0.09 | 0.02 |
| Dodecanoyl* | 0.12 | 0.03 | 0.06 | 0.01 |

CoA indicates coenzyme A; SEM, standard error of the mean; and SID-LC-MS/MS, stable isotope dilution-liquid chromatography-tandem mass spectrometry.

    b. Why might acetyl and succinyl be at higher concentrations than the other entries? (1-2 sentences)