Author's response to reviews

Title: Effects of coffee, smoking, and alcohol on liver function tests: a comprehensive cross-sectional study

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Manuscript Title: Effects of coffee, smoking, and alcohol on liver function test: a comprehensive cross-sectional study

We deeply thank editors and reviewers of the ‘BMC gastroenterology’ for taking time and efforts to review our paper. We have made corrections and clarifications in the manuscript after going over the reviewers’ comments as described below. A copy of questionnaire which the editor requested will be attached as a separate file.

We hope the revised manuscript will better meet publishing requirements of the ‘BMC gastroenterology’.

Responses to reviewer’s comments

Dr. Freedman:

The authors examine associations between coffee, smoking, and alcohol use with liver function tests in 500 Korean health check examinees. Although these exposures have been examined before, less information is available in Asian populations and thus the current results are of interest.

Major Compulsory Revisions:

1. Statistical analysis methods, paragraph 1—rather than performing chi-square tests for categorical variables, the authors should perform tests for trend (for table 1 and 2)
   → As you recommended, we performed tests for trend for the variables with ≥3 categories and annotated them in Table 1 and 2.

2. Did the authors have complete information on all exposures, was there any missing information and if so, how was it treated.
   → Several missing data were excluded from analyses about the missed variable. The number of analyzed cases represented in each tables.

3. Although the prevalence of hepatitis was low, were results similar if the patients testing positive were excluded?
There were 20 subjects who were infected by hepatitis viruses, but their viral titers were undetectable. Except a patient with anti-HCV positive whose serum HCV PCR was negative, 19 (3.8%) were inactive HBV carrier because Korea is HBV-prevalent country (about 4% in general population). According to our new analyses excluding those 20 patients, most results were similar with the present data (data not shown).

4. In addition, 45% of the eligible examinees refused to participate in the study which could affect the generalizability of the results. This should be mentioned as a limitation.

As you commented, we described the response refusal rate in our ‘Discussion’ part as a limitation (paragraph 6).

Minor Essential Revisions:

1. Discussion paragraph 2—c-reactive protein and total protein are very different exposures such that they should not be considered on the same causal pathway.

Although we used this expression since serum C-reactive protein is a part of serum total protein, you made a valid point. As you commented, the causal pathway of C-reactive protein and total protein might be different. The sentence was deleted from the ‘Discussion’ part.

2. The authors do not describe the proportion of instant and brewed coffee consistently. In paragraph 1 of the results section, the authors state that 57.8% of participants preferred instant coffee and 48.0% preferred brewed coffee. Whereas in the last paragraph of the discussion, they state that 81% of participants drank instant coffee. In Table 1, it looks like about 57.8% drank purely instant and 8% drank instant and brewed coffee. The results should be presented in a consistent way.

I apologize for several typographical errors. The proportion of subjects consumed brewed coffee was ‘8.0%’. In the last paragraph of the discussion, the proportion of participants who drank instant coffee was ‘71%’, as the sum of the proportions of instant coffee-only (57.8%) and mixed (instant and brewed, 13.2%) coffee consumers.

3. Instead of individual studies, the authors should cite one of the meta-analyses of coffee and liver cancer (introduction paragraph 2).

According to your recommendation, several meta-analyses about the relationship between coffee and liver cancer were cited in the ‘Introduction’ part (paragraph 2).

4. Please specify what the p-value is testing in tables 1 and 2. For table 1, is it the comparison between men and women?

P-values were specified and several p for trend values were annotated in Table 1 and 2.

5. Did the authors have information about decaffeinated or caffeinated coffee? If not, this should be added as a limitation.
We do not have information about the decaffeinated coffee consumption because many people do not know about the decaffeinated coffee and it is not available in many shops in Korea. This limitation was added in 'Discussion' part (paragraph 6).

**Discretionary Revisions:**

1. Did the authors have information on tea consumption? This would be an interesting addition as few studies of liver enzymes have been conducted in heavy tea drinking populations.

   → Unfortunately, information on tea or other caffeinated beverage consumption was not collected in the present study.

