Peer Review File

Review Comments

Comment #1: First, the authors focused on the effect of alkaline water supplementation and factors associated with this effect. The answered the two questions by using meta-analysis and an empirical study and the latter question was answered by the empirical study. In the analytic results, gender is also associated with the effect of alkaline water supplementation. So the title is not accurate and vague. Please revise the title to accurately reflect the work by the authors, i.e., baseline urine pH of ≤6.0 is not derived from the meta-analysis.

Response #1: We fully agree that this is a very important point and thank the reviewer for the helpful suggestion. Indeed, our title appears inaccurate and vague in terms of two aspects against a backdrop of "systematic review of literature plus self-controlled study in healthy volunteers": (1) mention of a specific baseline urine pH that was imputed from our empirical study rather than the meta-analyses, and (2) no mention of gender difference. The rationale for modification of our title was scrutinized by discussion among all authors, as stated below. Given that we answered the two questions in a two-pronged approach, we have to specify concomitant use of systematic review and human study (as mandated by STARD and PRISMA reporting checklists we were instructed to complete). Also, an imputed baseline urine pH of ≤6.0 (see ***Response #4***) from our human study seems not suitable to emphasize in the title lest readers be inadvertently misled. In our study, gender was associated with the effect of alkaline water supplementation but again, this was not derived from the meta analysis either. Likewise, the correlation between baseline urine pH and effect of alkaline water supplementation was not addressed in any other related studies, including any published meta-analyses. Finally, we modified our title as:

Baseline urine pH is related to effective urine alkalization by short-term alkaline water supplementation: Data from a self-controlled study in healthy Chinese volunteers following a systematic review of literature.

Changes in the text: Title page/Title, Page 1, lines 2-4.

Comment #2: Second, the abstract is not adequate. In the background, the authors did not explain why they focused on factors associated with the effect of alkaline water supplementation. The research objectives in this part are also not complete.

Response #2: We thank the reviewer for this criticism, and agree that this is a very useful reminder. We have added to the background that "***The inconclusive outcomes probably arise from difference in study design, ethnic group, or source of the alkaline water, which needs further clarification. With a systematic review of literature, followed by an empirical observation among healthy Chinese volunteers, we aim to investigate the outcomes of urine alkalization with alkaline water vs daily drinking water, and whether these outcomes are intersected by certain factors such as gender and body mass index.***". These two sentences, we believe, describes our research objectives and preludes our focus on factors associated with the effect of alkaline water supplementation.

Changes in the text: Abstract/Background, Page 3, lines 39-44

Response #3: Thanks for your professional advice. Rigorously pertaining to the reviewer comments on the methodology in the **Abstract**, we revised as:

We conducted a literature search of related studies on alkaline water supplementation and urine pH using the Pubmed, Embase, Medline and Cochrane Library databases from inception to February 01, 2021. The publication bias was assessed with inverted funnel plotting. Chi-square-based Q-test and I^2-statistic test were used to examine the data heterogeneity. The studies were evaluated for quality using the Cochrane risk of bias tool or Newcastle-Ottawa Scale.

Changes in the text: Abstract/Background, Page 3, lines 45-50

Comment #4: In the results part, it remains unclear how that the cut-off value of pH ≤6.0 is determined.

Response #4: Thank you for your comment. The optimal cut-off value of urine pH was determined using receiver operating curve (ROC) analysis and the Youden index. As per ROC analysis, the maximum Youden index of 1.548 was obtained, corresponding to a baseline urine pH cut-off of 5.977. In our general practice and clinical laboratories, a urine pH of 5.977 is usually reported as 6.0. Honestly, we have to acknowledge that "the cut-off value of pH ≤ 6.0" is an imputed value of the software-computed 5.977 rather than a field-work measurement (to the nearest 0.1). If the reviewer insists on using 5.977, we are happy to revise accordingly. At this moment, we clarified on this question as: "*… and ROC analysis suggested that subjects with more "acidic" urine, particularly those with a baseline urine pH ≤ 6.0 (maximum Youden index = 1.548, cut-off = 5.977), could show more pronounced outcome of urine alkalization from oral alkaline water.*".

Changes in the text: Abstract/Results, Page 4, lines 67-70

Comment #5: The conclusion seems did not reflect the findings, i.e., no effect of alkaline water supplementation in females. The authors should not use "benefit from alkaline water supplementation" because they did not examine the health benefits of alkaline water supplementation; the only tested the pH values after alkaline water supplementation.

Response #5: We respectfully agree with you. (1) In the conclusion (of **Abstract**), we added "*The outcomes seem not significantly pronounced in females, although more efforts warranted for validation.*" (2) We apologize for the improper use of "benefits" to refer to the usefulness of alkaline water supplementation in alkalizing the urine. In short, we have changed our description with "outcome" throughout the text wherever applicable.

Changes in the text: (1) Page 4, lines 72-73; (2) Page 3, lines 38, 39,42, 44, 56; Page 4, lines 69, 72; Page 14, lines 289, 293; Page 15, lines 303, 313; Page 17, lines 351, 362.

Comment #6: Third, the introduction talked a lot of information that is mot related to the research question, the effect of alkaline water supplementation and factors associated with this effect. The authors should have a brief review on the two, present the controversies regarding the effect of

**alkaline water supplementation, analyze the potential reasons, and explain why meta-analysis and an
empirical study are both needed to answer the two research questions. Please also have a brief review
on the factors associated with the effect, to inform the current study.**

Response #6: Thanks for your criticism and kind advice. In the revised manuscript, the Introduction part has
been carefully reformulated as instructed.

