ARTICLE DETAILS

TITLE (PROVISIONAL)	Hyperuricemia is Independently Associated with Endothelial Dysfunction in Postmenopausal Women but not in Premenopausal Women
AUTHORS	Higashi, Yukihito; Maruhashi, Tatsuya; Nakashima, Ayumu; Soga, Junko; Fujimura, Noritaka; Idei, Naomi; Mikami, Shinsuke; Iwamoto, Yumiko; Kajikawa, Masato; Matsumoto, Takeshi; Hidaka, Takayuki; Kihara, Yasuki; Chayama, Kazuaki; Goto, Chikara; Noma, Kensuke; Tomiyama, Hirofumi; Takase, Bonpei; Yamashina, Akira

VERSION 1 - REVIEW

REVIEWER	Mehmet KANBAY, Assoc. Prof. Department of Medicine, Division of Nephrology, Istanbul Medeniyet University School of Medicine, TURKEY Competing interest: None
REVIEW RETURNED	18-Aug-2013

GENERAL COMMENTS	In the present study, nothing is novel and already known that serum uric acid level is higher inelder /postmenoposal women due to decreased GFR (due to age, -decreased excretion of uric acid) and many other confounders. Many other well known factors might also influence the FMD of which they did not mention such as vitamins, phosphate, vit d, statins etc. They should clarify this. They should do an analysis and add this to result section to show the association between uric acid and GFR. Future studies should shed on light to answer, what should we do with hyperuricemia? should we treat or no? or whom should be treated? Many observational/cross-sectional studies showed such an association.

REVIEWER	Filipa Mascarenhas-Melo; PhD student; Laboratory of Pharmacology & Experimental Therapeutics, Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
REVIEW RETURNED	18-Sep-2013

THE STUDY	- In section of "study protocol" describing what devices/methods used for the determination of all biochemical parameters evaluated in the study. - Regarding the statistical analysis, saying what test used to exclude confounding factors; say if was made the determination of the sample normality for selection the significance test more appropriate; include an ANOVA post hoc test to see if there is

	specifically statistical difference between each group (Table 2) in order to confirm the results.
RESULTS & CONCLUSIONS	Make a test post hoc ANCOVA for table 2 would be important to justify the conclusions reported.
GENERAL COMMENTS	The authors describe a clinical study aimed to analyze the relationship between uric acid, endothelial function and cardiovascular risk factors in women and the differences between the menopausal status. The issue is of interest and up-to-date. This work is objective, with consistent results, due the sample size. The article gives a clear reading and enjoyable. However, in order to improve the publication I suggest that some changes are made and some additions are included. The main concerns are the following: **Comment 1:** - Throughout the manuscript, the authors refer several times to a female population that was analyzed "without established cardiovascular disease". However, the test sample comprises several women with various pathological conditions related to cardiovascular diseases (namely, diabetes mellitus, hypertension, metabolic syndrome and dyslipidemia). I suggest that this expression "without established cardiovascular disease" is reworded or deleted throughout the manuscript. **Comment 2:** - In the abstract, include the influence of menopause as one of the objectives of the study (and not only the female population) **Comment 3:** - In the abstract, in 44 line of results section, tell which population the FMD showed a gradual reduction in accordance with the serum uric acid level. **Comment 4:** - In the conclusions of the abstract, the two sentences should be joined, because somehow overlap. Then suggest: "These findings suggest that uric acid can be used as a risk marker of endothelial dysfunction in a female population, but particularly an independent risk factor in postmenopausal women, but not in premenopausal women" **Comment 5:** - In the Key messages, refer the population who report the results in the two first sentences. **Comment 6:** - In the introduction, explain the molecular mechanism by which the uric acid may contribute to cardiovascular disease (page 6, line 40). At the end of the introduction (page 7, line 22) include

the evaluation of the state of menopause as an aim of the study.

Comment 7:

- In the methods section, mean witch the reference used for the determination of hypertension and menopausal status (as is for diabetes, dyslipidemia and metabolic syndrome).

Comment 8:

- In section of "study protocol" describing what devices/methods used for the determination of all biochemical parameters evaluated in the study.

Comment 9:

- Regarding the statistical analysis, saying what test used to exclude confounding factors; say if was made the determination of the sample normality for selection the significance test more appropriate; include an ANOVA post hoc test to see if there is specifically statistical difference between each group (Table 2) in order to confirm the results.

Comment 10:

- In the discussion, page 15, line 13, the reference of epidemiological studies cited is missing.

Comment 11:

- indicate the registration number of the study.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Mehmet KANBAY, Assoc. Prof.
Department of Medicine, Division of Nephrology,
Istanbul Medeniyet University School of Medicine, TURKEY
Competing interest: None

We would like to thank the reviewer for the helpful comments and hope that we have now produced a more balanced and better account of our work.

Many other well known factors might also influence the FMD of which they did not mention such as

vitamins, phosphate, vit d, statins etc. They should clarify this.
Response: We agree with the reviewer's comment. However, we have no data on other factors, including vitamins, phosphate, vitamin d, and statin use, which might influence FMD. We have incorporated these comments into the Discussion section as limitations (page 18, lines 13-21).

They should do an analysis and add this to result section to show the association between uric acid and GFR.
Response: We analyzed the association between uric acid and eGFR in addition to the correlations between uric acid and other cardiovascular parameters. Serum uric acid level significantly correlated with eGFR (r=-0.27, P<0.001). We have added the results of univariate regression analysis of uric acid and cardiovascular parameters, including GFR, to the Results section (page 12, lines 7-12).

