Author’s response to reviews

Title: Association between serum uric acid levels and cardiovascular risk among university workers from the State of Mexico: a nested case-control study

Authors:

Patricia Cerecero (pcereceroa@uaemex.mx)
Bernardo Hernández-Prado (bhp3@u.washington.edu)
Roxana Valdés (rvaldesr@uaemex.mx)
Gilberto Vázquez (gfvazquezd@uaemex.mx)
Eneida Camarillo (escamarillo@uaemex.mx)
Gerardo Huitrón (gghuitronb@uaemex.mx)

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Author’s response to reviews: see over
Dear editorial board of BMC Public Health,

Please find enclosed revised version of the manuscript: “Association between serum uric acid levels and global cardiovascular risk among university workers from the State of Mexico”, by Patricia Cerecero, Bernardo Hernández Prado, Roxana Valdés, Gilberto Vázquez, Eneida Camarillo, and Gerardo Huitrón to be submitted as a Research Article to the BMC Public Health Journal for consideration of publication. We certify that the submission is original work and is not under review at any other publication. All co-authors have seen and agree with the contents of the new version of manuscript and have no commercial or other associations that might pose a conflict of interest in connection with the submitted article.

In this manuscript, we report the results of a nested case-control study in a Mexican population on the relationship between high levels of serum uric acid and global cardiovascular risk. The results from our study reveal that uric acid is positively associated with risk of developing cardiovascular disease in next ten years among people with a sedentary lifestyle. We believe that our findings could be of interest to the readers of BMC Public Health because even though the association between uric acid and risk of developing a cardiovascular disease has been observed among other ethnic groups, from results of this study it follows that uric acid can be considered as an early marker of the risk of developing an adverse cardiovascular event in Mexican population.

We have adopted all of the suggestions of the reviewers, including mention the possible pathophysiological mechanisms of interaction between physical activity and serum uric acid. We think that the manuscript has been greatly improved by these revisions and we hope that you will now find it suitable for publication in BMC Public Health. We detail the actions taken to attend the comments below.

**Major Compulsory Revisions**

1. The research question is not very clear, it is not clear if the authors were interested in causal relationship of SUA and CR (as mentioned in the background line 21) or they aimed to add SUA as another predictor to the previously built risk prediction models of cardiovascular disease or they aimed to show the association of SUA with CR? (Last line of background “uric acid constitute a risk marker of cardiovascular disease”). Based on the results of this study,
authors can suggest that SUA is a marker associated with CR but to conclude SUA is a risk marker for cardiovascular disease, observed events are required.

Response: In accordance with the comment of the reviewer, in last line of background section we have clarified that the purpose of the study were “…. to determine whether high levels of uric acid are a marker associated with 10-year CR in university workers from the State of Mexico.”

Also, in the background (line 21) we remove the lines that refer to the controversy on the causal relationship between serum uric acid and cardiovascular risk. On the other hand, we added a text that gives more emphasis to association of uric acid with atherosclerosis, endothelial dysfunction and lifestyle factors.

Current text: “A growing body of evidence has demonstrated that elevated SUA levels are associated with many of the risk factors for cardiovascular diseases (such as hypertension [11], obesity and hypertriglyceridemia [12]); with some of the factors that characterize atherosclerosis (such as inflammation, oxidative stress and endothelial dysfunction [8, 10]); and with lifestyle factors (i.e., physical inactivity, inadequate dietary habits and elevated alcohol intake [13, 14]).”

Former text removed: “….nonetheless, the causal relationship between uric acid and cardiovascular disease is controversial due to the tight relationship between uric acid and many of the risk factor for this illness, such as hypertension [11] obesity and hypertriglyceridemia [12].”

