# Dear Dr. Editor

# We’d like to thank the editors and reviewers for their thorough and insightful review of our manuscript. We have been able to make the vast majority of the suggested revisions, which we feel has improved the manuscript considerably. Below we present a point-by-point response to each of the editors’ and reviewers’ comments.

* All three reviewers agreed that the paper had the strength of longitudinal followup and would be of interest to readers of the JLR but they also noted that there have been earlier studies with similar outcomes and one reviewer noted that some of those papers were by the present group but were not mentioned here. The reviewers also thought that the discussion was too speculative and the authors need therefore, to moderate their enthusiasm. My additional comment is that although you quote one paper by Yki-Jarvinen and colleagues. you did not discuss/reference her more recent papers linking shorter chain saturated fatty acids in the liver to NAFLD/NASH. You should look at those papers and add them to the discussion. I look forward to a modified version.
* *Thank you for this suggestion. We have made additions to the manuscript based on the editor’s and reviewers’ suggestions, including the discussion around short chain SFA and NAFLD by Yki-Jarvinen. We believe this has strengthened the paper and hope it satisfactorily addresses the editor and reviewers comments.*

# Reviewer 1

1. This manuscript reports interesting and potentially valuable associations between fatty acid composition and metabolic endpoints in humans over time.
2. The associations are powerful and stand alone. The authors have no data directly implicating de novo lipogenesis, so the last sentence in the abstract about previous research should be removed or substantially modified.

* *We thank the reviewer for these comments. We agree with this suggestion and have removed the sentence from the abstract.*

1. There are statements in the Discussion that are overly speculative, dated, and misleading. At the bottom of page 13 the phrase “may lead to greater DNL in an attempt to control blood glucose” should be removed. On page 14, lines 8-9 the sentence “Higher insulin resistance may encourage greater DNL to handle the higher blood glucose” should be removed. In the next sentence on the same page, the authors discuss reference 47 by implying that weight loss improved fatty acid profiles through effects on DNL, but that is facile and does not consider the complex effects of improved insulin sensitivity on lipoprotein clearance associated with induction of lipoprotein lipase, so this sentence needs to be eliminated or modified.

* *These concerns and suggestions are appropriately raised. We have rephrased these sentences in the Discussion to be more cautious and less speculative.*

1. While the discussion emphasizes DNL, it does not allude to how DNL can mechanistically lead to the metabolic endpoints used in this study. It would be helpful to indicate that DNL is complicated, and in certain tissues is involved in the promotion of chronic inflammation leading to decreased insulin sensitivity and other adverse metabolic events (Wei X et al. Nature 2016; 539:294-298).

* *The discussion on DNL has been clarified in light of the reviewer’s comments.*

1. On page 9, line 11 from the bottom, the text omits the actual r values for the relationships between fatty acids and TG (should be in the 0.8 range for many) and waist circumference (should be in the 0.3 range for many). More omissions are made on lines 4 and 5 from the bottom when referring to the data in Figure 3.

* *We thank the reviewer for noticing those omissions. The r values have been included in the text.*

# Reviewer 2

1. It is a frustrating that this is seemingly the third in a series of papers that is looking at patterns in lipid fractions and association with metabolic outcomes. Thus, the TG, NEFA and phospholipid fractions all seem to be a ‘least publishable unit’. The authors must integrate the knowledge gained from the previous analysis of NEFA and phospholipids and discuss some of the seemingly contradictory findings from the previous studies. If the data were examined, would the DNL story ‘hold up’?

