Author’s response to reviews

Title: Pulse wave velocity and cardiac autonomic function in type 2 diabetes mellitus

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Author’s response to reviews:

Dear Ms Louise Maple-Brown,

Thank you for your letter of April 19th and for giving us the opportunity to revise our paper entitled “Pulse wave velocity and cardiac autonomic function in type 2 diabetes mellitus”, Ref: BEND-D-17-00055. We also thank the reviewers for their comments and the constructive criticism regarding this work. We hope we have successfully addressed all comments as follows:

Elif I. Ekinci, Reviewer #1:

Carotid femoral pulse wave velocity is independently associated with cardiovascular disease.
Pulse wave velocity is the gold standard method for assessing arterial stiffness.
Arterial stiffness is increased in people with diabetes.
PWV independently predicts mortality.
Chronic hyperglycaemia, hyperinsulinaemia, low grade inflammation and increased oxidative stress and AGEs may contribute to increased arterial stiffness in people with diabetes.

The hypothesis for the current study is that following adjustment for usual atherosclerosis risk factor, that CAD is independently associated with abnormal PWV in people with T2DM.

290 participants with type 2 diabetes were recruited.

Patients with AF, pacemaker were excluded, as were those with an eGFr < 30 and severe liver disease.

PWV was calculated from measurements of pulse transit time and the distance traveled between the common carotid artery and the common femoral artery. The distance measurements were taken with a measuring tape by subtracting the distance from the suprasternal notch to the carotid from the suprasternal notch to the femoral artery at the sensor location.

Patients with abnormal PWV were older, had higher arterial blood pressure and higher heart rate than those with normal PWV.

The investigators demonstrated that CAD assessed by determination of HRV was a significant determinant of abnormal PWV.

However, it is possible that both arterial stiffness and CAD simply share similar underlying aetiologies including chronic hyperglycemia and hyperinsulinemia, formation of AGEs and protein kinase C activation, low grade inflammation and endothelial dysfunction, as the authors have suggested.

Comment # 1:

Can the authors expand on the clinical implications of the study. Why would CAD lead to increased PWV? In particular can they expand more on the following statement please: "These data reveal an additional common pathophysiological pathway that may explain the relationship between arterial stiffness and autonomic neuropathy" and can they expand on the following: "Moreover, autonomic nervous system has been found to have a trophic influence on the structure of vessels and thus CAD may lead to arterial stiffening". Is there any information regarding the underlying biological pathway for this ie how CAD has a trophic influence on structure of vessels?
Our response:

Thank you for your suggestion. We have changed our discussion section according to your suggestions and we now provide more data about the possible pathophysiological link between cardiac autonomic dysfunction and arterial stiffness. Regarding the trophic influence of autonomic nervous system on arterial structure, since no biological pathway is known to date, the sentence has been removed from the manuscript.

Discussion section, pages 10-11, 2nd and 3rd paragraph: “The pathophysiological link between aortic stiffness and autonomic dysfunction and whether impaired cardiac autonomic function induces arterial stiffening or whether increased arterial stiffness leads to the impairment of the autonomic function remains obscure. Both arterial stiffness and cardiac autonomic dysfunction share common pathogenetic pathways including chronic hyperglycemia and hyperinsulinemia, formation of advanced glycation end-products (AGEs) and protein kinase C activation, low grade inflammation and endothelial dysfunction [2]. One hypothesis is that impaired cardiac autonomic function results in increased arterial stiffness. An explanation could be that patients with cardiac autonomic neuropathy present more often with calcification of the tunica media of the arterial wall [25]. It is noteworthy that the main determinant of the extent of arterial calcification is the severity of autonomic neuropathy [25]. On the other hand, arterial calcification has been suggested as an important determinant of arterial stiffness according to findings in humans and experimental models [26]. These data reveal that calcification of the arterial wall may be an additional common pathophysiological pathway that could explain the relationship between impaired cardiac autonomic function and arterial stiffness.

Another explanation could be that cardiac autonomic dysfunction may affect the elasticity of the arterial wall by changing the smooth muscle tone of large arteries [8, 27]. Interestingly, people without diabetes but with primary autonomic failure have been found to have stiffer aortas when compared with healthy control individuals [10]. Although this explanation is rather difficult to be proven in humans, experimental studies have shown that sympathectomized rats exhibit a significant reduction in the elastic properties of the aorta when compared with animals with intact sympathetic ganglia [28]. In humans on the other hand, high sympathetic activity has been associated with arterial stiffness in hypertensive patients with and without T2DM, as well as in healthy individuals. Increases in heart rate per se may lead to arterial stiffening independently of changes in activity of the autonomic nervous system [8]. Nevertheless, in the present study the association between autonomic dysfunction and arterial stiffness was not mediated by an increase in heart rate.”