2. To define cases, authors used the 10-year CR using the equation from the method proposed by Wilson et al. However, it is not very clear how they scored participants and which equation they used? For example in the analysis section they mentioned that they calculate the cardiovascular risk using these items : “The CR factors were defined as follows: age, men > 40 years, women > 45 years; high cholesterol, > 200 mg/dl; low HDL-chol, men > 45 mg/dl, women > 50 mg/dl; diabetes, fasting serum glucose > 110 mg/dl and/or treatment for diabetes; and systolic/diastolic hypertension, men > 140/90 mmHg, women > 130/85 mmHg”. However, in table 4 they added smoking and BMI to the table, it is not clear if they added them to the equation to define cases or they just checked them in the table (if it is just for checking then there is a discrepancy with the cited article, since they have smoking in their equation). Besides, BMI is not in the equation proposed by Wilson et al., what was the reason for adding BMI (If it is added).
Response: Thanks to the reviewer for noticing, since by mistake was not included smoking as a cardiovascular risk component in the analysis section. The mistake was corrected adding “smoking status, yes or no” to variables used to calculate the cardiovascular risk (analysis section, line 9).

In regard to the second point, really the BMI is not used for the construction of the global cardiovascular risk score proposed by Wilson et al. Anyway, we had the information on BMI available in this study, and we wanted to adjust for it because it may be a potential confounder of the association between CR risk and uric acid concentrations. We agree that including it in Table 3, after the components of cardiovascular risk, leads to confusion. In order to avoid this confusion we have changed the order in which the variable appears in Table 3. The change consisted pass the variable BMI to the first row, following the order observed in Tables 1 and 2.

3. The authors concluded that “SUA levels are positively associated to high 10-year global CR”, it seems that they draw this conclusion from the multivariate adjusted odds ratio of 1.48 from table 4. But this association became non-significant after adjusting for BMI 1.28 (0.89, 1.83). What was the rational for adjustment for BMI in the second multivariate model?

Response: Considering that the BMI is a potential confounder of the relationship between uric acid and the risk of developing cardiovascular disease, we decided to adjust for its effect. Adjustment for BMI caused a decrease in the strength of the association and loss of statistical significance.

In the abstract, results section line 3 we added.... “in the crude analysis, but the association was non-significant when adjusting for other covariates,

In the discussion section, second line, we added “Results show a positive association between SUA and CR in the crude analysis, but no association was found when the analysis was adjusted by physical activity, alcoholic beverages consumption, family history of myocardial infarction and body mass index. Among physically inactive individuals, SUA concentrations were positively associated with high CR”, and on page 12 line 7, we added.....“High levels of SUA were significantly associated with global CR in crude analysis. This association persisted after controlling for potential confounders including physical activity, alcoholic beverages intake, and family history of myocardial infarction. Nevertheless, further adjustment for BMI caused a decrease in the strength of the association and loss of statistical significance”.
4. Authors could justify why they are specifically interested in “joint effect” of physical activity and SUA. In addition, it is better to check the statistical interaction between physical activity and SUA, and then make this conclusion “This association is stronger for individuals with high levels of physical inactivity.”

Response: To facilitate understanding we have referred to this point in the article as interaction more than joint effect of physical activity and SUA. The figure presented in our results was done through a model that includes an interaction term between categories of physical activity and serum uric acid levels. In order to show more clearly the trend of the data, we prefer display results as a figure. The data from which was done the figure are:

Odds ratios of cardiovascular risk by serum uric acid concentrations and physical activity levels in university workers of State of Mexico, 2004

