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Vitamin D status and risk of chronic low back pain: a nested case-control analysis in the HUNT Study

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ABSTRACT

Objective: To explore potential associations between vitamin D status and risk of chronic low back pain (LBP) in a Norwegian cohort, and to investigate whether relationships depend on the season of blood sample collection.

Design: A nested case-control study in a prospective data set.

Setting: The Norwegian community-based Nord-Trøndelag Health Study (HUNT). Data were collected in the HUNT2 (1995-1997) and HUNT3 (2006-2008) surveys.

Main outcome measure: Chronic LBP, defined as LBP persisting at least 3 months continuously during the past year.

Participants: Among individuals aged 19-55 years without LBP in HUNT2, a data set was generated including 1685 cases with LBP in HUNT3 and 3137 controls without LBP.

Methods: Blood samples from the participants collected in HUNT2 were analysed for serum 25-hydroxyvitamin D (25(OH)D) level. Associations with LBP were evaluated by unconditional logistic regression analysis with adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking and BMI.

Results: No association between vitamin D status and risk of chronic LBP was found in the total data set (OR per 10 nmol/l 25(OH)D =1.01, 95% CI 0.97 to 1.06) or in individuals with blood samples collected in summer/autumn (OR per 10 nmol/l 25(OH)D =0.99, 95% CI 0.93 to 1.06). For blood samples drawn in winter/spring associations differed significantly between women and men (p=0.004). Among women a positive association was seen (OR per 10 nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20), but among men no significant association was observed (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01).

Conclusions: Overall, no association between vitamin D status and risk of LBP was demonstrated. The association suggested in women for the winter/spring season cannot be regarded as established.

KEYWORDS

back pain, musculoskeletal, vitamin D, epidemiology

Strengths and limitations of this study

-The study provides information about the potential association between vitamin D status and risk of chronic low back pain (LBP).

-The study is population-based and prospective.

-Season of blood sample collection for analysis of serum 25(OH)D is taken into account in the analysis.

-Vitamin D and LBP status were not registered in the intermediate period between baseline and end of follow-up.

INTRODUCTION

Low back pain (LBP) is one of the most common musculoskeletal disorders and often leads to sick leave and a high degree of disability with substantial costs for society.[1] The causes of non-specific LBP are not sufficiently understood.[2]

Vitamin D is required for absorption of calcium from the intestines, and vitamin D has shown positive health effects on the muscle and skeletal system.[3] Skeletal muscles have vitamin D receptors and may require vitamin D for maximum function.[4] Associations between vitamin D deficiency and incidence of chronic pain have been suggested in various studies, but the evidence is not conclusive.[5] Some studies have found associations between vitamin D deficiency and occurrence of non-specific musculoskeletal pain in patient materials[6] and in population-based data sets.[7]

Very few population-based studies have been carried out regarding associations between vitamin D status and occurrence of back pain,[8] in particular with a prospective design. Studies of associations between vitamin D status and back pain have mostly been based on relatively small data sets involving patients, with some studies[9, 10, 11, 12, 13] suggesting an association between low vitamin D levels and back pain, while other studies[14, 15, 16] were unable to demonstrate any relationships. A cross-sectional study among schoolchildren also indicated such an association.[17]

Potential associations between vitamin D deficiency and LBP have partly been ascribed to osteomalacia[11], with an accumulation of osteoid because of defective mineralization. Poor muscle strength induced by vitamin D deficiency[3] may also affect the experience of LBP. It is not certain, however, whether these factors play any role considering the incidence of low back pain at the population level.

Only prospective studies can show whether vitamin D levels affect the subsequent risk of experiencing LBP. It is possible that back pain conversely can affect vitamin D status,[10] perhaps through modified behaviour influencing exposure to sunlight or through nutritional factors, and for this reason it is essential to base conclusions on results from prospective studies. It is important to carry out adjustment for potential confounders such as obesity, which is related to both vitamin D levels and to risk of low back pain.[18, 19, 20] Vitamin D

levels are higher after sun exposure, with the prevalence of vitamin D deficiency varying seasonally,[21] so associations should also be assessed separately for the summer/autumn and winter/spring seasons.

This study will investigate whether an association can be established between vitamin D status and risk of LBP in a case-control study nested in a Norwegian cohort. Vitamin D status is based on measurement of the major circulating metabolite 25-hydroxyvitamin D (25(OH)D).[22] The importance of seasonality for blood sample collection for vitamin D measurement will be explored.

METHODS

Participants

The present work is based on information from the Nord-Trøndelag Health Study (HUNT). From 1995 to 1997, the large health survey HUNT2 was conducted in Nord-Trøndelag county in Norway. The entire adult population received a health questionnaire and participants underwent a clinical examination, including measurements of body weight and height.[23] One question dealt with chronic LBP defined as LBP lasting at least three months continuously within the past year. Blood samples were drawn at the clinical examination. In the HUNT3 survey, conducted in 2006 to 2008 in the same county with a corresponding target population, similar questionnaires were distributed.[23] Information about residence status was supplied by national registries and linked by use of the unique Norwegian personal identification numbers.

A nested case-control study was conducted using prospective data from HUNT2 regarded as baseline and HUNT3 regarded as follow-up, including 4822 individuals in the age range 19-55 years when attending HUNT2. Vitamin D status was assessed on the basis of blood samples from the participants collected in HUNT2. Participants belonged to three different subsamples. The first and second subsample consisted of random samples from HUNT2 and were established in connection with previous studies of the association between vitamin D and asthma and lung function,[24, 25] but in the current study all individuals with LBP in HUNT2 were excluded. The first subsample was analysed for 25(OH)D in 2010, and included altogether 1492 persons, comprising 247 cases (individuals with LBP in HUNT3) and 1245

controls (individuals without LBP in HUNT3). The second subsample with analysis date for 25(OH)D in 2015 included a total of 2051 persons, comprising 343 cases and 1708 controls. The third subsample had measurements of 25(OH)D carried out in 2014/2015 and consisted of 1279 persons without LBP in HUNT2. Of these, a total of 1095 cases were selected at random among persons suffering from LBP in HUNT3 and 184 controls were randomly selected among persons without LBP in HUNT3. The third subsample was solely established for the current nested case-control study. None of the individuals included in this study had missing values for any covariate considered.

The original plan was that the case-control study would consist of the combined first and third subsamples only, and the size of the third subsample was adapted to power requirements in this data set. The second subsample only became available at a later stage.

Exposure

Blood samples collected in HUNT2 were stored at -70 °C until analysis. The level of 25(OH)D in serum was measured in the years 2010 and in 2014/2015 by chemiluminescent immunoassay (CLIA) methodology, using Liaison 25-OH Vitamin D Total assay (DiaSorin Inc., USA). Another kit from DiaSorin was used from 2014, but a conversion factor was used to obtain comparable values.

Serum 25(OH)D levels were classified into three groups <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, which are widely used categories in studies of vitamin D levels. Values <50.0 nmol/l are usually regarded as representing vitamin D deficiency.[26]

Covariate assessment

Baseline age was categorized into 10-year intervals. Four categories of work status were defined, the first comprising people being employed or carrying out professional work. The second category included those temporarily out of work, students and individuals in military service. The third category included pensioners and people receiving social security support, and the fourth category represented women occupied full-time with housework. Those currently working supplied information about physical activity at work,[27] in four categories

representing substantially sedentary work, work involving extensive walking, work leading to both walking and lifting, and work involving particularly strenuous activities. For physical activity in leisure time, including going to work, one category included those engaged in light activity only or hard physical activity (leading to sweating or being out of breath) <1 hour per week.[28] Other categories represented hard physical activity 1-2 and ≥3 hours per week.

Education was grouped according to duration as ≤9, 10-12, or ≥13 years. Categories of cigarette smoking represented current daily smoking, previous daily smoking and never daily smoking. BMI, defined as weight/height^2 and computed in kg/m^2, was subdivided into three groups: <25, 25-29.9, ≥30.

