Author's response to reviews

Title: The effects of air pollution on vitamin D status in healthy women: A cross sectional study

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Author's response to reviews: see over
The effects of air pollution on vitamin D status in healthy women

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Editor-in-Chief, Dr. Melissa Norton

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Re: Revised manuscript submission MS # 1603266014394989 Version 1
Title “The effects of air pollution on vitamin D status in healthy women”

Dear Professor Melissa Norton

Thank you very much for your email message of July, 2010, about the outcome of the preliminary evaluation of our above-mentioned manuscript and the kind opportunity to revise and resubmit the paper. We have taken each critique and comment very seriously, and now submit a revised version in response to the reviewers’ comments.

As you have kindly instructed, we submit a point-by-point response to the reviewers’ comments and a revised paper with highlighted changes.

I hereby attest that I have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Thank you for your consideration.

Sincerely yours

_____________________
Farhad Hosseinpanah, MD
Reviewer #1:
In this paper authors have looked at the effect of air pollution on Vitamin D status of healthy women. Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

Comment: 1
Please explain the term “housekeeping women”, does it mean women who were housewives?

Action Taken: 1
Agreed and corrected. Thanks for your appropriate comment.

Comment: 2
What was the reference period for the FFQ? The validation should be briefly mentioned.

Action Taken:2
Agreed and corrected. In response to the valid point raised by the referee, we explained these parameters in the revised manuscript.

Comment: 3
The aim of the study is “To determine whether air pollution and low ground level of ultra-violet B light (UVB; 290-315) can deteriorate the body vitamin D status in healthy women”. However, authors have not measured air pollution and have used the ground level of UVB as a measure of pollution.

Action Taken:3
As we mentioned in our limitations, due to problems related to our equipment, it was impossible for us to determine the main chemical particles and the amount of air pollution in Tehran and Ghazvin. But regarding to previous researches that emphasize the relation between ground level of UVB and the degree of air pollution, we suppose that the lower ground levels of UVB in more polluted area are mainly due to atmospherics’ pollutant aerosols but some other parameters may also contribute in it.


Comment: 4
Authors should provide a reference for the fact that the ground level of UVB may be used as a function of air pollution. It would have been more appropriate if with the UVB, air pollution had been measured, or a reference should be provided stating that the mentioned cities were polluted or otherwise .

Action Taken:3
As it is discussed for the previous question, due to problems related to our equipment, it was impossible for us to determine the main chemical particles and the amount of air pollution in Tehran and Ghazvin. Previous researches
state the relation between ground level of UVB and the degree of air pollution (1, 2, 3). We supposed that the lower ground levels of UVB in more polluted area are mainly due to atmospherics’ pollutant aerosols but some other parameters may also contribute in it. In addition, previous study that reported the association between air pollution and vitamin D status used ground level of UVB ad a surrogate of air pollution (4).


Tehran’s air pollution is a major environmental crisis. Growing vehicle usages besides an increasing in people and economic activities have contributed to high levels of air pollution in Tehran. The main chemical compositions of Tehran air pollutions are particulate matter, nitrogen dioxide, ozone, sulfur dioxide, and carbon monoxide, suspended particulate matter, polycyclic aromatic hydrocarbons and heavy metals (1, 2, 3, 4, 5).

The second polluted cities in Iran are: 1. Ghazvin 2. Semnan 3. Karaj … (6)

1. The deterioration of urban environments in developing countries: Mitigating the air pollution crisis in Tehran, Iran, Volume 24, Issue 6, December 2007, Pages 399-409


**Comment: 5**

In methods, women’s clothing has not been described anywhere.

**Action Taken:5**

There is no significant variation in Iranian women clothing styles. This is mainly due to the governmental and Islamic rules.

**Comment: 6**
Authors mentioned that “nutritional data precisely collected, were analyzed by nutritionists....” How were the intakes of Ca, P, Vit D etc calculated? What was the reference?

**Action Taken: 6**

Agreed and corrected. In response to the valid point raised by the referee, we explained these parameters in the revised manuscript.

