Author's response to reviews

Title: Association of Inflammation and Endothelial Dysfunction with Metabolic Syndrome, Prediabetes and Diabetes in Adults from Inner Mongolia, China

Authors:

Angela M Thompson (athomps4@tulane.edu)
Yonghong Zhang (yhzhang@suda.edu.cn)
Weijun Tong (tongweijun@suda.edu.cn)
Tan Xu (xutan@suda.edu.cn)
Jing Chen (jchen@tulane.edu)
Li Zhao (nmtlzha2006@sina.com)
Tanika N Kelly (tkelly@tulane.edu)
Chung-Shiuan Chen (cchen1@tulane.edu)
Lydia A Bazzano (lbazzano@tulane.edu)
Jiang He (jhe@tulane.edu)

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Author's response to reviews: see over
Response to Comments from Reviewer 1:

General comments:

1) Introduction: please clarify the motivation to choose this population?

Very few studies have examined the metabolic syndrome and its risk factors in Inner Mongolia, an ethnic minority region in China. We have clarified this point in the introduction now (page 6, line 1-2).

2) Method: References need to be provided for the elevated level of the biomarkers. Is there any evidence that concentrations above the tertile level for these measures are associated with adverse outcomes?

These biomarkers are measured as continuous variables and are not normally distributed. Therefore, categorization of the biomarkers can help us to examine their association with our outcomes of interest. Prior studies have examined the association between these biomarkers and outcomes using tertiles, quartiles or quintiles of the distribution. In general, those studies have shown associations between the upper quantile and diabetes or the metabolic syndrome. A 2009 meta-analysis examined the association between CRP and diabetes comparing the odds of diabetes for those with CRP in the top third of the distribution versus those in the bottom third of the distribution. It included 16 studies, 3,920 incident cases of diabetes and 24,914 controls and reported that that those with CRP in the third tertile had a 72% increased risk of diabetes (RR 1.72, 95% CI: 1.54-1.92). In order to be consistent with this meta-analysis, we chose to rank the biomarkers by tertiles of distribution. We have cited the meta-analysis in the methods section (page 9, line 11-12).

3) Results: please keep consistent that which one was treated as exposure and which one was outcome measurement in the Tables and Figures.

We thank the reviewer for this comment. We have changed figure 1 so that it now shows the distribution of participants by tertile of biomarker concentration and disease category.

4) Discussion: how to explain the significant negative association between Angiotensin II and high glucose level? Thought the P value did not presented, from the 95%CI in Table 3, it should be significant.

In this study, we did not find Angiotensin II to be associated with diabetes, MetS or the combination of the two conditions. Although the linear trend was significant for the inverse association between tertile of Angiotensin II and mean blood glucose (table 2),
the association between elevated angiotensin II and diabetes, MetS or diabetes plus MetS was not significant (table 3). This may be explained by the fact that mean blood glucose differed by only 2.9 mg/dL between the first and third tertiles of Angiotensin II distribution. Also, the proportion of persons with elevated Angiotensin II did not differ greatly between outcome categories (Figure 1).

Specific comments:

1) Please reword "...risk of..." to "...odds ratio of..." throughout the manuscript because it is a cross-sectional observation. The authors also realized that "it is not possible to establish temporality or determine if elevated biomarkers leads to development of the prediabetes, diabetes or MetS or if development of the individual components of the MetS lead to inflammation and endothelial dysfunction."

We thank the reviewer for this observation. The wording has been changed throughout the manuscript to reflect this.

2) Why only adjust family history of hypertension, but not diabetes and other cardiovascular diseases?

Unfortunately, we do not have information on family history of diabetes or other cardiovascular disease (CVD) therefore we were not able to adjust for these in our analyses. However, hypertension is the most prevalent risk factor for CVD in Chinese population, so having a family history of hypertension likely provides information relevant to family history of CVD as well. Given that hypertension and diabetes are also closely associated, >60% of patients with diabetes also have hypertension, therefore, adjusting for family history of hypertension would also likely provide some level of adjustment for family history of diabetes even though that information is not available in our population.

3) The prediabetes and diabetes were NOT presented separately anywhere in the manuscript, so why not just in one group and simply in one name? Did the authors consider the limitation of define diabetes only based on fasting glucose?

We used prediabetes and diabetes together because of the small number of diabetes cases without the presence of the metabolic syndrome ([MetS] n=28). There are a total of 218 persons with prediabetes or diabetes without the presence of the MetS. Although the results are slightly attenuated when prediabetes and diabetes are used in combination, the results do not change much from the results using diabetes alone. For example, after adjustment for age, gender, smoking, drinking and family history of hypertension, and BMI, the odds associated with elevated hsCRP is 2.99 (95% CI: 1.37, 6.51) for those with diabetes compared to 2.29 (95% CI: 1.69, 3.10) for those with...
prediabetes or diabetes. The odds associated with elevated hsCRP is 2.85 (95% CI: 2.30, 3.54) for those with MetS but without diabetes compared to 2.38 (95% CI: 1.82, 3.11) for those with MetS but without prediabetes or diabetes. The odds associated with elevated hsCRP is 5.64 (95% CI: 3.19, 9.97) for those with MetS and diabetes compared to 4.82 (95% CI: 3.69, 6.31) for those with MetS and prediabetes or diabetes. Please see the supplemental table that accompanies this Response to Reviewers document.

We did not conduct 2 hour glucose tolerance test which is a limitation of this study. We have now discussed this limitation on page 13, lines 18-20.

4) Why not adjusted the dietary factors? As the author mentioned that this population’s diet was typically high fat and salt.

