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

Version: 2

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Reviewer: Ewout J Hoorn

Reviewer’s report:

Jensen et al. analyzed distal tubular sodium and water handling in human CKD. To do so, they infused hypertonic saline in patients with CKD and healthy volunteers and subsequently analyzed the response in plasma and urinary parameters, including vasoactive substances and immunoassays of urinary sodium and water transporters. After hypertonic saline, they observe an attenuated decrease in urinary output and free water clearance in patients with CKD. This response is accompanied by a (greater) increase in urinary AQP2 and NKCC2. They interpret this as a defective tubular response to volume expansion (abstract) and as an inability to concentrate urine (conclusions in discussion section).

This is an interesting study into human physiology and pathophysiology during CKD by a group with a track record in this type of studies.

However, I have three major comments that I would like the authors to address in a revised version:

1. I disagree with the main conclusion, namely that the CKD patients have an inability to concentrate urine. What the authors show is that the CKD patients excrete the sodium in the hypertonic saline normally (or at least without significant differences from controls). However, they have a problem to excrete the water load present in the hypertonic saline. This appears due to an exaggerated response in u-NKCC2 and u-AQP2, and therefore it seems the other way round: they concentrate their urine too well. This may have important implications for the tendency of CKD patients to develop hyponatremia (see emerging literature on this topic by for example Kovesdy et al., Circulation 2012 and Combs & Berl, AJKD 2014). Please comment and discuss. I also think the NKCC2 findings should be interpreted more in the context of vasopressin and concentrating mechanism rather than sodium handling. I would also suggest changing the “novelty” of this study (Page 17, Discussion), because mentioning that this group is the first to conduct such a study is not a merit by itself if the main findings are not emphasized.

2. Urinary transporter immunoassays: A paragraph should be added to discuss what the authors believed is picked up as a signal from the immunoassays. What do the authors believe to be the source of the transporters? Are these exosomes,
as alluded to once in the study? If so, do the authors believe the differences in transporters are due to higher protein abundance on the same number of vesicles or are more vesicles excreted? Any concern that non-specific signals are picked up because no (ultra)centrifugation was applied?

3. The presentation of the data is poor: The manuscript is much too long (> 7000 words) and the Methods section is almost a paper by itself (> 2000 words). Because the study’s main conclusions are limited, I think it should be possible to report these results aiming at half the words (~3500 words). Currently the lengthy manuscript distracts the reader from easily grasping the main message. There are also too many tables and figures. Table 1: many parameters are not relevant, including p-cholesterol, liver enzymes, and HbA1c. Table 1-2 may be combined if only relevant parameters are shown. Table 2: the baseline values of the transporters do not add in my opinion and may also be summarized as “no differences” in text. Table 2: instead of u-Na/K and FENa/K, I think UNa/K * V is a better measure of 24h urine and I suggest replacing this. Tables 3-5: difficult to digest the data, better to represent significant results graphically, u-NKCC2 is both in Table 3 and Figure 4. Table 6: can be removed, this is not really adding new information, because it was already shown that the CKD patients had an attenuated decrease in cH2O. Figure 1: may be converted to supplemental material. Figures 3, 4, and 6 may be combined to have one figure for all transporter assays, panel A could show AQP2, γ-ENaC, and NKCC2 for CKD vs patients, and panel B the distribution between CKD3 and 4. I suggest deleting U-NKCC2exr because this parameter is also not reported for the other transporters. Discussion: I would prefer a more focused review with an integrated discussion rather than different subsections which increase length and result in unnecessary textbook-like discussions.

**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:**

I declare that I have no competing interests