Report for the PhD Thesis of Luke Johnston:

Fatty acid composition in four serum lipid fractions and the pathogenesis of diabetes

Brief Synopsis of Thesis: This thesis investigates the relationships between individual fatty acids in different serum lipid fractions and their associations with insulin sensitivity / beta-cell function. Current clinical practice uses total triglycerides (TAG) and non-esterified fatty acids (NEFA) as risk markers for metabolic disorders, such as type-2 diabetes. Luke posits that studying individual fatty acids in TAG and NEFA fractions, as well as phospholipid (PL) and cholesterol ester (CE) fractions may identify more robust markers of insulin sensitivity and/or beta-cell function. Moreover, studying these individual fatty acids may also provide some insights into the underlying molecular dysregulations associated with changes in insulin sensitivity. This thesis consists of three studies. The first explores fatty acids in PL and CE fractions (published in JCEM). The second explores fatty acids in NEFA (under peer review), and the third explores fatty acids in TAG. All of these analyses used robust statistical and bioinformatic analyses to explore associations.

Objectives & Hypothesis: When I was about three quarters of the way through the thesis, I started to question whether these three studies were "enough" for a PhD. If I was to play the devil's advocate, I could say that the thesis essentially asks one question (i.e., what is the relationship between individual fatty acids and insulin sensitivity), but separates the answers across three different chapters/papers in a fraction-specific manner. I started to wonder if Luke had a role in the PROMISE clinical trial (e.g., participant recruitment, etc.) – although this would be unlikely given when baseline samples were collected. Consequently, I began to wonder if this was essentially a "data-mining" thesis. It wasn't until I reached the integrative discussion that I realized the Luke had to develop new statistical tools to conduct these analyses (codes which are now available for "R"). This is not trivial, but this important accomplishment isn't stated in the thesis until the very end. Luke may want to consider including a paragraph in Chapters 1 and 3 that outline the tools that he needed to develop in order to conduct his analyses. He may also want to include the actual codes in appendices.

It is my opinion that the research presented in this thesis is appropriate for a PhD, the objectives are clearly stated and the hypotheses are clearly described. Overall, the thesis is very well presented. The inclusion of the text above would, in my opinion, further strengthen the thesis and better clarify Luke's accomplishments.

Methodology / Analytical Assessment: The three studies use the same bioinformatic approach to study the associations between individual fatty acids and insulin sensitivity. Using a combination of general estimating equations and multivariate analysis (primarily PLS-DA) enabled Luke to explore the contribution of individual fatty acids in distinct serum lipid fractions on insulin sensitivity and beta-cell function. As indicated above, it would be beneficial if Luke incorporated text to clearly indicate that he had to develop new scripts for his analyses. Chapter 4 is published in an excellent journal, Chapter 5 is currently under review, and Chapter 6 is not yet submitted for peer review. Accordingly, I have provided more feedback on Chapter 6 that Luke may wish to incorporate / address when preparing this work for publication.

Originality and achievement: Luke's work is original and adds new insights regarding the associations between individual fatty acids and insulin sensitivity. In Chapter 6, Luke may wish to incorporate a stronger discussion in which he compares individual TAGFA and total TAG. Total TAG is a widely used clinical marker of health, but it is not clearly discussed in this chapter. A comparison between TAGFA and total TAG would help position if specific TAGFA are better predictors of changes in insulin sensitivity compared to total TAG.

Interpretation: The interpretation of results is both thoughtful and moderated. For example, the discussion about NEFA-FA versus total NEFA in Chapter 5 was well-balanced. The discussion about TAGFA in Chapter 6, as well as the Integrative Discussion, suggested a potential role for dietary carbohydrates. Given that the PROMISE cohort is a prospective study, it would surprise me that these dietary records are not available. If available, Luke should consider including carbohydrate intake into his models. If not available, then this should be addressed in the limitations section of the Integrative Discussion.

Presentation: The thesis is clearly presented. This is a highly polished document with virtually no typographical errors. Congratulations to Luke for submitting such a well-edited thesis.

Overall Recommendation: I recommend that the thesis be accepted for the degree of Doctor of Philosophy.

General comments:

- I was surprised to not see a list of abbreviations section in the thesis. I would suggest adding a list of abbreviations that you use commonly in your thesis after your Table of Contents (unless this is prohibited by the University of Toronto.
- List of Tables/Figures. Perhaps this is mandated by the University of Toronto, but I was surprised to see the entire legend appear in this section (as opposed to only the table or figure title). If not mandated, you could consider providing only titles to shorten this section up.
- As mentioned above, Luke should consider adding information regarding the R codes he had to develop. He may also wish to include the actual code as an appendix.

Specific comments:

Chapter 2:

- In section 2.2.3, you could consider adding the relative proportions of NEFA, PL, TAG, and CE in blood fractions. This could help provide a rationale for why you see stronger changes with individual fatty acids in TAG and PL fractions as compared to NEFA and CE fractions.
- You write both "diacylglycerol" (page 11) and "diacylglyceride" (page 12). I suggest choosing one (the former would align with your choice to use "triacylglycerol").
- Table 2.3: You should define what "X-sec" means in the legend, as this is not a conventional abbreviation.

Chapter 4: Since this chapter is published, I am only indicating minor typographical issues here.

- First line on Page 23: some text is missing "…declined by 8.3-19.4fully adjusted…"
- 4.3.1: You should include units after describing mean WC (i.e., WE of 98.5 cm).

Chapter 5:

- Table 5.1. Should this not be 1/HOMA-IR? Otherwise your data is indicating that insulin resistance has gone up over 6 years.

Chapter 6:

- It appears that you may have a few outliers as seen in Figure 6.1. Was a test for outliers conducted?
- Figure 6.7: Your legend indicates that R values are shown, but no values appear on the actual Figures.
- You may wish to incorporate the following article in this chapter: Zulyniak et al, Appl Physiol Nutr Metab. 2012 Oct;37(5):1003-7.
- A stronger presentation and discussion of the results you see when looking at total TAG would help to better position the importance of your findings about TAGFA. A clearer comparison regarding the predictive capacity of your TAGFA subset (of four) versus total TAG would be particularly useful.

Integrative Discussion:

- You may want to consider adding in a stronger discussion regarding the clinical implications (or lack thereof) of your research. Will the findings of your thesis change clinical practice? If yes, why. If no, why not? This relates to my comment on Chapter 6.