Future studies should shed on light to answer, what should we do with hyperuricemia? should we treat or no? or whom should be treated? Many observational/cross-sectional studies showed such an association.
Response: Given that this study was a cross-sectional study, it was difficult to establish a definitive causal relationship between hyperuricemia and endothelial dysfunction. We agree with the reviewer's comment that future studies are needed to obtain more specific conclusions as to whether hyperuricemia should be treated and which subgroup should be treated. We have incorporated these comments into the Discussion section as limitations (page 18, lines 13-21).

MS # BMJ-Open/2013/003659 R1

Reviewer: Filipa Mascarenhas-Melo; PhD student; Laboratory of Pharmacology & Experimental Therapeutics, Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.

We would like to thank the reviewer for the helpful comments and hope that we have now produced a more balanced and better account of our work.

Comment 1:
- Throughout the manuscript, the authors refer several times to a female population that was analyzed "without established cardiovascular disease". However, the test sample comprises several women with various pathological conditions related to cardiovascular diseases (namely, diabetes mellitus, hypertension, metabolic syndrome and dyslipidemia). I suggest that this expression "without established cardiovascular disease" is reworded or deleted throughout the manuscript.
Response: We have deleted "without established cardiovascular disease" (page 3, line 3, page 5, line 5, line 7, and line 20, page 7, line 8, and page 13, line 22 in the previous version). In the Methods section, we have changed "subjects with established cardiovascular disease" to "subjects who had been previously diagnosed with cardiovascualr diseases" (page 8, line 1).

Comment 2:
- In the abstract, include the influence of menopause as one of the objectives of the study (and not only the female population)
Response: We have added the part "and to investigate whether menopausal status was associated with the relationship between uric acid and endothelial function" into the Abstract section (page 3, lines 3-5).

Comment 3:
- In the abstract, in 44 line of results section, tell which population the FMD showed a gradual reduction in accordance with the serum uric acid level.
Response: We have added the phrase "in the entire study population" in the Abstract section (page 3,

line 21).

Comment 4:
- In the conclusions of the abstract, the two sentences should be joined, because somehow overlap. Then suggest: "These findings suggest that uric acid can be used as a risk marker of endothelial dysfunction in a female population, but particularly an independent risk factor in postmenopausal women, but not in premenopausal women"
Response: According to reviewer's suggestion, we have changed the Conclusions in the Abstract section (page 4, lines 2-4).

Comment 5:
- In the Key messages, refer the population who report the results in the two first sentences.
Response: We have added the phrase "in the entire study population" in the two first sentences in the Key messages in the Article summary (page 5, lines 9-11).

Comment 6:
- In the introduction, explain the molecular mechanism by which the uric acid may contribute to cardiovascular disease (page 6, line 40). At the end of the introduction (page 7, line 22) include the evaluation of the state of menopause as an aim of the study.
Response: Uric acid has been demonstrated to enhance the production of reactive oxygen species by activation of the local renin-angiotensin system, particularly angiontensin II, and the pro-oxidant effect of uric acid per se, after absorption into endothelial cells. These comments have been incorporated into the Introduction section (page 6, lines 17-19). We have added the part "and whether menopausal status was associated with the relationship between uric acid and endothelial function" into the Introduction section (page 7, lines 10-11).

Comment 7:
- In the methods section, mean witch the reference used for the determination of hypertension and menopausal status (as is for diabetes, dyslipidemia and metabolic syndrome).
Response: We have cited new references used for the determination of hypertension (reference 35, page 8, line 6) and menopausal status (reference 41, page 9, line 2). Therefore, references 35-39 in the previous version have been renumbered to 36-40 and references 40-58 in the previous version have been renumbered to 42-60.

Comment 8:
- In section of "study protocol" describing what devices/methods used for the determination of all biochemical parameters evaluated in the study.
Response: We have incorporated devices/methods used for the determination of all biological parameters evaluated in this study in the Methods section (page 9, lines 14-20).

Comment 9:
- Regarding the statistical analysis, saying what test used to exclude confounding factors; say if was made the determination of the sample normality for selection the significance test more appropriate;
Response: To exclude confounding factors, we performed stepwise multivariate logistic regression analyses to identify factors associated with endothelial dysfunction among potential confounders ($P<0.20$) in univariate analysis. To determine sample normality for selection, potential confounders ($P<0.20$) in univariate analysis were selected for stepwise multivariate logistic regression analyses. Using variables associated with endothelial dysfunction in stepwise multiple regressin analyses, we performed the final logistic regression analyses. These comments have been incorporated into the Statistical analysis section in the Methods section (Page 11, lines 11 and 13-16) and the Results section (Page 13, lines 3-4). Odds ratio and 95% CI have been changed to the new results in the Abstract section (Page 3, line 25), in the Methods section (Page 13, lines 5-6, Page 13, line 25-Page

14 line 1, and Page 14, line 5), and in Table 3 (Page 22) and Table 4 (Page 23).

include an ANOVA post hoc test to see if there is specifically statistical difference between each group (Table 2) in order to confirm the results.
Response: We have added the results of the ANOVA post hoc test to Table 2 (Page 21). Description of the post hoc test has been incorporated into the Statistical analysis section in the Methods section (Page 11, lines 8-10).

Comment 10:
- In the discussion, page 15, line 13, the reference of epidemiological studies cited is missing.
Response: We have cited the references of epidemiological studies in the Discussion section (page 16, line 2).

Comment 11:
- indicate the registration number of the study.
Response: Registration number of the study (UMIN000003409) has been incorporated into the Abstract (page 4, last line).

MS # BMJ-Open/2013/003659 R1

The detailed review of this manuscript is appreciated, and we have attempted to answer each of the questions raised. Changes made are shown in red in the revised manuscript.