Author’s response to reviews

Title: Epicardial Adipose Tissue Is Related to Arterial Stiffness and Inflammation in Patients with Cardiovascular Disease and Type 2 Diabetes

Authors:

Shaween Al-Talabany (s.altalabany@dundee.ac.uk)
Ify Mordi (i.mordi@dundee.ac.uk)
Graeme Houston (j.g.houston@dundee.ac.uk)
Helen Colhoun (helen.colhoun@igmm.ed.ac.uk)
Jonathan Weir-McCall (j.weirmcall@dundee.ac.uk)
Shona Matthew (s.z.matthew@dundee.ac.uk)
Helen Looker (h.c.looker@dundee.ac.uk)
Daniel Levin (d.levin@dundee.ac.uk)
Jill Belch (j.j.f.belch@dundee.ac.uk)
Fiona Dove (F.J.Dove@dundee.ac.uk)
Faisel Khan (f.khan@dundee.ac.uk)
Chim Lang (c.c.lang@dundee.ac.uk)

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Technical Comments:

1. Please include, at minimum the names, institutions, countries and email addresses of all authors, and the full postal address of the submitting author in the Title page.

These have now been added to the title page.

2. Please include Abstract heading in the Abstract section.

We have included this on page 2.
3. Please include a list of abbreviations used in the manuscript and their meanings. This should be placed in between the Conclusions and Declarations sections and should have its own sub-heading.

We have now included this as requested on page 16.

Reviewer 1: Al-Talabany et al examined the association between epicardial adipose tissue and markers of vascular function and inflammation in patients with high CVD risk. The manuscript is well written but several issues have to be address to support the conclusion

We thank the reviewer for their overall positive appraisal of our manuscript.

1. While EAT was higher in groups 1 and 3 (IL-6 was higher in Group 1), there was no significant difference in PWV among groups.

We agree with the reviewer that there was no significant difference in PWV between the groups. To the best of our knowledge we have not reported otherwise in the manuscript, and we believe that there is still an important message in our study. The lack of difference in PWV between groups may simply be a reflection of the myriad of different clinical factors which contribute to PWV. Additionally, for example in group 4, although the patients did not have overt CVD, there were still patients with hypertension etc. which may still contribute to altered PWV.

2. The associations between EAT and IL6 and CD40 were significant but extremely weak (r=-0.021 to 0.2). Please include figures to show the correlations. It is of interest to see whether the association was independent of obesity index.

We thank the reviewer for this suggestion. We have included these figures as suggested by the reviewer as Supplementary Figure 1.

We appreciate the reviewer’s point that regarding obesity, however in our study there was a significant correlation between EAT and BMI (used as a marker of obesity - r=0.28 p= 0.001). Given this, we didn’t feel it was appropriate to adjust for both in a relatively small cohort such as this due to problems with collinearity. For the reviewer, we did conduct an analysis regressing both EAT and BMI on IL6, however although in a univariate regression both were associated with IL6, in multivariate regression both associations became insignificant. We do not believe however that this is because neither are truly associated with IL6, but rather because of the problem of collinearity diluting the effects.

We have therefore, on balance, decided not to include this analysis in the manuscript.
3. Surprisingly, plasma adiponectin was not reported in the study that may modulate the reported association.

We agree with the reviewer that adiponectin would have been of interest in this study, given its associations with arterial stiffness. Unfortunately we were constrained in the number of biomarkers we could analyse. We acknowledge that the panel of biomarkers chosen, although large, is not exhaustive, and therefore this could be considered a limitation. As such, we have added this to the study limitations section as follows:

“Although we feel that our selection of 51 biomarkers is both pragmatic and broadly covers most pathophysiologically important biomarkers, we cannot completely exclude the possibility that there may be other biomarkers which could be important, for example adiponectin.” (page 15, paragraph 1, lines 3-6)

4. Differences in gender ratio, use of statin or other medication, blood pressures, smoking habit and lipid/lipoprotein (TG and HDL) among the groups could confound the association.

We presume the author is referring to the associations between EAT, IL6 and PWV. In order to prevent overfitting of our regression model, we only included clinical variables which were significantly associated with PWV in univariable analysis. Systolic BP was strongly associated with PWV (supp Table 2), however lipids and medications were not, hence these were not included in the multivariable regression.

Given this, we feel that we have excluded for confounders as much as possible, although we recognise and accept the reviewer’s point that as an observational study, residual confounding may remain.

