Author’s response to reviews

Title: Foetal aortic flow velocity waveforms in healthy and hypertensive pregnant women

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Author’s response to reviews: see over
Cover Letter

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Editor-in-Chief,
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We would like to thank you and the reviewers of CARDIOVASCULAR ULTRASOUND for taking the time and effort to review our manuscript. The revised version and the response to both reviewers’ remarks is attached to this letter, after being approved by all authors. We hope it is addressing all raised concerns and will be looking forward to your response.

Yours sincerely,

Signature     Date

January 5, 2014

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**Reviewer:** Francesco Stea

**Reviewer's report:**

- **Major Compulsory Revisions**

1- **Abstract, Conclusions:** this does not seem the main finding. I would say that the main finding is that pulsatility indexes trend up during pregnancy in both normotensive and hypertensive women. The increase could be said to be less pronounced only if a formal statistical comparison is performed (see further on).

We appreciate the reviewer’s important comment. The 'conclusions' section was reformulated. (lines 46-49, revised version)

2- **Results:** The study is a comparison between normotensive and hypertensive women, but blood pressure is not reported. If the BP difference between groups is small, it could explain the non-significance of difference in results with this number of subjects.

Thank you for your positive criticism. The 'blood pressure' was not measured at the moment of Doppler studies were made and so was not used to correlate with them; indeed it was part of the regular pregnancy follow-up procedures. Stage 1 essential hypertension was an *explanatory variable* and, therefore, no correlations between blood pressure and indices were established.

3- **It is hard to infer curves, especially curves other than a straight line, from three points only.** Using the log of time in the analyses, rather than the time, may seem artificial. The authors should clarify why they choose a non-linear relationship and why they opted for log-transforming the time. It is not even clear if “time” in the analyses refers to trimester 1-2-3 or to the exact gestational week. In the first case, it would be hard to treat it as a continuous variable, not to mention log-transforming it.

Concerning this comment, several remarks ought to be made:

a) With the considered log transformations, the transformed response depends linearly on the transformed time; in particular, this is a linear model and is (easily) fitted as such. No theory from non-linear models is required.

b) We have 3 time points and a longitudinal study consisting of 101 women. As a straight line is totally defined by two points and as our model is linear, the 3 time points in the study are good enough.

c) In the analyses, “time” refers to gestational trimesters: 1<sup>st</sup> (11-14 weeks), 2<sup>nd</sup> (19-22 weeks) and 3<sup>rd</sup> (28-32 weeks). As consecutive differences between the three evaluations are roughly the same, the variable may be given a continuous character. Moreover, its coding is redundant, as far as the two consecutive differences can be computed and are (approximately) the same.

d) We agree that using time directly would have given more interpretable models, but the fact is that those models did not satisfy the requirement of residuals normality. We first started by log-transforming the response only, but then better models were found once time was also log transformed (better in the sense of both fitting and diagnostic analysis).

e) The equation in the original variables has multiplicative effects; therefore, on those variables, the coefficients have to be interpreted accordingly. For example, for a fixed index, a fixed hypertensive status and a fixed time point t, the response value obtained for kt is k<sub>total</sub> times the response value obtained for t, for any k.

4- **Results, multivariate analysis / Figure 3:** it is hard to infer from figure 3 that RI is different between HT and NT. No p-value is mentioned. See also comment in the next section. No statistical analysis and p-value are shown to support the statement that the curves and the evolution over time are significantly different and different from a flat line.

Although the visual inspection may suggest otherwise, in fact RI was different when NT and HT women were compared as stated in the first manuscript version. This statement follows from the statistical significance of the effect of the first 4 variables in Table 3, once variable PI equals 0 when the index is RI, and log(Time) equals 0 when Time is the 1<sup>st</sup> trimester. The fact that all curves are significantly different from the flat line follows again from the significant effects in Table 3. More precisely:
i) For PI and HT women: progression along Time is given by the effects of log(Time), log(Time)×PI, log(Time)×HT and log(Time)×PI×HT, and they are all significant in the model

ii) For PI and NT women: progression along Time is given by the effects of log(Time) and log(Time)×PI, and both are significant in the model

iii) For RI and HT women: progression along Time is given by the effects of log(Time) and log(Time)×HT, and both are significant in the model

iv) For RI and NT women: progression along Time is given directly by the effect of log(Time), which is statistical significant

Therefore the original sentence was changed to:” At baseline, in the first trimester, and for RI only, a significant difference between its mean values in hypertensive and normotensive women was identified - Table 3”. (lines 295-296, revised version)

5- Discussion, page 12, second paragraph: no formal statistical analysis was performer to say that the PI trends are divergent.