**Comment: 7**

Was the questionnaire for sunshine exposure validated? Details need to be provided for how sun exposure was assessed. How was the reference period decided? It is usually given as hours or minutes per day or week. Also the time of the day and type of clothing (Percentage skin exposed) are vital. How was the sun exposure index calculated?

**Action Taken: 7**

This is a valid comment but considering the facts that Iranian women clothing styles have no significant variations and all of the women in this study were housewives, we did not calculate sun exposure index. We defined simple questions for housing status (i.e. apartment or villa), time spent outdoor whether over or less than 3 days in a week, and the usage pattern of sunscreen (i.e. always or sometimes or never) instead of SEI we added a point in this regard in discussion.

**Comment: 8**

From table 1, authors have shown that 25 OH-D levels were significantly higher in women from Ghazvin than from Tehran. However, both levels are still very low and though there is a statistically significant difference between 11 and 15.5, this difference may not be significant clinically.

**Action Taken: 8**

It is worthwhile to mention that 4-5 point increase in serum vitamin D has significant effects on BMD (1). In young Caucasians, BMD continued to rise with increasing 25(OH)D beyond the upper end of the reference range (1). In addition, in the binary logistic regression, the odds ratio of living in Tehran and having serum 25-OH-D less than 20 ng/ml was 5.22 (95% confidence interval 2.2-12.2, P-value < 0.001). This odds ratio and confidence interval is really valuable. FIG 1 shows that the prevalence of vitamin D deficiency and insufficiency were higher in Tehranians (36%, 54% vs. 31%, 32%).


**Comment: 9**

Table no. 3, is not very relevant and the correlation of 25 OH-D has only been shown with age. Very few of the co-relations are significant and may thus be mentioned in the text.

**Action Taken: 9**

Agreed and corrected. This table is removed from the revised manuscript.

**Comment: 10**
It is surprising that 25 OHD levels did not show any association with housing, time in the sun or sunscreen.

**Action Taken:10**

We tried to determine the probable correlation of vitamin D and housing, time in the sun or sunscreen. But in our analyses there was no significant correlation between vitamin D status and these factors. It is mainly due to Islamic culture and dressing styles. In addition, all of the women in this study were housewife and the total percent of sunscreen usage was low. It means that low variation of above mentioned variables among participants probably can dilute possible associations. However it is important to note that, using more sophisticated instrument such as SEI could strength our analysis.

**Comment: 11**

The sun shine exposure in women from Ghazvin was higher than for the women in Tehran, though the difference is not statistically significant. It is likely that this may also be a contributing factor to higher 25 OH-D levels.

**Action Taken:11**

In ANCOVA models, we considered all of the probable factors and adjusted them. The adjusted mean difference of 25-OH-D in Ghazvin and Tehranian participations was 4 ng/ml (13 vs. 17 ng/ml, P-value = 0.04). This means that, after excluding all the other contributing factors, mean of 25-OH-D in Ghazvin was 4 ng/ml higher than Tehranian that was statistically significant.

**Comment: 12**

Results on page 12, the authors mention that “The age and BMI of the two groups were statistically different. But their values were clinically comparable.” What does this mean?

**Action Taken:12**

This means that these differences in this range have no biological and clinical importance.

**Comment: 13**

In conclusions, the authors mention that the place of living as a surrogate of air pollution has a significant influence on vitamin D status. Since air pollution was not measured and the sun light exposure has not been very accurately captured, the conclusions are not well supported by the data.

**Action Taken:13**

As we mentioned as a limitation, similar to other studies, we used ground levels of UVB as function of air pollution. Moreover Tehran and Ghazvin are totally different in terms of air pollution. However, it would be better to quantify exact values of air pollution along with ground level of UVB.
There is an increase in solar UVB doses from a difference in surface elevation. The amount seems to be between 20% per 1000 m at the elevation of the two cities. The elevation difference between the two cities is 640 m, implying a difference of 12%. This should translate directly to differences in serum 25(OH)D levels. It would indirectly affect the fraction of the populations below specified 25(OH)D levels. Changes in solar UVB doses with change in surface elevation should be factored into the analysis.

From Table 2: 25-OH-D (ng/ml) 11 (8-14) vs. 15.5 (8.5-26)
The ratio of the two locations is 1.41 plus uncertainty 12/41 = 0.29, so 30% of the difference has to be attributed to difference in solar UVB due to elevation difference.

