Author's response to reviews

**Title:** The Association of Serum Lipids with the Histological Pattern of Rectosigmoid Adenoma in Taiwanese Adults

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**Author's response to reviews:** see over
Dear Editors,

Thank you very much for providing us the comments to refine our paper. Here follows are the revisions made responding to the editorial and reviewers’ concerns. Adjustments have also been marked in red in the manuscript.

**Editorial comments:**

*Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.*

**Reply from Sun et al.:**

Our study had been reviewed and approved by Institutional Review Board of National Cheng Kung University Hospital in Taiwan that it followed all applicable institutional and governmental regulations concerning ethics. The approval statement has been added in the Methods section of the manuscript. Please see page 9.

“All authors declare that this research has followed all applicable institutional and governmental regulations concerning ethics and has been approved by the Institutional Review Board of National Cheng Kung University Hospital in Taiwan.”

**Reviewer's comments:**

**Reviewer:** Patricia Thompson

**Minor Essential Revisions:**

*The one concern that I think the authors really need to address to make this a higher impact paper is the issue of age. Clearly, the no adenoma group is considerably younger. Age is both a major risk factor for adenoma and elevated serum lipids. The authors have adjusted for age as a confounder, which is appropriate. However it would be informative to evaluate the relationship between TG and adenoma by age strata and also by gender. While the study may be underpowered for the more advanced histology, results of stratified analyses, even if exploratory in nature, would be informative as it is unclear if these relationships are present in younger individuals or restricted to older populations. This would help to inform on when interventions targeting TGs or other serum lipids would make sense.*
Reply from Sun et al.:

Thanks for reviewer’s to-the-point finishing touch comment, we evaluate the relationship between TG and adenoma with different pathological type as shown in Table 1-4.

Table 1 and table 2 below show the results of multinomial logistic regression analyses of different gender. With adjustment for age, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total cholesterol, triglyceride, and HDL-C, high TG (≥200 mg/dL) was highly related to villous-rich adenoma in women (OR=17.27, 95%CI: 4.40-67.75). There was a trend toward villous-rich adenoma in men with high TG (OR=2.50, 95%CI: 0.90-6.96), although not reaching statistical significance (p=0.078) due to small sample size of male subjects with villous-rich adenoma.

Table 1 - Multinomial logistic regression analyses of female subjects (n=995)

<table>
<thead>
<tr>
<th>Lipid abnormality</th>
<th>Tubular adenoma (n=100)</th>
<th>Villous-rich adenoma (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200 mg/dL</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥200 mg/dL</td>
<td>0.50</td>
<td>17.27</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.11-2.19)</td>
<td>(4.40-67.75)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.355</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adjusted for age, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total cholesterol, triglyceride, and HDL-C.

Table 2 - Multinomial logistic regression analyses of male subjects (n=1511)

<table>
<thead>
<tr>
<th>Lipid abnormality</th>
<th>Tubular adenoma (n=233)</th>
<th>Villous-rich adenoma (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200 mg/dL</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥200 mg/dL</td>
<td>1.03</td>
<td>2.50</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.59-1.80)</td>
<td>(0.90-6.96)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.920</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Adjusted for age, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total cholesterol, triglyceride, and HDL-C.

On the other hand, table 3 and table 4 show the results of multinomial logistic regression analyses by age strata. With adjustment for gender, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total
cholesterol, triglyceride, and HDL-C, high TG (\( \geq 200 \text{ mg/dL} \)) was related to villous-rich adenoma in both older subjects (OR=4.03, 95%CI: 1.68-9.66) and in younger subjects (OR=5.24, 95%CI: 0.95-28.90), although the later showed marginal statistical significance (p=0.057).

Because of limited subject numbers with villous-rich adenoma, it is difficult to perform detailed cross-tabulated analyses of age and gender.

Table 3 - Multinomial logistic regression analyses of older subjects (Age\( \geq \)50 years, n=1287)

<table>
<thead>
<tr>
<th>Lipid abnormality</th>
<th>Tubular adenoma (n=229)</th>
<th>Villous-rich adenoma (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200 mg/dL</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>200 mg/dL</td>
<td>0.76</td>
<td>4.03</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.39-1.49)</td>
<td>(1.68-9.66)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.428</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Adjusted for gender, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total cholesterol, triglyceride, and HDL-C.

Table 4 - Multinomial logistic regression analyses of younger subjects (Age<50 years, n=1219)

<table>
<thead>
<tr>
<th>Lipid abnormality</th>
<th>Tubular adenoma (n=104)</th>
<th>Villous-rich adenoma (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200 mg/dL</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>200 mg/dL</td>
<td>1.13</td>
<td>5.24</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.50-2.52)</td>
<td>(0.95-28.90)</td>
</tr>
<tr>
<td>P Value</td>
<td>0.775</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Adjusted for gender, general obesity, abdominal obesity, hypertension, diabetes, smoking, alcohol consumption, total cholesterol, triglyceride, and HDL-C.

