PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Is there an association between vitamin D status and risk of chronic low back pain? A nested case-control analysis in the Nord-Trøndelag Health Study
AUTHORS	Heuch, Ingrid; Heuch, Ivar; Hagen, Knut; Mai, Xiao-Mei; Langhammer, Arnulf; Zwart, John-Anker

VERSION 1 – REVIEW

REVIEWER	BABITA GHAI POST GRADUATE INSTITUTE OF MEDICAL EDUCATION AND RESEARCH (PGIMER), CHANDIGARH, INDIA
REVIEW RETURNED	26-Jul-2017

GENERAL COMMENTS	The study explores an interesting potential association/no assocaition, using a nested case controlled analysis. The authors may want to look at the following issues related to the manuscript Comments for authors 1. The study indicates that the collection of the blood samples were done at the time of HUNT2 data collection (1995-97) and the LBP assessment was done at least lasting 3 months with in the past year prior to follow up in HUNT 3 (2006-8), this strongly conflict the confounding effect of the 10 years of age added during follow-up and vitamin d measurement. This probably nullifies the hypothesis mentioned by the authors. The changes in weight, intake of vitamin D, and physical activity have been reported to be related to change in in serum 25(OH)D levels. 2. The HUNT 3 study omit the LBP cases developed during the time period of HUNT 2 study to one year prior to the follow up during HUNT 3 study. This is one of the major limitations of this study and needs to be addressed. 3. It would be better to present the flow of events of participants in a flow diagram for better depiction.

REVIEWER	William B. Grant Sunlight, Nutrition and Health Research Center United States I receive research funding from Bio-Tech Pharmacal, Inc.
REVIEW RETURNED	01-Aug-2017

GENERAL COMMENTS	p. 4, line 48: case-control studies conducted with blood draw at time of onset of LBP could also be used to determine the effect of 25OHD on risk of LBP. In fact, that would probably be a better way (see discussion on lag in the following). The discussion of the participants does not make clear the timeline between blood draw for 25OHD determination and when LBP may have occurred. The interval between blood draw and health event is very important since 25OHD changes with season as noted, as well as with time such as due to lifestyle change, taking vitamin D supplements, etc. See these two papers: Grant WB. Effect of interval between serum draw and follow-up period on relative risk of cancer incidence with respect to 25-hydroxyvitamin D level; implications for meta-analyses and setting vitamin D guidelines. Dermatoendocrinol. 2011;3(3):199-204. Grant WB. Effect of follow-up time on the relation between prediagnostic serum 25-hydroxyitamin D and all-cause mortality rate. Dermatoendocrinol. 2012;4(2):198-202. Also consider analyzing results with respect to follow-up time. It may be the case that the HUNT Study data did not find a correlation between vitamin D status and LBP due to long intervals between blood draw and diagnosis of LBP. p. 6, line 18 - is it case-control or nested case-control? Lines 32-35. The city for DiaSorin should be given. Also, the measurement precision and accuracy. Also, it would be worthwhile to discuss the conversion factor used. See, also, Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattni P, Sempos CT, Twomey P, Williams EL, Wise SA. HYDROXYVITAMIN D ASSAYS: AN HISTORICAL PERSPECTIVE FROM DEQAS. J Steroid Biochem Mol Biol. 2017 Jul 19. pii: S0960-0760(17)30183-8. doi: 10.1016/j.jsbmb.2017.07.018. [Epub ahead of print] Durazo-Arvizu R, Tian L, Brooks S, Sarafin K, Cashman K, Kiely M, Merkel J, Myers G, Coates P, Sempos C. The Vitamin D Standardization Program (VDSP) Manual for Retrospective Laboratory Standardization of Serum 25-Hydroxyvitamin D Data. J AOAC Int. 2017 Jul 18. doi: 10.5740/jaoacint.17-0196. [Epub ahead of print] Table 4, What is known about the difference in vitamin D supplementation between men and women in winter? p. 13, line 40, there are very few 25OHD U-shaped relationships that may not be explained by a difference in vitamin D supplementation history: Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, Garland CF, Holick MF. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D–health outcome relationships reflect adverse effects? Dermato-Endocrinology, 2016;8(1):

	e1187349. As stated in the abstract for Ref. 34: LIMITATIONS: A causal relationship between either low or high 25(OH)D levels and increased mortality can not necessarily be inferred from this observational study.

