Author's response to reviews

Title: Reproductive factors and its association with intima media thickness and carotid plaques in a cross sectional study of postmenopausal women enrolled in the population-based KORA F4 study

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Author's response to reviews: see over
Dear Editor:

We would like to thank the 3 reviewers for the amount of work they did put into their reviews and their valuable comments. We have made changes accordingly and feel that the manuscript has considerably improved. In the following we respond to the comments point by point. Our answers are presented in italics and in blue colour.

Comments to Reviewer 1:

Reviewer: enis ozkaya

Reviewer's report:

First # want to thank you for this opportunity, In this article authors indicate a possible association between some reproductive factors and atherosclerosis determined by carotid plaques and intima media thickness. And authors concluded that the early menarche is associated with the carotid plaques and ever use of hrt is inversely associated with the carotid plaques. However they did not show any association between the variables and the IMT. This is very well designed and written article with appropriate statistical analyses indicating a data which was not assessed before in this perspective. I think most important drawback in this paper is the retrospective design of the study. However authors tried to overcome this problem by the large number of study population they included in the study. I would suggest publication

1. Is the question posed by the authors well defined: Definition of the problem and the hypothesis were well defined.

2. Are the methods appropriate and well described?: Methods of data collection, measurements were appropriate.

3. Are the data sound? Gathered data is good enough to draw a conclusion

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? .Manuscript is well written

5. Are the discussion and conclusions well balanced and adequately supported by the data? :Authors have discussed all the issues about the subject.

6. Are limitations of the work clearly stated? The most important drawback of the study was clearly mentioned in discussion section.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes, it is clear.

8. Do the title and abstract accurately convey what has been found? yes

9. Is the writing acceptable? Yes it is OK
Dear Dr. Enis Ozkaya, thank you very much for the positive review of our manuscript. We made changes to the manuscript as suggested by the other two reviewers, which did to our opinion really improve the manuscript. We hope you agree.

Comments to Reviewer 2:

Reviewer: Waqas Qureshi

Dear Dr. Waqas Qureshi, thank you for putting so much time and effort in the review of this manuscript. Thank you for your valuable suggestions. We think that we could improve our manuscript considerable.

Reviewer’s report:

Stockl et al reported in their cross-sectional analysis of a population based study of postmenopausal women, the association of carotid intimal thickness and presence of carotid plaques with reproductive parameters. They demonstrated that age at menarche and use of hormone replacement therapy was associated with these outcomes.

Major Revision:

The authors studied the association of carotid IMT/plaques with reproductive parameters, however this is an old question and as authors very well stated that this has been studied in the past in quite much detail in regards to the same findings that the authors found (ref Acta Obstet Gynecol Scand 2007 vol 86, 995 – 1002.

The reviewer is right this topic has been studied in general before. Nevertheless, we pointed out in the introduction that there are some conflicting results and a few reproductive parameters have not been studied before.

Another point is the certain lack of population-based studies on this issue therefore the data of the population-based KORA F4 study adds new information to the current knowledge. The association between reproductive parameters and cardiovascular disease has been studied extensively and there are a lot of population-based samples. But to our opinion, as we pointed out in the introduction, it is a difference if you study the association between reproductive parameters and cardiovascular disease or with carotid plaques and intima media thickness, an early marker of atherosclerosis. There is not a complete concordance. And there are slight different results. Therefore, to our opinion, our study adds to the current knowledge.

Their conclusion that use of HRT was associated with lower risk of carotid plaques in age and multivariable model also seems overstated as they did not adjust for multiple comparisons and these findings might be just by chance.

Thank you for this hint. We revised the conclusions in the abstract and at the end of the discussion accordingly (see lines 61-63, and lines 329-331).
Several of the p-values are missing for carotid plaque associations with the several of the variables.

*We added the missing values.*

The authors used logistic regression for analyzing the associations, however logistic regression tends to overestimate outcomes in situations where the outcome occurs in >10% of the individuals. In such situations, generalized linear regression model using binomial distribution and log link might be appropriate. This will reduce the effect size even further but may provide a better sense of the association.

*Generalized linear regression models were used for analysing the associations with carotid plaques.*

One of the greatest limitations of these data is the missing data for which authors did not provide adequate methods.

