Reviewer’s report

Title: Abnormal Urinary Excretion of NKCC2 and AQP2 in Response to Hypertonic Saline in Chronic Kidney Disease. An Intervention Study in Patients with Chronic Kidney Disease and Healthy Controls

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Reviewer: Thomas Larsen

Reviewer’s report:

Jensen et al report results of an interventional case-control study investigating the renal tubular response to hypertonic saline infusion in CKD stage III-IV compared with healthy controls. The trial was well-designed with utilization of a standardized diet prior to the experimental examination adding additional strength to the study. By means of quantifying the urinary excretion of the NKCC2, ENaC# and AQP2 membrane transport proteins, the authors document pathophysiologic changes in renal sodium and water handling. The paper highlights important alterations in renal tubular function, and illustrates the kidney’s inability to effectively compensate for acute volume expansion in later stages of CKD.

- Major Compulsory Revisions

1. Regarding analysis of FENa, NKCC2, AQP2 and ENaC, the authors performed a post hoc sub-analysis dividing the CKD population into two groups consisting of those with stage III and IV disease, resp. These results offer important contributions to the overall conclusion of the study. However, it should be clearly stated in the discussion whether this was a pre-planned analysis.

- Minor Essential Revisions

1. Regarding “inclusion criteria” on page 3, paragraph 3: Please correct “stage II-IV” to “stage III-IV”.
2. Three CKD patients and three healthy controls were excluded from urine analyses due to incomplete voiding. Were these subjects reasonably matched regarding gender and age, or could this represent a potential cause of confounding in the study?
3. The abbreviations NKCC2 and NK2CC are used interchangeably on pages 10 and 11. I would suggest using only NKCC2.
4. On page 14, paragraph 3 “Figure 2B” should read “Figure 3B”
5. Labels for figure “A” and “B” does not appear on figures 2 and 3.
6. The label of the Y-axis in figure 4B should read “ng/mmol” instead of “ng/min”.
7. Please revise grammar in the legend for Figure 4.
8. On page 12, paragraph 2 it states that 11 patients were on loop diuretics.
However, on page 13, paragraph 2 and on page 19, paragraph 3, it appears that 8 patients were treated with furosemide. Were 3 patients then on a non-furosemide loop diuretic? If so, it would be reasonable to include those in the analysis of u-NKCC2 in “furosemide vs no-furosemide” users. If not, please correct accordingly.

- Discretionary Revisions

1. How did the authors ensure that none of the patients included in the study had chronic heart failure?

2. On page 19, the authors write in paragraph 2 “The quantity of excreted NKCC2 in urine reflects the activity of sodium transport via NKCC2, just as…” Is that an assumption or is the statement supported by previous studies? If so, please add a reference. Without this clarification the statement in paragraph 4 on the same page that reads “Thus, the lack of change in urine [NKCC2] does not always reflect a lack of change within the plasma membrane of kidney epithelial cells”, may appear contradictory.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

From 2009-2012, I was employed at the institution where the current trial was conducted. Although having worked with Dr. Jensen and some of the co-authors in the past, I have not had any involvement in the current trial. Additionally, I have no financial competing interests in relation to this paper.