**Discretionary Revisions:**

The data suggest the primary factor of interest is TG and not 'insulin' related features. The discussion might benefit from focus around those factors that are mechanistically related to TGs such as increased fecal bile acid content and effects on the colonic environment including effects on gut flora. This would also slightly shorten a fairly long discussion text.
Reply from Sun et al.:  
Thanks for comments and we have shorten the description about insulin related  
features, and added some sentences about the increased fecal bile acid content and  
their effects on the colonic environment.  
Revisions have been made accordingly and were marked in red in the manuscript.  
Please see page 14.  
“Secondary bile acids are potent nonsubstrate inhibitors of glutathione  
sulfotransferase activity which is involved in the detoxification of exogenous  
carcinogens, and some of their potentially toxic biological activities might lead to  
mutagenicity, transforming activity, and DNA-strand breakage [50]. Deoxycholic acid,  
a secondary bile acid, was found to stimulate colorectal epithelial proliferation and  
promote adenoma formation”

Reviewer: Georgia Lazaraki  
Major compulsory revisions:  
The authors should clearly state in the first paragraph of the methods part whether  
this is a retrospective study or the examinees were prospectively included. When  
reading the subjects part one comes to the conclusion that this is a retrospective study  
since finally from ~5000 examinees only one half were finally included. If this was a  
prospective study the authors should comment on this poor inclusion percentage.  
After that, in the study design part, it is stated that all participants filled a  
questionnaire regarding medical history, lifestyle and demographic data: was this  
prospective or this is part of a standard visit?

Reply from Sun et al.:  
Thanks for your reminding.  
This is a retrospective study, which has been added in the manuscript. Please see page  
5. Further, it is part of a standard visit that all participants need to fill out a  
questionnaire containing the above data.

Same comment as above regarding the blood sample: the authors describe the  
conditions the blood sample was taken, but again this is only the proper way to take  
blood sample for lipid levels. Were these instructions given in a prospective way to  
include patients or these are the standard instructions given for a correct blood  
sample and were estimated retrospectively by extracting data from patients' files?

Reply from Sun et al.:  
These were the standard instructions given for a correct blood sample and were
estimated retrospectively by extracting data from patients’ files.

Discussion part: “…We found that subjects with adenoma were older, and had higher fasting glucose and blood pressure than normal subjects. Male gender, diabetes, hypertension, general obesity, abdominal obesity, smoking, and alcohol consumption were also more common in those with adenoma (data not shown)”. I find a little bit odd the fact that the authors, in the first paragraph of the discussion part, comment on data that are not presented in the results part. I think that these data, that were may be expected, should be presented in a table in the results part.

Reply from Sun et al.: Data that was not shown in the previous version has been provided in a new table (see new table 1 on page 25 and 26) of revised manuscript. Original table 1 and table 2 were merged to form a new table 2. Please see page 27. The Methods part (see page 8) and Results part (see page 9 and 10) were also revised in the manuscript.

Discussion part: “…One possible explanation is that, different to the previous studies, we had fewer subjects with pure villous adenoma and we utilized a different statistical methodology”. Since the authors think it is the different statistical analysis used in the study that provides different results, they should explain here why it is preferable compared to that used in previous studies.

Reply from Sun et al.: Explanations on why our statistical methodology is preferable compared to that used in previous studies has been clearly made in the manuscript. Please see page 13. “More confounding factors were considered and entered into our final regression analysis. On the other hand, we had adopted ATP III criteria that were commonly used in clinical practice but were unusually applied for analysis in other studies.”

Minor essential comments: Abstract, Results, the first sentence “…The eligible examinees were classified into three groups: tubular adenoma (3333 subjects), villous-rich (tubulovillous/villous) adenoma (53 subjects) and normal (2,120 subjects)”. I think that population classification into groups should be included in the patients methods part.

Reply from Sun et al.: Population classification into groups was stated in the methods part of abstract. Please
see page 2. Some revisions were made in the results part of abstract. Please see page 3.

*Page 4, introduction, last paragraph: “…Previous studies on the relationship between serum lipids and colorectal adenoma shows conflicting results…” I think that “shows” should be replaced by “show”.*

**Reply from Sun et al.:**
Revision has been made according to the suggestion. Please see page 4.

*Page 5, Methods –Subjects part, GPT serum levels: I believe that the authors should replace this term either by SGPT or ALT levels, terms that are internationally more easily recognizable.*

**Reply from Sun et al.:**
Revision has been made according to the suggestion. Please see page 5.

*Page 7, Methods-Study Design part, “automatic biochemical analyzer (Roche Modular D&P)”, the authors should state the exact model, the manufacturer and country where this was manufactured.*

**Reply from Sun et al.:**
Revision has been made according to the suggestion. Please see page 7.
“an automatic biochemical analyzer (Model 7600, Hoffmann-La Roche Inc., USA)”

*Page 7: what is this electronic scale to count the height? If this is a device, please provide the exact model, the manufacturer and country where this was manufactured. If not, please provide a reference.*

**Reply from Sun et al.:**
Revision has been made according to the suggestion. Please see page 7.
“an electronic scale (Model HM-586, Jeng Jyi Co. Ltd, TAIWAN)”

*Page 7, last line: I believe that “21” goes with the beginning of the next paragraph and next sentence so it would be better if it was stated like this: “Twenty-one experienced gastroenterologists…”*

**Reply from Sun et al.:**
We are sorry that “21” is not a beginning word of the paragraph but a reference guide. The negligence has been corrected. Please see page 8.

_Discretionary Revisions:_
_The authors could comment in the discussion on the data presented in a recent similar Taiwanese study (Liu CS, BMC Gastroenterol. 2010 May 27;10:51)_

Reply from Sun et al.:
Comments on the data presented by Liu CS et al. were stated in the discussion part. Please see page 12.

“In Taiwan, there were studies showing that low HDL-C and high TG in metabolic syndrome are associated with increased risk for colorectal adenoma [31,44]. However, they weren't aimed at the histological patterns of adenoma. Our study further investigated the association of serum lipids with different histological patterns of adenoma.”

Thanks a lot for reviewers’ great comments and precise corrections.
We are glad to answer any of your other questions at any time.

Sincerely,

Yi-Ching Yang