REVIEWER	Howard Amital Sheba Medical Center, Israel No Competing Interest
REVIEW RETURNED	08-Aug-2017

GENERAL COMMENTS	This is an interesting and properly designed paper assessing the association between serum vitamin D levels and the odds developing low back pain. A few minor issues It is not clear whether the definition of LBP relied on self-report or by proactive physician periodical assessment. This issue should be clarified. The discussion lacks data regarding the manner by which vitamin D supplementation may affect chronic pain such as low back pain. There are several publications dealing with this issue and this should also be presented to the readership.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 (Babita Ghai)

Reviewer's remark:

The study explores an interesting potential association/no assocaition, using a nested case controlled analysis.
The authors may want to look at the following issues related to the manuscript

Comments for authors
1. The study indicates that the collection of the blood samples were done at the time of HUNT2 data collection (1995-97) and the LBP assessment was done at least lasting 3 months with in the past year prior to follow up in HUNT 3 (2006-8), this strongly conflict the confounding effect of the 10 years of age added during follow-up and vitamin d measurement. This probably nullifies the hypothesis mentioned by the authors. The changes in weight, intake of vitamin D, and physical activity have been reported to be related to change in in serum 25(OH)D levels.

Our response:

We recognize that there is a potential problem with the long period between collection of blood samples and LBP assessment. As indicated in our response to the associate editor, these aspects are now dealt with more comprehensively in the fourth paragraph of the discussion. In that revised

paragraph we also refer more clearly to the problem of confounders changing their values during follow-up.

Reviewer's remark:

2. The HUNT 3 study omit the LBP cases developed during the time period of HUNT 2 study to one year prior to the follow up during HUNT 3 study. This is one of the major limitations of this study and needs to be addressed.

Our response:

The fact that LBP cases which occurred in the intervening period were not recorded, was noticed as a limitation of this study already in the second paragraph of the discussion in the original version of this paper. However, we agree with the reviewer that this is a major issue, and for this reason we have now expanded this part of the discussion in the third paragraph of the revised manuscript.

Reviewer's remark:

3. It would be better to present the flow of events of participants in a flow diagram for better depiction.

Our response:

As indicated above in our response to the associate editor, a flow chart has now been included.

Reviewer 2 (William B. Grant)

Reviewer's remark:

p. 4, line 48: case-control studies conducted with blood draw at time of onset of LBP could also be used to determine the effect of 25OHD on risk of LBP. In fact, that would probably be a better way (see discussion on lag in the following).

Our response:

We certainly agree that a study carried out with such a case-control design would also be informative. It would be a purely cross-sectional study, however, so any association observed could be caused by effects of back pain on vitamin D levels (not the other way around, as we are studying). In our situation, the number of individuals that could be included with measurements of vitamin D levels was limited, and because of the resources available we made the decision at the outset of our work to concentrate on a case-control study nested in a prospective design. In the discussion in the revised version of our manuscript we have now included a short section at the end of the fourth paragraph dealing with these design issues.

Reviewer's remark:

The discussion of the participants does not make clear the timeline between blood draw for 25OHD determination and when LBP may have occurred. The interval between blood draw and health event is very important since 25OHD changes with season as noted, as well as with time such as due to lifestyle change, taking vitamin D supplements, etc. See these two papers:

Grant WB. Effect of interval between serum draw and follow-up period on relative risk of cancer incidence with respect to 25-hydroxyvitamin D level; implications for meta-analyses and setting vitamin D guidelines. Dermatoendocrinol. 2011;3(3):199-204.

Grant WB. Effect of follow-up time on the relation between prediagnostic serum 25-hydroxyitamin D and all-cause mortality rate. Dermatoendocrinol. 2012;4(2):198-202.