<table>
<thead>
<tr>
<th>Factors</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiles of uric acid (UA) concentrations</td>
<td></td>
</tr>
<tr>
<td>Tertile 3 of UA (n = 319)</td>
<td>1.41 (0.60, 3.31)</td>
</tr>
<tr>
<td>Tertile 2 of UA (n = 320)</td>
<td>1.07 (0.60, 2.03)</td>
</tr>
<tr>
<td>Physical inactivity (n = 319)</td>
<td>1.66 (0.91, 3.00)</td>
</tr>
<tr>
<td>Insufficient physical activity (n = 318)</td>
<td>1.26 (0.64, 2.48)</td>
</tr>
<tr>
<td>Tertile 3 of UA Physical inactivity (n = 100)</td>
<td>2.35 (1.24, 4.45)</td>
</tr>
<tr>
<td>Tertile 2 of UA Physical Inactivity (n = 101)</td>
<td>1.78 (0.93, 3.42)</td>
</tr>
<tr>
<td>Tertile 1 of UA Physical inactivity (n = 143)</td>
<td>1.25 (0.68, 2.28)</td>
</tr>
<tr>
<td>Tertile 3 of UA Insufficient physical activity (n = 99)</td>
<td>1.57 (0.81, 3.05)</td>
</tr>
<tr>
<td>Tertile 2 of UA Insufficient physical activity (n = 104)</td>
<td>1.68 (0.88, 3.22)</td>
</tr>
<tr>
<td>Tertile 1 of UA Insufficient physical activity (n = 91)</td>
<td>1.26 (0.64, 2.48)</td>
</tr>
<tr>
<td>Tertile 3 of UA Recommended physical activity (n = 119)</td>
<td>1.35 (0.71, 2.56)</td>
</tr>
<tr>
<td>Tertile 2 of UA Recommended physical activity (n = 115)</td>
<td>1.07 (0.56, 2.03)</td>
</tr>
<tr>
<td>Tertile 1 of UA Recommended physical activity (n = 85)</td>
<td>1 (reference)</td>
</tr>
</tbody>
</table>

Interaction

In order to attend this comment, in analysis section page 8, line 4 we added “Furthermore, we evaluated the interaction of physical activity level and uric acid concentrations on CR by
introducing an interaction term in the logistic regression models. In the background section (page 4, line 2) we include references about the evidence of the association between uric acid and lifestyle factors, including physical activity “.....and with lifestyle factors (i.e., physical inactivity, inadequate dietary habits and elevated alcohol intake [13, 14]”.

Likewise, en la discussion section (page 13, line 4) we have enhanced the information about the possible pathophysiological mechanisms of interaction between physical activity and serum uric acid levels, as well as the possible pathophysiological mechanisms which link high serum uric acid levels with cardiovascular risk.

Current text: “On the other hand, our results suggest an interaction between uric acid levels and physical inactivity on the likelihood of developing cardiovascular disease in the next ten years. Physically inactive workers with high SUA levels were two fold more likely to have high CR in comparison with those with low SUA levels who reported carrying out moderate to vigorous activity. Concerning these relationships, previous studies reveal that physical activity is inversely related to both the cardiovascular morbidity [39] as the uric acid levels [40-42]. There is a remarkable similarity between the features associated with elevated levels of uric acid and those associated with physical inactivity. Endothelial dysfunction by decreased nitric oxide bioavailability (8, 43), inflammation [44], oxidative stress and insulin resistance are some of the features which they share.

It has been proposed that the relationship between SUA and physical activity is mediated by the latter’s effect on insulin sensitivity [40, 45]. Nonetheless, since elevated levels of uric acid decrease the bioavailability of nitric oxide, and insulin requires endothelial nitric oxide to stimulate glucose uptake in skeletal muscle [46], it has also been posited that elevated uric acid levels may have a causal role in the pathogenesis of insulin resistance [47]. Due to the characteristics associated with insulin resistance, this is probably of major importance in the global cardiovascular risk increase”.

Former text removed: “On the other hand, our results suggest that the association between uric acid and the presence of CR is higher among individuals with a sedentary lifestyle. Physically inactive workers with high SUA levels were more likely to have high CR in comparison with those with low SUA values who reported carrying out moderate to vigorous activity. Observational [37, 38] and experimental [39] studies report lower uric acid concentrations among individuals that carry out moderate physical activity regularly and suggest that the decrease of the values could be from 0.3 to 3.2 mg/100 ml [39]. It has been posited that the relationship between SUA and physical activity is mediated by the latter’s effect on insulin sensitivity. Apart from improving insulin sensitivity, physical activity reduces low-grade inflammation markers as well as adiposity [38, 40]”.
5. Last paragraph of result section is about ROC for SUA. Cases in this study were defined based on a model (there was no extra observation), therefore no strong conclusion can be draw from this analysis. In addition, there is no explanation about applying ROC curve in the analysis section, method.

