

Peer Review File

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Reviewer A

This manuscript by Xu et al. looks at the relationship between serum uric acid and hand grip strength in about 1100 Chinese adults over 40 years of age. Given the accumulating reports of associations of uric acid concentrations with chronic diseases that are often coincident with muscle wasting, this may be an interesting study. However, this association between UA and grip strength has been done numerous times before, and it is not clear what is novel here. Numerous points need to be clarified to improve the utility of this study.

1. Title. The phrasing of the title, “serum uric acid ... contributes to a greater ...” imply a cause and effect relationship. The current data does not support this. This needs to be rephrased to state that UA and handgrip strength are associated, similar to the phrasing at the end of the Introduction.

Reply: Thank you for your comment. Following the suggestion of you and other reviewer’s comments, the title has been changed to " Cross-sectional analysis of the association between serum uric acid levels and handgrip strength among Chinese adults over 45 years of age". The running title has been added to " The association between serum uric acid levels and handgrip strength".

Changes in the texts: Please see Page 1, line 1-3.

2. Introduction. It is not sufficient to cite a single review from 15 years ago as “several recent investigations.” Provide citations of the primary literature to support the statement that UA is a strong antioxidant.

Reply: Thank you for your suggestion. As suggested, we added some citations and references to support the statement that UA is a strong antioxidant in revised manuscript.

Changes in the texts: Please see Page 2-3, line 47-54.

3. Introduction. While it is true that uric acid has some anti-oxidant properties, it is also true that production of uric acid by xanthine oxidase generate reactive oxygen species. For balance, this should be added to the Introduction and the implications discussed in the Discussion.

Reply: Thank you for your comment. As suggested, some related content was added in revised manuscript.

Changes in the texts: Please see line 43-53 and line 190-191.

4. Introduction, third paragraph. The authors should give the age ranges (not just statements of “elderly” or “middle aged”) and nationality of subjects to define these introductory studies of UA. Clearly these matter.

Reply: Thank you for your comments concerning our relevant content . We made a detailed introduction to relevant studies in “Introduction, third paragraph”.

Changes in the texts: Please see Line 61-64.

5. Introduction. The authors need to be much more explicit about the purpose of this study. Why is this study novel and important? If, as suggested in the Introduction, that the main novelty is that this was done with Chinese, then explain why it might be expected that Chinese may be different (or the same) than other nationalities. And then explain the implication in the Discussion.

Reply: We found the referee’s comments most helpful and have revised the manuscript in the section “Introduction and Discussion”.

Changes in the texts: Please see Line 64-67 and Line 151-152.

6. Methods. How were the laboratory tests of Table 1 & 2 measured? What methodology was used? What instrument was used?

Reply: We are sorry we didn't mention methodology and instrument used for the laboratory tests in the manuscripts.

Changes in the texts: As suggested, we added detailed presentation in line 79-84.

7. Methods. Was the hand grip strength done at the same time as blood collection? Please clarify.

Reply: We are sorry we didn't explain procedure of this research clearly.

Changes in the texts: As suggested, we added detailed presentation in line 91.

8. Methods. Was the dynamometer calibrated for hand size? Please explain.

Reply: We are sorry we didn't introduce method of electronic dynamometer clearly. As suggested, we have supplemented.

Changes in the texts: As suggested, we added detailed presentation in line 92-93.

9. Methods. The authors must rationally describe why the data was split into quartiles. Why not tertiles or quintiles? And why were sexes combined when making the quartiles?

Reply: Thank you for your comment. In contemporary epidemiologic practice, it is more customary to group continuous variables into quantiles - most often tertiles, quartiles or quintiles - based on the exposure's distribution. We held the opinion that it was difficult to show the trend changes between UA and hand grip strength with detail when UA was divided by tertiles. In addition, when we used the quartile and quintile of serum UA levels to assess the relationship between uric acid and grip strength respectively, the same trend was observed. So we selected the quartiles which was most commonly used in clinical research. We have adopted the opinions of several reviewers, we recalculated the quartiles of uric acid for men and women separately, so that each group had the same number of participants. We are sorry for my fault.