The confidence intervals for the mean prediction in Table 3 indeed support that.

6- Even assuming that the trends are significantly divergent, since PI and RI do not reflect arterial impedance only, but depend on several factors – as the authors have correctly stated – I would soften statements in the patho-physiological explication, and above all I would not attribute the changes to the aorta only.

We appreciate the reviewer’s important comment and as consequence, the paragraphs were reorganized. (lines 360-379, revised version)

7- Conclusions: as said above, this does not seem to be the main finding of the study; no formal statistical comparison was performed to say that trends are different; it is hard to conceive that adaptive mechanisms would be “local” and selectively concern the aorta only.

Thank you again for this remark. We replaced 'a local adaptive' for 'an adaptive'. (lines 391-396, revised version)

• Minor Essential Revisions

8- Abstract, Background: “aimed to compare” -> “aimed at comparing”
9- Throughout the abstract and the manuscript: “indexes” and “indices” are both acceptable, but you should stick to one form.
10- Introduction, first paragraph: “It’s application”... The period is not clear, there are probably some misspellings. Could “It’s” be “Its”?
11- Paragraph two: “foetal circulations because they” -> “foetal circulations, because they”
12- Third paragraph: Resistance and pulsatility indexes should both begin either with upper or lower case letters.
13- Page 4, last paragraph: i would use “chronic arterial hypertension” rather than “chronic hypertension”.
14- In a medical context I would say “a highly prevalent disease” rather than “a prevalent disease”.
15- Page 5, first line: “impinge enhanced risk” -> “impinge on the risk”
16- Last paragraph: “we aimed in to compare” -> “we aimed at comparing”. See above for “indices”.

Thank you again. All suggested changes were made.

17- Patients and Methods: Acetylsalicylic acid is not entirely neutral to hemodynamics or maternal biochemistry, even at low doses. Since women taking this drug were included, this subject is to be discussed. If all hypertensive and no normotensive were taking the drug, this should be clearly stated; if the proportions are different from 100 and 0% respectively, they should be reported and used as confounders in the analysis.

We appreciate the reviewer’s important comment. In fact, the proportions are 100 and 0%, respectively. The sentence was reworded and this aspect has been clarified. (lines 131-134, revised version)
18- Patients and Methods, Subjects, paragraph two: “taking other medication” -> “taking other medications”
19- Paragraph three: a comma should be placed after “the first ultrasound evaluation”
20- Page 6: “which corresponds closely to” -> “which corresponds _quite_ closely to”

Thank you again. All suggested changes were made.

21- If growth <10th or >90th percentile is an exclusion criteria, it should be stated previously rather than here. Note that it excludes nearly 20% of pregnancies: the number of subjects excluded due to this criteria should be stated. It is not clear if this is actually an exclusion criteria at baseline and/or a reason for quitting the follow-up. Please make more clear.

Thank you for your positive criticism. Foetal growth <10th and >90th percentile is, in fact, a reason for quitting the follow-up. (lines 150-153, revised version)

22- Page 6: numbers about follow-up and exclusions – actually more a dropout – should go in the Results section.

We thank the suggestion made. The paragraph was presented in the results section (1st paragraph).

23- Patients and Methods, Clinical data..., first paragraph: since a trimester is too long, weeks chosen for evaluation should also be provided.

Thank you again. We added this information by writing a new sentence.
New sentence:
Doppler flow study of foetal proximal descending aorta (AoF) was performed in the 101 women included in the study immediately before performing the regular obstetrical ultrasound evaluation at the 1st (11-14 weeks), 2nd (19-22 weeks) and 3rd (28-32 weeks) trimesters. (lines 157-160, revised version)

24- Results, first paragraph, and Table 1: it is questionable to report and compare age and BMI as intervals rather than mean and SD if normally distributed or median and interquartile range if not.