5. Table 2 I wonder EAT should be the independent variable rather than a dependent variable in relation to IL-6 level.

The reviewer is correct in their assertion and we thank them for pointing out this error. It is logical in our hypothesis that EAT should be an independent variable as we suggest that EAT might release IL-6. Given this, we have reported this as such in the results as follows:

“In a univariate regression analysis, IL6 and CD40L were significantly associated with EAT (IL6: beta 0.2 p=0.019; CD40L: beta -0.21 p= 0.01).” (page 10, paragraph 3, lines 6-7)
Additionally, we have reported amended table 2 as follows:

Table 2. Multivariable Linear Regression of PWV and Biomarkers

<table>
<thead>
<tr>
<th>PWV (m/s)</th>
<th>β</th>
<th>95% CI</th>
<th>β (S)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40L</td>
<td>-0.5</td>
<td>-0.90 -0.10</td>
<td>-0.21</td>
<td>0.014</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.67</td>
<td>0.23-0.1.12</td>
<td>0.26</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Reviewer 2: (Page: 3, lines : 40-45) Abstract- Results section.

There is:

EAT measurements were significantly higher in the groups with CVD, with or without T2DM compared to patients without CVD or T2DM (group 1 EAT 15.9 ± 5.5 cm² vs. group 4 EAT 11.8 ± 4.1 cm², p=0.001; group 3 EAT 15.1 ± 4.3 cm² vs. group 4 EAT 11.8 ± 4.1 cm², p=0.024).

Comment:

The definition of 4 distinguished subgroups is not fully clear. Is group 1 composed of CVD patients with diabetes? Similar questions to each other group.

(Page: 5, lines: 57-59 and Page: 6, lines 4-5)

We apologise if the reviewer feels that the description we had given in the methods section was not clear. As they correctly state, group 1 had CVD patients with DM, group 2 DM patients with no CVD, group 3 CVD patients with no DM, and group 4 patients with no overt CVD or DM. We have now altered the description to hopefully clarify this as follows:

“Group 1 comprised of type 2 diabetes mellitus patients (T2DM) with clinical CVD including cerebrovascular disease, CAD and/or lower extremity arterial disease (LEAD); Group 2 included volunteers with T2DM and no clinical evidence of CVD; Group 3 included subjects with clinical
evidence of CVD and no T2DM; and Group 4 was a group in whom there was no clinical evidence of either T2DM or CVD.” (page 6, paragraph 3, lines 6-9 and page 7, paragraph 1, lines 1-2)

There is:

Tentative supportive evidence of a systemic effect of EAT is provided by the association between EAT and peripheral arterial stiffness, a known predictor of adverse CV outcome.[12]

Comment:

Cited reference by Willum-Hansen et al. is dedicated to prognostic value of aortic PWV, which is large artery stiffness index. I am not sure if it is correct to use the term: 'peripheral' to this index, because represents the stiffness of aorta not peripheral (usually muscular) arteries, however I understand that in the context of heart, coronary arteries and EAT aorta might be considered as a 'peripheral' artery.

In the discussion (Page: 11, lines: 57-59) the authors wrote:

Second, we have shown that EAT is significantly associated with PWV, a marker of systemic arterial stiffening…

This is definitely a better term than 'peripheral', previously used, however ESC expert consensus document counts carotid femoral PWV rather to proximal stiffness indices and precisely to regional stiffness indices. I hope this document will be helpful for authors in terminological issues. See: Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European Heart Journal 2006: 27; 2588-2605.

We thank the reviewer for identifying this. For clarity, we have simplified these sentences as follows:

“Tentative supportive evidence of a systemic effect of EAT is provided by the association between EAT and arterial stiffness, a known predictor of adverse CV outcome.” (page 1, paragraph 2, lines 7-8 and page 6, paragraph 1, line 1)

“Second, we have shown that EAT is significantly associated with PWV, a marker of arterial stiffness that is linked to adverse CVD outcome.” (page 12, paragraph 1, lines 2-4)
There are significant differences in age between distinguished subgroups, not mentioned in the Baseline Characteristics on page 9. Looking on the Table 1, group 1 and 3 seems to be older than 2 and 4. This might be important because EAT is higher in group 1 and 3 than in 2 and 4. What is surprisingly this difference in age is not reflected by the difference in PWV, the variable typically age dependent. Please consider to describe these findings.

We agree with the reviewer that this finding was slightly unexpected, although given the size of the study it is certainly possible that these types of results could be found but may be different in a larger study. We have now mentioned this in the results as follows:

“CVD patients were significantly older than those without CVD.” (page 9, paragraph 2, lines 4-5)

Given the relatively small numbers of patients in each group however, in our opinion it is difficult to draw any conclusions about the relationship between PWV and age in this cohort, particularly because there are likely to be other differences between the groups (both measured and unmeasured) which will also have had an effect.