* *We thank the reviewer for these comments. We agree that ideally the results of all the fatty acid pools would have been included together, but given the number of fatty acids, the longitudinal visits, the depth and volume of the analyses, and the complex biology involved, it simply wasn’t possible to contain everything in a single journal paper given word count and table/figure limitations. We have included a brief discussion of the results from the other fractions in the Discussion section. The complete and integrated discussion and presentation of the results is found in the first author’s (LWJ) PhD thesis (*[*http://hdl.handle.net/1807/80893*](http://hdl.handle.net/1807/80893)*). As to a combined analysis, we have presented these results at the European Diabetes Epidemiology Group Meeting in April, 2018 (*[*https://doi.org/10.6084/m9.figshare.6159293*](https://doi.org/10.6084/m9.figshare.6159293)*), and the proposed DNL mechanism remained clearly associated and had a strong contribution to associations with diabetes pathogenesis compared to other fatty acids/compositions. A short aside, these currently unpublished findings do not invalidate or undermine the previous work and the current manuscript. The combined analysis uses a powerful statistical technique (PLS), but it has a number of major limitations, not least of which is it can only handle cross-sectional data and cannot adjust for potential confounders. The present analysis in this manuscript uses PLS in addition to the powerful longitudinal technique GEE.*

1. The discussion of GEE and its limitations is well done. However, it seems that the analysis suggests that total triglycerides are predictive of HOMA2-%S and the other parameters. Do we need to subfractionate to get insight into risk for type 2 diabetes?

* *This is excellent question. For a couple of reasons, no we don’t need to subfractionate TAG to understand risk. Firstly, we found that the four TGFA also highly positively correlated with clinical TAG. Secondly, clinical TAG had a much higher magnitude of association compared to any individual TGFA. Measuring clinical TAG is enough to assess risk. However, our results provide insight into the potential mechanisms regarding how TAG influences risk for diabetes, which we show could be due to higher amounts of the four TGFA and that higher amounts of these four TGFA alone may strongly contribute to the higher clinical TAG.*

1. Supplemental figures 2 and 3 are not discussed at all. They appear to be interesting and informative in the development of the model. While the process is described in the earlier NEFA paper, the authors should help the reader understand what information is being provided without having to go back to a previous paper.

* *We thank the reviewer for this comment. We agree, this information is useful and have added a brief discussion of it to the Methods section.*

# Reviewer 3

1. The authors noted that cluster of four TGFA that correlated negatively with IS (14:0, 16:0, 14:1n-7, 16:1n-7) are products of DNL, and are therefore likely reflecting a connection between DNL, CHO intake and diabetes risk. This conclusion seems a bit overstated. Clearly, a link between this cluster and DNL is possible, but the signature could be also be reflecting dietary intake, hepatic steatosis and/or TGFA clearance by tissues such as muscle and adipose tissue. The authors should include a more balanced discussion of potential explanations/mechanisms beyond DNL.

* *We thank the reviewer for these comments. We have revised the discussion to include alternative possibilities.*

1. Are there any additional analyses that can be offered to strengthen evidence the TGFA composition is indeed a biomarker of DNL and/or CHO intake is this specific cohort? For example, did the PROMISE study include food recall records?

* *PROMISE did collect food frequency questionnaires, however this was done at the 3rd year visit while the fatty acids where measured at the baseline visit. So while we could technically use these dietary intake data to help understand the fatty acid data, we would likely obtain inaccurate or biased results because of the time separation. We therefore decided to not analyze these data.*

1. Also, the discussion should mention and consider that blood samples were acquired after an overnight fast. Does fasting/fed state impact the correlation between TGFA composition and DNL?

* *Excellent question. In the fed state, DNL would increase due to conversion of excess carbohydrates to fatty acids. This in turn would impact the composition of primarily the TGFA. In order to control for this source of variability in our cohort we only measured TGFA during fasting. We have included a brief description of this fed vs fasted state in the limitations section.*

Minor comments.

1. The authors should comment on how results reported here combine with (consistencies/contradictions) those reported in their recent Diabetalogia paper (the parallel NEFA analysis).

* *We have included a comment in the discussion on how our current analysis fits with our previous NEFA analysis.*

1. Authors underscore the strength of the statistical modeling used for the analysis, including the use DAG modeling to identify potential confounders. Readers might benefit from a brief comment on the basic principles and strengths of the method.

* *We agree with this suggestion, but initially excluded it from the methods to limit word count. We have included a section in the methods that briefly explains this method.*