*Thank you for this hint. Due to the suggestions from reviewer 3, only postmenopausal women aged 50 or older were now included in the analyses. Women not included in this analysis due to missing variables (which was mainly the variable IMT) were 140 women. These women did not differ significantly regarding age, BMI and other main characteristics, from the included women. We have added this information in the manuscript (see lines 120-121).
Reviewer 3 had also some questions regarding this issue. Please see also our answers to him. There is a table to compare the included and excluded women. We hope we have clarified this issue satisfactorily.*

Minor Revision:

It is important to describe the measures of exposure even though they were described previously for the better readability and understanding of the paper.

*The reviewer is right, we added more information in the methods section regarding this point.*

Several of the percentages in the table 1 are missing.

*We changed table 1 now.*

Minor discretionary revision:

Scientific writing needs improvement.

*A fluent English speaker corrected the manuscript after we performed this revision of the paper.*

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

*Comments to Reviewer 3:*
Reviewer: Samar El Khoudary

Dear Dr. El Khoudary, thank you for putting so much time and effort in the review of this manuscript. We answered to your review point by point. And we think that we could improve our manuscript considerably thanks to your suggestions.

Reviewer’s report:

Using data from the population-based KORA F4 study of 843 postmenopausal women (35-81 years old); the authors evaluated the cross-sectional associations between several reproductive parameters and early measures of atherosclerosis (intima-media thickness and plaque of the common carotid artery). The authors reported independent associations between younger age at menarche and ever use of hormone therapy with presence of carotid plaque. In order to ensure clarity of presenting these results the following changes are recommended.

Major Compulsory Revisions

1. The wide age range of women included in this analysis may impact the results. The study included postmenopausal women between 35-81 years old. According to the results’ section, first paragraph “the youngest women were included in the analysis based on their postmenopausal status after bilateral oophorectomy”. It is well known that surgical menopause women differ from natural menopause women in several aspects related to the menopausal transition (as the authors acknowledged in reference 17: Oophorectomy before natural menopause increases IMT independent of age and time since menopause). In addition, the young age of part of the study population indicates the possibility of premature menopause or the existence of other conditions that lead to bilateral oophorectomy in such young age. It would be of interest to know the distribution of type of menopause in this study. I am wondering why the authors did not create a menopausal status variable and adjust for it in their analysis. In order to insure that the results were not biased by including young postmenopausal women, it would be of great interest to reanalyze the data excluding women younger than 50 years old (with surgical menopause) and check if the results are the same. Please provide a table showing how women younger than 50 years old differs from those older than 50 years old in relation to study outcomes and main independent variables.

This is an interesting suggestion to see if there is an influence of the type of menopause, especially bilateral oophorectomy on the analysed association between reproductive parameters and IMT or CP. The reviewer is right about the fact, that women with surgical menopause are different to women with natural menopause, there exists good literature. The reason why we decided to include all postmenopausal women in this analysis, is, that we wanted to show an overview over a population-based sample of postmenopausal women, so how it would be in “real life”. But of course it is interesting to have a look under the surface and see if there are differences in these women due to the type of menopause.

5 women (0.59%) in this study were classified as postmenopausal before the age of 40 and 43 women before the age of 50 years (5.21%).

The table shows an overview over the study sample.
Based on the results in this table we decided to include only women aged 50 years and older in the analysis for this manuscript.

2. The description of the study population as well as the timeline of the reproductive factors was not clear. In specific, it was not clear if the reproductive factors were collected as part of KORA S4 or KORA F4. If the data was collected at both visits, please clarify which time point was used and why? What was the level of agreement between the two time points? Also did all women included in this analysis came from KORA F4? The method section gave the impression that some of the data were derived from the baseline visit as well. This needs to be clearly stated.

Thank you for this useful hint. This needs to be clear to the reader where the data exactly comes from. All women included in this analysis were from the follow-up study F4. All of them have therefore also been included in the baseline study S4. Some reproductive parameters like age at menarche have only been asked in the S4 study and have not been asked again in F4 since there is not expected to be a change. All other reproductive parameters like use of hormone replacement therapy were asked in both studies and of course the newest information (from F4) was used for these analyses. We added this information in the method section to clarify where the data came from exactly (see lines 149-159).
3. Methods’ section, page 5, first paragraph, the authors specified that the sample size of KORA S4 was 6640 subjects. Then the authors stated in the second paragraph of the method section, page 5 “of the 4261 participants in S4, 3080 took part in the F4”. Please clarify which number is the correct one for the sample size of KORA S4.