Age and BMI were considered as explanatory variables in the regression model (as no significant effect was detected, they did not appear in the final model). With this purpose in mind, the authors thought that an age or BMI class effect would be much more plausible, and with a clearer understanding, than a continuous age or BMI effect.

25- Results, first paragraph: the sentences about the age of the population and the mean gestational age at birth are a duplicate of the data in the table so they should be omitted, or different information could be conveyed here.

As a suggestion made, we removed these sentences from the results section.

26- Results / Table 2 / Figure 2: Table 2 and Figure 2 partially show the same data, so they are redundant. My suggestion is to show data with figures only (and possibly to write means and SD in the context of the graph) marking significant differences between groups.

Figure 2 shows the results only from the first two lines of Table 2, together with the results from an ANOVA on repeated measurements analysis. If the HT and NT groups were to be included in Figure 2, the assessment of the crude effect of the gestational trimester would not make sense, as that effect would have to be corrected for the hypertensive status. We therefore prefer to leave Table 2 and Figure 2 as they are; the latter illustrates data and results from a crude analysis while the former describes data that is stratified by hypertensive status (and for which a detailed analysis will be given by the regression model).

27- Page 10, last line: “puts impedance data at a lower level” is not clear.

We agree that the sentence is not necessary. Therefore, we decided to remove it.
28- Discussion, page 11, last paragraph: the sentence about foetal stimulation and maternal mobilization should be in the Methods section.

Thank you again. The sentence is now in the *Methods* section. (lines 178-182, revised version).

29- Page 12, first paragraph: since no analysis was performed on the fluxometric spectra themselves, the sentence about them should be omitted or softened to “no GROSS morphological differences were identified”.

Thanks for the suggestion. The sentence was adjusted. (lines 348-350, revised version)

30- Table 1: as already said, “pregnancy outcomes” is probably not a correct term; age and BMI would better be shown with numbers rather than intervals. It seems doubtful that “equality of population frequencies amongst the different categories of a variable” is of any interest in this study, so I would omit it.

The table was reformulated according to the suggestions provided. The analysis of ‘equality of population frequencies amongst the different categories of a variable’ was removed.

31- Table 2: as already said, I would merge it with figure 2. If the table is maintained, significant differences should be marked.

Thank you again. The answer to this suggestion is presented jointly with the answer to question number 26.

32- Table 3: it is not clear what the “expected indices” are. Is it RI? As PI and RI are so highly correlated, it makes little sense to put PI into a multivariate model for RI. If * is used to multiply, it can’t be used to mark a significance, and vice versa. A “x” could be used for multiplications.

As the indexes are highly correlated (they are actually read in the same women), two independent regression analysis, one for each index, would produce biases results and highly correlated residuals. We therefore model everything at once, including a dummy variable in the regression that distinguishes RI from PI values. We acknowledge your suggestion and have altered the multiplication sign to “x”.

33- Figure 3: as already said, significant differences should be clearly marked. This kind of graph can’t anyway used to show differences between HT and NT.

We acknowledge your suggestion. We have added the confidence intervals for the mean prediction for each trimester in the legend of the figure. As time is continuous and our curves are all significantly different from the flat line, there is no way to mark this fact on the plot.

• Discretionary Revisions

34- Please use the same format for all author affiliations (some have the full address, some do not include the country…)
35- The word “index” is repeated several times; synonyms could be used.
36- Introduction: “intra uterine” should better be spelt “intrauterine” or “intra-uterine”.
37- Patients and Methods, Subjects, paragraph two: the number of recruited subjects should be in the Results section.
38- “We studied women… hypertension” -> “We studied singleton pregnancies in healthy women or in women with chronic arterial hypertension”
39- Patients and Methods, Statistical analysis: I would not call “standard” the statistical methods that are rather “appropriate”, neither call “usual” the p-value of 0.05.
40- Results, first paragraph, and Table 1: the term “pregnancy outcomes” to refer to GA at examination and delivery only is questionable.

All suggested changes were made.

