**Author's response to reviews**

**Title:** A randomized trial of sodium-restriction on kidney function, fluid volume and adipokines in CKD patients

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**Version:** 2  
**Date:** 13 January 2014

**Author's response to reviews:**

Please see below response to Editor, Reviewer 1 and Reviewer 2 comments.

**Editorial Comments:**

In addition to the comments of the reviewers, I have one comment. I would leave out the ACE polymorphism data, as the study is clearly underpowered to report valuable data on the polymorphism and the sodium-induced changes.

**Authors response:**

Thanks to the Editor and Reviewers for their helpful comments.

The authors have agreed to remove the data related to ACE polymorphism due to lack of power.

**Editorial Request:**

1) Please can you include full name of the ethics committee (Completed)
2) Please can you include a patient consent to participate statement in the Methods section. (Completed)
3) We note that some of the authors have not been included in the Authors' Contributions (DJ, JB, CH, NI, MS). Please can each author be named (using initials) in this section. (Completed)
4) Please can you include the TRN at the bottom of the abstract in both the manuscript and submission system (Completed)

**Reviewer 1 report:**

This is an important study: in a group of Stage 3 and Stage 4 CKD patients, who have been sodium restricted, compares the effect of continued salt restriction in
one subgroup to a second subgroup supplemented with addition of 120 mmol sodium daily.

Reviewer 1 Comment 1:
Results are provided for each sub group after run in and washout but baseline data are not given.

Response: Baseline data for available variables has now been listed in Table 1

Reviewer 1 Comment 2:
The paper could benefit by containing a flow diagram as advised by CONSORT.

Response: The CONSORT diagram for the present study was included in a previous paper. The manuscript has been amended to include a reference to this:

Original text: “Participant characteristics were detailed in a previous publication.”
Amended text: “Participant characteristics and CONSORT diagram were detailed in a previous publication.”

Reviewer 1 Comment 3:
The results are of great interest, including the lack of effects on inflammatory markers by high sodium intake and the different behavior following salt restriction according to gene polymorphism for angiotensin in regard to various markers.

However, the paper is somewhat confusing: One of the primary aims stated is to assess effects of sodium restriction on kidney function and metabolic markers yet a significant part of the paper deals with sodium loading and the results read as changes in results from the high sodium period to the low sodium period but we do not see results from the low sodium to the high sodium periods, completing the crossover. Actually the study design itself states: “Following a 1-week run-in period, participants were randomized to a high sodium diet (achieved via slow release sodium tablets providing an additional 120 mmol sodium/day) or low sodium diet (placebo) with a 1-week washout” which would not permit comparison of the same subjects under two situations of sodium intake. I am sure that there is an easy answer to this question.

Response:
This is a blinded, randomized cross-over study which was designed to compare a ‘usual sodium intake’ (one normally seen in CKD patients) (high sodium period) with one in line with recommended intakes (a low sodium diet) in an attempt to replicate what would occur in practice. Following a 1 week washout, patients crossed over to the alternate group. Patients contribute data to both groups and are therefore able to be compared under both low and high salt conditions. To assess for variance due to treatment order, an analysis of covariance has been included.

The following text has been included in the methods section: “To test for variation due to treatment order, analysis of covariance was conducted with treatment type
and treatment order included in the model and observations clustered by study number to account for correlation of within-patient results.”

The following text has been included in the results section: “Effect of sodium restriction on outcomes did not vary according to treatment order (p > 0.05).”

Reviewer 1 Comment 4:
Other aspects: The abstract refers to improvement in kidney function with sodium restriction. Perhaps this refers to reduction in supposed ultrafiltration but is not shown by the GFR measurements as being improved.

Response:
The conclusion wording has been amended to state ‘change’ in kidney function and fluid volume.

Reviewer 1 Comment 5:
The Discussion contains the following: “We also found a significant change in kidney function parameters with a decrease in eGFR mirrored by an increase in creatinine and urate. This is consistent with findings from other studies investigating the effect of sodium load on creatinine clearance showing that high sodium intake can result in increased creatinine clearance, at least in the short term [24].”

The first sentence surely relates to sodium restriction but is not consistent with the second sentence.

Response:
This section of the discussion has been amended to improve clarity – the first sentence now reads:
“We also found a significant change in kidney function parameters with a low sodium diet resulting in a decrease in eGFR mirrored by an increase in creatinine and urate, compared with the high sodium diet.”

Reviewer 1 Comment 6:
Given the many effects occurring it would be useful to give the relevant albumin, protein and creatinine concentrations rather than the ratios only in order to understand what might be changing.

Response: 24-hour urinary albumin and protein are presented in the original publication (Reference 2). The serum creatinine is listed in Table 1 and is also reflected in the eGFR calculation.

Reviewer 1 Comment 7:
The bioimpedance studies are a little puzzling to have a 0.87 ECF/ICF ratio suggests over hydration even during sodium restriction however the method may have inherent errors.

Response:
We agree this reflects a state of overhydration, which reflects the common finding of extra-cellular fluid excess in this population, and similar to levels at baseline. Bioimpedance Spectroscopy using Cole-Cole plot in the quantification of body fluid compartments was the best available methodology to use at the bedside at the time of this investigation.

Reviewer 1 Comment 8:
These are minor criticisms compared to the overall message especially that related to the effect of gene polymorphism's but they should be responded to.

Response:
This section has now been removed from the manuscript at the request of the Editor.

Reviewer 2 report:
In this manuscript Campbell et all show a post-hoc analysis of ACE polymorphism, inflammatory markers and adipokines during low en high sodium intake in chronic kidney disease.
Although the manuscript is well- written and the trail is neatly performed there are some major issues the authors should assess:

Reviewer 2 Comment 1:
This is a post-hoc analysis which is a first impressions adds no major new issues to the original paper the recently published. Therefore the data seems to offer little additional information. Maybe something for a short communication or letter?

Response:
As per Editor recommendation – this manuscript is being revised and will be submitted in its current form. The manuscript is considered a post-hoc analysis and has separate aims from the original paper.

Reviewer 2 Comment 2:
As the authors already stated in the discussion part the study is grossly underpowered to assess the issues regarding ACE polymorphism. This is however one of the main questions of the study.

Response:
This component has now been removed at request of the Editor

Reviewer 2 Comment 3:
There was no significant effect of dietary sodium intake found on plasma adiponectin levels. The possible link between sodium and adiponectin levels suggested in literature is the RAAS. To make a proper judgment it would be helpful to know the extent of ACE/ ARB use in the patients
Response:
ACE/ARB use was prevalent in this population and may have impacted this relationship. Patients were prescribed a mean 3.15 antihypertensive medications at baseline with 90% prescribed RAAS blockade (30% prescribed ACE inhibitors (n = 6/20), 70% A2 receptor-blockers (n = 14/20). This has been highlighted in the discussion on RAAS blockade potentially impacting on response of adipokines to high sodium diet.

Reviewer 2 Comment 4:
Inflammatory parameters were measured during both diets as a high sodium diet is known to induce pro-inflammatory status. However this hypothesis is not supported by these data. Probably the study period is not long enough to see such subtle changes. Could the authors please elaborate on this?

Response:
In healthy men a 1 week sodium restriction was sufficient to alter adiponectin (Krikken et al, 2012 & Lely et al 2007), however given this subjects of this study are CKD patients, as now highlighted in the discussion comorbid factors and background medications, including RAAS blockade may have impacted the response to sodium load. In addition it may be the intervention was too short for the panel of inflammatory markers assessed. This has been added to the discussion.

We trust that the above response and corresponding amendments are satisfactory to meet the Editors requirements for future publication.