Author's response to reviews

Title: In vivo Assessment of Arterial Stiffness in the Isoflurane Anesthetized Spontaneously Hypertensive Rat

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Author's response to reviews: see over
Dear Dr. Sicari,

Enclosed you will find the revised manuscript entitled “In vivo Assessment of Arterial Stiffness in the Isoflurane Anesthetized Spontaneously Hypertensive Rat” for your consideration for publication in Cardiovascular Ultrasound. We appreciate the time and comments the reviewers have provided and have addressed their concerns point by point below (responses are in bold face):

Reviewer 1 Comments:

1) PWV is influenced by blood pressure. How do the investigators conclude that the higher PWV measured in SHR rats is independent of the elevated arterial pressure? Lack of blood pressure normalization is a significant limitation of this study. If the investigators are recommending that PWV can be used as a measure of stiffness in SHR rats regardless of blood pressure influences, then this should be explicitly stated. However, blood pressure is a critical component of PWV and it thus cannot be ignored. Indeed, PWV is influenced by blood pressure. To address this issue, we conducted additional studies to determine the systolic blood pressure (SBP) in the younger and older animals (age matched for the time points at which PWV was obtained). We found that there was no difference in the SBP at these time points. Thus, we are able to conclude that the observed differences in PWV are attributable to changes in arterial elasticity and are not to changes in blood pressure.

2) Using isoflurane for non-invasive PWV measurement is a standard lab procedure and using PWV to measure stiffness in SHR rats is thus not entirely novel. Thus, expanding the introduction by addressing the following questions would provide better context for the study: What methods were used previously to measure stiffness in SHR rats? Was isoflurane used in these methods? Is E arterial PWV better than the other methods that have perhaps been utilized? Why would PWV and E arterial be better or more convenient laboratory measures of stiffness? The introduction has been expanded somewhat to better illustrate the relatively new concept of utilizing ultrasound to determine PWV and to compare it to older methods. We have also attempted to provide better context for the study by clarifying the importance of understanding the effect of isoflurane on in vivo measurements of arterial elasticity in the Spontaneously Hypertensive Rat.

Reviewer 2 Comments:

1.) It is wrong to report data as “mean +/- SEM”. Please express variability with standard deviation all throughout the manuscript and the tables. Data are now expressed as mean ± SD throughout the manuscript.
2.) Page 7, Methods, Doppler US... last paragraph: variability in Doppler-derived PWV is mainly due to path length measurement and probe placing and angling. Measuring the time intervals on a frame, i.e. counting the pixels, is a much smaller source of error (as your data do confirm). Therefore, measuring variability of time intervals is far from “assessing intra- and inter-observer variability of PWV” and this section should be restated. Did the authors perform a real repeatability test? If not, this should be stated as a limitation. This section has been rewritten to reflect that we are measuring the variability of the determination of the time intervals and not of PWV itself. We have now included a separate independent experimental set of 14 SHR animals at 32 weeks to determine repeatability. We found that there were no differences when comparing PWV between the two age matched groups (p = 0.77).

3.)Page 9, Results, Intra and Inter-... and figure 2: units of measurements in the text are wrong since the error is expressed either as a percentage or as a time, not m/s. We thank the reviewer for noting this error. The units have now been corrected to %.

4.)Linear regression can’t be performed since here we are dealing with two distinct and different populations and this violates the basic assumptions of the test. Please remove scatter plots from Figure 2 and rely on the Bland-Altman plot. Pearson’s coefficients (with 2 decimals) could just be incidentally cited in the text, bearing in mind that it’s not a formally correct statistics. The scatter plots have been removed from Figure 2 and the Pearson’s coefficients are now cited in the text as suggested.

5.)Page 11, Discussion: it is not surprising at all that the correlation between readings of time intervals is "statistically significant". Actually, the opposite would have been worrying. Please remove this sentence. This sentence has been removed.

6.)Table 1: there should be something wrong. What’s in columns "Carotid", "Iliac"and "PTTci"? It seems milliseconds (ms) rather than m/s. Anyway, "Carotid" and "Iliac" (presumably the time intervals between the R wave and the Doppler waves) are not defined in a table caption, which is actually missing. Table 1 has been changed to reflect the reviewer’s comments with definitions provided below the table.

7.)Figure 1: why there’s a table imbedded in the figure (with wrong units of measurement and column titles not defined)? Why there’s a 160 mm length? Please remove and replace with the formula used to calculate time interval (R to iliac - R to carotid). In the caption, "the mean pulse wave was subtracted" is meaningless. The embedded table has been removed from Figure 1 and the PWV formula inserted in panel C.

8.)Page 2, Abstract, Background: the Background is the largest section in the abstract. Please reduce this section and expand the Methods part, where no mention is made to the actual methods used. The abstract now includes the PWV measurement and Pressure-volume loop methods as suggested.

9)Page 2, Abstract, Conclusions: "demonstrates" is a strong term, since the population is small but, above all, involves only two age points. I'd replace with "suggests". "Demonstrates" has now been replaced with "suggests".

10.)Page 7, Methods, Doppler US...: probe placing and angling is a major source of error in Doppler-derived PWV. This should be stated as a limitation later on. Why did the authors choose to use a tape meter, greatly decreasing precision in length measurement as compared to measuring with a caliper? This should also be stated as a limitation. To assess repeatability, accuracy and intra-operator consistency in probe placement, angling and path measurement, we repeated the PWV assessment in a second group of older SHR animals (n=14) and compared the two data sets. We found that there was not a difference between the two groups (p = .77)
11.) Page 9, Results, PWV, and later on all throughout the manuscript: please do not repeat "12 weeks old" and "32 weeks old" every time the groups are cited, as "younger" and "older" have already been defined. This has been changed throughout the paper to younger and older.

12.) Please do not repeat "The mean X was Y and values ranged from Z to W" for every measure, but shorten to ... "and Y for X (range Z-W)" or similar after the first occurrence, to improve readability and avoid repetitions. This has been changed as suggested throughout the paper.

13.) For non-significant P’s, 2 decimals are enough (i.e. p=0.62 rather than p=0.6153). Please check here and the rest of the manuscript. P-values have been adjusted as suggested.

14.) Page 10, Results, PWV and EaI: also for Pearson’s coefficient 2 decimals are enough (0.53, not 0.5286). These have been changed throughout the manuscript.

15.) Page 11, Discussion, last 4 lines: for the reasons already stated, the conclusions should be softened: no data is provided to say that PWV values are "highly reproducible" or "accurate", (and the correlation between PWV and EaI is not strong enough to say they measure the same thing). Page 13, Conclusion: see above, "precise", "accurate" and "repeatable" are terms not supported by the data shown in the paper. Please soften. The text has been modified to reflect the findings of our additional experiments and softened as suggested.

16.) Tables 1 and 2: replace SEM with SD. This has been changed as suggested.

17.) All throughout the manuscript, PWV, being a velocity, should be defined "high" or "higher", rather than "fast" or "faster". Please change. This has been changed as suggested.

We thank the reviewers for their careful critique and helpful comments, and we hope that you find the revision satisfactory for publication in Cardiovascular Ultrasound.

Sincerely,

[Signature]

Andrea L. Kalinoski, Ph.D.