Author's response to reviews

Title: No effect modification of serum bilirubin or coffee consumption on the association of gamma-glutamyltransferase with glycated hemoglobin in a cross-sectional study of Japanese men and women

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Author's response to reviews: see over
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Editor
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Effect modification of serum bilirubin and coffee consumption on the association of gamma-glutamyltransferase with glycated hemoglobin in a cross-sectional study of Japanese men and women

Dear Editor,

Thank you very much for your consideration to the above-referenced manuscript. We revised the manuscript in response to the reviewers’ comments and would like to submit the revised version for publication in BMC Endocrine Disorders. Changes made in the revision are described below and shown in red. The comments were numbered by class of comments for each reviewer, if not numbered.

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Reviewer 1: Vidar Hjellvik
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Minor Essential Revisions

Methods, "Laboratory measurements":
1. As I understand, GGT was measured up to one year earlier than HbA1c and bilirubin (and the reports of coffee consumption) for 3924 persons. Could high GGT-measurements for some of these result in life-style changes or medication which affected the levels of HbA1c / bilirubin / coffee consumption at the survey? It would be reassuring if the authors could show that the association between GGT and HbA1c / bilirubin / coffee was the same for those with GGT measurements done at and before the survey.

[Response] This point is important in hindsight. We repeated the analysis for those with GGT measured at and before the survey. The GGT-HbA1c association was found to be weaker in men with the past measurement (page 12), but the repeated analysis for those with the measurement at the survey produced almost the same results for Tables 2 and 3. The results for those with the past measurement were rather subject to fluctuation because the number in some subcategories were much fewer. Thus we referred to only the results for those with the measurement at the survey (page 12).

2. How many of the blood samples for HbA1c was fasting and non-fasting, respectively? And what about the GGT measurements from within the past year? Were they fasting or non-fasting?

[Response] We described details of fasting status in the Methods (paragraph 2 on page 6).

3. Methods, alcohol (in sub-sections "Lifestyle questionnaire" and "Statistical analysis"): - The authors say that "alcohol users were defined as those who had consumed alcoholic beverages at least once per week over a period of one year" (in sub-section "Lifestyle questionnaire"). Further, they classify alcohol consumption as "never, past and current drinking with a consumption < 30, 30-59 or ≥ 60 ml/day" (in sub-section "Statistical
analysis"). Was a person who had not consumed alcoholic beverages at least once per week over a period of one year classified as 'never-drinker'? And I assume the limits of 30 and 60 ml/day is pure alcohol? 30 ml/day seems high as a lower limit. Should it be ml/week? As the GGT-level is affected by alcohol consumption I would like to see more detailed information on alcohol consumption in Table 1.

[Response] The definition of alcohol users was such as described, and lifelong abstainers included those who had drunk less than once per week or for less than one year in life. Alcohol amount is the amount of PURE alcohol (ethanol) per DAY. To avoid confusion, we used "ethanol" in the test (line 2 from the bottom on page 7). In fact, alcohol consumption seems high in Japan. According to the Japan Heath and Nutrition Survey in 2005, among men at age of 50-69 years, 40-45% were daily alcohol users, and 40-50% consumed ≥1 go of sake per day with a frequency of ≥3 times per week (1 go of sake = approximately 30 ml of ethanol). We did not refer to alcohol consumption in Japan, because this is another matter. In Table 1, however, we presented percentages of alcohol categories and also percentages of smoking categories for consistency.

4. Methods, "Statistical analysis":
- lines 2-4: Were the cut-off points for bilirubin chosen to have "groups as equal a size as possible" - i.e. the gender-wise medians - or because they "appeared to be a threshold in the relationship with HbA1c levels"?

[Response] Actually, the latter was a background information when the cutoff was decided. The text was modified accordingly (lines 3-4 in the Statistical analysis on page 7).

5. Results, second paragraph:
- line 6: I suspect the upper limit of 5.13 in the last CI is wrong: 5.06 (95% CI 5.04-5.13) as it makes the CI asymmetric and the corresponding P-value improbable.

[Response] We appreciate a very careful inspection. Yes, "5.13" was WRONG, and it was "5.07". The CI seems to be still asymmetric, but this is due to the rounding; geometric mean (95% CI) was 5.058 (5.044-5.072) in 3 decimal digits. P-value was correct; it derived from T = −6.12 with DF of 6226. On this occasion, we rechecked the presented values. All but one were correct; P-value described on the same line (bilirubin in men) was actually P<10^(-4), derived from T = −4.19 with DF of 4476 (the last line on page 8).

6. Results, third paragraph / table 3:
- Even though the p-value for trend in women is < 0.05, the authors should be careful to conclude that there is an association between coffee and Hb1Ac in (and only in) women with low bilirubin. As there are four p-values in table 3, there is 15% chance that at least one of them will be 0.04 or less if there is no association between coffee and Hb1Ac (1 - 0.96^4). This should be mentioned.