We do not have detailed information on individual dietary factors, so we were not able to adjust for any aspect of diet in our analyses. However, people of this region eat a fairly homogenous diet; therefore we would not expect large variability between individuals with regards to dietary factors. Diet is more consistent in rural areas than in urban areas and persons in this study were from 32 rural villages (page 6, line 10). We have stated that “The majority of local residents were Mongolians who had lived there for many generations, their professions were farmers and herdsmen and they maintained a traditional diet that was high in fat and salt” (page 6, line 10-12).

5) How did other lifestyle factors that maybe confounded the association, such as socioeconomic status or physical activity?

We also do not have detailed information on the other possible confounders suggested by the reviewer. The people of this region are traditional farmers and herdsmen and have similar socioeconomic status and heavy physical activity patterns; therefore, we would not expect large variability between individuals with regards to these factors.
Response to Comments from Reviewer 2:

1) In the introduction section, author stated that the prevalence of prediabetes and diabetes were 2.1% and 3.2%, respectively, in Mongolian population (Line 6, Introduction section). However, the proportions of participants with prediabetes or diabetes are even more than 22% ([218+343]/2536) in this study. What make this difference?

In our study population, there were 91 study participants with diabetes and 470 with prediabetes; therefore, the prevalence was 3.6% for diabetes and 18.5% for prediabetes. Although the study previously cited took place in Inner Mongolia, it was conducted in healthy steel workers and the workers were predominantly of Han ethnicity. Therefore, we have no longer included this citation.

2) In this study, the authors combined the phenotypes of prediabetes and diabetes. How about the associations between the biomarkers and diabetes alone?

We used prediabetes and diabetes together because of the small number of diabetes cases without the presence of the metabolic syndrome ([MetS] n=28). There are a total of 218 persons with prediabetes or diabetes without the presence of the MetS. Although the results are slightly attenuated when prediabetes and diabetes are used in combination, the results do not change much from the results using diabetes alone. Please see responses to reviewer 1 specific comment 3 for details.
Please note that the following Table **does not include pre-diabetes** in the categorization and uses the following categorizations:

1. **No Metabolic Syndrome, No Diabetes**
2. **Diabetes Only**
3. **Metabolic Syndrome without Diabetes**
4. **Metabolic Syndrome with Diabetes**

Supplemental Table. Odds ratios of metabolic or glycemic abnormality associated with elevated biomarkers of inflammation and endothelial dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>No Diabetes or MetS</th>
<th>Diabetes only</th>
<th>MetS without Diabetes</th>
<th>MetS with Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hsCRP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, gender</td>
<td>1.00</td>
<td>3.03 (1.42, 6.48)</td>
<td>3.42 (2.81, 4.15)</td>
<td>7.00 (4.02, 12.19)</td>
</tr>
<tr>
<td>Multivariable</td>
<td>1.00</td>
<td>2.83 (1.31, 6.12)</td>
<td>3.37 (2.77, 4.10)</td>
<td>6.72 (3.83, 11.76)</td>
</tr>
<tr>
<td>Multivariable + BMI</td>
<td>1.00</td>
<td>2.99 (1.37, 6.51)</td>
<td>2.85 (2.30, 3.54)</td>
<td>5.64 (3.19, 9.97)</td>
</tr>
<tr>
<td><strong>sICAM-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, gender</td>
<td>1.00</td>
<td>2.26 (1.07, 4.78)</td>
<td>1.60 (1.32, 1.94)</td>
<td>3.05 (1.83, 5.10)</td>
</tr>
<tr>
<td>Multivariable</td>
<td>1.00</td>
<td>2.06 (0.96, 4.41)</td>
<td>1.54 (1.28, 1.88)</td>
<td>2.81 (1.67, 4.73)</td>
</tr>
<tr>
<td>Multivariable + BMI</td>
<td>1.00</td>
<td>2.10 (0.98, 4.49)</td>
<td>1.38 (1.11, 1.72)</td>
<td>2.43 (1.42, 4.13)</td>
</tr>
<tr>
<td><strong>sE-selectin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, gender</td>
<td>1.00</td>
<td>1.39 (0.65, 2.99)</td>
<td>1.51 (1.25, 1.83)</td>
<td>1.48 (0.88, 2.49)</td>
</tr>
<tr>
<td>Multivariable</td>
<td>1.00</td>
<td>1.41 (0.65, 3.05)</td>
<td>1.52 (1.26, 1.85)</td>
<td>1.52 (0.90, 2.56)</td>
</tr>
<tr>
<td>Multivariable + BMI</td>
<td>1.00</td>
<td>1.56 (0.72, 3.90)</td>
<td>1.24 (1.00, 1.53)</td>
<td>1.18 (0.69, 2.02)</td>
</tr>
<tr>
<td><strong>Angiotensin II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, gender</td>
<td>1.00</td>
<td>0.90 (0.41, 2.01)</td>
<td>1.10 (0.90, 1.33)</td>
<td>1.28 (0.76, 2.16)</td>
</tr>
<tr>
<td>Multivariable</td>
<td>1.00</td>
<td>0.92 (0.41, 2.05)</td>
<td>1.07 (0.88, 1.30)</td>
<td>1.21 (0.71, 2.06)</td>
</tr>
<tr>
<td>Multivariable + BMI</td>
<td>1.00</td>
<td>0.90 (0.40, 2.01)</td>
<td>1.12 (0.90, 1.39)</td>
<td>1.26 (0.74, 2.17)</td>
</tr>
</tbody>
</table>

Abbreviations: hsCRP, high sensitivity C-reactive protein; sICAM-1, soluble Intercellular adhesion molecule-1; sE-selectin, soluble E-selectin. The multivariable models are adjusted for age, gender, smoking, drinking and family history of hypertension.