Author's response to reviews

Title: Correlation of Renin Angiotensin and Aldosterone System Activity with Subcutaneous and Visceral Adiposity: the Framingham Heart Study

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

We are grateful to you, and your external reviewers, for your helpful comments and suggestions, which we believe have greatly strengthened our paper. What follows is an itemized response to each reviewer comment, referenced to the relevant edit in the manuscript.

Sincerely,

Conall O’ Seaghdha MD
Clinical Research Fellow, the Framingham Heart Study
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Caroline S. Fox MD MPH
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National Heart, Lung, and Blood Institute
Response to reviewer comments:

Reviewer 1: Guido Lastra

In this paper, the authors evaluated adiposity by computed tomography and measured systemically aldosterone, plasma renin activity and calculated the aldosterone to renin activity ratio. They did not find any significant correlation between SAT, VAT and their measurements of systemic RAAS activity. This paper is based on a large and well established population from the Framingham Heart Study and certainly is interesting and provocative. The topic studied and discussed is indeed important and has potentially a high impact on public health. However, in my opinion the paper in its present form is not suitable for publication and I would suggest a major revision. I would be willing to review a corrected manuscript.

1. The authors have to clearly elaborate and differentiate the systemic versus tissue (local) RAAS activities. Not finding correlations between SAT, VAT or systemic activity of the RAAS does not necessarily mean that there is not inappropriate activation of the system at a local level, which could affect several tissues. This has been demonstrated in multiple experimental conditions.

Response: We agree with Dr. Lastra that the absence of evidence of systemic RAAS activity does not preclude the existence of significant localized tissue activation. To underscore the importance of this point, we have added additional text to the manuscript (page 12, paragraph 3, line 2).

2. The study should include analysis of subpopulation. The average reported BMI is 26, so the population studied is in a category of overweight.

   a. Specific analysis in obese, overweight and lean individuals should be presented in this paper, and would contribute to improving the accuracy of the study. Even further, differential analysis specific for BMI, waist circumference, and percentiles of VAT/SAT should be entertained and reported.

Response: We now present correlations of VAT / SAT and renin activity and aldosterone levels by category of BMI in reviewer table 1. The categories used are standard definitions of lean (BMI < 25), overweight (BMI 25.1 – 29.9) and obese (BMI >30). In general, results were similar to the primary analysis, with the exception of a weak association between renin activity and renin: aldosterone ratio and SAT in lean individuals. We now describe this secondary analysis in the Methods (page 9, paragraph 2) and the results (page 11, paragraph 3).
Reviewer table 1. Age-, and sex-adjusted Pearson correlation coefficients (r) of adiposity measures and log-aldosterone to renin ratio, log-aldosterone and log-renin presented by BMI category. P values are in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Lean (BMI &lt;25) (n = 832)</th>
<th>Overweight (BMI 25.1-29.9) (n = 644)</th>
<th>Obese (BMI &gt; 30) (n = 411)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAT</td>
<td>VAT</td>
<td>WC</td>
</tr>
<tr>
<td>Log aldosterone</td>
<td>-0.01</td>
<td>-0.01</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.8)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Log renin</td>
<td>0.08</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.3)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>Log renin: aldosterone</td>
<td>0.08</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.3)</td>
<td>(0.08)</td>
</tr>
</tbody>
</table>

b. Equally it would be interesting to evaluate correlations with blood pressure

Response: Correlations with blood pressure have been previously reported. We have now included this information and the reference in the introduction on page 5, paragraph 1.

3. The authors should present markers of local activation of the RAAS. I understand this would imply more invasive procedures and taking samples of adipose tissue, so it might be technically challenging, but would add value to the paper and allow to better support the conclusions.

Response: Unfortunately, this undertaking is not feasible in a large, observational epidemiologic study such as the Framingham Heart Study. We have added this as a limitation to the manuscript (page 15, para 1, line 3).