10. Results. The upper and lower bound ranges of serum uric acids for Q1 and Q4 should be reported.

Reply: Following the suggestion, we added the upper and lower bound ranges of serum uric acids for Q1 and Q4. The UA quartiles in males were 1.26-4.20 mg/dL (Q1), 4.21-5.34 mg/dL (Q2), 5.35-6.41 mg/dL (Q3), and 6.24-11.24 mg/dL (Q4). The UA quartiles in females were 1.02-3.39 mg/dL (Q1), 3.40-4.42 mg/dL (Q2), 4.43-5.21 mg/dL (Q3), and 5.22-10.28 mg/dL (Q4).

Changes in the texts: Please see in line 111-113.

11. Results. To allow the reader to better appreciate the correlations and compare to the bar graph of figure 1, a scatter plot must be shown for UA vs hand grip strength. Put men and women on the same plot with different symbols for each sex. Add the calculated line of fit for each.

Reply: As suggested, we have added a scatter plot and calculated line in a resubmitted manuscript.

Changes in the texts: Please see the Figure 1 for the detailed information in revised manuscript.

12. The table describing the female subjects is labeled as table 1. It should be table 2. Also, the distribution of subjects across quartiles (i.e. the N per group) are identical between tables. Presumably the N's differ between males and females, as reported in Table 3. Please correct.

Reply: We are very sorry that I failed to submit Table 1 and Table 2 was marked as Table1 by mistake in original manuscript. We have corrected this mistake in resubmitted manuscript. In addition, we recalculated the quartiles of uric acid for men and women separately, so that each group had the same number of participants.

Changes in the texts: Please see the Table 1 and Table 2 for the detailed information in revised manuscript.

13. Discussion. The trends for the males and females, as described in Figure 1, are fundamentally different. Given that the association of HGS and UA is the main topic of the manuscript, this discrepancy between sexes must be thoroughly discussed. Provide specific literature that might explain the differences for the topics alluded to (i.e. age distribution, daily exercise, food intake, drug, sex hormones). Additionally, do the sexes

differ in the production and/or clearance of uric acid. Are there sex differences in purine degradation in general?

Reply: As suggested, we recalculated the quartiles of uric acid for men and women separately, so that each group had the same number of participants. Once again, the statistical reanalysis showed an inverted J-shaped association between serum UA levels and HGS in both men and women. For men, the third quartile (Q3) UA (5.35-6.41mg/dL) had the highest HGS, while for women, the Q2 UA (3.40-4.42 mg/dL) had the highest HGS. In our study, the gender-specific difference was discussed. Additionally, Serum UA reflects a balance between endogenous production, external sources, and pathways for elimination. The level of serum UA is intimately linked to age and age-related changes in sex hormones, lifestyle, nutrition, comorbidities, and therapies. These were also discussed in our article. However, in fact, the exact mechanism is still unclear how the UA level changes with age and gender.

Changes in the texts: Please see the line 154-162 and 182-193.

14. Discussion. A related point that must be discussed is why the distribution of subjects across quartiles differ wildly between sexes. For example 34% of males are in Q4, while only 11% of females are in Q4. Can the differences in the trends be explained because of this vastly different distribution?

Reply: We are sorry for different distribution in men and women of each group due to our mistake for grouping (men and women were not calculated separately in quartile). In the new submission, we recalculated the quartiles of uric acid for men and women separately, analyzed and discussed the relationship between serum uric acid and muscle strength. Because of the new grouping, we reanalyzed the data and discussed again.

Changes in the texts: Please see the Table 1, Table 2 and section “Results” for the detailed information in revised manuscript.

Reviewer B

GENERAL COMMENT

This is an interesting study which is not adequately reported and discussed. I would suggest editorial reworking to an extensive amount based on those suggestions appended below.

SPECIFIC COMMENT

MAJOR

Introduction

“Increasing evidence has demonstrated that low muscle strength is a strong and consistent predictor of mortality in middle-aged and elderly persons.”◇ A reference must be added.

Reply: Following the suggestion, we have added a reference.

Changes in the texts: Please see the line 56-57 and 14th reference.

“The different conclusions were based on different countries and populations.”◇ These conflicting findings may result from studies being conducted in varying countries and populations”

Reply: Thank you for your comment and we have changed the sentence according to your suggestion.

Changes in the texts: Please see the line 64-65.

