Author's response to reviews

Title: Cross sectional associations of epicardial fat with coronary calcification, insulin resistance, markers of inflammation and fibroblast growth factor-23 and in stage 3-5 chronic kidney disease patients

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

Thank you for the opportunity to revise our manuscript “Cross sectional associations of epicardial fat with coronary calcification, insulin resistance, markers of inflammation and fibroblast growth factor-23 and in stage 3-5 chronic kidney disease patients”. As requested, we are included a detailed letter explaining and referencing changes as per the reviewers suggestions. Reviewer 1’s comments are highlighted in turquoise, and reviewer 2’s comments are highlighted in yellow. Comments are also provided to guide the reviewers to the area of the manuscript where changes have been made, and line numbers are also provided.

We hope our efforts are satisfactory, as we completely revised and re-organized the discussion, based on their constructive comments.

We look forward to hearing from you,

Kind regards,

Jocelyn Garland, MD

Response to reviews:

Reviewer 1:

Major Comments:

1) In results section, the data about fetuin A, IL-6 and OPG should be written in the text.

Response: In revising the manuscript, we tried not to duplicate data available from the tables in the written text, as we were conscious of the length of the paper. Unless we wished to emphasize a point, we attempted not to duplicate results. Since neither fetuin A nor OPG were associated (significantly) with EFV, we did not add these values in the text (line 224). However, the section on IL-6 has been completely revised in the discussion, and hopefully this will address the request (line 247, 275-277, 286-300)

2) In the discussion you can summarize the main results in the first paragraph

Response: We have added a summary, as suggested (lines 243-252)

3) I do not understand why you performed multivariable regression analysis in patients who were not using insulin? If the reason is appropriate you have to discuss this in the discussion section.
Thank you for the comment. We apologize for this omission. The HOMA IR formula requires a blood insulin level, and thus blood insulin levels obtained from patients treated with insulin would be confounded, as results would not represent only endogenous insulin. Thus HOMA-IR was only measured in those patients not treated with insulin. This stipulation has been added to the method section of the paper (lines 162-164). Based on this comment, however, we removed the original Table 4 including insulin resistance and instead included insulin resistance (HOMA-IR) as a covariate to the fully adjusted model (line 234-237, Results, in text). The association no longer remained. In addition, we added a second regression model including the metabolic syndrome, as per the second reviewer (line 599; Table 4). We also added a statement in the limitations part of the paper that the lack of association between HOMA-IR and EFV may reflect inadequate study power, as 72 patients instead of 94 are included (lines 344-345).

4) You mentioned the relation between inflammatory markers and EFT. Please discuss this issue more.

Response: Thank you for the suggestion. This issue was extensively revised in the discussion. Please refer to the manuscript, as numerous changes were made to expand on this point, and also to describe the issue of inflammation, EFV, and CKD patients. (Lines 270-300)

Minor Comments:

1) Please correct the spelling errors in the introduction part.
Response: This has been completed. Thank you.

2) Correct the reference 13 as Tonbul HZ, Turkmen K, Kayikcioglu H.........
Response: This has been corrected. Thank you.

3) You used HOMA-IR formula to define IR. However, this formula is not the best tool to define IR. Please mention this as a limitation.
Response: We have added the following section:

(Lines: 340-343) The optimal measures of insulin resistance are obtained by the euglycemic hyperinsulinemic clamp procedure; however, it was not possible to perform clamp procedures in this study. In addition, we wished to employ a tool for measuring insulin resistance which could be easily applied to clinical settings, and thus the use of HOMA-IR was the preferred method.

Reviewer 2:

Minor Essential Revisions:

1. The aim of the study is to explore the association of EFV with CAC and
conventional risk factors including Metabolic syndrome and Insulin Resistance. The authors have suggested that in the multivariate analysis, EFV is higher in patients with Metabolic syndrome and Insulin Resistance. However as shown in the study by Yerramasu et al (reference 11) and Rosito et al (Circulation. 2008 Feb 5;117(5):605-13), associations between EFV and Metabolic syndrome were not significant once adjusted for other measures of obesity. I would suggest repeating the multivariate analyses using Metabolic syndrome, Insulin resistance and CAC score as outcome variables and EFV along with other measures of obesity (waist circumference or BMI) and relevant conventional risk factors as the variants in the model. This will help us to show if EFV is independently associated with metabolic risk factors and coronary calcification.

I would also suggest including the findings from the above studies in discussion, especially the fact that EFV is not independently associated with metabolic syndrome after adjusting for other measures of obesity but it is associated with CAC score, suggesting a local paracrine effect.