Season of blood sample collection was categorized as either the summer/autumn season (June through November) or the winter/spring season (December through May). This subdivision gives a marked difference in vitamin D deficiency between 6-months periods.[21]

Statistical methods

Associations between 25(OH)D levels in serum and risk of chronic LBP were assessed by unconditional logistic regression analysis with adjustment for potential confounders. Analyses were performed separately with 25(OH)D levels as categorical and continuous variables. All other variables were considered categorical. First, adjustments were carried out for age and sex and a factor indicating which subsample each individual belonged to. Additional adjustments for work status, physical activity at work and in leisure time, education, smoking, BMI and season of blood sample collection were then introduced. Separate tests were performed for interaction between continuous 25(OH)D level and all variables adjusted for. Linearity in the association with continuous 25(OH)D level was tested for by adding a quadratic term to the statistical model. All statistical analyses were carried out using IBM SPSS version 23 (IBM Corp., Armonk, New York).

Separate analyses were limited to participants who had the blood samples drawn in the summer/autumn season and the winter/spring season.

RESULTS

The age distribution was quite similar among cases and controls (table 1). The percentage of women was higher among cases than among controls. Within the three separate groups of serum 25(OH)D levels categorized as <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, the percentage of persons with and without LBP at end of follow-up were almost the same among cases and controls (table 1).

Table 1 Number of individuals by age, sex, vitamin D status and case-control status

	Cases, with LBP at end of follow-up		Controls, without LBP at end of follow-up	
	Number	Percent	Number	Percent
Age groups (years)				
19-29	246	15	504	16
30-39	492	29	951	30
40-49	687	41	1191	38
50-55	260	15	491	16
Sex				
Female	1043	62	1677	54
Male	642	38	1460	47
25(OH)D (nmol/l)				
<50.0	925	55	1689	54
50.0-74.9	619	37	1126	36
≥75.0	141	8	322	10

LBP, low back pain.

In the total data set, the mean serum 25(OH)D levels were quite similar among cases and controls (table 2). Both in women and men the serum 25(OH)D levels were higher in the summer/autumn than in the winter/spring season among cases and controls.

Table 2 Descriptive statistics of 25(OH)D level by case-control status

	Cases, with LBP at end of follow-up				Controls, without LBP at end of follow-up			
	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)
Total data set								
All seasons	1685	49.4	18.2	47.9	3137	50.0	19.1	48.0
Summer/autumn	773	54.1	18.4	52.0	1424	56.7	19.0	55.2
Winter/spring	912	45.4	17.0	43.8	1713	44.4	17.4	41.9
Women								
All seasons	1043	50.2	18.6	48.9	1677	50.5	19.0	49.0
Summer/autumn	480	53.8	18.7	52.9	784	56.2	18.7	54.4
Winter/spring	563	47.1	17.9	45.9	893	45.4	17.9	43.0
Men								
All seasons	642	48.0	17.4	45.8	1460	49.4	19.3	46.6
Summer/autumn	293	54.5	18.0	51.3	640	57.4	19.4	56.1
Winter/spring	349	42.6	14.9	40.8	820	43.2	16.7	40.4

LBP, low back pain; SD, standard deviation.

In the overall data set no association was found between vitamin D status and risk of LBP, neither with adjustment for age, sex and subsample nor with complete adjustment (OR per 10 nmol/l 25(OH)D= 1.01, 95% CI 0.97 to 1.06) (table 3). No significant interaction was found, but the interaction with sex was marginally significant (p=0.06). A weak positive association was suggested among women, although the estimated relation among men was in the opposite direction (table 3). Results did not differ significantly between subsamples.

Table 3 Associations between vitamin D status and risk of chronic LBP

	With adjustment for age, sex and subsample		With complete adjustment*	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.64†		0.97†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.94 (0.80 to 1.11)		1.01 (0.85 to 1.20)	
≥75.0	0.90 (0.68 to 1.18)		0.98 (0.73 to 1.31)	
Per 10 nmol/l	0.99 (0.95 to 1.03)	0.59	1.01 (0.97 to 1.06)	0.59
Women				
25(OH)D (nmol/l)		0.54†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.09 (0.88 to 1.36)		1.20 (0.96 to 1.51)	
≥75.0	0.91 (0.64 to 1.30)		1.05 (0.72 to 1.52)	
Per 10 nmol/l	1.02 (0.97 to 1.08)	0.48	1.06 (1.00 to 1.12)	0.054
Men				
25(OH)D (nmol/l)		0.10†		0.16†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.75 (0.57 to 0.98)		0.76 (0.57 to 1.01)	
≥75.0	0.90 (0.58 to 1.39)		0.88 (0.55 to 1.41)	
Per 10 nmol/l	0.94 (0.88 to 1.01)	0.10	0.94 (0.88 to 1.02)	0.13

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI, subsample and season of blood sample collection.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Separate analyses of the association between vitamin D status and risk of LBP by season of blood sample collection revealed no effect in either sex in the summer/autumn season (table 4). However, in the winter/spring season associations differed significantly between women and men (p=0.004). In women a significant positive association was observed (OR per 10

nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20) (table 4). In men the estimated association was negative (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01) but did not reach statistical significance. Considering the different OR values from the categorical analyses, a consistent trend was suggested in each sex.

Table 4 Associations between vitamin D status and risk of chronic LBP by season of blood sample collection*

	Summer/autumn		Winter/spring	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.87†		0.79†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.93 (0.72 to 1.20)		1.08 (0.85 to 1.38)	
≥75.0	0.96 (0.66 to 1.39)		0.96 (0.58 to 1.58)	
Per 10 nmol/l	0.99 (0.93 to 1.06)	0.78	1.03 (0.97 to 1.10)	0.37
Women				
25(OH)D (nmol/l)		0.56†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.13 (0.81 to 1.57)		1.26 (0.92 to 1.73)	
≥75.0	0.89 (0.53 to 1.47)		1.34 (0.76 to 2.35)	
Per 10 nmol/l	1.00 (0.92 to 1.09)	0.94	1.11 (1.02 to 1.20)	0.012
Men				
25(OH)D (nmol/l)		0.15†		0.20†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.70 (0.46 to 1.05)		0.84 (0.56 to 1.27)	
≥75.0	1.01 (0.58 to 1.76)		0.37 (0.12 to 1.17)	
Per 10 nmol/l	0.97 (0.88 to 1.08)	0.58	0.90 (0.81 to 1.01)	0.08

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI and subsample.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

No significant deviations from linearity were observed in the relationships with 25(OH)D considered as a continuous variable.

DISCUSSION

The association between vitamin D status and chronic LBP was examined in a case-control study nested in a population-based follow-up of a Norwegian cohort. No association between vitamin D status and risk of LBP was found overall. For measurements in the winter/spring season, associations differed significantly between women and men. Among women a positive association was seen, but among men no significant association was observed.

A strength of the study is that the overwhelming majority of participants belonged to a homogeneous ethnic group.[29] Information was available on potential confounders, which made it possible to carry out accurate adjustments. The risk factor considered is represented by the vitamin D status measured in blood samples drawn at the clinical examination in HUNT2. However, the blood samples were stored for many years at low temperature, and the measurements of serum 25(OH)D levels were carried out in 2010 or in 2014/2015. A limitation is the lack of information about back pain occurring at other times in the 11-year follow-up interval between HUNT2 and HUNT3. Furthermore, the extent of back pain was not characterized by the participants. Another potential problem is the relatively long period between collection of information about risk factors and the recording of LBP status. However, vitamin D levels have been shown to be relatively stable over long periods in the Norwegian population.[30]

There are few population-based studies of relationships between vitamin D status and LBP.[8] Some studies have investigated such relationships among patients,[10, 12, 15] but population-based studies of risk of LBP with a prospective design have been lacking. The present study is to our knowledge the only prospective study of the association between vitamin D status and risk of LBP.

In cross-sectional population-based studies, an association between low level of 25(OH)D and prevalence of back pain has been found in older women[8] and in schoolchildren.[17] Some case-control studies have shown an association between low levels of vitamin D and occurrence of back pain,[11, 13] but others do not support an association.[14, 16] In a recent small Swedish case-control study no difference in vitamin D levels could be established

between participants with chronic LBP and matched controls.[16] This is consistent with the overall results found in our study.

Among women with blood samples drawn in the winter/spring season, we found that high levels of vitamin D were associated with an increased risk of LBP, which is the opposite of what was initially hypothesized. In a Danish cross-sectional study of LBP patients an association in the same direction was found, and normal levels of vitamin D, as opposed to vitamin D deficiency, were associated with more Modic changes in the lumbar vertebral end plates as seen on MRI.[15]

An association between vitamin D deficiency and adiposity has been found in several studies.[19] This was also confirmed in a cross-sectional study in the HUNT population.[21] In a prospective study using data from HUNT2 and HUNT3, overweight and obesity were found to be associated with a predisposition to chronic LBP.[20] This suggests that BMI could be a confounder in the association with 25(OH)D. However, our complete analyses included adjustment for BMI, and it is not to be expected that any substantial relationship should remain because of the association with adiposity.