Sorry for not explaining it better. The numbers are all correct, but S4 had a response of 64.2%, therefore we changed the methods section and added the following sentence: Of the randomly selected 6640 subjects, 4261 (64.2%) participated in the S4 baseline study. (see lines 118-120)

In the same section, the authors indicated that 943 subjects were included. According to the exclusion criteria which were listed in first paragraph of page 6, the final study population sample should be 715 and not 843. This needs to be clarified. Please show how those excluded differ from included and discuss the ramifications of the exclusion criteria on the results.

Thank you for this hint. We revised the respective paragraph on page 6 to make it clear, how many women were included in the analysis (n=800). Due to your first comment, now only women 50 years or older were included. The postmenopausal women aged 50 or older who were not included in this analysis due to missing variables (which was mainly the variable IMT) were 140 women. The following table shows basic characteristics of the included women and the 140 excluded women. These women did not differ significantly regarding age, BMI and other main characteristics from the included women. We have added this information in the manuscript (see lines 120-121).
4. Data collection: “a fasting venous blood sample was obtained from all study participants”. Please clarify what was the purpose of these blood specimens, and how the related data were used in the current paper. If the participants had lipid profile, insulin resistance index, inflammatory markers, it would be very important to assess if the detected associations are independent of these potential covariates.

We additionally included the following variables now in the models: Triglycerides, total cholesterol and fasting glucose. Unfortunately inflammatory markers are not available for this study. We added the description in the methods section and added the parameters in table 1. (see lines 142-143)

5. Page 7, 2nd paragraph, the authors stated the following: “Women were classified postmenopausal at the absence of menstrual bleeding for 12 consecutive months, if they had bilateral oophorectomy (either alone or in combination with hysterectomy) and had hysterectomy without bilateral oophorectomy and were above 50 years (without reported menopause before hysterectomy)”. What about those who were younger than 50 years old with hysterectomy with/out bilateral oophorectomy? How those were classified and what was the distribution of the different categories of post menopause in this study (natural post menopause, hysterectomy with/without bilateral oophorectomy, bilateral oophorectomy).
Women with bilateral oophorectomy (regardless of a hysterectomy) were classified postmenopausal. Women with only one or a partially removed ovary and a uterus in situ were classified postmenopausal after absence of menses for more than 12 consecutive months. Women with hysterectomy but without bilateral oophorectomy were included in this analysis. Of course it would be possible to assess the menopausal status with blood tests, but unfortunately this data is not available for the F4 study.

We have discussed extensively in our working group what to do with hysterectomized women, but with intact ovaries, because we don’t want to lose all of them for the analysis, therefore we decided to include these women in the analysis. But we want to leave this to your decision and the decision of the editor, if you can agree to this exclusion and inclusion criteria.

Of the now included 800 women, 270 (33.8%) had a hysterectomy and 54 (6.8%) were oophorectomized. 49 of these 54 women had a hysterectomy and bilateral oophorectomy.

6. Please clarify how absence of menstrual bleeding for 12 consecutive months was verified. Was that self-reported or based on menstrual bleeding data

This information was gained in the personal interview by trained interviewers. The women were asked to recall their age at the last menstrual period. This means that self-reported data was used for this manuscript. We added this information in the method section (see lines 153-154)

7. Statistical analyses, page 9. Since the study included both continuous and dichotomous outcomes, I assume that the authors meant to say “PROC GENMOD” generalized linear models were used rather than “PROC GLM” general linear models were used. This needs to be corrected.

This is corrected now in the methods section, thank you for pointing this out. We wrote now: Generalized linear models were used for the analysis of IMT and logistic regression analyses for CP. (see lines 198-199)

8. Table 1 should be presented for the total population as well as by menopausal status category. P value should be provided. It was not clear why the authors chose to present differences of study covariates by plaque but not by IMT categories. Also why IMT was not listed in the table? What was the mean IMT of the current study population? Please include summary statistics of both outcomes for the full cohort as well as by menopausal status. Also, please provide “n” along with “%”. Please clarify what the superscript “2” stand for in Table 1?. Although the variable “menarche” is a continuous variable, the symbol “%” was listed after it to indicate that it is a categorical variable. Please correct.

Thank you for pointing the errors in table 1 out. We corrected this now. Menarche is shown in years and not % of course, sorry for that error. And 2 has an explanation what actual hypertension is defined as – while we changed the table to fit to the manuscript guidelines this sentence was somehow lost, which of course should not happen. (see Table 1)

We added a sentence in the results section of the manuscript to describe the mean IMT of the study population. Since we use IMT as a continuous and not as a categorized variable in the analysis we show the covariates by plaque and not by IMT categories. (see lines 212-213)

We added the n’s to the shown percentages and the p-values in table 1.