Our response:

This is an important point which has now been taken it into account in the new fourth paragraph in the discussion. In particular, we have introduced a citation to the paper from 2012 referred to above. We have also introduced new references to other papers discussing the stability or lack of stability of 25(OH)D levels over time. We hope this part of the discussion now provides a balanced point of view with regard to the use of 25(OH)D measurements from the baseline in HUNT2.

Reviewer's remark:

Also consider analyzing results with respect to follow-up time. It may be the case that the HUNT Study data did not find a correlation between vitamin D status and LBP due to long intervals between blood draw and diagnosis of LBP.

Our response:

The hypothesis suggested by the reviewer here should certainly be taken into account. These aspects are now dealt with more fully in the expanded section about the long follow-up period. The variation in follow-up time between participants in this data set is unfortunately not large enough to make it possible to analyse results with respect to the length of the follow-up period.

Reviewer's remark:

p. 6, line 18 - is it case-control or nested case-control?

Our response:

The reference to a plain "case-control study" at this point was an unfortunate oversight. The correct term "nested case-control study" is used in the revised version.

Reviewer's remark:

Lines 32-35. The city for DiaSorin should be given. Also, the measurement precision and accuracy. Also, it would be worthwhile to discuss the conversion factor used. See, also,
Carter GD, Berry J, Durazo-Arvizu R, Gunter E, Jones G, Jones J, Makin HLJ, Pattni P, Sempos CT, Twomey P, Williams EL, Wise SA. HYDROXYVITAMIN D ASSAYS: AN HISTORICAL PERSPECTIVE FROM DEQAS. J Steroid Biochem Mol Biol. 2017 Jul 19. pii: S0960-0760(17)30183-8. doi: 10.1016/j.jsbmb.2017.07.018. [Epub ahead of print]

Durazo-Arvizu R, Tian L, Brooks S, Sarafin K, Cashman K, Kiely M, Merkel J, Myers G, Coates P, Sempos C. The Vitamin D Standardization Program (VDSP) Manual for Retrospective Laboratory Standardization of Serum 25-Hydroxyvitamin D Data. J AOAC Int. 2017 Jul 18. doi: 10.5740/jaoacint.17-0196. [Epub ahead of print]

Our response:

A more detailed specification for the manufacturer DiaSorin is now supplied in the Exposure part of the Methods section, and intraassay and interassay coefficients of variation are specified. The procedure used to generate a conversion factor by remeasuring a small subsample is also described in more detail. Additionally, the problems associated with the measurements carried out in two different periods are now described in the second paragraph of the Discussion. A warning is given against a direct comparison of the 25(OH)D values found here and values found by other procedures, and a new reference is included to the paper by Carter et al. (2017) mentioned by the reviewer.

Reviewer's remark:

Table 4, What is known about the difference in vitamin D supplementation between men and women in winter?

Our response:

Considering the contrast between men and women seen for the winter/spring season in Table 4, this is a very natural question. Traditionally, vitamin D supplementation in Norway would largely represent use of cod liver oil, and there are indications that about the same percentages of men and women would use such supplementation, but we cannot state whether this is true in particular in winter. However, the characteristics of Norwegian women using this kind of supplementation are known in general because of the study of Brustad et al. (2004). We have now included a brief discussion of these issues, with a new reference to this paper, in the last paragraph of the manuscript before the Conclusion.

Reviewer's remark:

p. 13, line 40, there are very few 25OHD U-shaped relationships that may not be explained by a difference in vitamin D supplementation history:
Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, Garland CF, Holick MF. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D–health outcome relationships reflect adverse effects? Dermato-Endocrinology, 2016;8(1): e1187349.

As stated in the abstract for Ref. 34:
LIMITATIONS:
A causal relationship between either low or high 25(OH)D levels and increased mortality can not necessarily be inferred from this observational study.

Our response:

We completely understand the arguments used by the reviewer here. It was not our intention to indicate that any U-shaped relationship reflected causality, only that such apparent relationships had been found in some cases. The reference given was merely meant as an example. We have now added a qualification to the description of the result of Amrein et al. (2014), stating that a causal relationship could not be inferred from that study.