Comment # 2:

It is interesting that there were no significant associations between PWV and conventional risk factors like age, smoking, microalbuminuria and lipid profile. Why might this be?
Our response:

Thank you for your comment. We have added in the revised discussion section (page 11, 3rd paragraph) the following: “It was suggested that in the early phases of atherosclerosis increased arterial stiffness is caused not by the atherosclerotic process itself and the formation of the atherosclerotic plaque, for which gender, smoking and lipids are powerful risk factors, but by an alternative pathophysiological mechanism, in which increased blood pressure is one of the most important factors.”. In addition, in the same paragraph we state that “It could be hypothesized that the presence of diabetes per se has a cardinal impact on arterial stiffness, overcoming the potential effect of other factors [11]. However, it should be noted that almost 80% of the participants in our study were on statin treatment, while more than 60% received antihypertensive medications and these factors may have influenced our results.”.

Comment # 3:

The introduction is too long and it could be cut down by half.

Our response:

Thank you for your suggestion. Our introduction section (pages 3-4) has been modified and shortened.

Comment # 4:

One limitation is that there are no non diabetic controls.

Our response:

Thank you for your remark. We have modified the limitation section of our manuscript that now reads (discussion section, page 12, last paragraph): “Another limitation is that we did not recruit participants without diabetes as a control group to investigate potential differences in the associations of PVW with cardiac autonomic dysfunction between persons with and without T2DM. However, cardiac autonomic dysfunction is not common in persons without diabetes.”.

Michael Skilton, Reviewer #2:

This study seeks to determine the association of abnormal pulse wave velocity (PWV; >90th percentile from age-appropriate charts), as the gold-standard non-invasive measure of arterial stiffness, with cardiac autonomic dysfunction. Previous studies have detailed these associations, but with PWV as a continuous variable. As such, the findings of the current study are somewhat
incremental, although are relevant from the perspective of identifying the putative mechanistic markers that co-exist with a high vascular risk phenotype in type 2 diabetes.

Comment # 1:

I have concerns regarding the statistical analysis, in particular the potential for false positives due to multiple analyses. Analyses of the same autonomic measures have been repeated using both parametric (log transformed) and non-parametric analyses, possibly increasing the likelihood of false positives due to multiple comparisons. In my opinion, it would be more appropriate to consistently apply one analytic approach.

Our response:

Thank you for your comment. As we mention in the statistical analysis section of our manuscript BRS and parameters of HRV were skewed and thus their values were log-transformed to improve normality for statistical testing. According to your suggestion we changed the results section of our manuscript and Tables 2 and 3 and in the revised manuscript we present only the results of the parametric analyses with the log transformed values.

Comment # 2:

Please don't use "CAD" as an acronym for cardiac autonomic dysfunction, given that it is commonly used for coronary artery disease, and thus may cause confusion readers.

Our response:

Thank you for your remark. We removed this abbreviation in the revised manuscript.

Comment # 3:

The measures of autonomic activity are continuous, and not dichotomized into normal / dysfunctional. The language used should reflect that you are detailing cardiac autonomic function or activation, but not dysfunction itself.

Our response:

Thank you for your comment. We have changed the term “cardiac autonomic dysfunction” in the revised manuscript and we now use the term “impaired cardiac autonomic function”.
Comment # 4:

The study is cross-sectional, and therefore unable to determine causality. Statements that indicate or infer causality should be tempered accordingly. For example, the rationale for the study is that "the effect of cardiac autonomic dysfunction on pulse wave velocity is not known"; yet the study design used does not inform on this question.

Our response:

Thank you for your comment. As we state in the limitation section of our manuscript: “A limitation is, however, the cross-sectional design that does not allow determination of a causal relationship between PWV and cardiac autonomic function. Although a non-causal association cannot be ruled out, causality could only be determined if the question of which of the two events (impaired cardiac autonomic function or arterial stiffening) appears first could be answered”. Statements in the manuscript that indicated causality are modified according to your suggestion. For example, in the revised abstract (page 2, 1st paragraph) it is now stated that: “However, the association of cardiac autonomic dysfunction with PWV is not known. In this study we examined the association between cardiac autonomic function and PWV in subjects with type 2 diabetes mellitus.”, while the last paragraph of the introduction section (page 4, 1st paragraph) now reads: “Based on the above literature data, the research hypothesis we examined in this study is that impaired cardiac autonomic function is associated with abnormal PWV in people with T2DM, when diabetes-related and classical risk factors for atherosclerosis are taken into consideration.”.

Thank you very much for your time.

Sincerely,

Associate Professor N. Tentolouris

On behalf of the co-authors