Author's response to reviews

Title: Serum 25-hydroxyvitamin D levels Are Associated with Carotid Atherosclerosis in Normotensive and Euglycemic Chinese Postmenopausal women: The Shanghai Changfeng Study

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Author's response to reviews: see over
Response letter

Dear editors,

We really appreciate the reviewers’ careful and thoughtful suggestions to our previous draft entitled “Serum 25-hydroxyvitamin D levels Is Associated with Carotid Atherosclerosis in Normotensive and Euglycemic Chinese Postmenopausal women: The Shanghai Changfeng Study” (MS: 1509363424137354). We have carefully taken their comments into consideration in preparing our revision, which has resulted in a paper that is clearer and more compelling.

The followings are our point-by-point response to reviewers’ comments. Changes in the text are made accordingly (highlighted in red in the text).

Reviewer(s)’ Comments to Author:

Reviewer: 1

Comments to the Author

1) Major Compulsory Revisions

(1) A comparison with the current literature on the topic is missing.

This study builds on multiple previous studies examining the association of 25-OH-D with carotid IMT or plaque, which have been mostly null. Two recent examples, along several other, include Deleskog A (ATVB 2013) and Blondon M (ATVB 2013). The manuscript would be much improved by adding a thorough discussion of differences between the presented positive findings and the overall null results from the literature.

Response: Thanks for the valuable suggestions. Previous studies have indicated that there is debatable for an independent association between serum 25-hydroxyvitamin D (25(OH)D) and carotid atherosclerosis. As we know, the 25(OH)D is associated with hyperglycemia and hypertension that may themselves be linked with atherosclerosis. However, recent studies enrolled study populations with different proportions of hypertension and/or abnormal glucose metabolism. In contrast, our study was conducted...
in a population with normal glucose tolerance and without hypertension. We found that the 25(OH)D was independently associated with carotid atherosclerosis after full adjustment for traditional cardiovascular diseases (CVD) risk factors. The studies of Deleskog A (ATVB 2013)[1] and Blondon M (ATVB 2013)[2] are very important. We add the discussion of differences between our positive findings and the null results from the literatures in line 262-283.

(2) Low levels of 25(OH)D

About 75% of subjects in this sample have 25(OH)D levels below 50nmol/L, which has been defined by many, including the Institute of Medicine, as deficient levels (mostly based on Caucasian populations, though). This is even more surprising as the studied sample appears very healthy and non-obese. Further I anticipated a stronger association between 25(OH)D levels and BMI. Authors should make a convincing point in explaining these somewhat unexpected data.

Response: Thanks for the valuable suggestions. Actually, 69.9% of subjects have 25(OH)D levels below 50nmol/L. Similarly, another study in China evaluated vitamin D status of healthy adults living in Guiyang (latitude 26.5° north). The study showed that the average serum 25(OH)D level of 20.4 ng/mL(51nmol/L) and serum 25(OH)D was below 50 nmol/L in 52.3% [3]. Lu L et al [4] measured plasma 25(OH)D was in a cross-sectional sample of 1,443 men and 1,819 women aged 50-70 years from Beijing (latitude 40° north) and Shanghai (latitude 31° north). The median value of plasma 25(OH)D was 35.6 nmol/l in Beijing and 47.6 nmol/l in Shanghai, and the percentages of vitamin D deficiency, insufficiency, and sufficiency were 69.2, 24.4, and 6.4%, respectively. Indeed, poor vitamin D status in middle and older Chinese individuals was also reported previously in two small bone related studies conducted in Beijing [5] and Shenyang [6]. The above data suggested that vitamin D deficiency was common in middle-aged and elderly Chinese individuals. Another explanation is the exclusion criteria of the intake of the vitamin D supplements in our study. Additionally, unlike in the United States and other western
countries, a racial/ethnic difference may be exist in the levels of 25(OH)D concentrations. Although little is known regarding to the high prevalence of vitamin D deficiency in our population, the criteria of vitamin D deficiency in Chinese may differ from that in the western population.