**Dr. Medici:**

Jang et al. conducted a cross-sectional study on 500 subjects who responded to a self-administered questionnaire about their alcohol and coffee drinking and smoking habits. In addition, BMI was calculated and subjects underwent abdominal ultrasound for determination of presence of fatty liver and blood tests for measurement of serum total cholesterol, total protein, serum albumin, alkaline phosphatase, total bilirubin, AST, ALT, and GGT. Coffee drinking was associated with lower total serum protein, albumin, and AST. Smoking was associated with higher GGT and lower serum protein, whereas alcohol drinking was associated with higher GGT.

**Major critiques:**

1) the study provides some useful information but overall it lacks of originality. In addition, the finding of inverse correlation between coffee drinking and serum protein and albumin levels is unexpected and the authors do not have a convincing explanation for their findings. This result in particular is concerning for selection bias where potentially coffee drinkers were also drinking more alcohol and may had lower serum protein levels.

   → As you commented, we could not find valid mechanism of the decreased protein and albumin levels in high-coffee consumers. However, those findings were very consistent in our study. When we performed the same multivariable analyses in 137 subjects who were never-drinker and never-smoker to evaluate the possible selection bias, high coffee drinking had negative associations with serum protein (p for trend=0.055) and albumin (p for trend=0.008) levels. These findings were commented in the ‘Discussion’ part (paragraph 2).

2) The definition used for heavy and non-heavy drinking is very questionable. The authors included among non-heavy drinkers men all subjects with alcohol consumption of < 60 grams/daily. Given that one alcoholic drink is 14 grams of alcohol, the non-heavy drinkers men included subjects who were drinking 3-4 drinks/day which is already classified as heavy drinking. This most likely represents a major confounder. The authors should perform a new analysis with a new classification of drinkers.
We defined the heavy drinker according to several references (Schiff’s disease of the liver, 10th ed. Page 884; Gut 1997; 41: 845-850) which commented an increased risk of developing cirrhosis with the ingestion of more than 60 to 80 g/day of alcohol in men and more than 20 g/day in women. Moreover, one alcoholic drink in Korea usually contains ~10 g of alcohol. It means about 6 drinks/day. According to your recommendation, we performed several multivariable analyses using a new criteria of heavy alcoholics (M>40 g/day, F>20 g/day) about the effect of daily coffee consumption and smoking amounts on the liver function tests. As a results, the negative correlation between daily coffee consumption amount and serum protein/albumin/AST levels were consistent with previous analyses using the original criteria for heavy alcoholics (M>60 g/day, F>20 g/day). Results about the effect of smoking on the liver function test were remained significant after changing the criteria. A supplementary tables is attached about the estimated liver function test results depending on the daily coffee consumption or smoking amount after adjustments for age, gender, BMI, regular medication, daily alcohol drinking amount.

**Minor essential revisions:**

The authors definition of “liver function tests” is questionable. In the background, they say that LFTs consist of total cholesterol, total protein, albumin, ALP, total bilirubin, AST, ALT, and GGT. It would be more appropriate to consider protein and albumin, liver function tests. AST, ALT, and ALP are liver enzymes. There are no data on INR which would have been a useful information among other liver function tests.

We agree your point that AST, ALT and ALP are liver enzymes. However, serum total cholesterol, protein and albumin levels were used to estimate long-term synthetic function of the liver. Thus, the ‘liver panel’ in our clinical laboratory contains total cholesterol, protein and albumin as well as liver enzymes. Unfortunately, prothrombin time was measured in about only two third participants since our subjects were health-check examinees.
Supplementary Table for Dr. Medici’s comment 2)

The effect of daily coffee drinking and smoking on the liver function tests by multivariable analyses

