DOCTORAL DISSERTATION REVIEW

STUDENT: Patrycja Bielawiec

THESIS: Evaluation of cannabidiol influence on lipid metabolism in the skeletal muscle in a rat model of obesity induced by a high-fat diet.

OVERVIEW: This thesis is comprised of 3 published manuscripts. The first chapter corresponds to a review describing different cannabis derivatives and outlining how cannabinoids impact key metabolic tissues whose functions are disrupted with obesity. The second- and third-chapters stem from the same animal study but explore different signaling pathways in skeletal muscle. Briefly, rats were allocated to 1 of 4 diet groups: standard rodent diet (12.4% kcal from fat), standard rodent diet plus CBD, a high-fat diet (60% kcal from fat), or a high-fat diet plus CBD. CBD was administered during the final 2 weeks of the 7-week diet feeding study. In Chapter 2, the focus is on the ceramide synthesis pathway. Whole-body and skeletal muscle (red gastroc) insulin sensitivity were assessed. Briefly, rats fed a high-fat diet plus CBD experienced a partial or full reversal in study outcomes, e.g., sphinganine, ceramides, sphingosine, spingosine-1-phosphate and the S1P/ceramide ratio. Changes in the sphingolipid (and related) metabolites were generally supported by key proteins. In Chapter 3, the focus was on the fatty acid composition of various lipid fractions (FFA, DAG, PL, and TAG) in both white and red gastroc muscle. The 3rd chapter is largely descriptive in nature, revealing that consuming a HFD affected fatty acid composition in a lipid fraction-dependent manner, and that in some instances these changes were attenuated with CBD. Notably, there were instances where the effects of CBD varied between the two muscle fibers. A HFD generally increased the expression of markers of oxidative stress, inflammation, and eicosanoid production, and these changes were often rescued by the inclusion of CBD. The 3 articles are well written, informative, and provide new insights that support the growing interest in CBD. The thesis is equally well presented, with some comments for consideration outlined below.

COMMENTS FOR CONSIDERATION: In general, the thesis is very well written, easy to follow, and logically presented. The quality of the 3 manuscripts is excellent. Since the manuscripts are all published, my feedback relates to the unpublished material. Additionally, I will preface my comments and say that I am applying a Canadian perspective to the thesis. As such, please feel free to ignore my feedback if this is not typical of a PhD thesis in Poland.

1. I felt that the Aims outlined on Page 15 did not fully capture/align your study objectives. For example, you don't mention inflammation or oxidative stress in your Aims, but this is big part of your 3rd paper. You also don't mention looking at 2 muscle fiber types. In contrast, you do indicate fatty acid transporters, but you did not measure fatty acid transporters in your 2 research papers. It would also be helpful for you to add hypotheses for each Aim. In other words, what did you expect/anticipate to see in relation to each Aim?

2. I was surprised to not read a final integrative discussion (4-6 pages) that positions the thesis in relation to the broader field. Identifying gaps and limitations in the PhD thesis, proposing studies that could address these gaps (i.e., suggest next steps based on this PhD), discussing the overall implications of these findings in the "big picture", etc. This would be very useful to see how the PhD candidate would build on these results.

3. I was surprised by some of the changes observed in n-3 and n-6 PUFA in the various lipid fractions. This prompted me to wonder as to the fatty acid composition of the standard chow and high-fat diet. It would be very useful to add a supplementary table to the thesis that

provides a detailed breakdown of the diet compositions (both macronutrients and micronutrients, as well as individual fatty acids).

4. Page 17. Rats were fasted prior to euthanization, but I didn't see it indicated for how long they were fasted (apologies if I missed this).
5. What did you use to confirm that you loaded equivalent protein in your Western blots? Did you run Ponceaus or a housekeeping protein? I didn't see this indicated on Page 19 or in your chapters.

Minor typographical errors:

Abbreviations:

Δ9-THC – Δ9-tetrachydroCannabinol

DAG, FAME, FFA, LCFA, PL, TAG – all are written out in a pluralized form. Is this correct? Generally, people refer to TAGs rather than TAG (where the latter is a pluralized term). However, if you want these abbreviations to indicate the pluralized form, then in the Introduction you should remove the "s" when you use these abbreviations.

Introduction:

Page 10, Line 8: "FAs level is elevated" → FA levels are elevated

Line 19: "where it is are rapidly" → where it is rapidly

Line 22: "phosphorylates docking protein" → phosphorylates the docking protein

Line 25: "phosphatydilinositol" → phosphatidylinositol

Page 12, Line 2: "undergoes the esterification process" → undergoes esterification

Page 13, Line 15: "components of *Cannabis* plant" → components of the *Cannabis* plant

Line 25: "shown to decline mitochondrial" → I understand what you mean here, but suggest using the word "reduce" or "decrease" rather than decline. There are several instances where you use "decline" in the Introduction that could be similarly revised.

FINAL EVALUATION: Excellent, the candidate should be commended for their research output (in particular given that past 2 years of COVID-19 disruptions). The candidate should consider my suggested edits outlined above, but the thesis is strong. I recommend that the thesis progresses to the oral defense.