In view of the seasonal variation in sun exposure, it is important to look at the time when blood samples are collected to define vitamin D deficiency.[31] Thus in a study of bone mineral density the summer season was found to be the best period to determine the serum 25(OH)D level.[32] This contrasts with our results, with a positive association between LBP and vitamin D status observed in the winter/spring season for women only. Elevated levels of 25(OH)D may be harmful in other respects.[33] In a study of mortality among hospitalized patients a U-shaped relationship was found, with both low and high values of 25(OH)D associated with increased mortality.[33, 34] However, the 25(OH)D levels had to be considerably above the typical values in the present study to be associated with higher mortality, so such effects at the upper end of the range for 25(OH)D are hardly relevant here.

Gender and sex hormone levels are important variables that can influence a possible effect of vitamin D in rheumatic diseases.[35] In a Danish population receiving UVB treatment, the decline in 25(OH)D varied over time between sexes, with women maintaining a greater half-life of 25(OH)D.[36] It is not evident, however, how such differences can explain the sex contrast seen in the present study in the association with risk of LBP.

Measurements of 25(OH)D levels made during the winter/spring season may possibly capture variability of vitamin D in a better way, because vitamin D values in winter are not so dependent on sunlight exposure. It is possible, however, that women tending to report LBP in this population are particularly health-conscious and use more vitamin D supplements during the winter/spring season, creating a false positive association. Moreover, the significant relationship observed may be spurious due to the multiple statistical tests carried out. Thus this association cannot yet be regarded as causal.

CONCLUSIONS

In this population-based nested case-control study, no overall association between vitamin D status and risk of LBP was found. The positive association observed in women with blood samples drawn in the winter/spring season needs confirmation from other studies.

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Contributors IngridH, IvarH, KH, XMM, AL and JAZ contributed to the study design. IngridH and IvarH contributed to analysis and interpretation of data. IngridH wrote the paper. IvarH, KH, XMM, AL and JAZ all revised the manuscript. All the authors have read and approved the paper.

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Competing interests None declared.

Ethics approval The work was approved by the Regional Committee for Medical and Health Research Ethics in Central Norway, and HUNT was also approved by the Norwegian Data Inspectorate. Each participant in the HUNT2 and HUNT3 surveys signed a written informed consent regarding the collection and use of data for research purposes.

Data sharing statement The data set analysed belongs to a third party, the HUNT study (the Nord-Trøndelag Health Study). The authors of the current manuscript are not affiliated with the project as such, but have been given permission to analyse the data after obtaining the necessary Norwegian permits. Because of the confidentiality requirements according to Norwegian law, a data set of this kind with information from a complete county at the individual level cannot be made public. However, research groups wishing to analyse data from the HUNT study may apply to the HUNT organisation (http://www.ntnu.edu/hunt) to get access to the data, after having obtained the permits needed according to Norwegian law.

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	12-13
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	8
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	6-7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

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

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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

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ABSTRACT

Objective: To explore potential associations between vitamin D status and risk of chronic low back pain (LBP) in a Norwegian cohort, and to investigate whether relationships depend on the season of blood sample collection.

Design: A nested case-control study in a prospective data set.

Setting: The Norwegian community-based Nord-Trøndelag Health Study (HUNT). Data were collected in the HUNT2 (1995-1997) and HUNT3 (2006-2008) surveys.

Main outcome measure: Chronic LBP, defined as LBP persisting at least 3 months continuously during the past year.

Participants: Among individuals aged 19-55 years without LBP in HUNT2, a data set was generated including 1685 cases with LBP in HUNT3 and 3137 controls without LBP.

Methods: Blood samples from the participants collected in HUNT2 were analysed for serum 25-hydroxyvitamin D (25(OH)D) level. Associations with LBP in HUNT3 were evaluated by unconditional logistic regression analysis with adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking and BMI.

Results: No association between vitamin D status and risk of chronic LBP was found in the total data set (OR per 10 nmol/l 25(OH)D =1.01, 95% CI 0.97 to 1.06) or in individuals with blood samples collected in summer/autumn (OR per 10 nmol/l 25(OH)D =0.99, 95% CI 0.93 to 1.06). For blood samples drawn in winter/spring associations differed significantly between women and men (p=0.004). Among women a positive association was seen (OR per 10 nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20), but among men no significant association was observed (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01).

Conclusions: Overall, no association between vitamin D status and risk of LBP was demonstrated. The association suggested in women for the winter/spring season cannot be regarded as established.

KEYWORDS

back pain, musculoskeletal, vitamin D, epidemiology

Strengths and limitations of this study

-The study is prospective and the medical condition considered, chronic low back pain at end of follow-up, cannot have influenced vitamin D status at baseline.

-Season of blood sample collection for analysis of serum 25(OH)D is taken into account in the analysis.

-Vitamin D and back pain status were not registered in the intermediate period between baseline and end of follow-up.

-The mean length of time between blood sample collection and assessment of final back pain status was 11 years, and individual vitamin D status may have changed considerably in the meantime.

INTRODUCTION

Low back pain (LBP) is one of the most common musculoskeletal disorders and often leads to sick leave and a high degree of disability with substantial costs for society.[1] The causes of non-specific LBP are not sufficiently understood.[2]

Vitamin D is required for absorption of calcium from the intestines, and vitamin D has shown positive health effects on the muscle and skeletal system.[3] Skeletal muscles have vitamin D receptors and may require vitamin D for maximum function.[4] Associations between vitamin D deficiency and incidence of chronic pain have been suggested in various studies, but the evidence is not conclusive.[5] Some studies have found associations between vitamin D deficiency and occurrence of non-specific musculoskeletal pain in patient materials[6] and in population-based data sets.[7] A meta-analysis[8] concluded that vitamin D supplementation can decrease pain scores in chronic widespread pain.

Very few population-based studies have been carried out regarding associations between vitamin D status and occurrence of back pain,[9] in particular with a prospective design. Studies of associations between vitamin D status and back pain have mostly been based on relatively small data sets involving patients, with some studies[10-14] suggesting an association between low vitamin D levels and back pain, while other studies[15-17] were unable to demonstrate any relationships. A cross-sectional study among schoolchildren also indicated such an association.[18] A small randomized clinical trial of patients with chronic LBP failed to show any effect of vitamin D supplementation.[19]

Potential associations between vitamin D deficiency and LBP have partly been ascribed to osteomalacia[12], with an accumulation of osteoid because of defective mineralization. Poor muscle strength induced by vitamin D deficiency[3] may also affect the experience of LBP. It is not certain, however, whether these factors play any role considering the incidence of low back pain at the population level.

Only prospective studies can show whether vitamin D levels affect the subsequent risk of experiencing LBP. It is possible that back pain conversely can affect vitamin D status,[11] perhaps through modified behaviour influencing exposure to sunlight or through nutritional factors, and for this reason it is essential to base conclusions on results from prospective studies. It is important to carry out adjustment for potential confounders such as obesity, which is related to both vitamin D levels and to risk of low back pain.[20-22] Vitamin D

levels are higher after sun exposure, with the prevalence of vitamin D deficiency varying seasonally,[23] so associations should also be assessed separately for the summer/autumn and winter/spring seasons.

This study will investigate whether an association can be established between vitamin D status and risk of LBP in a case-control study nested in a Norwegian cohort. Vitamin D status is based on measurement of the major circulating metabolite 25-hydroxyvitamin D (25(OH)D).[24] The importance of seasonality for blood sample collection for vitamin D measurement will be explored.

METHODS

Participants

The present work is based on information from the Nord-Trøndelag Health Study (HUNT). From 1995 to 1997, the large health survey HUNT2 was conducted in Nord-Trøndelag county in Norway. The entire adult population received a health questionnaire and participants underwent a clinical examination, including measurements of body weight and height.[25] In particular, each participant provided information in the questionnaire showing whether he or she had experienced chronic LBP during the preceding 12 month period, defined as LBP lasting at least three months continuously in that period. Blood samples were drawn at the clinical examination. In the HUNT3 survey, conducted in 2006 to 2008 in the same county with a corresponding target population, similar questionnaires were distributed.[25] Information about residence status was supplied by national registries and linked by use of the unique Norwegian personal identification numbers.

