Author's response to reviews

Title: Early atherosclerosis and cardiac autonomic responses to mental stress: a population-based study of the moderating influence of impaired endothelial function

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

Thank you for the interest you have shown in our study. Enclosed please find the revised manuscript entitled "Early atherosclerosis and cardiac autonomic responses to mental stress: a population-based study of the moderating influence of impaired endothelial function". The responses to the issues raised by the reviewers are included below.

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Cordially,

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Editorial Comments:

Abstract: Please elaborate the Background section of the abstract to place the study in the context of the current knowledge in the field, in addition to stating the aims of the research. Please remember to also update the abstract details on the submission page.

Response: We’ve now corrected the Background in the Abstract as follows (p. 3): “Acute mental stress may contribute to the cardiovascular disease progression via ANS-controlled negative effects on the endothelium. The joint effects of stress-induced sympathetic or parasympathetic activity and endothelial function on atherosclerosis development have not been investigated. The present study aims to examine the interactive effect of acute mental stress-induced cardiac reactivity/recovery and endothelial function on the prevalence of carotid atherosclerosis.”

Reviewer 1.

In this study by Chumaeva et al. of a young subgroup of 61 men and women enrolled in the Cardiovascular Risk in Young Finns Study, the interactions between acute mental stress-induced cardiac reactivity/recovery surrogated by changes in each of RSA and PEP, and endothelial function surrogated by FMD, on subclinical atherosclerosis as measured by carotid IMT was examined. While there was no significant cross-sectional relationship between each of the baseline measure of RSA and PEP as well their reactivity with IMT, there was significant interaction between each of RSA recovery and PEP recovery with FMD in predicting IMT. Specifically, among the supra-median FMD subgroup, enhanced RSA recovery was predictive of lower IMT, whereas among the intra-median FMD subgroup, better PEP was associated with lower IMT. The results are interesting and of academic relevance to better understand the role of stress on the development of subclinical atherosclerosis.

major Comments:
(1) In examining interactions, the variables of interest together with their interaction term should often be entered simultaneously into the multivariable regression model. The robustness of the interaction is more assured when significance was demonstrated for both the variables as well as their interaction term. Apparently, only the interaction term was entered in the linear regression model. Was this the case? What are the results when the variables of interest, together with their interaction term are simultaneously entered? Are the interaction terms still significant?

Response: The main effects were included in the model. We have now mentioned this in the note in Table 2 as follows: “Each interaction is examined in a separate analysis including the main effects of sex, age, brachial baseline diameter, FMD, and the reactivity/recovery measure in question, but the main effects are not presented in the Table” (please, see p. 27).
Response: This is an important point. We have discussed this issue as follows: (pp. 15-16):

"Our results showing delayed RSA and PEP recovery may reflect the autonomic imbalance. We can suggest that ANS dysregulation is a very essential independent reason for cardiovascular health problems, and it may increase a risk of cardiovascular diseases independently on endothelium functioning. In line with this suggestion, ANS imbalance has been shown to be associated with ineffective cardiovascular functioning or with a risk of atherosclerosis even if the endothelium works properly, i.e., among healthy individuals (Cacioppo et al. 1995; Jae et al. 2008). On the other hand, we can suggest, that harmful influence of ANS dysregulation may be accelerated in impaired endothelium (reflected by low FMD in the present study). Our results reflect both these situations. In our study, slow RSA recovery was shown to be related to an increased IMT in normal endothelium, whereas slow PEP recovery was related to higher IMTs in impaired endothelium. These results are in line with the findings, that elevated sympathetic activity (Cacioppo et al. 1995; Grassi et al. 2001; Liao et al. 1995) and impaired parasympathetic control (Jae et al. 2008; Lucini, Norbiato, Clerici, Pagani 2002) have been shown to exert a harmful effect on cardiovascular functioning or to be related with atherosclerotic processes in both healthy individuals (Cacioppo et al. 1995; Jae et al. 2008) and in individuals with cardiovascular and metabolic disease states (Grassi et al. 2001; Liao et al. 1995). Cardiac recovery seems to play an important role in atherosclerosis development in persons with high and low FMD; however, we can suggest different impact of sympathetic and parasympathetic nervous system in atherosclerosis risk in individuals with normal or impaired endothelium. Thus, the role of sympathetically mediated cardiac activity seems to be more important in those with impaired FMD, and parasympathetically mediated in those with relatively high FMD.”
Given blood pressure is a known correlate with each of FMD and IMT, it should be adjusted for in the multivariable models. While as the author stated that BP responses were not measured, however, is there any other BP measurement available, such as pre-examination baseline BP, or any other BP measurement taken around the time of examination? Are the results modified by introduction of BP measurement?

**Response:** In our laboratory experiments, we focused on ECG and facial EMG measures and thus we have no blood pressure measures of our participants. In the Cardiovascular Risk in Young Finns Study, we have collected blood pressure data at several points in time but the data collection has been conducted in resting situations and the laboratory data collection is separated by years from the other follow-ups as it was conducted as a separate study at a different time point. Therefore, blood pressure measurements were considered rather inaccurate to be used in the current study. Furthermore, including blood pressure would be likely to reduce the number of participants even further.

Minor comments:
(4) Given the relatively limited sample size and as such the limited number of permissible covariate adjusted, the author should acknowledge that residual confounding as a potentially important limitation.

**Response:** We have now stressed this in the Methodological considerations (p. 17) as follows: “In addition, we cannot exclude residual confounding as an important limitation because of the limited number of covariates we were able to control for.”

**Reviewer 2.**

Minor Essential Revisions
Nice experimental study. Please, provide some p-values in the abstract, and result section of abstract seems to brief. So, conclusions seem not to be supported by the results and statistical significance

**Response:** We have now improved the reporting of the results in the Abstract by adding p-values and by explaining the results in more detail as suggested (p. 3): “We found a significant interaction of FMD and cardiac RSA recovery for IMT (p = 0.037), and a significant interaction of FMD and PEP recovery for IMT (p = 0.006). Among participants with low FMD, slower PEP recovery was related to higher IMT. Among individuals with high
FMD, slow RSA recovery predicted higher IMT. No significant interactions of FMD and cardiac reactivity for IMT were found”.

We’ve clarified and corrected the Conclusions as follows: “Cardiac recovery plays an important role in atherosclerosis development in persons with high and low FMD. The role of sympathetically mediated cardiac activity seems to be more important in those with impaired FMD, and parasympathetically mediated in those with relatively high FMD” (please, see pp. 3-4).
List of references


