Author's response to reviews

Title: Correlation of Omega-3 Levels in Serum Phospholipid from 2053 Human Blood Samples with Key Fatty Acid Ratios

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Author's response to reviews: see over

Dear Editorial Staff,

Thank you for taking the time to read our submission. We apologise for the delay in resubmitting the article, however we had some difficulty in contacting our statistician. We would also like to thank the reviewers for taking the time to review our manuscript and for their helpful comments. Below are our responses to the reviewers comments.

Reviewer #1: Jeffrey Yao

Major Compulsory Revisions:

1) Page 7, line 4 from the bottom The book entitled "Basic Practice of Statistics" by Moore was referenced for Pearson product-moment correlation coefficients to measure the correlations. The Pearson correlation is essentially used for a linear regression, whereas authors applied the "non-linear" regressions to all their figures (Figs. 1-3). This discrepancy needs to be verified in the Method section. Also, the book by Moore had 2004 and 2007 editions, not 2006 edition. Please verify.

Response: We have checked with our statistician (Dr. B. Allen, Professor, Univ. of Guelph) and verified the statistical methods used for this manuscript and have determined that a Pearson correlation was not used and that the following methods were used: 'Different calculations using the correlation function were made using linear, polynomial, log, exponential and power curve fits. In every case, the power curve provided the highest correlation coefficient and Power was there fore selected to model the data. A curve based on a Power function was plotted and the R^2 was recorded. This curve is used for graphical purposes only. The R^2 are used to compare between the different Y-values (i.e. AA/EPA ration, n-6/n3 ratio, etc) so that a determination could be made as to which ratio "fit" the data best. The Power Function was not used in determining the cut-offs referred to in the paper.' Changes to reflect the appropriate methods and the appropriate references have been made to the manuscript.

2) In Table 1, the cut-off AA:EPA ratio was listed as < 1.1 under the low risk category (DHA # 4.5%). However, this cut-off value appears to be over 5.0according to the figure 2. Please clarify.

Response: As is indicated in our updated methods section, the lines in the figures are a representation of the mean values, and the cut-offs are based on the distribution of scores; thus, the cut-offs as reported are correct.

Minor Essential Revisions:

1). The terms omega- and n- were used sparsely in the text, figures and table. Perhaps, authors can synchronize one term throughout the manuscript.

Response: Thank you for this comment. We have made our notation consistent throughout the manuscript.

2). Page 9, 2nd paragraph, line 4 ….. : omega-6 fatty acids (sum) ratio …… omega-6 was incorrect and should be omega-3 instead. Also in the same sentence, the AA:EPA+DHA is better changed to AA:(EPA+DHA) to avoid mathematical confusion.

Response: We have made the appropriate correction and notation changes.

Discretionary Revisions:

1). In Results section, additional table summarizing complete fatty acid profile by gender, age, and other confounding factors would be desirable as reference values for comparisons by other published or future studies.

Response: The authors' agree that the categorizing of fatty acid data by age and gender would add information to the paper. However, age and gender information is not available to the authors as multiple collection sites through our commercial omega-3 blood testing services were used to achieve this rather large number of samples and the subjects' demographic information was not consistently collected across all blood sampling sites at the time of sampling. Our subjects including both genders with widely-varying omega-3 intakes. However, the main contribution (s) of this paper are the provided/extensive correlations of the blood levels of the various omega-3 fatty acid parameters in a mixed population with the key ratios including the risk 'cut-offs' for the latter.

Reviewer #2: Sarah M. Conklin

2). Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

No. Participant recruitment is unclear. What % of the participants were female? Did all of the participants have diagnosed CVD? If no, why did they request a CVD fatty acid risk assessment? What is the average age and standard deviation? Do values differ as a function of age? Are there gender differences in relation to the FA percentages for risk? Why is this method of data collection not a potential limitation of the study? Wouldn't self-selection bias of patients requesting a fatty acid profile bias these participants that they may have levels that are much higher (or lower) than a typical North American? Why or why not?

Response: Participant recruitment is outlined on pg. 6 lines 1-6, under the 'Study Population and Blood Samples' heading in the Methods section. To elaborate, serum samples which were collected through our commercial omega-3 blood testing services were used with the permission of the clients for analysis/data purposes of this paper. Since the samples were collected and sent to us from numerous sites, we could not

readily record demographic information such as age, sex etc.(e.g., when samples were collected by physicians who maintained their private charts). In terms of self selection bias, most individuals in North America are not aware of food sources of omega-3 fatty acids or even different fatty acid species so it is difficult to determine if self-selection bias is an issue. However, given the large number of samples (2503) in this study self-selection bias should not be an issue compromising our overall results and conclusions.

3. Are the data sound and well controlled? Yes. Although, some details relating to variable merging in Excel may be unnecessary.

Response: We have revised our statistical methods section.

5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes, however with this unprecedented access to such a large number of samples it is unclear why additional descriptives regarding the sample were not made or discussed.

Response: Please see response to comment #2.

7. Is the writing acceptable? The manuscript would benefit from revision with attention to grammar. For example, the last paragraph of page 5 is one sentence.

Response: We have revised the manuscript and made appropriate grammatical changes where necessary. We reviewed the last paragraph on pg. 5; and since it is separated with a colon and a list was used, this sentence is not a run-on sentence and is grammatically correct in our opinion.