Since we now changed the study sample for this analysis and excluded women younger than 50 years, we think it is not necessary to separate women due to the reason of postmenopause. It is an interesting question to separate cause of menopausal status with the research question in our study. But we think this should be looked at in a further publication. But of course we leave this point for discussion for the reviewer and editor.
9. Table 2, were lipids, insulin resistance and/or inflammatory markers available? If yes, please provide models that are additionally adjusted for these potential covariates. I assume that what was presented in this table were the estimated means of IMT from the linear regression analysis. Please add unit of IMT to the table and indicate that estimated means of IMT was presented. The presented means (estimates) of IMT are higher than what was previously reported. This could be an indication of old age. What was the proportion of participants who were older than 60 years in the current study? The authors should discuss the compatibility of IMT level of their study population as compared to other study populations. Please discuss the clinical implication of the reported effect size (differences of IMT level between groups) in light of other clinical studies.

Of the 800 women included in the analyses 517 were 60 years or older. We added the unit of IMT to the headline of the table. We added that estimated means are presented. The analysis was done with adjusting for 3 more potential confounders: Total cholesterol, triglycerides and fasting blood glucose levels. We changed therefore the methods section and table 2. (see table 2).

10. Given the emerging concept of “timing hypothesis”, did the authors tested possible interactions between current use of HT and time since menopause as well as age at menopause?

For the analysis we used age at menopause and time since menopause in years as variables, which did not show an association with IMT or CP. And we examined duration of fertility in years, with no association as well.

11. Did the study collected information about CVD events? were the study population healthy women with no reported CVD events? If these data are not available then this should be discussed as one important limitation of the study.

We added this to the limitations section of the discussion: Another limitation of the study is, that information on CVD events was not yet available for the analysis (see lines 315-316)

12. The following statement was not clear “however, it could explain why the association between ever use HRT and IMT loses its statistical significance in the multivariable adjust model in this study”. Please clarify.

Thank you for this hint. Because the mentioned statement was confusing and not very informative we omitted the sentence from the text.

13. The authors stated that IMT is a less reliable marker for early atherosclerosis than CP and that is why they were not be able to report significant associations between reproductive parameters and IMT. This explanation is not convincing given that IMT is widely used in clinical studies as an early marker of atherosclerosis or vascular remodeling. As the authors acknowledged in reference # 2, IMT predict CVD in the general population based on a recently published meta-analysis.

Thank you for this hint. We corrected this now. As discussed, we are still of the opinion, that IMT is a less reliable marker compared to plaques. But of course this is no reason for the nonsignificance of our results. Therefore we changed the text. (see lines 295-298)
14. The final conclusion in the abstract as well as in the manuscript “in general, more gender based studies on cardiovascular risk factors in population based samples are necessary to better” is not directly related to the reported finding. Please provide a more clinically related conclusion.

We changed it in the abstract and in the conclusion section of the manuscript. We feel that it is more specific now: Further research, especially in studies with prospective population-based study design, is necessary to assess in detail what events in women’s life lead to increased IMT or CP. (see lines 61-63 and 329-331)

Minor Essential Revisions

1. Some of the statements made by the authors were too strong and should be modified. For example, in the introduction section, the author stated that “the menopausal transition is associated with CVD”. It would be better to say “the menopausal transition has been shown to be associated with CVD” instead.

This is right. We don’t want to have too strong statements. Therefore we changed this special sentence and we checked the rest of the manuscript for sentences which need to be modified accordingly.

Additionally the reference which was used assessed association between metabolic syndrome and menopausal status (cross-sectional) and not specifically CVD.

We added the correct reference.

2. Table 2: was generalized linear model used for both table 2 and 3 or general linear model for table 2 and logistic regression for table 3. Please clarify in the method section and under each table.

Generalized linear model was used for table 2 and logistic regression analysis for table 3, we clarified this in the methods section (see lines 198-199)

3. Table 2 and 3, please remove the symbol “%” for evaluated independent variables as this is not related to the tables contents.

The reviewer is right, we removed the %’s.

Discretionary Revisions

1. Please state what “KORA” is stand for.

KORA stands for Cooperative Health Research in the Region of Augsburg. We added this to the methods section. (see lines 105-106)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being Published

After performing the revisions, we showed this manuscript to a fluent English speaker.

Statistical review: No, the manuscript does not need to be seen by a statistician.