Reviewer 3 (Howard Amital)

Reviewer's remark:

This is an interesting and properly designed paper assessing the association between serum vitamin D levels and the odds developing low back pain.

A few minor issues

It is not clear whether the definition of LBP relied on self-report or by proactive physician periodical assessment. This issue should be clarified.

Our response:

We recognize that this point was not adequately dealt with in our original manuscript. We have now rephrased the relevant statement in the first paragraph of the Participants section so that it clearly indicates that the definition relies on a self-report made by each participant in the questionnaire.

Reviewer's remark:

The discussion lacks data regarding the manner by which vitamin D supplementation may affect chronic pain such as low back pain. There are several publications dealing with this issue and this should also be presented to the readership.

Our response:

We agree that it is reasonable to deal briefly with the issue of vitamin D supplementation and its effect on pain. We feel that this is an issue which is slightly different from the one studied by us, as it relates to treatment of individuals who are already affected by pain, whereas we are studying the risk of chronic low back pain among those who are not currently afflicted. In any case, the issue raised by the reviewer is of course relevant in the broader area of vitamin D and LBP. Thus we feel it is most natural to refer to studies about vitamin D supplementation in the general description of the background to our study provided in the introduction. We have now added a statement in the second paragraph of this section referring to the recent review and meta-analysis of Yong et al. (2017) on vitamin D supplementation in connection with chronic widespread pain. In the third paragraph, we now also refer explicitly to the randomized clinical trial of Sandoughi et al. (2015) about the effect of vitamin D supplementation on non-specific chronic low back pain.

VERSION 2 – REVIEW

REVIEWER	BABITA GHAI POST GRADUATE INSTITUTE OF MEDICAL EDUCATION AND RESEARCH (PGIMER), CHANDIGARH, INDIA No Competing Interest
REVIEW RETURNED	01-Oct-2017

GENERAL COMMENTS	Authors have looked into and replied to all the comments made.

REVIEWER	William B. Grant Sunlight, Nutrition and Health Research Center San Francisco, CA, USA
REVIEW RETURNED	15-Sep-2017

GENERAL COMMENTS	The manuscript is improved. A key argument in favor of the findings is the fact that HUNT2 found an inverse correlation between 25(OH)D and all-cause mortality rate with a follow-up period of 18.5 years. Regarding the discussion elevated 25(OH)D concentrations, this paper should be added as it points out that a likely reason for many U-shaped 25(OH)D concentration-health outcomes is that some enrollees may have only recently started supplementing with vitamin D and, thus, are put into the wrong 25(OH)D category. Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, Garland CF, Holick MF. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D–health outcome relationships reflect adverse effects? Dermato-Endocrinology, 2016;8(1): e1187349.

REVIEWER	Howard Amital Sheba Medical Center, Israel No Competing Interest
REVIEW RETURNED	16-Sep-2017

GENERAL COMMENTS	accept this revision

VERSION 2 – AUTHOR RESPONSE

Reviewers 1 and 3 did not make any remarks at this stage suggesting further changes in the manuscript, so we will only respond to Reviewer 2.

Reviewer 2 (William B. Grant)

Reviewer's remark:
The manuscript is improved. A key argument in favor of the findings is the fact that HUNT2 found an inverse correlation between 25(OH)D and all-cause mortality rate with a follow-up period of 18.5 years.

Regarding the discussion elevated 25(OH)D concentrations, this paper should be added as it points out that a likely reason for many U-shaped 25(OH)D concentration-health outcomes is that some enrollees may have only recently started supplementing with vitamin D and, thus, are put into the wrong 25(OH)D category.
Grant WB, Karras SN, Bischoff-Ferrari HA, Annweiler C, Boucher BJ, Juzeniene A, Garland CF, Holick MF. Do studies reporting 'U'-shaped serum 25-hydroxyvitamin D –health outcome relationships reflect adverse effects? Dermato-Endocrinology, 2016;8(1): e1187349.

Our response:

We have now adopted the suggestion made by the reviewer. The additional reference appears as no. 44 in the revised manuscript (with the subsequent references being renumbered). The paper is cited in the text at the end of the second paragraph on page 14 in a new statement about potential false U-shaped relationships