However, ground level of UVB was significantly higher in Ghazvin as compared with Tehran (mean (SE), 0.31(0.07) and 0.16(0.03) W/m² respectively, P-value = 0.003).

Thank you so much for your nice comment, but we can conclude that at least 70% percent of serum vitamin D difference is due to air pollution.

Please discuss the role of housing type, hours spent out of doors, and sunscreen use affect the results. From Table 1, it appears as if these factors have significant impacts on serum 25(OH)D levels.

Considering the facts that Iranian women clothing styles have no significant variations, mainly due to the governmental and Islamic rules and all of the women in this study were housewives, we defined simple questions for housing status (i.e. apartment or villa), time spent outdoor whether over or less than 3 days in a week, and the usage pattern of sunscreen (i.e. always or sometimes or never) and analyzed them. We tried to determine the probable correlations of vitamin D and housing, time in the sun or sunscreen. But there was no significant correlation between vitamin D status and these factors. As it is shown in table 1, the sun exposure indices were higher in Ghazvinian participations. But, in ANCOVA models, the adjusted mean difference of 25-OH-D in Ghazvin and Tehranian participations was 4 ng/ml (13 vs. 17 ng/ml, P-value = 0.04). This means that, after excluding all the other contributing factors, mean of 25-OH-D in Ghazvin was 4 ng/ml higher than Tehranian that was statistically significant. In addition, in the binary logistic regression, the odds ratio of living in Tehran and having serum 25-OH-D less than 20 ng/ml was 5.22 (95% confidence interval 2.2-12.2, P-value < 0.001). This odds ratio and confidence interval is really valuable. FIG 1 shows that the prevalence of vitamin D deficiency and insufficiency were higher in Tehranians (36%, 54% vs. 31%, 32%).

Why aren’t serum 25(OH)D levels in the same ratio? What are other sources of vitamin D?
Skin synthesis of vitamin D, under the influence of UVB, includes about 90% of all the body’s requisites. Meanwhile, dietary sources of vitamin D (e.g., fish liver oils, egg yolks, and vitamin D fortified foods) are only responsible for a small portion of body requirements.

**Comment: 4**

Effect of clothing?

**Action Taken:4**

There is no significant variation in Iranian women clothing styles. This is mainly due to the governmental and Islamic rules.

**Comment: 5**

What is known about air pollution in Tehran – types, loads, etc.?

**Action Taken:5**

Tehran’s air pollution is a major environmental crisis. Growing vehicle usages besides an increasing in people and economic activities have contributed to high levels of air pollution in Tehran. The main chemical compositions of Tehran air pollutions are particulate matter, nitrogen dioxide, ozone, sulfur dioxide, and carbon monoxide, suspended particulate matter, polycyclic aromatic hydrocarbons and heavy metals.

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1. The deterioration of urban environments in developing countries: Mitigating the air pollution crisis in Tehran, Iran, Volume 24, Issue 6, December 2007, Pages 399-409

**Comment: 6**

Conclusion: air pollution may play a role in differences in serum 25(OH)D levels, but the effects of other factors have not been carefully evaluated to determine their contributions.
In ANCOVA models, we considered all of the probable factors and adjusted them. The adjusted mean difference of 25-OH-D in Ghazvin and Tehranian participations was 4 ng/ml (13 vs. 17 ng/ml, P-value = 0.04). This means that, after excluding all the other probable contributing factors, mean of 25-OH-D in Ghazvin was 4 ng/ml higher than Tehranian that was statistically significant. In addition, in the binary logistic regression, the odds ratio of living in Tehran and having serum 25-OH-D less than 20 ng/ml was 5.22 (95% confidence interval 2.2-12.2, P-value < 0.001). This odds ratio and confidence interval is really valuable. FIG 1 shows that the prevalence of vitamin D deficiency and insufficiency were higher in Tehranians (36%, 54% vs. 31%, 32%). It is a valid comment that we did not consider elevation difference between these 2 places. But regarding to your really very nice calculations, the effects of other factors are more prominent.