Obesity is known to be associated with decreased bioavailability of vitamin D, which is sequestered in body fat [7]. Currently, the relationship of body fat index with 25(OH)D levels remains controversial [8]. Most of the reports showed that the 25(OH)D concentration was negatively correlated with BMI and total body fat [8,9]. In the current study, the subjects were selected to help minimize the effects of potential confounding factors (as detailed above); for example, each subject had NGT and normal blood pressure, resided in a single geographic region, was of a single race. The results indicated that serum 25(OH)D levels were negatively correlated with BMI (22.8±2.9 kg/m\(^2\)); the finding is consistent with another study in Shanghai [10]. In the study serum 25(OH)D3 levels were measured in 567 men (BMI=23.8±2.8 kg/m\(^2\)) with normal glucose tolerance by electrochemiluminescence immunoassay and 25(OH)D3 levels showed a significantly inverse correlation with BMI [10]. The BMI in our study ranged from 16.1 to 38.8 kg/m\(^2\), 33 subjects (4.9%) had a BMI ≥28 kg/m\(^2\), 166 subjects (24.7%) had a BMI between 24 kg/m\(^2\) and 27.9 kg/m\(^2\). We mainly excluded the confounding factors of hypertension and hyperglycemia, so the subjects included a small proportion of obesity. Therefore, our data still found an inversely relationship between 25(OH)D and BMI. It is worth noting that we used cross-sectional data, and thus, our finding together with existing evidence call attention to the need for longitudinal studies to examine this association. To address the issue more clearly, we take the advice and make the revisions in line 284-299 and line 300-312 accordingly.

(3) Statistical handling / models

A few points need to be enhanced or revised:

- No information is available about possible missing data.
Response: Thanks for the valuable suggestions. In this article, we have already excluded the subjects who were lack of physical examination and laboratory assessments (n=51) in line 131-132. Compared with the 51 subjects, participants in our study had similar blood glucose, blood pressure, BMI and lipid profiles. Measurements of CIMT and 25(OH)D were similar in these 2 groups.

- If authors want to use analysis of covariance, some assumptions of this method, in particular homoskedasticity, should be tested. I’d personally advise using linear regression with robust standard errors instead of analysis of covariance for alleviation of such assumptions and to come up with more meaningful results (differences in CIMT per one unit increase in 25(OH)D or doubling of 25(OH)D [if log2-transformed]).

Response: Thank you for the valuable suggestion. We want to apologize for our carelessness. In fact, we use a general linear model/univariate analyses in SPSS, in which the outcome is the results of comparison among quartiles in table 3. In the analyses, we complete the homogeneity tests. We test the null hypothesis that the error variance of the dependent variable is equal across groups and get the result that P=0.366. To address the issue more clearly, we take the advice and make the revisions in line 198-200 accordingly. According to reviewer’s helpful suggestion, we reanalyze the data using linear regression with robust standard errors. The results showed that a 1-unit increase in the log25(OH)D corresponded to a standardized beta of -0.009 (p=0.053) of CIMT value after adjusting for CVD risk factors. There was a trend of inverse relationship between 25(OH)D and CIMT. We also found that postmenopausal women with 25(OH)D level in the fourth quartile had lower CIMT than those in the first, second and third quartiles and the subjects with 25(OH)D in the fourth quartile had a 0.421-fold decreased risk of carotid plaques relative to those in the lowest quartile even after adjusting for CVD risk factors. Sincerely thank you for your useful suggestions.
• The potential for residual confounding remains, regardless of the restriction to non-hypertensive and non-diabetic women. Why did the authors not consider socio-economic status and physical activity as possible confounding variables? The rationale to include liver enzymes levels in covariates appears low, on the other hand.

Response: Thank you for the valuable suggestion. Indeed, we should consider socio-economic status and physical activity as possible confounding variables. In terms of physical activity, our middle-aged and elderly female adults usually do housework at home. Therefore, it is quite difficult to estimate the physical activity in this population. The Changfeng community is a middle-class community in Shanghai [11] and the socio-economic status was similar among the residents living in this community. So we didn’t select the socio-economic status as the independent variables in the article.

The effect of the liver disease on the 25-hydroxylation of pre-vitamin D in the liver is not known. An article reported the association of elevated ALT with vitamin D deficiency that suggested the hepatic dysfunction could impair vitamin D metabolism in youth with T2DM [12]. Considering the possibility that liver enzymes levels may be associated with vitD levels, we added it as the covariates in the analyses.

(4) Lack of basic description of cIMT and plaques

It would really help to have complete descriptions of mean cIMT, including some measures of its dispersion, and prevalences of plaques, including their numbers per individual. Do the authors have any data on plaque surface?