[Response] We should have been more modest in interpreting the finding. In the Results, lack of the interaction between coffee and bilirubin in women was more emphasized (lines 5-6 of paragraph 2 on page 9). A problem from the multiple comparisons was referred to in the Discussion (lines 5-8 on page 11).

7. Spelling:
1) HbA1c is sometimes written with 1c as subscript, sometimes not. In the 5th paragraph of
the discussion, line 2, "Hb" is missing.
2)- Abstract: "Meethods" should be "Methods"
3)- Abstract, results: Something is missing in the last sentence
4) - Background, §2, line 4: An "of" is missing in "GGT may be a marker oxidative stress.."
5) - Background, §2, line 9: "indiv

[Response] All of the above points were corrected. We consistently used "HbA1c" throughout the text.

Discretionary Revisions

Methods, "Statistical analysis":
1. - line 5: I suggest categorizing coffee consumption as <1, 1-3 and #4.

[Response] We used <1 rather than 0 in the text and Tables.

2. - line 9: Please specify which association in "Trend of the association was evaluated…"

[Response] We specified it as "the associations of GGT and coffee with HbA1c" (the last line on page 7).

Results:
I can not find any information about how the variables adjusted for (age, BMI, smoking, alcohol) are related to GGT, HbA1c, bilrubin, and coffee consumption. I suggest that the authors
3. - include curves of the crude associations in Figure 1 to see the effect of the adjustments.

[Response] In response to Minor Essential Revisions comment 1 of Dr Lin (reviewer 3), we deleted Figure 1. Yet we described age-adjusted and multivariate-adjusted geometric means of HbA1c in the text so that figure can be easily created. The reviewer suggests presenting crude means, but age-adjustment is usually essential (the latter half of paragraph 2 on page 8).

4. - include a table similar to table 1, but with the columns representing categories of GGT, HbA1c, bilrubin, and coffee consumption in stead of gender.

[Response] It is known that smoking, alcohol use and BMI are important covariates for HbA1c and GGT, and the adjustment for these covariates seems essential although the degree of confounding may vary with populations. We did not add a suggested table because it seemed voluminous.

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Reviewer 2: Siamak Bidel
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1. In the discussion section p.10 paragraph 1: The study finding does not exclude the possibility of decreasing risk of type 2 diabetes in high normal GGT levels! Please describe it better.

[Response] We did not address the risk of type 2 diabetes, and the present findings cannot exclude the possibility that the reviewer suggests. This point was referred to in the Discussion
(paragraph 2 on page 10).

2. The language of the text should be checked.

[Response] Thanks to Dr Hjellvik's corrections and suggestions, English in the revision was much better. We also made some additional minor corrections.

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Reviewer 3: Wen-Yuan Lin

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Major Compulsory Revisions:

1. Since the authors found no effect modification of either bilirubin or coffee consumption on the association between GGT and HbA1C, how authors use the title of “effect modification of serum bilirubin and coffee consumption on the association of gamma-glutamyltransferase with glykaed hemoglobin”? From authors’ group’s previous studies, they have reported that the associations between bilirubin/GGT and HbA1C. Can authors tell us what the manuscript add the current evidences on glucose metabolism?

[Response] The title was slightly changed using the reviewer's words. We previously reported the association between bilirubin and HbA1c (ref 10) and between coffee and GGT (ref 28) in the present study population. We have not reported on either the GGT-HbA1c association or the effect modifications of coffee and bilirubin. To emphasize the new findings, we modified the first paragraph of the Discussion (page 9).

2. The authors found that the sex difference existed between coffee consumption/bilirubin and HbA1C. Please describe why so in discussion section.

[Response] Honestly we do not have a plausible explanation for the sex-difference if it is true. Dr Hjellvik (reviewer 1) suggests that this may be a chance finding due to the multiple comparisons (Minor Essential Revisions comment 6), and we referred to this point in the Discussion (lines 5-8 on page 11). We also modified the sentence in the conclusion relevant to this finding (Abstract and page 12).

3. The exclusion criteria for participants included total bilirubin>3 mg/dl, AST/ALT/GGT greater than 3-fold of upper limit of the normal range and serum creatine> 2mg/dl. Please explain why the authors choose these criteria.

[Response] The laboratory criteria were rather arbitrary, but were employed to exclude the subjects with pathological conditions which possibly affected lifestyles and/or glucose metabolism. This point was added in the Methods (the last 2 lines on page 5 to the first 2 lines on page 6).

Minor Essential Revisions:

1. The figure is redundant. Please delete it.

[Response] We deleted the Figure, and described the geometric means of HbA1c according to GGT levels in the Results section (paragraph 2 on page 8) because Dr Hjellvik suggests that crude values be presented (Discretionary Revision comment 2).
2. Authors excluded those participants with medication for diabetes but didn’t exclude those with fasting glucose greater than 126 mg/dl (diabetes’s definition). Can authors analyze the effect modification among hyperglycemic patient and euglycemic patients?

[Response] Plasma glucose was not measured in the study, and we were unable to address the raised question.