Discussion

- “Uric acid is the final product of purine metabolism. It has been reported that there are molecular switches in different parts of the human body to regulate the role of uric acid as an antioxidant or oxidant[16]. It is a

powerful endogenous antioxidant, which can protect the body from oxidative stress[17]. In addition, when the serum UA level rises to a certain level, uric acid can change from an antioxidant to an oxidant, which leads to organ dysfunction by affecting the release of vasodilatory factors[18]. Therefore, the significance of uric acid to human health remains a debate. In recent years, with the acceleration of the aging process, a variety of age-related diseases have attracted people's attention. The World Health Organization estimates that in 2050, the number of people aged 65 and over will reach at least 2 billion[19]. The aging process causes a series of changes in body composition, such as decreased skeletal muscle mass and strength. Sarcopenia is a type of syndrome associated with aging that is related to the impairment of muscle metabolism and cognitive function, which is manifested by the age-related decline in skeletal muscle quality and low level of muscle function (muscle strength and physical activity) [20]. Commonly used methods to evaluate muscle strength are grip strength and walking speed. The European Working Group on Sarcopenia in Older People (EWGSOP) and the Asian Working Group for Sarcopenia (AWGS) agree that there is a good correlation between hand grip strength and lower extremity muscle strength, and because the hand grip strength test is simple and measures easily and repeatably, it is currently recommended by various sarcopenia working groups as the preferred method to evaluate muscle strength.”◇ This section must be either deleted or encapsulated (in a shorter form) in the Introduction. The discussion must start with novel findings.

Reply: Thank you for your comment and we encapsulated this section according to your suggestion.

Changes in the texts: Please see the Section “Discussion” from line 143.

- In reworking discussion, please use either indentation or subheadings to facilitate reading.

Reply: We are sorry for bothering you due to the format of the manuscript. We use indentation to facilitate reading in resubmitted manuscript.

Changes in the texts: Please see the Section “Discussion” from line 143.

- Address the following topic: sarcopenia has been associated with NAFLD; and hyperuricemia has also been associated with NAFLD. Not having evaluated NAFLD is a limitation of the study design.

Reply: I have read the referee's comments very carefully. Understanding the interplay between sarcopenia, UA and NAFLD remains a challenge. Skeletal muscles have been recognized as an important endocrine organ. Loss of muscle mass can cause insulin resistance, which is an important risk factor for NAFLD. Sarcopenia play a role in NAFLD, not only in the pathogenesis but also severity and prognosis. Moreover, insulin resistance not only increased uric acid synthesis, but also decreased uric acid excretion. This effect of insulin resistance on UA metabolism may partly explain elevated SUA levels in NAFLD patients. Our research focus on the relationship between uric acid and the grip strength. Although we excluded the influence of many confounding factors, no considering effects of IR and fatty liver on uric acid and muscle strength is a limitation of the study design. This is explained in the newly submitted manuscript. Thank you for your advice. We will consider it in future work.

Changes in the texts: Please see the line188-189.

- Again, NAFLD is a sexually dimorphic disease and the association of uric acid with sarcopenia also showed a dimorphic pattern in the present study. Could these authors comment on this?

Reply: We found the reviewer's comments most helpful. Sarcopenia is complex involving multiple biological processes. The underlying molecular pathology may include decline of muscle insulin sensitivity, elevation in oxidative stress, the inflammatory response, as well as mitochondrial dysfunction. Many of these age-related changes in skeletal muscle appear to be influenced by sex. Although controversy exists regarding how sex influences each aspect of the aging process of skeletal muscle, sex differences have been reported in age-related loss of muscle mass and strength. Thus, it is reasonable to assume that the aging of muscle is regulated through different mechanisms in men and women. A better understanding of the potential sexually

dimorphic disease would help us gain a greater insight into the different susceptibilities to sarcopenia and metabolic diseases for men and women, and could therefore promote customized strategies to alleviate the aging-related health problems.

Conclusions

This section is too long and must be dramatically shortened. In doing so, eliminate the section on the limitations of the study design and report this among the heading “Discussion”.

Reply: We are sorry for bothering you in original manuscript. As suggested, we simplified Section “Discussion” and shortened Section “Conclusion” in resubmitted manuscript.

Changes in the texts: Please see the Section “Discussion” and “Conclusion” from line 141 to the end of manuscript.