Response: This suggestion is quite valuable. We take the advice and make the revisions in line 207-208. The mean value of C1MT was 0.703±0.123mm and the prevalence of carotid plaques was 15.4%. Our study procedure involved scanning the near and far walls of both common carotid arteries, the carotid bifurcation, and the internal carotid artery for the presence of plaques. If the carotid plaque exists in any segment, the plaque is considered as positive in the article. However, the study procedure did not include
recording the plaque numbers per individual. In addition, we do not have data on plaque surface limited to the large sample and finite time. Taking the carotid plaque as the marker of atherosclerosis into account, we would discuss the possibility to enroll recording the plaque numbers per individual in our follow-up procedure. Thank you for your valuable suggestion.

(5) Abstract

Please report results from the main analysis. Readers will not be interested about association with 25(OH)D here, but will be interested about inference from the main analysis (25(OH)D with cIMT and with plaque).

Response: Thank you for the valuable suggestion. We take the advice and make the revisions accordingly in the abstract.

Discretionary Revisions

Line 184 – what does the ICC of 95% relate to (plaque, pathological IMT, other)? Given the difficulty in measuring ICC, more data on reliability of the measures of this study would be valuable.

Response: Thank you for the valuable suggestion. In the study procedure, a single examiner took all measurements. Repeated measurements on the same subjects (performed in 104 subjects) for CIMT and carotid plaque yielded an intraclass correlation coefficient (ICC) of 95% (95% confidence interval, 0.91 to 0.97) and 96% (95% confidence interval, 0.92 to 0.97), respectively. We take the advice and make the revisions in line 182-185 accordingly.

The 25(OH)D assay is not the gold-standard (mass spectrometry). Do the authors believe this may have influenced their results?
Response: Thank you for the valuable suggestion. Current assays can generally be divided into either immunoassay-based methods or chromatographic approaches with either UV absorbance or mass spectrometric (MS) detection [13]. The serum 25(OH)D was measured by electrochemiluminescence immunoassay using an immunoassay analyzer (Roche Cobas-6001, Switzerland; coefficient of variation <4.0%) in our study. Recently, the population studies reported by YQ Bao [10] and Nguyen HT [14] published their researches using electrochemiluminescence immunoassay to measure 25(OH)D3 levels. The 25(OH)D concentration was assayed with the immunoassay-based methods in many other studies [1,4]. Although the electrochemiluminescence immunoassay is not as precise as the mass spectrometry, the results obtained by this way were admitted widely and could be comparable with other studies.

The significance of IMT measured in the CCA remains to be clarified. Although many use it as a marker of atherosclerosis, it may be more related to hypertensive remodeling. I have less worries about plaques. Overall, I’d advise to at least use « markers of atherosclerosis » more than « atherosclerosis » throughout the manuscript.

Response: This suggestion is quite valuable. CIMT may be affected both by atherosclerosis and wall hypertrophy, however, carotid plaque may be more representative of atherosclerosis than CIMT and more informative for predicting cardiovascular risk. We take the advice and use « markers of atherosclerosis » instead of « atherosclerosis » throughout the manuscript.

Minor Essential Revisions

Title – « are » instead of « is »

Line 101 - Reference [7] appears to be wrong.

Line 222 – « fourth » instead of « forth »
Response: Thank you for the valuable suggestion. We take the advice and make the revisions accordingly.

References


Reviewer: 2

Comments to the Author

The manuscript presents quite a nice piece of work, however some important issues have to be clarified and some informations are missing. The study was performed in quite a high number of apparently healthy postmenopausal nonobese women however, what is striking, with a very low 25(OH)D concentrations. It might be specific for the Chinese women which is not surprising having in mind a high worldwide prevalence of vitamin D insufficiency or deficiency. Mean 25(OH)D concentration in the whole group was very low, in the first to third quartile in the range of severe deficiency 12.4-30.7 nmol/L or deficiency 40.6-53.0 nmol/L. Only these in the fourth quartile were in the range of insufficiency or optimal level (>75 nmol/L). Despite of this, CIMT in all quartiles was well within a normal range, meaning below 0.9 mm. So the authors describe the situation in women with more or less normal lipidemia where CIMT is normal.

The associations of 25(OH)D with CIMT are interesting however, it would be even more interesting to show whether there are differences in CIMT between women with optimal D concentration >75 nmol/L and these with insufficiency 50-75 nmol/L and deficiency. These are the important data which are missing in this paper.