Reviewer 2: Gian Paolo GP Rossi

In a large, community-based sample from the Third Generation Framingham Heart Study O’Seaghdha et al sought for correlations between indexes of the renin-angiotensin-aldosterone system (RAAS) and indexes of regional adiposity. At variance with previous studies that described a correlation between BMI and plasma aldosterone in salt-loaded normotensive individuals and in overweight and obese essential hypertensive subjects they found no correlation in either crude or covariate-adjusted analyses. The manuscript is nicely organized and written, but unfortunately, albeit probably adequately powered, it is a negative study. Strengths of this study entail the accurate CT-based method for assessing regional adiposity indexes and the large sample size. Unfortunately the latter is not a valid surrogate for the poor patients preparation at the time of RAAS measurement and for paying no attention to major determinants or renin and aldosterone.

Major Compulsory Revisions

1. In my view a likely potential bias entails the facts that the indexes of the RAAS were not measured under the proper conditions as they should have. Moreover, no attention to ongoing pharmacological treatment and sodium intake, which are major determinant of renin and aldosterone, was given. Hence, it is uncertain if the findings are real or just deriving from a poor
study design. In my opinion the Authors should report data on sodium intake and/or urinary sodium excretion and should confine their analysis to the patients without pharmacologic treatment. With this large sample size even after exclusion of the subjects not fulfilling tighter inclusion criteria they should be able to provide valuable data.

Response: We agree that data on sodium balance, particularly intake and urinary excretion, would enhance this analysis, but unfortunately these were not available. We now state this as a limitation on page 14, paragraph 3.

Reviewer table 2 below presents correlation coefficients for VAT / SAT and renin / aldosterone in participants not taking hypertension treatment. These are similar to the primary analysis, with the exception of a borderline association between renin: aldosterone ratio and BMI. We now include details of this secondary analysis in the methods on page 9, paragraph 1 and in the results (page 11, paragraph 4).

Reviewer table 2. Age-, and sex-adjusted Pearson correlation coefficients (r) of adiposity measures and log-aldosterone to renin ratio, log-aldosterone and log-renin in participants not taking hypertension treatment (n = 1,748). P values in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>SAT</th>
<th>VAT</th>
<th>BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log aldosterone</td>
<td>-0.01</td>
<td>-0.02</td>
<td>-0.04</td>
<td>-0.01</td>
</tr>
<tr>
<td>(0.8)</td>
<td>(0.3)</td>
<td>(0.9)</td>
<td>(0.7)</td>
<td></td>
</tr>
<tr>
<td>Log renin</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(0.7)</td>
<td></td>
</tr>
<tr>
<td>Log renin: aldosterone ratio</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>(0.2)</td>
<td>(0.07)</td>
<td>(0.04)</td>
<td>(0.06)</td>
<td></td>
</tr>
</tbody>
</table>

2. A further point regards the possibility, which was largely neglected in Introduction and Discussion, that factors other the component of the RAS can account for a secretagogues effect on aldosterone. For example, Adiponectin receptors were described in the human adrenocortical zona glomerulosa and some CTRPs were suggested to stimulate aldosterone in vitro (Wang et al Faseb J).

Response: We are grateful to Dr. Rossi for bringing this interesting and relevant paper to our attention. We now discuss it on page 5, paragraph 1.

Minor Essential Revisions
1. Despite quoting an excessive number of references some more relevant references documenting a relationship between BMI and aldosterone are overlooked.

Response: We now include 2 new references that report an association between BMI and aldosterone in normotensive and hypertensive patients; these are included in the introduction and discussion. We have also trimmed down the number of original references such that the total number of references is also reduced by ~10%.

2. Some sentences are odd and need to be changed. For example, the sentence “Internal validity is supported by prior reports using these data,
including associations with sodium excretion and pulse pressure,28 as well as the observation that increased aldosterone levels within the physiologic range increase the risk of incident hypertension in normotensive individuals” is unacceptable from the standpoint of validation of any assay.

Response: This sentence has been removed, as has a similar sentence from the limitations section on page 15.

Discretionary Revisions
1. The statement on page 12 “Larger studies in obese individuals with hypertension have generally failed to replicate this association between obesity and circulating RAAS components.” Is not correct. I can recall at least two papers published in JCEM in 2008 that depicted a correlation between aldosterone and BW or BMI. The Authors should use their judgement to better select the relevant papers to be quoted.

Response: Please see response 2 under “Minor Essential Revisions” above. We now include these 2 references from JCEM, and have rephrased this statement to better reflect the literature on this subject (page 13, paragraph 1).

References: