

## Peer Review File

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### Reviewer A

Fan et al have derived reference intervals (Ris) for selected cardiometabolic related tests in China. This is an important study as Ris for some analytes are known to have regional variation and use of inappropriate Ris could result in sub-optimal care. They recruited over 8000 subjectively healthy individuals and centralized the analysis which aids in reducing analytical related variation in results. A non-parametric approach was used to derive the Ris, and the need for partitioning was determined using standard deviation ratios (SDRs).

#### Methods

Comments 1: Line 155-157: Participants were also required to meet the following criteria: GluG<7.0 mmol/L, TC <5.2 mmol/L, TG <1.7 mmol/L, HDL-C  $\geq$ 1.0 mmol/L, and LDL-C<3.4 mmol/L. What was the justification for excluding individuals based on these cut-offs? Are these clinical decision limits derived from a Chinese population or at the very least verified to be appropriate for identifying people with either overt or sub-clinical disease in the Chinese population?

Reply 1: These cut-off values of lipid parameters were derived from the Chinese guidelines for lipid management for the purpose of excluding the population with dyslipidemia.

Changes in the text: None.

Comments 2: Line 164: How was 'continued use of alcohol, tobacco' defined?

Reply 2: 'Continued use of alcohol, tobacco' refers to those who had not abstained from drinking or smoking upon the medical history collection in the present study.

Changes in the text: None.

Comments 3: Line 168: Provide reference number for ethics approval.

Reply 3: The reference number for ethics approval had been provided in the revised manuscript.

Changes in the text: Line 169.

Comments 4: Line 173: "Fasting blood was collected from participants" check grammar

Reply 4: "Fasting blood" are incorrect expression and had been replaced by "Cubital venous blood".

Changes in the text: Line 173.

Comment 5: Line 175: Was the same brand of vacuum tubes used across all study sites? Were samples for glucose collected in separate tubes?

Reply 5: Varied brand of vacuum tubes were used across the study sites and the sample for glucose were collected in separate tubes.

Changes in the text: None.

Comments 6: Line 184-185: Provide a table listing the tests, methodology and within and between run coefficient of variation Statistics

Reply 6: The tests, methodology and within and between run coefficient of variation Statistics had been listed in a Table in the Methods section.

Changes in the text: Line 202-204.

Comments 7: Line 195-197: "Data from individuals with abnormal results outside the respective reference intervals were removed." Were reference intervals recomputed after removal of outliers? How many times was this repeated?

Reply 7: The reference intervals were recomputed once after removal of outliers.

Changes in the text: None.

Comments 8: Line 197-198: Was the use of the non-parametric method for RI determination done before or after transformation of data?

Reply 8: The non-parametric method for RI determination was used before the transformation of data.

Changes in the text: None.

Comments 9: Line 207: More recently an SDR >0.4 has been used for determining when to partition. Using SDR alone has a weakness as it focuses on the central distribution of reference values yet for reference intervals, distribution of data around the upper and lower limits is probably more important. Partitions may have similar SDRs but have significant differences at the lower and upper limits. Use of SDR alone could also result in over partitioning. It is worth pointing this out as a limitation.

Reply 9: Thank you very much for your valuable comments. The statistical methods used in this study were limited by the dataset and more reasonable methods would be employed in future studies.

Changes in the text: None.

Comments 10: Line 216: How was it determined that 15 years age intervals would be appropriate yet recruitment was not based on 15 years age stratifications? It is important to provide this detail to enable reproducibility of the work.

Reply 10: Thank you very much for your comments. We have described it in our revised manuscript. Which list as follows:

According to the age classification standards of the United Nations World Health Organization,

people under 44 years old are young aged, 45-59 years old are middle-aged, 60 to 74 years are younger elderly, 75-90 years are elderly. In order to maintain a consistent 15-year age group distance, age group 30-44 was identified. Because minors are in the physical development stage, many substances in the body are different from the adult, so the starting point of the first group is not 15 years old, but 18 years old.