Response: Thanks for the valuable suggestions. The mean value of 25(OH)D was 43.6±9.2nmol/L. Actually, 69.9% of subjects have 25(OH)D levels below 50nmol/L. Similarly, another study in China evaluated vitamin D status of healthy adults living in Guiyang (latitude 26.5° north). The study showed that the average serum 25(OH)D level of 20.4 ng/mL(51nmol/L) and serum 25(OH)D was below 50 nmol/L in 52.3% [1]. Lu L et al [2] measured plasma 25(OH)D was in a cross-sectional sample of 1,443 men and 1,819 women aged 50-70 years from Beijing (latitude 40° north) and Shanghai (latitude 31° north). The median value of plasma 25(OH)D was 35.6 nmol/l in Beijing and 47.6 nmol/l in Shanghai, and the percentages of vitamin D deficiency, insufficiency, and sufficiency were 69.2, 24.4, and 6.4%, respectively. Indeed, poor vitamin D status in middle and older Chinese individuals was also reported previously in two small bone related studies conducted in Beijing [3] and Shenyang [4]. The above data suggested that vitamin D
deficiency was common in middle-aged and elderly Chinese individuals. Another explanation is the exclusion criteria of the intake of the vitamin D supplements in our study. Additionally, unlike in the United States and other western countries, a racial/ethnic difference may be exist in the levels of 25(OH)D concentrations. Although little is known regarding to the high prevalence of vitamin D deficiency in our population, the criteria of vitamin D deficiency in Chinese may differ from that in the western population. A change in diagnostic criteria has important public health implications. The identification of individuals with abnormal 25(OH)D is important because early interventions could be adopted. We still require larger Chinese prospective studies to determine whether 25(OH)D cutoff point is appropriate. We take the advice and make the revisions accordingly.

The mean value of CIMT was 0.703±0.123mm and the prevalence of carotid plaques was 15.4% in this apparently healthy postmenopausal nonobese women. Total cholesterol (TC) is 5.3±0.8mmol/L, low-density lipoprotein cholesterol (LDL-C) is 3.0±0.7mmol/L, high-density lipoprotein cholesterol (HDL-C) is 1.6±0.4mmol/L and triglyceride (TG) is 1.4±0.7mmol/L. The above risk factors for carotid atherosclerosis including blood pressure, lipid profile and blood glucose are pretty good. This could explain that the CIMT in all quartiles was well within a normal range, meaning below 0.9 mm.

It would be more meaningful to compare the differences in CIMT between women with optimal 25(OH)D concentration and these with insufficiency and deficiency. The nutritional status of vitamin D was divided into 3 groups based on 25(OH)D levels [5]: vitamin D deficiency (25(OH)D<50.0nmol/L), vitamin D insufficiency (50.0nmol/L ≤ 25(OH)D<75.0 nmol/L) and vitamin D sufficiency (25(OH)D ≥ 75.0nmol/L). As for our data, 469(69.9%) of subjects have 25(OH)D levels below 50nmol/L. 160(16.6%) subjects have vitamin D insufficiency. Only 42(6.3%) subjects have vitamin D sufficiency. After adjusting for conventional CVD risk factors, liver enzymes and the GFR, the subjects with 25(OH)D in the sufficiency and insufficiency groups had significantly thinner CIMTs compared with the subjects in the vitamin D deficiency group (0.686±0.018mm and 0.691±0.009mm vs 0.714±0.005mm), and the subjects in the sufficiency group had
0.579-fold decreased risks for carotid plaques relative to those in the deficiency group. However, the vitamin D sufficiency group may be too small to provide more compelling data. Additionally, considering that the criteria of vitamin D sufficiency in Chinese may differ from that in the western population, we still use 25(OH)D quartiles in statistical analyses. If the reviewer thinks it as meaningful, we will add it into the manuscript. Thank you again for your comments.

References


Editorial comments and request:

All reviewers’ comments. In particular the authors should improve the Discussion by including recent studies evaluating relationships between VitD and carotid atherosclerosis, as suggested by one of the reviewers (M Blondon).
We recommend that you copyedit the paper to improve the style of written English.

Response: Thank you for the valuable suggestions. We have improved the discussion by including recent studies. A professional in English writing had completely revised the article.

Editorial request:

(1) Please place your "Abstract" section after the Title Page.
(2) Competing interests, Authors? contributions and Acknowledgments section should be re-arrange as follows:
- Competing interests
- Author details
- Authors? contributions
- Acknowledgments
(3) Figure File and Figure Legends
Please remove the duplicate figure file from the main manuscript. Figure Legends should be after references list.

Response: We have made the revisions accordingly.