A nested case-control study was conducted using prospective data from HUNT2 regarded as baseline and HUNT3 regarded as follow-up, including 4822 individuals in the age range 19-55 years when attending HUNT2. Vitamin D status was assessed on the basis of blood samples from the participants collected in HUNT2 in 1995 to 1997, but the actual measurements were only carried out later. Participants belonged to three different subsamples. The first and second subsample consisted of random samples from HUNT2 and were established in connection with previous studies of the association between vitamin D and asthma and lung function,[26, 27] but in the current study all individuals with LBP in HUNT2 were excluded. The first subsample was analysed for 25(OH)D in 2010, and included

altogether 1492 persons (figure 1), comprising 247 cases (individuals with LBP in HUNT3) and 1245 controls (individuals without LBP in HUNT3). The second subsample with analysis date for 25(OH)D in 2015 included a total of 2051 persons (figure 1), comprising 343 cases and 1708 controls. The third subsample had measurements of 25(OH)D carried out in 2014/2015 and consisted of 1279 persons without LBP in HUNT2. Of these, a total of 1095 cases were selected at random among persons suffering from LBP in HUNT3 and 184 controls were randomly selected among persons without LBP in HUNT3 (figure 1). The third subsample was solely established for the current nested case-control study. None of the individuals included in this study had missing values for any covariate considered.

The original plan was that the nested case-control study would consist of the combined first and third subsamples only, and the size of the third subsample was adapted to power requirements in this data set. The second subsample only became available at a later stage.

Exposure

Blood samples collected in HUNT2 were stored at -70 °C until analysis. The level of 25(OH)D in serum was measured in the years 2010 and in 2014/2015 by chemiluminescent immunoassay (CLIA) methodology, using Liaison 25-OH Vitamin D Total assay (DiaSorin Inc., Saluggia, Italy). The method has an intraassay coefficient of variation of 4% and an interassay coefficient of variation of 8%. Another kit from DiaSorin was used from 2014. A total of 118 samples with measurements available from 2010 were reanalysed in 2015 with the new kit. After exclusion of 2 outliers, a conversion factor for measurements from 2010 to new values from 2014/2015 was established by Passing and Bablok regression.[28]

Serum 25(OH)D levels were classified into three groups <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, which are widely used categories in studies of vitamin D levels. Values <50.0 nmol/l are usually regarded as representing vitamin D deficiency.[29]

Covariate assessment

Baseline age was categorized into 10-year intervals. Four categories of work status were defined, the first comprising people being employed or carrying out professional work. The

second category included those temporarily out of work, students and individuals in military service. The third category included pensioners and people receiving social security support, and the fourth category represented women occupied full-time with housework. Those currently working supplied information about physical activity at work,[30] in four categories representing substantially sedentary work, work involving extensive walking, work leading to both walking and lifting, and work involving particularly strenuous activities. For physical activity in leisure time, including going to work, one category included those engaged in light activity only or hard physical activity (leading to sweating or being out of breath) <1 hour per week.[31] Other categories represented hard physical activity 1-2 and ≥3 hours per week.

Education was grouped according to duration as ≤9, 10-12, or ≥13 years. Categories of cigarette smoking represented current daily smoking, previous daily smoking and never daily smoking. BMI, defined as weight/height^2 and computed in kg/m^2, was subdivided into three groups: <25, 25-29.9, ≥30.

Season of blood sample collection was categorized as either the summer/autumn season (June through November) or the winter/spring season (December through May). This subdivision gives a marked difference in vitamin D deficiency between 6-months periods.[23]

Statistical methods

Associations between 25(OH)D levels in serum and risk of chronic LBP were assessed by unconditional logistic regression analysis with adjustment for potential confounders. Analyses were performed separately with 25(OH)D levels as categorical and continuous variables. All other variables were considered categorical. First, adjustments were carried out for age and sex and a factor indicating which subsample each individual belonged to. Additional adjustments for work status, physical activity at work and in leisure time, education, smoking, BMI and season of blood sample collection were then introduced. Separate tests were performed for interaction between continuous 25(OH)D level and all variables adjusted for. Linearity in the association with continuous 25(OH)D level was tested for by adding a quadratic term to the statistical model. All statistical analyses were carried out using IBM SPSS version 23 (IBM Corp., Armonk, New York).

Separate analyses were limited to participants who had the blood samples drawn in the summer/autumn season and the winter/spring season.

RESULTS

The age distribution was quite similar among cases and controls (table 1). The percentage of women was higher among cases than among controls. Within the three separate groups of serum 25(OH)D levels categorized as <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, the percentage of persons with and without LBP at end of follow-up were almost the same among cases and controls (table 1).

Table 1 Number of individuals by age, sex, vitamin D status and case-control status

	Cases, with LBP at end of follow-up		Controls, without LBP at end of follow-up	
	Number	**Percent**	**Number**	**Percent**
Age groups (years)				
19-29	246	15	504	16
30-39	492	29	951	30
40-49	687	41	1191	38
50-55	260	15	491	16
Sex				
Female	1043	62	1677	54
Male	642	38	1460	47
25(OH)D (nmol/l)				
<50.0	925	55	1689	54
50.0-74.9	619	37	1126	36
≥75.0	141	8	322	10

LBP, low back pain.

Table 2 Descriptive statistics of 25(OH)D level by case-control status

	Cases, with LBP at end of follow-up				Controls, without LBP at end of follow-up			
	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)
Total data set								
All seasons	1685	49.4	18.2	47.9	3137	50.0	19.1	48.0
Summer/autumn	773	54.1	18.4	52.0	1424	56.7	19.0	55.2
Winter/spring	912	45.4	17.0	43.8	1713	44.4	17.4	41.9
Women								
All seasons	1043	50.2	18.6	48.9	1677	50.5	19.0	49.0
Summer/autumn	480	53.8	18.7	52.9	784	56.2	18.7	54.4
Winter/spring	563	47.1	17.9	45.9	893	45.4	17.9	43.0
Men								
All seasons	642	48.0	17.4	45.8	1460	49.4	19.3	46.6
Summer/autumn	293	54.5	18.0	51.3	640	57.4	19.4	56.1
Winter/spring	349	42.6	14.9	40.8	820	43.2	16.7	40.4

LBP, low back pain; SD, standard deviation.

In the total data set, the mean serum 25(OH)D levels were quite similar among cases and controls (table 2). Both in women and men the serum 25(OH)D levels were higher in the summer/autumn than in the winter/spring season among cases and controls.

In the overall data set no association was found between vitamin D status and risk of LBP, neither with adjustment for age, sex and subsample nor with complete adjustment (OR per 10 nmol/l 25(OH)D= 1.01, 95% CI 0.97 to 1.06) (table 3). No significant interaction was found, but the interaction with sex was marginally significant (p=0.06). A weak positive association was suggested among women, although the estimated relation among men was in the opposite direction (table 3). Results did not differ significantly between subsamples.

Table 3 Associations between vitamin D status and risk of chronic LBP

	With adjustment for age, sex and subsample		With complete adjustment*	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.64†		0.97†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.94 (0.80 to 1.11)		1.01 (0.85 to 1.20)	
≥75.0	0.90 (0.68 to 1.18)		0.98 (0.73 to 1.31)	
Per 10 nmol/l	0.99 (0.95 to 1.03)	0.59	1.01 (0.97 to 1.06)	0.59
Women				
25(OH)D (nmol/l)		0.54†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.09 (0.88 to 1.36)		1.20 (0.96 to 1.51)	
≥75.0	0.91 (0.64 to 1.30)		1.05 (0.72 to 1.52)	
Per 10 nmol/l	1.02 (0.97 to 1.08)	0.48	1.06 (1.00 to 1.12)	0.054
Men				
25(OH)D (nmol/l)		0.10†		0.16†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.75 (0.57 to 0.98)		0.76 (0.57 to 1.01)	
≥75.0	0.90 (0.58 to 1.39)		0.88 (0.55 to 1.41)	
Per 10 nmol/l	0.94 (0.88 to 1.01)	0.10	0.94 (0.88 to 1.02)	0.13

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI, subsample and season of blood sample collection.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Separate analyses of the association between vitamin D status and risk of LBP by season of blood sample collection revealed no effect in either sex in the summer/autumn season (table 4). However, in the winter/spring season associations differed significantly between women and men (p=0.004). In women a significant positive association was observed (OR per 10 nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20) (table 4). In men the estimated association was

negative (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01) but did not reach statistical significance. Considering the different OR values from the categorical analyses, a consistent trend was suggested in each sex.