Changes in the text: Introduction, Page 5, lines 119-139 and Page 6, lines 140-150

**Comment #7: Fourth, the methodology of the main text should be divided into two parts: the
meta-analysis and the pre-post comparison in the sample of volunteers.**

Response #7: Thank you for this suggestion. The methodology of the main text is now presented in two
parts as advised.

Changes in the text: Methods, Page 5, lines 152 - Page 11, line 358.

**Comment #8: In the part of meta-analysis, all the essential elements and details of the meta-analysis
should be reported, including literature search, inclusion of studies according to the PICOS principles,
data extraction, risk of bias assessment, and statistical pooling methods. This current version is very
inadequate on these details.**

Response #8: We thank the reviewer for this comment. (1) As suggested, in this revision we have
supplemented on details regarding the literature search, the PICOS principles, data extraction, risk of bias
assessment, and statistical pooling methods. (2) We added details on quality assessment of included studies
in the **Results**.

Changes in the text: (1) Methods, Page 6, lines 112- Page 8, lines159 (2) Results, Page 12, lines 230- Page
13, lines 235.

**Comment #9: For the second part, the authors should provide their considerations for the sample size
of these volunteers, in particular the small sample size of females, which may be related to the
negative findings on females.**

Response #9: Thank you for your incisive criticism. (1) In the second part, we have carefully presented our
considerations for the sample size. (2) Since the lower proportion of females vs males finally included was
not foreseen at study design, we did not talk about the small sample size of females in the methodology, but
frankly explained on this in the **Discussion**. (3) To inspire future clarification, we preserved and toned down
our finding about gender difference in the outcome of alkaline water supplementation. We acknowledged
the small sample sizes of all subjects and of females as a study limitation to be addressed in the **Discussion**.

Changes in the text:

(1) Method/The pre-post comparison in human volunteers/Study population, Page 8, lines 163- Page 9, lines
176: "*Several months after completion of our meta-analysis, we tentatively approached 100 medical
students from Guangzhou Medical University to be recruited as healthy volunteers in early October of
2021. Among these, there were 51 males and 49 females, with a male-to-female ratio close to 51.25%
vs 48.76% as released by the Chinese Population Census 2021. Given the paucity of reference data on
this topic, our consideration for initial sample size (n=100) was based on the total number of subjects*

in healthy subjects (5.5 to 7.5), measured to the nearest 0.1, corresponds to a spectrum of twenty 0.1-pH units, such that we speculated that 100 subjects with 51 males and 49 females (nearly 2.5-fold of twenty) could be suitable. Furthermore, as a convenience sample, the initial sample size of 100 healthy subjects was considered proper, regarding the number of students readily available in our institution who would be voluntary but not obliged to participate in a human study."

(2) Discussion, Page 15, lines 312- Page 16, lines 321: "***Our gender-based subgroup analysis showed that only male subjects showed favorable outcomes from alkaline water supplementation. At a first glance, it was speculated that this could be due to the higher urine pH in normal women compared with men (27). On a second thought, the fewer females compared with males (31.5% vs 68.5%) in this study could be a confounding factor. As per our protocol, we excluded females with a menstrual period projected to occur during the 2-week human study, to avoid interference with urine pH measurements. The lower inclusion rate of females (23 out of 49, 46.9%) could be explained by the theoretical probability of exclusion (50.0%) based on the designed study duration and the mean cycle of menstruation (14 days vs 28 days). We have to inform that this was not forseen at study design.***"

(3) Discussion, Page 17, lines 350-257: "…***Moreover, while gender was likely linked to the outcome of urine alkalization and hence the AGU-pH or baseline urine pH, the small sample size and relatively lower proportion of females in this study disabled the reliability of a multivariate regression to account for this. Adjusting for these confounders, and including more factors into a regression model to develop a prediction model and determine the cut-off value based on the model scores, should be encouraged in future studies. In the context of these caveats, our findings should be interpreted with prudence.***"

Comment #10: In statistics, I do not agree with the focus on the baseline pH value only. Because other factors such as gender are also associated with the AGU pH, the authors should include more factors into a regression model to develop a prediction model and decide the cut-off value based on the model scores.

Response #10: We fully agree that a multivariate regression is important to account for more factors, and thank you for these professional suggestions. In this study, we found a negative correlation between AGU-pH and baseline urine pH in the study population (males and females combined), which preliminarily suggested a greater increase in urine pH from a lower baseline value after alkaline water supplementation.

We were interested to have noted that the AGU-pH was not statistically significant in females. To the best of our knowledge, very rare data, if any, have looked at the gender difference in this aspect, such that our study could inspire further investigations. However, as the reviewer pointed out, our human study was empirical and with a small-size convenience sample; in addition, the porportion of females was relatively low (as explained in **Response #9**). These settings gave rise to a difficulty in performing a reliable multivariate regression analysis. Because of these, we have been toning down our finding about gender difference since the very beginning of paper writing. We added to aknowledge this difficulty as one of the study limitations, declaring that our findings should be interpreted with prudence. By any means, your advices should be addressed in future studies of our team and others. We plead for your kind understanding.

Changes in the text: Discussion, Page 17, lines 350-357.

"…***Moreover, while gender was likely linked to the outcome of urine alkalization and hence the AGU-pH or baseline urine pH, the small sample size and relatively lower proportion of females in this study disabled the reliability of a multivariate regression to account for this. Adjusting for these confounders,***

cut-off value based on the model scores, should be encouraged in future studies. In the context of these caveats, our findings should be interpreted with prudence."

Comment #11: Please ensure P<0.05 is two-sided.

Response #11: Thank you for your reminding. In the previous and revised versions of our manuscript, we ensure that only two-sided P value < 0.05 was deemed statistically significant.

Changes in the text: Null.