41- Figure 2: box-and-whiskers plots are preferable to histograms here.
Figure 2 presents sample means and respective 95% confidence intervals, and is therefore not a histogram. If we were to use box-and-whisker plots, the indication of the statistical significance of mean comparisons would not make much sense.
Reviewer’s report:

- Major Compulsory Revisions

1) An English revision is recommended: there are several misspellings, such as “It’s” instead of “its” at page 4, 2nd line and so on

Thank you for your positive criticism. An English revision was performed.

2) From the methods section it seems that the study enrolled on the HT group only women with hypertension before the 20th week of gestation. Did anyone develop hypertension or preeclampsia after the 20th week? Where they excluded or, if not, in which group were they included? Did you measure maternal blood pressure values during the study?

Thank you again. The Methods section has been clarified. As you can see only women with hypertension prior to pregnancy were included. The exclusions that were made are now reported in the results section. The ‘blood pressure’ was not measured at the moment of Doppler studies were made and so was not used to correlate with them; indeed it was part of the regular pregnancy follow-up procedures. Stage 1 essential hypertension was an explanatory variable and, therefore, no correlations between blood pressure and indices were established.

3) Methods: Please add formulae used for RI and PI.

Pulsatility index (PI) was used as a measure of impedance of the flow of blood distal to the sampling point and automatically calculated according to the formula $PI = \frac{(s-d)}{d}$ where $s$ is the peak $d$ is the minimum and the average is the mean maximum Doppler shift frequency over the cardiac cycle. Resistance index (RI) was automatically calculated using the formula $RI = \frac{(s-d)}{s}$.

Formulae were added. (lines 172-176, revised version)

3) In the studied population how frequently did you find a value of 0 for end-diastolic velocity? Are RI and PI normally distributed?

RI=1: 1st trimester: 3 women; 2nd and 3rd trimesters: 0 women.
At each of the trimesters, the empirical distributions were most of the times positively skewed.

4) Page 2. Abstract, results: please add more detailed information (numbers, p values …).

Thanks for your suggestion.
The abstract and results section were restructured.

5) Discussion is quite poor. The authors mentioned that an increase in phoetal aortic RI and PI in healthy pregnancies is expected. Which is the possible mechanism?

Thank you for your positive review. The discussion section was restructured and we hope that these changes have met the concerns of the reviewer.

Minor Essential Revisions

1) Page 2. Abstract, methods: The following statement is unclear and can be removed from the abstract: “Multiple linear regression models, fitted using generalized least squares and with errors that were allowed to be correlated and/or to have unequal variances, were applied”.

The above sentence has been replaced. (lines 37-41, revised version)

New sentence:
Multivariate regression had to be considered due to the experiment’s nature: two different indexes were read on the same set of individuals, once at each trimester of the pregnancy [1st (11-14 weeks), 2nd (19-22 weeks) and 3rd (28-32 weeks) trimesters]. The response variable was denoted as index d, in a subject with hypertensive status h (hypertensive or normotensive), at continuous time t.
2) Please check for use of abbreviations (i.e. Page 2. Abstract, results: weas write PI and RI instead of pulsatility and resistance indices or later on AoF-PI instead of PI) 

This aspect was taken into consideration. Substantial changes were made in the Abstract.

3) What is the intraobserver variability for the sonographer?

Employing procedures included in *de novo* added references (ref. 34-35) the intra-observer reliability was calculated and data is now added to the manuscript: «The reliability coefficients were 0.053 and 0.153 for the pulsatility and resistance indexes respectively. The intraclass correlation coefficient for the evaluation of intraobserver reliability concerning PI (respectively RI) measurements was very high, namely 0.996 (resp. 0.936) with correspondent 95% confidence interval ranging from 0.994 to 0.998 (resp. from 0.895 to 0.961).» (lines 215-223; 272-276; 331-335, revised version)

4) The example shown in figure 1 seem to show a decrease of RI and PI form the first to the third trimester. I suggest to show a more typical example.

Figure 1 was reformulated in agreement to the suggestion made.

5) Add p values on Table 2 and Figure 3.

Table 2 describes data that is stratified by *hypertensive status* and for which a detailed analysis will be given by the regression model. We added the confidence intervals for the mean prediction for each trimester in the legend of the figure 3. As *time* is continuous and our curves are all significantly different from the flat line, there is no way to mark p-values on the plot.