No significant deviations from linearity were observed in the relationships with 25(OH)D considered as a continuous variable.

Table 4 Associations between vitamin D status and risk of chronic LBP by season of blood sample collection*

	Summer/autumn		Winter/spring	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.87†		0.79†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.93 (0.72 to 1.20)		1.08 (0.85 to 1.38)	
≥75.0	0.96 (0.66 to 1.39)		0.96 (0.58 to 1.58)	
Per 10 nmol/l	0.99 (0.93 to 1.06)	0.78	1.03 (0.97 to 1.10)	0.37
Women				
25(OH)D (nmol/l)		0.56†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.13 (0.81 to 1.57)		1.26 (0.92 to 1.73)	
≥75.0	0.89 (0.53 to 1.47)		1.34 (0.76 to 2.35)	
Per 10 nmol/l	1.00 (0.92 to 1.09)	0.94	1.11 (1.02 to 1.20)	0.012
Men				
25(OH)D (nmol/l)		0.15†		0.20†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.70 (0.46 to 1.05)		0.84 (0.56 to 1.27)	
≥75.0	1.01 (0.58 to 1.76)		0.37 (0.12 to 1.17)	
Per 10 nmol/l	0.97 (0.88 to 1.08)	0.58	0.90 (0.81 to 1.01)	0.08

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI and subsample.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

DISCUSSION

The association between vitamin D status and chronic LBP was examined in a case-control study nested in a population-based follow-up of a Norwegian cohort. No association between vitamin D status and risk of LBP was found overall. For measurements in the winter/spring season, associations differed significantly between women and men. Among women a positive association was seen, but among men no significant association was observed.

A strength of the study is that the overwhelming majority of participants belonged to a homogeneous ethnic group.[32] Information was available on potential confounders, which made it possible to carry out accurate adjustments. The risk factor considered is represented by the vitamin D status measured in blood samples drawn at the clinical examination in HUNT2. However, the blood samples were stored for many years at low temperature, and the measurements of serum 25(OH)D levels were carried out in 2010 or in 2014/2015. Despite attempts to standardize the measurements, use of different instruments in the two periods may have led to minor systematic deviations in the 25(OH)D levels. To some extent, this was accounted for by adjusting all statistical analyses for subsample. This procedure was also essential in view of the different sampling procedures applied to establish the subsamples. The Liaison immunoassay method may still underestimate true 25(OH)D levels[33] and a direct comparison with values found by other methods may not be justified.[34]

A limitation is the lack of information about back pain occurring at other times in the 11-year follow-up interval between HUNT2 and HUNT3. The case definition only refers to the last year before collection of information in HUNT3. Thus all cases must have experienced incident chronic LBP during follow-up but so may some of the controls in the intervening period if they later recovered. Any real association between vitamin D status and LBP should be present anyhow but may be more difficult to detect. Furthermore, the extent of back pain was not characterized by the participants.

Another potential problem is the relatively long period between collection of information about risk factors and the recording of LBP status. In view of the weaker associations seen between 25(OH)D levels and mortality and cancer risk in prospective studies with longer follow-periods, it has been suggested that 25(OH)D levels should be measured at regular

intervals in prospective studies, perhaps every 2-4 years.[35] Such information was not available in the present study. However, vitamin D levels have been shown to be relatively stable over periods as long as 14 years in the Norwegian population,[36] and large data sets in other countries have shown a definite degree of stability over somewhat shorter periods.[37, 38] In data with measurements from the HUNT2 survey, low 25(OH)D levels showed a clear association with all-cause mortality in a prospective study with a median follow-up time of 18.5 years.[39] Thus potential relationships between 25(OH)D levels and disease should still persist in a study such as the current one with 11 years of follow-up, although associations may be attenuated. The state of relevant confounders may also change during a long follow-up. An alternative to the design used here would be a cross-sectional case-control study based on both 25(OH)D levels and reports of chronic LBP from the HUNT2 survey. Problems concerning 25(OH)D levels changing over time would be eliminated but it would not be possible to rule out an influence of disease on 25(OH)D status.

There are few population-based studies of relationships between vitamin D status and LBP.[9] Some studies have investigated such relationships among patients,[11, 13, 16] but population-based studies of risk of LBP with a prospective design have been lacking. The present study is to our knowledge the only prospective study of the association between vitamin D status and risk of LBP.

In cross-sectional population-based studies, an association between low level of 25(OH)D and prevalence of back pain has been found in older women[9] and in schoolchildren.[18] Some case-control studies have shown an association between low levels of vitamin D and occurrence of back pain,[12, 14] but others do not support an association.[15, 17] In a recent small Swedish case-control study no difference in vitamin D levels could be established between participants with chronic LBP and matched controls.[17] This is consistent with the overall results found in our study.

Among women with blood samples drawn in the winter/spring season, we found that high levels of vitamin D were associated with an increased risk of LBP, which is the opposite of what was initially hypothesized. In a Danish cross-sectional study of LBP patients an association in the same direction was found, and normal levels of vitamin D, as opposed to vitamin D deficiency, were associated with more Modic changes in the lumbar vertebral end plates as seen on MRI.[16]

An association between vitamin D deficiency and adiposity has been found in several studies.[21] This was also confirmed in a cross-sectional study in the HUNT population.[23] In a prospective study using data from HUNT2 and HUNT3, overweight and obesity were found to be associated with a predisposition to chronic LBP.[22] This suggests that BMI could be a confounder in the association with 25(OH)D. However, our complete analyses included adjustment for BMI, and it is not to be expected that any substantial relationship should remain because of the association with adiposity.

In view of the seasonal variation in sun exposure, it is important to look at the time when blood samples are collected to define vitamin D deficiency.[40] Thus in a study of bone mineral density the summer season was found to be the best period to determine the serum 25(OH)D level.[41] This contrasts with our results, with a positive association between LBP and vitamin D status observed in the winter/spring season for women only. Elevated levels of 25(OH)D may be harmful in other respects.[42] In a study of mortality among hospitalized patients a U-shaped relationship was found, with both low and high values of 25(OH)D associated with increased mortality.[42, 43] However, the 25(OH)D levels had to be considerably above the typical values in the present study to be associated with higher mortality and a causal relationship was not inferred, so such effects at the upper end of the range for 25(OH)D are hardly relevant here.

Gender and sex hormone levels are important variables that can influence a possible effect of vitamin D in rheumatic diseases.[44] In a Danish population receiving UVB treatment, the decline in 25(OH)D varied over time between sexes, with women maintaining a greater half-life of 25(OH)D.[45] It is not evident, however, how such differences can explain the sex contrast seen in the present study in the association with risk of LBP.

Measurements of 25(OH)D levels made during the winter/spring season may possibly capture variability of vitamin D in a better way, because vitamin D values in winter are not so dependent on sunlight exposure. It is possible, however, that women tending to report LBP in this population are particularly health-conscious and use more vitamin D supplements during the winter/spring season, creating a false positive association. Cod liver oil supplement has traditionally represented a major source of vitamin D in Norway,[46] with about 35% of both the male and female populations using such supplement in the 1990s.[47] Whole-year usage among females is associated with poor perceived health but is otherwise associated with a healthy diet,[46] suggesting that use of supplements is not in general matched to the vitamin

D needs.[46] In any case, the significant relationship observed among women may be spurious due to the multiple statistical tests carried out. Thus this association cannot yet be regarded as causal.

CONCLUSIONS

In this population-based nested case-control study, no overall association between vitamin D status and risk of LBP was found. The positive association observed in women with blood samples drawn in the winter/spring season needs confirmation from other studies.

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Competing interests None declared.

Ethics approval The work was approved by the Regional Committee for Medical and Health Research Ethics in Central Norway, and HUNT was also approved by the Norwegian Data Inspectorate. Each participant in the HUNT2 and HUNT3 surveys signed a written informed consent regarding the collection and use of data for research purposes.