MINOR

Introduction

“and so on.” Delete this. Or list what else is to be said.

To be to be

Discussion

“and so on,” Either delete it or say what you have to.

“Abovementioned” above mentioned

“We speculated the reason the conclusion of these two studies differed from previous studies was related to the grouping of uric acid levels.” Sentence not clear, please rephrase.

Reply: We are sorry for bothering you because of inappropriate wording. Following the suggestion, we revised the wording and the main text was checked by a native-English speaker.

Reviewer C

1) Table 1 should include 634 male subjects. You seem to have written wrong numbers (females' number instead of male).

Reply: We are sorry for bothering you due to the wrong table. We redraw Table 1 and Table 2 in resubmitted manuscript.

Changes in the texts: Please see the Table 1 and 2 for the detailed information.

2) There must be different distribution of UA between male and female. I wonder why you did not calculate quartiles separately (so that every quartile has same numbers of subjects in each sex, I suppose it is better to analyze).

Reply: We found your comments most helpful. As your and other reviewers' suggestion, the level of serum UA between male and female is different, the quartiles of uric acid were best calculated separately for men and women. In the newly submitted manuscript, we corrected it and reanalyzed the data.

Changes in the texts: Please see the Table 1, Table 2 and section “Results” from line 109 for the detailed information in revised manuscript.

3) It is interesting to read reference paper about the hypothesis of UA as anti- and oxidative substance, but still your discussion. You described that UA can become a useful indicator for sarcopenia, but how?

Reply: Thank you for your comments concerning our manuscript. Our data show that an optimal level of serum uric acid has a better HGS. But the mechanisms responsible for this effect remain to be clarified. The mechanism is not the focus of our current research, but our work will focus on it in the future. Thanks for your suggestion.

Reviewer D

The title notes that subjects were over 40 years old, which is technically true, but the average age of the cohort was ~60-70y. I'd change the title to better indicate that this is a study of older adults.

Reply: Thank you for your comments concerning our manuscript. We carefully checked the original data and found that subjects ≥ 40 years old and < 60 years old accounted for 38.3% of the total subjects. which resulted in a relatively high average age. Following your and other reviewers' suggestion, we have modified included subjects to middle-aged and elderly people aged 45 years, excluding young subjects aged ≥ 40 years and < 45 years.

Changes in the texts: Please see the line 73.

Introduction

Line 2-instead of saying "and so on", replace with all-cause mortality risk (PMID 31650159, PMID 29694719).

Line 3-"tobe" should be 2 words.

Line 11-"the relationship between serum uric acid levels *and*". And should be replaced with "with".

Reply: Following the suggestion, we revised the manuscript and the wordings of the main text is be checked by a native-English speaker.

Changes in the texts: Please see line 46,68.

Statistical Analysis

Line 3-should be "hs-CRP".

I see that some of the blood analytes were log transformed-this should also be indicated in Tables 1 and 2.

Line 4: delete the extra space before the comma

Page 5, Line 1: "formedical" should be 2 words.

Reply: Following the suggestion, we revised the manuscript and the wordings of the main text is be checked by a native-English speaker.

Changes in the texts: Please see line 99,132,288,296.

Results

"table 1 and table 2": Table should be capitalized

Reply: Following the suggestion, we revised the manuscript and the wordings of the main text is be checked by a native-English speaker.

Changes in the texts: Please see line 114,117,119,282,290,302.

Where is Figure 1A? I don't see it in the manuscript.

Reply: Figure1 in the new manuscript refers to scatter plots for UA against handgrip strength by gender.

Changes in the texts: Please see the Figure 1 in revised manuscript.

Table 3-footnote 3 says that the model was adjusted for age, BMI, and age again?

Reply: I'm sorry for my carelessness. We corrected it in the new manuscript.

Changes in the texts: Please see the explanatory legends in line 304.

Because uric acid increases in association with decreased kidney function, shouldn't the models be adjusted for eGFR? I'd like to see data for a model that includes age, BMI, and eGFR

Reply: In the new manuscript, we analyzed the association between muscle strength and serum UA quartiles after adjusting for the potential confounding factors. The potential confounding factors for Model 1 were age and BMI, and Model 2 were Model 1 plus eGFR.