Response: We thank the reviewer for these comments regarding the analysis. We agree completely that patients with the metabolic syndrome have increased epicardial fat, and we reported that finding in the manuscript (lines 211, 230-234, 243, 260-269). The reviewer also points out that in the paper by Yerramsasu et al, patients with increased burden of epicardial fat had a higher prevalence of metabolic syndrome. The reviewer also discusses in the paragraph 3.3, page 226 of the paper cited (Yerramasu et al) that because waist circumference, systolic and diastolic BP, HDT and TG level are characteristics of the metabolic syndrome, that these components were not entered in the logistic regression models. We agree, and used a similar approach in the statistical analysis for this paper (Lines 260-269).

In designing the analysis, we were focused on the primary objective which was to explore the relationship between EFV and CAC (Line 228, 249-252, 301-314, (Line 579; Table 2), (Line 590; Table 3) and (Line 599; Table 4).

First, we demonstrated a univariate association between EPV and CAC (Line 221, Table 2, Line 590). Next, we wished to determine if this association remained in the multivariable analysis. (Line 590; Table 3 and Line 599, Table 4). We adjusted for the components of metabolic syndrome, including abdominal obesity (table 3). As the reviewer requests, we have included a second model using metabolic syndrome as a covariate (table 4). It is difficult to know whether it is necessary to adjust for BMI as well. Since obesity is included in the definition of metabolic syndrome, and is assessed by abdominal obesity in the definition, adjusting again for BMI may result in adjusting twice for the same component. Without adjusting for BMI, metabolic syndrome remains a predictor for EFV in the adjusted model (B = 1.8; 95% confidence interval 0.44 – 3.2; P=0.01)(full data not shown). In including BMI in the adjusted model, metabolic syndrome just misses statistical significance (B = 1.2; 95% confidence interval -0.07 – 2.45; P=0.06). We have included the latter model in the revised manuscript, including metabolic syndrome and BMI, in the paper (table 4).

Discretionary Revisions
1. On page 12, the authors state that "The CAC score detected by MSCT
represents calcification arising from both the intimal blood vessel layer (where traditional CVD risk factors are known to impact) and the medial blood vessel layer (where kidney-related CVD risk factors including abnormal bone and mineral metabolism parameters are known to impact) [36]”. I thought that calcification in coronary arteries is predominantly intimal and represents atherosclerosis unlike other vascular beds where medial calcification due to other factors is possible. Is this different in patients with CKD? Perhaps the authors can elaborate on this.

Response: There are many studies demonstrating that CKD patients develop accelerated medial and intimal vascular calcification. The “kidney related” cardiovascular disease risk factors, in particular abnormalities in bone and mineral metabolism specific to the CKD population, are associated with increased medical calcification. By the statement that is mentioned above, we were meaning to convey that the MSCT result describes the total burden of coronary calcification, but does not differentiate between intimal versus medial calcification. However, because the location of the calcification is not a research objective for this study, and because the paper is approaching 4000 words, we opted to remove this thought from the discussion in the revised manuscript.

2. If the authors have the entire CAC volume dataset, why did they measure EFV from a single slice? I guess saving time could be one reason, but they have quoted only one study that showed single slice EFV is related to total EFV. Further evidence in this regard would be useful and could the authors consider measuring total EFV in a subset of patients and see how well it correlates with single slice EFV in this study?

Response: To measure the total epicardial fat volume, the reviewer is correct that this would have required the determination of EFV for each separate MSCT slice. The software that we used, GE workstation software (ADW version 4.3), requires this be completed manually per slice. Thus it was not feasible from a time perspective which is why we used our described method (Lines 178-187). Moreover, for others interested in this measure, this same issue would likely be a limitation if similar software is being used. The Oyama et al paper that we referenced (Manuscript reference 17) has already completed what the reviewer is suggesting. The total EFV was quantified, and separately, a measure reported at the level of each of the coronary arteries. The correlation between the EFV total and the EFV at the left main coronary artery was excellent, and so therefore we chose a similar method in our study. In discussion with Dr Nolan, the radiologist who measured the ssEFV, in order to determine 1 patient’s total EFV using the software available at Kingston General Hospital would take 5 hours per patient. Thus, preforming total EFV was not feasible for us to complete.

3. The authors could consider including a figure that shows the method of EFV measurement from single slice CT (a cross sectional image with the EFV highlighted), that would be of help to the readers not familiar with the cross-sectional CT anatomy.
Response: We have added a Figure to the manuscript as suggested with relevant landmarks (Figure 1).

4. Most of the other studies on this subject included Hounsfield units -30 to -190 to represent fat voxels, unlike the current study which included the range of -30 to -230 (is there any specific reason for this?)

Response: We used the method of Oyama et al, (manuscript reference 17) who described a method of estimating the total EFV by MSCT scan, using a single MSCT slice located at the level of the LMCA. In following this technique, pixels in the pericardium with a density from -30 to -230 Hounsfield units were considered fat, and thus we used the same parameters. In the original manuscript, we stated -230 to -30 but we have amended this to -30, to -230 as per our radiologist’s suggestion.