Data sharing statement The data set analysed belongs to a third party, the HUNT study (the Nord-Trøndelag Health Study). The authors of the current manuscript are not affiliated with the project as such, but have been given permission to analyse the data after obtaining the necessary Norwegian permits. Because of the confidentiality requirements according to Norwegian law, a data set of this kind with information from a complete county at the individual level cannot be made public. However, research groups wishing to analyse data from the HUNT study may apply to the HUNT organisation (http://www.ntnu.edu/hunt) to get access to the data, after having obtained the permits needed according to Norwegian law.

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Figure legend

Figure 1 Flow chart showing participants for statistical analysis in the nested case-control study.

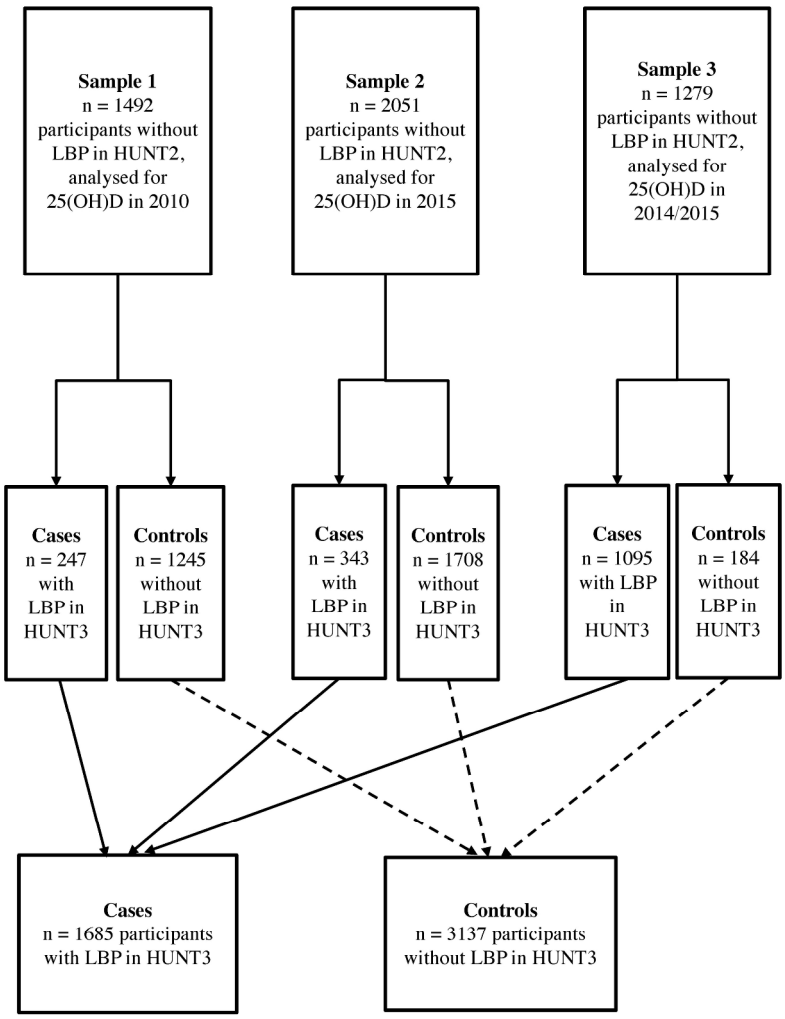

Figure 1 Flow chart showing participants for statistical analysis in the nested case-control study.

254x338mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	12-13
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	8
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	6-7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

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

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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

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ABSTRACT

Objective: To explore potential associations between vitamin D status and risk of chronic low back pain (LBP) in a Norwegian cohort, and to investigate whether relationships depend on the season of blood sample collection.

Design: A nested case-control study in a prospective data set.

Setting: The Norwegian community-based Nord-Trøndelag Health Study (HUNT). Data were collected in the HUNT2 (1995-1997) and HUNT3 (2006-2008) surveys.

Main outcome measure: Chronic LBP, defined as LBP persisting at least 3 months continuously during the past year.

Participants: Among individuals aged 19-55 years without LBP in HUNT2, a data set was generated including 1685 cases with LBP in HUNT3 and 3137 controls without LBP.

Methods: Blood samples from the participants collected in HUNT2 were analysed for serum 25-hydroxyvitamin D (25(OH)D) level. Associations with LBP in HUNT3 were evaluated by unconditional logistic regression analysis with adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking and BMI.

Results: No association between vitamin D status and risk of chronic LBP was found in the total data set (OR per 10 nmol/l 25(OH)D =1.01, 95% CI 0.97 to 1.06) or in individuals with blood samples collected in summer/autumn (OR per 10 nmol/l 25(OH)D =0.99, 95% CI 0.93 to 1.06). For blood samples drawn in winter/spring associations differed significantly between women and men (p=0.004). Among women a positive association was seen (OR per 10 nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20), but among men no significant association was observed (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01).

Conclusions: Overall, no association between vitamin D status and risk of LBP was demonstrated. The association suggested in women for the winter/spring season cannot be regarded as established.

KEYWORDS

back pain, musculoskeletal, vitamin D, epidemiology

Strengths and limitations of this study

-The study is prospective and the medical condition considered, chronic low back pain at end of follow-up, cannot have influenced vitamin D status at baseline.

-Season of blood sample collection for analysis of serum 25(OH)D is taken into account in the analysis.

-Vitamin D and back pain status were not registered in the intermediate period between baseline and end of follow-up.

-The mean length of time between blood sample collection and assessment of final back pain status was 11 years, and individual vitamin D status may have changed considerably in the meantime.

INTRODUCTION

Low back pain (LBP) is one of the most common musculoskeletal disorders and often leads to sick leave and a high degree of disability with substantial costs for society.[1] The causes of non-specific LBP are not sufficiently understood.[2]

Vitamin D is required for absorption of calcium from the intestines, and vitamin D has shown positive health effects on the muscle and skeletal system.[3] Skeletal muscles have vitamin D receptors and may require vitamin D for maximum function.[4] Associations between vitamin D deficiency and incidence of chronic pain have been suggested in various studies, but the evidence is not conclusive.[5] Some studies have found associations between vitamin D deficiency and occurrence of non-specific musculoskeletal pain in patient materials[6] and in population-based data sets.[7] A meta-analysis[8] concluded that vitamin D supplementation can decrease pain scores in chronic widespread pain.

Very few population-based studies have been carried out regarding associations between vitamin D status and occurrence of back pain,[9] in particular with a prospective design. Studies of associations between vitamin D status and back pain have mostly been based on relatively small data sets involving patients, with some studies[10-14] suggesting an association between low vitamin D levels and back pain, while other studies[15-17] were unable to demonstrate any relationships. A cross-sectional study among schoolchildren also indicated such an association.[18] A small randomized clinical trial of patients with chronic LBP failed to show any effect of vitamin D supplementation.[19]

Potential associations between vitamin D deficiency and LBP have partly been ascribed to osteomalacia[12], with an accumulation of osteoid because of defective mineralization. Poor muscle strength induced by vitamin D deficiency[3] may also affect the experience of LBP. It is not certain, however, whether these factors play any role considering the incidence of low back pain at the population level.

Only prospective studies can show whether vitamin D levels affect the subsequent risk of experiencing LBP. It is possible that back pain conversely can affect vitamin D status,[11] perhaps through modified behaviour influencing exposure to sunlight or through nutritional factors, and for this reason it is essential to base conclusions on results from prospective studies. It is important to carry out adjustment for potential confounders such as obesity, which is related to both vitamin D levels and to risk of low back pain.[20-22] Vitamin D

levels are higher after sun exposure, with the prevalence of vitamin D deficiency varying seasonally,[23] so associations should also be assessed separately for the summer/autumn and winter/spring seasons.

This study will investigate whether an association can be established between vitamin D status and risk of LBP in a case-control study nested in a Norwegian cohort. Vitamin D status is based on measurement of the major circulating metabolite 25-hydroxyvitamin D (25(OH)D).[24] The importance of seasonality for blood sample collection for vitamin D measurement will be explored.