<table>
<thead>
<tr>
<th>Daily amount of coffee consumption (cups/day)</th>
<th>Total cholesterol, g/dL</th>
<th>Total protein, g/dL</th>
<th>Albumin, g/dL</th>
<th>Alkaline phosphatase (ALP), IU/L</th>
<th>AST, IU/L</th>
<th>ALT, IU/L</th>
<th>Ln(GGT)</th>
<th>Total bilirubin, mg/dL</th>
</tr>
</thead>
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<td>No</td>
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<td>4.55</td>
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<td>26.3</td>
<td>3.25</td>
<td>1.05</td>
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<td>(7.17, 7.38)</td>
<td>(4.48, 4.61)</td>
<td>(64.4, 75.0)</td>
<td>(21.3, 28.2)</td>
<td>(21.3, 31.2)</td>
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<td>(189.5, 202.6)</td>
<td>(7.04, 7.18)</td>
<td>(4.39, 4.47)</td>
<td>(60.0, 66.8)</td>
<td>(26.1, 30.5)</td>
<td>(26.4, 32.7)</td>
<td>(3.14, 3.36)</td>
<td>(1.02, 1.18)</td>
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<td>4.44</td>
<td>61.6</td>
<td>22.8</td>
<td>24.3</td>
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<td>(21.3, 24.4)</td>
<td>(22.1, 26.5)</td>
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<td>7.06</td>
<td>4.43</td>
<td>62.6</td>
<td>22.2</td>
<td>24.1</td>
<td>3.20</td>
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<td>(6.99, 7.13)</td>
<td>(4.39, 4.47)</td>
<td>(59.2, 66.1)</td>
<td>(20.0, 24.4)</td>
<td>(20.9, 27.2)</td>
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<td>(0.94, 1.09)</td>
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<tr>
<td><em>p for trend</em></td>
<td>0.222</td>
<td>0.006</td>
<td>0.033</td>
<td>0.053</td>
<td>0.002</td>
<td>0.049</td>
<td>0.540</td>
<td>0.346</td>
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</table>

Daily smoking amounts (packs/day)

<table>
<thead>
<tr>
<th>Daily smoking amounts (packs/day)</th>
<th>Total cholesterol, g/dL</th>
<th>Total protein, g/dL</th>
<th>Albumin, g/dL</th>
<th>Alkaline phosphatase (ALP), IU/L</th>
<th>AST, IU/L</th>
<th>ALT, IU/L</th>
<th>Ln(GGT)</th>
<th>Total bilirubin, mg/dL</th>
</tr>
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<tbody>
<tr>
<td>No</td>
<td>199.9</td>
<td>7.20</td>
<td>4.49</td>
<td>62.4</td>
<td>22.3</td>
<td>23.2</td>
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<td>(194.7, 205.2)</td>
<td>(7.15, 7.26)</td>
<td>(4.45, 4.52)</td>
<td>(59.7, 65.2)</td>
<td>(20.5, 24.0)</td>
<td>(20.7, 25.8)</td>
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<td>&lt;0.5</td>
<td>199.6</td>
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<td>(188.4, 210.7)</td>
<td>(6.94, 7.18)</td>
<td>(4.33, 4.47)</td>
<td>(55.8, 67.6)</td>
<td>(19.2, 26.7)</td>
<td>(19.5, 30.3)</td>
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<td>(0.88, 1.14)</td>
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<td></td>
<td>(186.8, 205.7)</td>
<td>(6.92, 7.12)</td>
<td>(4.39, 4.51)</td>
<td>(59.2, 69.1)</td>
<td>(26.0, 32.4)</td>
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<td>195.1</td>
<td>6.95</td>
<td>4.36</td>
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<td>(25.6, 33.3)</td>
<td>(3.29, 3.55)</td>
<td>(0.96, 1.14)</td>
</tr>
</tbody>
</table>

Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; Ln(GGT), natural logarithmic scale of gamma-glutamyltransferase

aData are presented as mean (95% C.I.). Estimated mean and 95% CI were calculated by using multivariable linear regression model adjusted for age, gender, body mass index, regular medication, daily alcohol drinking (M < 40 g or F < 20 g, M ≥ 40 g or F ≥ 20 g) and smoking (none, < 0.5 pack, 0.5-1 pack, > 1 packs) amounts.

bData are presented as mean (95% C.I.). Estimated mean and 95% CI were calculated by using multivariable linear regression model adjusted for age, gender, body mass index, regular medication, daily alcohol drinking (M < 40 g or F < 20 g, M ≥ 40 g or F ≥ 20 g) and coffee consumption (none, < 1 cup, 1-2 cups, ≥ 3 packs) amounts.