Response: We agree with the reviewer's comment and recognize that we really have no strong evidence to reach a conclusion based on this analysis; we therefore decided to remove it from the last lines of the results section. In addition to changes in the results section, we have made corresponding adjustments to discussion removed the paragraph on page 11, line 10.

Text removed in results section: “....The final model displayed a ROC area under the curve of 0.56 and it correctly classified 58.6% of the observations. Sensitivity and specificity of the SUA levels as a diagnostic test for CR were 41.1% and 71.0%, respectively (data not shown)”.

Text removed in discussion section: “....In this study, SUA levels had low sensitivity (41.0%) and good specificity (71.0%) as a CR indicator. Given that the average probability (8.3%) of university workers of having an adverse cardiovascular event is located within the classification of latent CR (<10.0%) [15], it is likely that the low sensitivity observed reflects a low CR profile in the study sample”.

Minor Essential Revisions

1. It is informative to mention the number of participants in intermediate and high CR categories. In addition, please provide the number of participants in each tertile of uric acid (table 3).

Response: We added this information to both in Table 3, as the beginning of the results section: “The study sample was constituted by 957 university workers, 319 of which were CR cases (244 in intermediate CR, 75 in high CR)”

2. Analysis section line 8 “sistolic” should be replaced by systolic.

Response: Thanks to the reviewer for noticing, the mistake was corrected.
3. In result section paragraph one, it will be more consistent to report baseline characteristics with percentage rather than terms like “a little more than one third”, “seven out of ten”, ...

Response: In response to this suggestion we changed the drafting of paragraph as follows:

Current text: “Most workers were overweight or obese (72.0%) and declared consuming at least one alcoholic beverage a day (84.2); 36.0% reported being physically inactive. Out of the factors that constitute global CR, 75.6% of the sample displayed low HDL-chol values and nearly half displayed high cholesterol or hypertension (Table 1)”.

Former text removed: “Seven out of ten workers were overweight; a little more than one third (36.0%) reported being physically inactive and more than four fifths declared consuming at least one alcoholic beverage a day (84.2%). Out of the factors that constitute global CR, three quarters of the sample displayed low HDL-chol values and nearly half displayed high cholesterol or hypertension (Table 1)”.

4. Result section paragraph 5 line 10, “There was a linear trend in the association between these variables and uric acid levels” please make this sentence more clear.

Response: In order to improve clarity, we have included the variables for which odds ratios showed a linear trend in increasing tertiles of uric acid.

Current text (page 10, line 4): “There was a linear trend of the ORs for high CR, hypertension, high total cholesterol, low HDL-chol, and having three or more risk factors across increasing tertiles of uric acid (ρ < 0.05)”. 

Former text removed: “There was a linear trend in the association between these variables and uric acid levels (ρ < 0.05)”. 

Discretionary Revisions

1. In the population and sample section authors mentioned that they included 2,065 participants for the present analyses, but final number of participants is 957. I assume this difference is due to matching? (I suggest a flow diagram to explain how 957 participants were selected from the source population)
Response: Since the total number of cases classified with cardiovascular risk is introduced for the first time in article in the results section, as you mention it, is not known as the sample of 957 workers is obtained. To correct this inconvenience, the information is included at the end of the study design section on page 5. “In total 319 cases were classified (Figure 1)”, and as you suggest we included a flow chart (Figure 1).

2. Adding the components of CR score in table 2 would be informative.

Response: This suggestion was attended by adding the components of cardiovascular risk score in Table 2. As one might expect, there were statistically significant differences between groups for all risk factors, since through them were identified the cases in cardiovascular risk.

Sincerely yours,

D. Sc. Patricia Cerecero Aguirre

Corresponding author: D. Sc. Patricia Cerecero, Centro de Investigación en Ciencias Médicas.

Jesús Carranza 205, col. Universidad. Toluca, México. CP 50130. Email pcerecero@uaemex.mx