Author's response to reviews

Title: Hypovolaemia was associated with clustering of major cardiovascular risk factors in general population

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Author's response to reviews: see over
Dear Mr Gilbert Taebobo:

Thank you for your potential interest in our manuscript. We also greatly appreciate the thoughtful and helpful comments from the three peer reviewers.

In our response, we have responded to each comment from the reviewers and have revised the manuscript accordingly. Each reviewer comment is listed verbatim, followed directly by our response. When the manuscript was altered according to a comment, we have included both the location of the change and a quotation of the change. We believe that the reviewer’s suggestions have substantially enhanced our manuscript. We have also checked the writing of the manuscript thoroughly and have polished the language.

We hope you find this revision suitable for publication in the *BMC Cardiovascular Disorders*.

Sincerely,

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**Reviewer 1 (Dr. Luxia Zhang)**

1) There are certain factors known to be associated with clustering of CVD risk factors (eg. lifestyle, insulin resistance), and those were not captured by the analyses. Potential confounding by those factors is one of the major concerns.

- We agree that those potential confoundings may be associated with clustering of CVD risk factors. Unfortunately, we do not have those data in present study.
2) Between-individual variation exists when using ECW/TBW to define volume status. The authors should mention that in the limitation part.

-- We agree that, and we have made corresponding revisions in the limitation part.

3) Regarding Logistic regression models in Table 3, the authors should state clearly that who did they include (“none” and “cluster”, excluding “single”)?

- We are sorry that we did not make the regression model clear. We have made corresponding revisions in Table 3. Indeed, we excluded the “single” group.

Reviewer 2 (Dr. Chih-Yu Yang)

Major:

1. The diagnosis of volume status should be accurate and reproducible, and it may be influenced by fasting duration, diuretic medications, meal intake, and etc. How many times did these people receive BIA test? The accuracy might be low if there was only one measurement because volume status varies at different time points even within one day. Additionally, the authors mentioned that the study subjects were from a health examination department and they were told to undergo a 10hr minimum fasting period for a venipuncture. The authors also mentioned that these people were told not to take a heavy meal. So what is the fasting or dietary status of these people? Were these people allowed to take meal? Were some people remained fasting when he or she received a BIA test? If it is not unified, the BIA results might not truly reflect the long-term volume status.

- We agree that between-individual variation exists when using BIA test to
define volume status receiving one measurement. In our study, if the patient was
enrolled in the study, the collecting of blood and BIA test would be arranged in the
morning in order to facilitated the study. In fact, they were kept on an empty
stomach in order to unify.

2. Regarding the hypothesis and discussion of the association between hypovolemic
and CVD risk factor clustering, the authors proposed that their findings might be
explained by RAAS activation. In my opinion, this proposed mechanism for health
hazards should be a "chronic" or “long-term” RAAS activation. Could the authors
convince the readers that these patients were exposed to chronic hypovolemia? I think
it will be difficult due to the cross-sectional study design. In the last paragraph of
discussion, the authors suggested to increase fluid consumption for patient with CVD
risk factor clustering. This comes back to the fundamental question: were those
patients diagnosed as hypovolemia used to drink less water or just on that
examination day?

- We are sorry for the wrong statement, and we have revised that in the last
paragraph of discussion, which now reads:

“Future clinical trials need to be implemented to confirm whether increased
consumption of fluids would be feasible for controlling the clustering of CVD risk
factors”.

We agree that our data was cross-sectional and do not provide an insight into the
mechanisms that are responsible for the observed associations.

Minor:
1. Line 72: What is the meaning of "the mean of the three readings was greater than 10 mmHg"? Did the authors referred to the difference between three mean BP data?

- We are sorry that we did not make the sentence clear, and we have revised that in Page 6, the first paragraph, which now reads:

  “The mean of the three readings was calculated, unless the difference between the readings was greater than 10 mmHg, in which case the mean of the two closest measurements was used”


- We have made those corresponding revisions.

Reviewer 3 (Dr. Chun-Fu Lai)

Major points:

1. Background: before describing the aim of this study, please specify the rationale why you investigate the association between volume status and clustering of CV risks. (“little is known…” is not a good rationale)

- We have provided information of the rationale in the background, at Page 4, the second paragraph, which now reads:

  “Clustering of CVD risk factors was positively associated with Chronic kidney disease (CKD) [11]. Recent observations indicated that higher levels of water intake were associated with slower progression of CKD [12,13]. Epidemiologic evidence suggests that the balance of water intake and output may have implications for development of CKD. Hypovolaemia caused by arduous physical labor or high ambient temperature may be associated with CKD [14,15]. Arginine vasopressin...
(AVP), a crucial peptide hormone that regulates water homeostasis, may contribute to CVD progression. In a rat model of 5/6 nephrectomy, increased water intake decreases AVP and reduces histological damage [16,17]. However, little is known about the direct association between volume load and clustering of CVD risk factors in general population. Therefore, we performed a cross-sectional study on a large scale population to examine the relationship between volume load and clustering of CVD risk factors, which was evaluated based on bioelectrical impedance analysis.”

2. Method: this study excluded patients who were taking diuretics. Those who take diuretics are often patients with hypertension and diabetes. So the prevalence of clustering CV risks may be underestimated. Besides, these patients may be over-hydrated so that they should take diuretics. The association between fluid status and clustering CV risks may be quite different with those included in this study. Could the authors analyze this subgroup? or they should make a critical discussion at least.