Changes in the texts: Please see the explanatory legends in line 305.

Reviewer E

Overall, this is an interesting trial of an important area. However, several areas need attention and I do have a few issues to address:

Was this review performed according to a review protocol? Was this protocol preregistered at e.g. clinical trials.gov?

Reply: As an observational study, this protocol was not conducted a clinical trial registration. But we performed this study following the clinical trial procedure strictly. Thanks for your advice.

2. The participants in the study were people over 40 years old. How did the authors eliminate the related confounding biases (combined diseases, eating habits) and get a specific correlation between uric acid and grip strength?

Reply: Thank you for your comment. As described in the manuscript, to avoid the effects of drugs, daily diet and renal failure on the uric acid level, participants with gout, an unbalanced diet, diuretics, uric acid-lowering medications, and renal failure (eGFR<30 mL/min per 1.73 m²) were excluded. Furthermore, we analyzed the association between muscle strength and serum UA quartiles after adjusting for the potential confounding factors through ANCOVA.

3. The ethical approval number should be added.

Reply: Thank you for your valuable advice and the ethical approval number was added.

Changes in the texts: Please see the explanatory legends in line 89.

4. Some details in the table should be paid attention to and modified, such as the missing units in Group Q3 in Table 1.

Reply: We are sorry that there are so many mistakes in the details. These mistakes have been corrected in resubmitted manuscript.

Changes in the texts: Please see the Table 1 in revised manuscript.

5. The author seems to have omitted Table 2. There are two table 1 at the end of the manuscript.

Reply: We are sorry for giving you some trouble because of our fault. Table 1 and 2 have been corrected in resubmitted manuscript.

Changes in the texts: Please see the Table 1 and 2 in revised manuscript.

6. The authors should pay attention to grammar and language mistakes among the whole manuscript. In particular, please check tenses throughout the text.

Reply: I apologize for the grammar and language mistakes among the whole manuscript. The wordings of the main text has be checked by a native-English speaker.

Reviewer F

Authors aimed at investigating the relationship between uric acid and handgrip strength. The topic is worthy of discussing. However, the research is not rigorous. Several problems should be corrected.

1. Why did authors choose patients age over 40 years old. It is not reasonable, because patients aged over 40 years old includes the young, middle-aged and elderly patients.

Reply: Thank you for your comment. Following your suggestion and age range divided by WHO, we selected middle-aged and elderly people ≥ 45 years old as the research subjects.

2. What are inclusion and exclusion criteria of this study.

Reply: We are sorry that we did not clearly indicate inclusion and exclusion criteria in the original article. We have rewritten this part in resubmitted manuscript.

Changes in the texts: Please see the line 85-87 in revised manuscript.

3. Authors enrolled all patients with various diseases in this study. However, some disease would significantly interfere levels of uric acid and handgrip strength. Subgroup analysis is necessary.

Reply: I greatly appreciate your help in concerning improvement to this paper. We analyzed the association between muscle strength and serum UA quartiles after adjusting the potential confounding factors (including hypertension, diabetes and kidney disease), so subgroup analysis was not in consideration. And in future studies, we will consider the effects of uric acid on grip strength in different populations with a certain disease. Thanks for your suggestion.

4. What are confounding factors in each model in linear regression model? It is not clearly described in the manuscript.

Reply: For the description of confounding factors in each model, we have made a detailed description in the explanatory legends of Table 3.

Changes in the texts: Please see the line 304-309 in revised manuscript.

5. The analysis is too simple. Further investigations should be performed.

Reply: Thank you for your comments concerning our manuscript. In the revised manuscript, a scatter plot and calculated line are added to further illustrate the relationship between UA and hand grip strength. More over, in future studies, we will consider the effects of uric acid on grip strength in populations with a particular disease.

Changes in the texts: Figure 1 showed scatter plots for UA against handgrip strength by gender in resubmitted manuscript.

6. Where is Figure 1?

Reply: We are sorry for bothering you in original manuscript because of carelessness.

Changes in the texts: Figure1 was resubmitted in the revised manuscript.

7. There are two Table 1 in the manuscript. Please check.

Reply: We are very sorry for my fault due to careless check. we have resubmitted Table 1.

Changes in the texts: Table 1 was resubmitted in the revised manuscript.