METHODS

Participants

The present work is based on information from the Nord-Trøndelag Health Study (HUNT). From 1995 to 1997, the large health survey HUNT2 was conducted in Nord-Trøndelag county in Norway. The entire adult population received a health questionnaire and participants underwent a clinical examination, including measurements of body weight and height.[25] In particular, each participant provided information in the questionnaire showing whether he or she had experienced chronic LBP during the preceding 12 month period, defined as LBP lasting at least three months continuously in that period. Blood samples were drawn at the clinical examination. In the HUNT3 survey, conducted in 2006 to 2008 in the same county with a corresponding target population, similar questionnaires were distributed.[25] Information about residence status was supplied by national registries and linked by use of the unique Norwegian personal identification numbers.

A nested case-control study was conducted using prospective data from HUNT2 regarded as baseline and HUNT3 regarded as follow-up, including 4822 individuals in the age range 19-55 years when attending HUNT2. Vitamin D status was assessed on the basis of blood samples from the participants collected in HUNT2 in 1995 to 1997, but the actual measurements were only carried out later. Participants belonged to three different subsamples. The first and second subsample consisted of random samples from HUNT2 and were established in connection with previous studies of the association between vitamin D and asthma and lung function,[26, 27] but in the current study all individuals with LBP in HUNT2 were excluded. The first subsample was analysed for 25(OH)D in 2010, and included

altogether 1492 persons (figure 1), comprising 247 cases (individuals with LBP in HUNT3) and 1245 controls (individuals without LBP in HUNT3). The second subsample with analysis date for 25(OH)D in 2015 included a total of 2051 persons (figure 1), comprising 343 cases and 1708 controls. The third subsample had measurements of 25(OH)D carried out in 2014/2015 and consisted of 1279 persons without LBP in HUNT2. Of these, a total of 1095 cases were selected at random among persons suffering from LBP in HUNT3 and 184 controls were randomly selected among persons without LBP in HUNT3 (figure 1). The third subsample was solely established for the current nested case-control study. None of the individuals included in this study had missing values for any covariate considered.

The original plan was that the nested case-control study would consist of the combined first and third subsamples only, and the size of the third subsample was adapted to power requirements in this data set. The second subsample only became available at a later stage.

Exposure

Blood samples collected in HUNT2 were stored at -70 °C until analysis. The level of 25(OH)D in serum was measured in the years 2010 and in 2014/2015 by chemiluminescent immunoassay (CLIA) methodology, using Liaison 25-OH Vitamin D Total assay (DiaSorin Inc., Saluggia, Italy). The method has an intraassay coefficient of variation of 4% and an interassay coefficient of variation of 8%. Another kit from DiaSorin was used from 2014. A total of 118 samples with measurements available from 2010 were reanalysed in 2015 with the new kit. After exclusion of 2 outliers, a conversion factor for measurements from 2010 to new values from 2014/2015 was established by Passing and Bablok regression.[28]

Serum 25(OH)D levels were classified into three groups <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, which are widely used categories in studies of vitamin D levels. Values <50.0 nmol/l are usually regarded as representing vitamin D deficiency.[29]

Covariate assessment

Baseline age was categorized into 10-year intervals. Four categories of work status were defined, the first comprising people being employed or carrying out professional work. The

second category included those temporarily out of work, students and individuals in military service. The third category included pensioners and people receiving social security support, and the fourth category represented women occupied full-time with housework. Those currently working supplied information about physical activity at work,[30] in four categories representing substantially sedentary work, work involving extensive walking, work leading to both walking and lifting, and work involving particularly strenuous activities. For physical activity in leisure time, including going to work, one category included those engaged in light activity only or hard physical activity (leading to sweating or being out of breath) <1 hour per week.[31] Other categories represented hard physical activity 1-2 and ≥3 hours per week.

Education was grouped according to duration as ≤9, 10-12, or ≥13 years. Categories of cigarette smoking represented current daily smoking, previous daily smoking and never daily smoking. BMI, defined as weight/height^2 and computed in kg/m^2, was subdivided into three groups: <25, 25-29.9, ≥30.

Season of blood sample collection was categorized as either the summer/autumn season (June through November) or the winter/spring season (December through May). This subdivision gives a marked difference in vitamin D deficiency between 6-months periods.[23]

Statistical methods

Associations between 25(OH)D levels in serum and risk of chronic LBP were assessed by unconditional logistic regression analysis with adjustment for potential confounders. Analyses were performed separately with 25(OH)D levels as categorical and continuous variables. All other variables were considered categorical. First, adjustments were carried out for age and sex and a factor indicating which subsample each individual belonged to. Additional adjustments for work status, physical activity at work and in leisure time, education, smoking, BMI and season of blood sample collection were then introduced. Separate tests were performed for interaction between continuous 25(OH)D level and all variables adjusted for. Linearity in the association with continuous 25(OH)D level was tested for by adding a quadratic term to the statistical model. All statistical analyses were carried out using IBM SPSS version 23 (IBM Corp., Armonk, New York).

Separate analyses were limited to participants who had the blood samples drawn in the summer/autumn season and the winter/spring season.

RESULTS

The age distribution was quite similar among cases and controls (table 1). The percentage of women was higher among cases than among controls. Within the three separate groups of serum 25(OH)D levels categorized as <50.0 nmol/l, 50.0-74.9 nmol/l and ≥75.0 nmol/l, the percentage of persons with and without LBP at end of follow-up were almost the same among cases and controls (table 1).

Table 1 Number of individuals by age, sex, vitamin D status and case-control status

	Cases, with LBP at end of follow-up		Controls, without LBP at end of follow-up	
	Number	**Percent**	**Number**	**Percent**
Age groups (years)				
19-29	246	15	504	16
30-39	492	29	951	30
40-49	687	41	1191	38
50-55	260	15	491	16
Sex				
Female	1043	62	1677	54
Male	642	38	1460	47
25(OH)D (nmol/l)				
<50.0	925	55	1689	54
50.0-74.9	619	37	1126	36
≥75.0	141	8	322	10

LBP, low back pain.

Table 2 Descriptive statistics of 25(OH)D level by case-control status

	Cases, with LBP at end of follow-up				Controls, without LBP at end of follow-up			
	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)	Number	Mean (nmol/l)	SD (nmol/l)	Median (nmol/l)
Total data set								
All seasons	1685	49.4	18.2	47.9	3137	50.0	19.1	48.0
Summer/autumn	773	54.1	18.4	52.0	1424	56.7	19.0	55.2
Winter/spring	912	45.4	17.0	43.8	1713	44.4	17.4	41.9
Women								
All seasons	1043	50.2	18.6	48.9	1677	50.5	19.0	49.0
Summer/autumn	480	53.8	18.7	52.9	784	56.2	18.7	54.4
Winter/spring	563	47.1	17.9	45.9	893	45.4	17.9	43.0
Men								
All seasons	642	48.0	17.4	45.8	1460	49.4	19.3	46.6
Summer/autumn	293	54.5	18.0	51.3	640	57.4	19.4	56.1
Winter/spring	349	42.6	14.9	40.8	820	43.2	16.7	40.4

LBP, low back pain; SD, standard deviation.

In the total data set, the mean serum 25(OH)D levels were quite similar among cases and controls (table 2). Both in women and men the serum 25(OH)D levels were higher in the summer/autumn than in the winter/spring season among cases and controls.

In the overall data set no association was found between vitamin D status and risk of LBP, neither with adjustment for age, sex and subsample nor with complete adjustment (OR per 10 nmol/l 25(OH)D= 1.01, 95% CI 0.97 to 1.06) (table 3). No significant interaction was found, but the interaction with sex was marginally significant (p=0.06). A weak positive association was suggested among women, although the estimated relation among men was in the opposite direction (table 3). Results did not differ significantly between subsamples.