- We agree that those information would be helpful. Unfortunately, we excluded patients who were taking diuretics, and we do not have those data in present study. We have made corresponding revisions in the limitation part, at Page11, the second paragraph, which now reads:

“ This study excluded patients who were taking diuretics. Those who take diuretics are often patients with hypertension and diabetes, so the prevalence of clustering CV risks may be underestimated. And the association between fluid status and clustering of CVD risks in this subgroup who taking diuretics deserve further investigation. ”
3. Method: Did this cohort include data of [Na] and plasma glucose levels? These two parameters may have influence on subjects’ volume status. These data should be listed in Table 1 and be adjusted in the multivariate logistic regression analysis.

   - We agree that information about plasma [Na] is useful to understand subjects’ volume status. Unfortunately, we do not have the data of [Na] in present study. We have provided the data of glucose levels in Table 1. In the study, the clustering of CVD risk factors was defined as two or more of the following: hypertension, diabetes, dyslipidemia and overweight. Plasma glucose level is important basis of diagnosis of diabetes, which can be seen as “dependent” variable. Hence, we did not included in the multivariable model.

4. Method: The regression analysis used “age, sex, hypovolemia, Hb, decreased eGFR, and serum uric acid” as covariates. How did these selected and the rationale? Why [Na], glucose level, and blood pressure not included? Are these factors chosen before study initiation?

   - Age and sex are traditional risk factors, which included in the multivariable model. Many studys confirms that high uric acid hematic disease and chronic kidney disease (defined as decreased eGFR) are independent risk factors of CVD. So we selected age, sex, hypovolemia, Hb, decreased eGFR, and serum uric acid” as covariates before study initiation. But why we selected the Hb? Because in general population, the rise of Hb is associated with atherosclerosis (Kawamoto R, Tabara Y, Kohara K, et al. Clinical and experimental hypertension. 2012;34(2):92-98.), its specific mechanism is unknown. Studies have shown that hemoglobin was associated
with the ratio of triglyceride/cholesterol (Shimizu Y, Nakazato M, Sekita T, et al. Internal medicine. 2014;53(8):837-843), the latter was a risk factor for atherosclerosis. That other possible mechanism was that the elevated hemoglobin may increase blood viscosity and injury the vascular endothelial.

We do not have the data of [Na] in present study. A few studies demonstrated that when sufficient extra water is given to increased urine volume without a reduction in the plasma sodium concentration (Wang C.J et al, Kidney Int, 2013, 84(1):45-53.). Clustering of CVD risk factors was defined as two or more of the following: hypertension, diabetes, dyslipidemia and overweight. Plasma glucose level and blood pressure are important basis of diagnosis of diabetes and hypertension, which can be seen as “dependent” variable. Hence, we did not included glucose level and blood pressure in the multivariable model.

5. Discussion: The last 3 sentences of the first paragraph (Multiple unhealthy lifestyles, ...........to reduce the burden of CVD) are completely the same as that in the Background session. Please delete the redundancy.

- We agree that those sentences were redundant, and we have made corresponding revisions.

6. Discussion: in the last paragraph: “Increased consumption of fluids to improve blood volume would be feasible and cost-effective for controlling the clustering of CV risk factors”, this description could not be supported by the data of this observational study.

- We have revised the sentence as the followings:
“Future clinical trials need to be implemented to confirm whether increased consumption of fluids would be feasible for controlling the clustering of CVD risk factors”.

Minor points:

1. General: The grammar should be modified by a native English speaker, especially the discussion session.
   - We have checked the writing of the manuscript thoroughly and have polished the language by a native English speaker.

2. Method: This study included 7900 adults who visited the health checkup clinics. Are they all otherwise healthy and included during health checkup? or are they outpatients of the hospital and included in the clinics? Please specify it.
   - We are sorry that we did not make the “study population” clear, and we have revised that in Page 4, the second paragraph, which now reads:
     “A total of 7900 adults who visited the Health Checkup Clinic consecutively in Qianfoshan Hospital of Shandong University were enrolled in the study. This study excludes outpatient or clinical patients.”

3. Method: Do the authors have data about smoking in this cohort?
   - We agree that potential confoundings may be associated with clustering of CV risk factors. Unfortunately, we do not have the data of smoking in present study.

4. Method: This study use TBW/TBWwatson ratio as an indicator of hypovolemia. Does the Watson’s formula predict fluid status well in Chinese population? And why TBW/TBWwatson ratio<1 indicate volume deficit?
- So far, the Watson’s formula was not validated in Chinese population.

Alternatively, the TBW$_{BIA}$/TBW$_{watson}$ ratio, which can be calculated using anthropometric formulas, may be useful for assessing fluid volume because TBW$_{BIA}$ provides the value of fluid volume and TBW$_{watson}$ provides the adequacy of fluid volume. So TBW/TBW$_{watson}$ ratio<1 may indicate volume deficit. (Yasushi Ohashi, MD et al. Journal of Renal Nutrition, Vol 23, No 1 (January), 2013: pp 28-36)

5. Discussion: 3rd paragraph, “First, this study used a convenience sample which was not based on a community-based screening and could introduce bias”: please specify and discuss what kind of bias and how to interpretation.

- We have revised the first limitation, which now reads:

“Firstly, it was implemented on a voluntary bias and was not based on a community-based screening. There were kinds of selecting bias in the study which limited the extension of the results from this study.”