Changes in the text: 165-173.

## Results

Comments 11: Line 232: Change 'variants' to 'variation'

Reply 11: 'variants' had been replaced by 'variation'.

Changes in the text: Line 233

Comments 12: Line 238-240: Delete this explanation as it is already provided below figure 2.

Reply 12: The explanation for figure 2 in the manuscript had been removed.

Changes in the text: Line 239.

Comments 13: Line 240-241: Delete this sentence as there is no need to mention that it will be discussed in the discussion section.

Reply 13: The sentence you mentioned in the comments had been deleted.

Changes in the text: Line 239

Comments 14: Figure 2: Needs to be clearer.

Reply 14: A revised Figure 2 with higher resolution had been provided.

Changes in the text: None.

Comments 15: Figures 3 and 4: These figures are too small hence difficult to read.

Reply 15: Figure 3 and 4 with higher resolution had been provided as separated profiles from the manuscript.

Changes in the text: None.

Comments 16: Table 3: For LDL, glucose and APO-A how did you decide to partition the RIs based on cities? Was the SDRreg done after partitioning the regions as 7 cities or 2 regions?

Reply 16: The LDL, glucose and APO-A are parameters affected by dietary habit which was

diversity between north and southern China, so we decided to partition the Ris based on cities. The SDRreg were performed after partitioning the regions.

Changes in the text: None.

#### Discussion

Comments 17: Line 275: Edite “Chinese’s” to “Chinese”.

Reply 17: “Chinese’s” had been replaced by “Chinese”.

Changes in the text: Line 278

Comments 17: Line 277: Abbreviation CVD not defined.

Reply 17: Abbreviation CVD had been defined as CVD (cardiovascular disease).

Changes in the text: Line 280

Comments 18: Line 298: Add “to” before “the incidence”.

Reply 18: “to” had been added before “the incidence”.

Changes in the text: Line 302

Comments 19: Line 305-310: Could these regional variations be linked to BMI and/or the presence of metabolic syndrome?

Reply 19: These regional variations were most likely attributed to dietary habits and climate.

Changes in the text: None.

Comments 20: Line 313-318: You cannot justify partitioning data based on a speculation. If there is no data to back the statement on CVD I would suggest deleting these sentences.

Reply 20: We agree with your comments that the partitioning data should not be justified based on a speculation and the relative sentences had been deleted. So that, the references 30 and 31 had also been removed and the references in the revised manuscript had been re-numbered.

Changes in the text: Line 317

Comments 21: Line 346: This sentence contradicts an earlier sentence. If BMI data wasn't collected how did you exclude participants with a body mass index (BMI)  $\geq 28$  or  $< 18.5$  kg/m<sup>2</sup>? Does it mean that no attempt was made to assess any vitals to determine whether the participants were healthy yet it is said that the participants were undergoing health checkups?

Reply 21: We agree with your valuable comments and the contradict sentence had been deleted.

Changes in the text: Line 346

Conclusion

Comments 22: Line 357: For most lipoproteins, many laboratories report risk based clinical decision limits (CDLs) based on guidelines. A comment on whether one should adopt RIs or CDLs should be made and whether in China you recommend RIs instead of CDLs.

Reply 22: The Conclusion had been revised according to your suggestions.

Changes in the text: Line 352-353

Additional comments

Comments 23: It is recommended that you use sex instead of gender if referring to male and female as biological constructs.

Reply 23: The “gender” in the manuscript had been replaced by “sex” according to your suggestion.

Changes in the text: Line 39, 44, 56, 92, 121, and 127

Comments 24: It would be useful to include a table summarizing participant characteristics e.g. age, sex, blood pressure, BMI, regional distribution of recruitment etc.

Reply 24: Thank you very much for your suggestions. The information regarding age, sex, and regional distribution had been listed in Table 1. BMI and blood pressure are very important variables associated with blood lipids and CVD, but unfortunately these variables were not collected in our present study.

Changes in the text: None.

## **Reviewer B**

I only have minor remarks and recommend accepting the manuscript.

Comments 1: Introduction - I suggest dividing the text into more than 2 paragraph.

Reply 1: The Introduction section had been divided into 3 paragraphs.

Changes in the text: Line 106

Methods

Comments 2: I suggest combining and numbering exclusion criteria (currently several sentences).

Reply 2: The exclusion criteria had been combined and numbered accordingly.

Changes in the text: Line 157-164.

Comments 3: "fasting blood... after they had fasted"- perhaps could be rephrased?

Reply 3: "fasting blood" had been rephrased as "Blood was collected at cubital venous".

Changes in the text: Line 172.

Comments 4: outlier removal - unclear formula (I think percentage points are meant by Pxy, where xy is number)

Reply 4: The abbreviation of P had been explained as "percentage" in the formula.

Changes in the text: Line 194-195.

Comments 5: I lack expertise to assess the statistical methodology, a statistician may best be one of reviewers.

Reply 5: Thank you very much for your comments.

Changes in the text: None.

## Results

Comments 6: as per lines 225-6 -please provide the number of outliers and define 'abnormal results', i.e, are these below/above manufacturers' reference range? target concentration? dylipidemia is mentioned in 2600 excluded volunteers, no definition for it given;

Reply 6: Thank you very much for your comments which definitely make us recognize our manuscript's defect. We have described it in our revised Figure 1 and manuscript, which list as follows:

As shown in Figure 1, 0 outliers were detected in TG; 66 outliers were detected in TC; 161 outliers were detected in LDL-C; 156 outliers were detected in HDL-C; 574 outliers were detected in sd-LDLC; 600 outliers were detected in GluG; 59 outliers were detected in ApoA1; 200 outliers were detected in ApoB; 691 outliers were detected in LpA; 948 outliers were detected in LpA II; 505 outliers were detected in HCY II. These outliers were excluded from further analysis..

According to 2016 Chinese guidelines for the management of dyslipidaemia in adults, Dyslipidemia was defined as one or more following abnormal blood lipid concentrations: TC  $\geq 5.20$  mmol/L (200 mg/dL), LDL-C  $\geq 3.40$  mmol/L (130 mg/dL), HDL-C  $< 1.00$  mmol/L (40

mg/dL), TG $\geq$  1.70mmol/L (150 mg/dL). Abnormal results are these outside the respective reference intervals.

Changes in the text: 233-239

Comments 7: line 246 - missing 's' in year?

Reply 7: The missing “s” had been added in the “year”.

Changes in the text: Line 242

Comments 8: line 247 'lower in females in males'->than

Reply 8: The missing “than” had been added before “in males”.

Changes in the text: Line 243

Discussion

Comments 9: first part repeats introduction...

Reply 9: The first paragraph of Discussion was a summary of the whole study.

Changes in the text: None.

Comments 10: 'Cis'? line 288

Reply 10: 'Cis' had been replaced by “CIs (Confidence Intervals)”.

Changes in the text: Line 285

Comments 11: 337 - 'inphysical'

Reply 11: 'inphysical' had been replaced by “in physical”.

Changes in the text: Line 330

Comments 12: Conclusions -biochemical...chemistry

Reply 12: “biochemistry” is a noun so that “biochemical” could not be replaced by “biochemistry”.

Changes in the text: None.

Comments 13: Figure 2 - Beijing missing in ApoA1 graph

Reply 13: Beijing had been added in ApoA1 graph and therefore a revised Figure 2 was provided.

Changes in the text: None. (see Figure 2-revised)

Comments 14: Figure 4 - impossible to read even zoomed-in

Reply 14: A revised Figure 4 with higher resolution had been provided.

Changes in the text: None.