Table 3 Associations between vitamin D status and risk of chronic LBP

	With adjustment for age, sex and subsample		With complete adjustment*	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.64†		0.97†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.94 (0.80 to 1.11)		1.01 (0.85 to 1.20)	
≥75.0	0.90 (0.68 to 1.18)		0.98 (0.73 to 1.31)	
Per 10 nmol/l	0.99 (0.95 to 1.03)	0.59	1.01 (0.97 to 1.06)	0.59
Women				
25(OH)D (nmol/l)		0.54†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.09 (0.88 to 1.36)		1.20 (0.96 to 1.51)	
≥75.0	0.91 (0.64 to 1.30)		1.05 (0.72 to 1.52)	
Per 10 nmol/l	1.02 (0.97 to 1.08)	0.48	1.06 (1.00 to 1.12)	0.054
Men				
25(OH)D (nmol/l)		0.10†		0.16†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.75 (0.57 to 0.98)		0.76 (0.57 to 1.01)	
≥75.0	0.90 (0.58 to 1.39)		0.88 (0.55 to 1.41)	
Per 10 nmol/l	0.94 (0.88 to 1.01)	0.10	0.94 (0.88 to 1.02)	0.13

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI, subsample and season of blood sample collection.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

Separate analyses of the association between vitamin D status and risk of LBP by season of blood sample collection revealed no effect in either sex in the summer/autumn season (table 4). However, in the winter/spring season associations differed significantly between women and men (p=0.004). In women a significant positive association was observed (OR per 10 nmol/l 25(OH)D =1.11, 95% CI 1.02 to 1.20) (table 4). In men the estimated association was

negative (OR per 10 nmol/l 25(OH)D =0.90, 95% CI 0.81 to 1.01) but did not reach statistical significance. Considering the different OR values from the categorical analyses, a consistent trend was suggested in each sex.

No significant deviations from linearity were observed in the relationships with 25(OH)D considered as a continuous variable.

Table 4 Associations between vitamin D status and risk of chronic LBP by season of blood sample collection*

	Summer/autumn		Winter/spring	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Total data set				
25(OH)D (nmol/l)		0.87†		0.79†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.93 (0.72 to 1.20)		1.08 (0.85 to 1.38)	
≥75.0	0.96 (0.66 to 1.39)		0.96 (0.58 to 1.58)	
Per 10 nmol/l	0.99 (0.93 to 1.06)	0.78	1.03 (0.97 to 1.10)	0.37
Women				
25(OH)D (nmol/l)		0.56†		0.27†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	1.13 (0.81 to 1.57)		1.26 (0.92 to 1.73)	
≥75.0	0.89 (0.53 to 1.47)		1.34 (0.76 to 2.35)	
Per 10 nmol/l	1.00 (0.92 to 1.09)	0.94	1.11 (1.02 to 1.20)	0.012
Men				
25(OH)D (nmol/l)		0.15†		0.20†
<50.0	1.00 (Reference)		1.00 (Reference)	
50.0-74.9	0.70 (0.46 to 1.05)		0.84 (0.56 to 1.27)	
≥75.0	1.01 (0.58 to 1.76)		0.37 (0.12 to 1.17)	
Per 10 nmol/l	0.97 (0.88 to 1.08)	0.58	0.90 (0.81 to 1.01)	0.08

*Adjustment for age, sex, work status, physical activity at work and in leisure time, education, smoking, BMI and subsample.

†For categorical effect.

LBP, low back pain; OR, odds ratio; CI, confidence interval; BMI, body mass index.

DISCUSSION

The association between vitamin D status and chronic LBP was examined in a case-control study nested in a population-based follow-up of a Norwegian cohort. No association between vitamin D status and risk of LBP was found overall. For measurements in the winter/spring season, associations differed significantly between women and men. Among women a positive association was seen, but among men no significant association was observed.

A strength of the study is that the overwhelming majority of participants belonged to a homogeneous ethnic group.[32] Information was available on potential confounders, which made it possible to carry out accurate adjustments. The risk factor considered is represented by the vitamin D status measured in blood samples drawn at the clinical examination in HUNT2. However, the blood samples were stored for many years at low temperature, and the measurements of serum 25(OH)D levels were carried out in 2010 or in 2014/2015. Despite attempts to standardize the measurements, use of different instruments in the two periods may have led to minor systematic deviations in the 25(OH)D levels. To some extent, this was accounted for by adjusting all statistical analyses for subsample. This procedure was also essential in view of the different sampling procedures applied to establish the subsamples. The Liaison immunoassay method may still underestimate true 25(OH)D levels[33] and a direct comparison with values found by other methods may not be justified.[34]

A limitation is the lack of information about back pain occurring at other times in the 11-year follow-up interval between HUNT2 and HUNT3. The case definition only refers to the last year before collection of information in HUNT3. Thus all cases must have experienced incident chronic LBP during follow-up but so may some of the controls in the intervening period if they later recovered. Any real association between vitamin D status and LBP should be present anyhow but may be more difficult to detect. Furthermore, the extent of back pain was not characterized by the participants.

Another potential problem is the relatively long period between collection of information about risk factors and the recording of LBP status. In view of the weaker associations seen between 25(OH)D levels and mortality and cancer risk in prospective studies with longer follow-periods, it has been suggested that 25(OH)D levels should be measured at regular

intervals in prospective studies, perhaps every 2-4 years.[35] Such information was not available in the present study. However, vitamin D levels have been shown to be relatively stable over periods as long as 14 years in the Norwegian population,[36] and large data sets in other countries have shown a definite degree of stability over somewhat shorter periods.[37, 38] In data with measurements from the HUNT2 survey, low 25(OH)D levels showed a clear association with all-cause mortality in a prospective study with a median follow-up time of 18.5 years.[39] Thus potential relationships between 25(OH)D levels and disease should still persist in a study such as the current one with 11 years of follow-up, although associations may be attenuated. The state of relevant confounders may also change during a long follow-up. An alternative to the design used here would be a cross-sectional case-control study based on both 25(OH)D levels and reports of chronic LBP from the HUNT2 survey. Problems concerning 25(OH)D levels changing over time would be eliminated but it would not be possible to rule out an influence of disease on 25(OH)D status.

There are few population-based studies of relationships between vitamin D status and LBP.[9] Some studies have investigated such relationships among patients,[11, 13, 16] but population-based studies of risk of LBP with a prospective design have been lacking. The present study is to our knowledge the only prospective study of the association between vitamin D status and risk of LBP.

In cross-sectional population-based studies, an association between low level of 25(OH)D and prevalence of back pain has been found in older women[9] and in schoolchildren.[18] Some case-control studies have shown an association between low levels of vitamin D and occurrence of back pain,[12, 14] but others do not support an association.[15, 17] In a recent small Swedish case-control study no difference in vitamin D levels could be established between participants with chronic LBP and matched controls.[17] This is consistent with the overall results found in our study.

Among women with blood samples drawn in the winter/spring season, we found that high levels of vitamin D were associated with an increased risk of LBP, which is the opposite of what was initially hypothesized. In a Danish cross-sectional study of LBP patients an association in the same direction was found, and normal levels of vitamin D, as opposed to vitamin D deficiency, were associated with more Modic changes in the lumbar vertebral end plates as seen on MRI.[16]

An association between vitamin D deficiency and adiposity has been found in several studies.[21] This was also confirmed in a cross-sectional study in the HUNT population.[23] In a prospective study using data from HUNT2 and HUNT3, overweight and obesity were found to be associated with a predisposition to chronic LBP.[22] This suggests that BMI could be a confounder in the association with 25(OH)D. However, our complete analyses included adjustment for BMI, and it is not to be expected that any substantial relationship should remain because of the association with adiposity.

In view of the seasonal variation in sun exposure, it is important to look at the time when blood samples are collected to define vitamin D deficiency.[40] Thus in a study of bone mineral density the summer season was found to be the best period to determine the serum 25(OH)D level.[41] This contrasts with our results, with a positive association between LBP and vitamin D status observed in the winter/spring season for women only. Elevated levels of 25(OH)D may be harmful in other respects.[42] In a study of mortality among hospitalized patients a U-shaped relationship was found, with both low and high values of 25(OH)D associated with increased mortality.[42, 43] However, the 25(OH)D levels had to be considerably above the typical values in the present study to be associated with higher mortality and a causal relationship was not inferred, so such effects at the upper end of the range for 25(OH)D are hardly relevant here. False U-shaped relationships between 25(OH)D levels and health problems may easily appear when individuals with poor health have only recently started using vitamin D supplement and are thus essentially misclassified.[44]

Gender and sex hormone levels are important variables that can influence a possible effect of vitamin D in rheumatic diseases.[45] In a Danish population receiving UVB treatment, the decline in 25(OH)D varied over time between sexes, with women maintaining a greater half-life of 25(OH)D.[46] It is not evident, however, how such differences can explain the sex contrast seen in the present study in the association with risk of LBP.

Measurements of 25(OH)D levels made during the winter/spring season may possibly capture variability of vitamin D in a better way, because vitamin D values in winter are not so dependent on sunlight exposure. It is possible, however, that women tending to report LBP in this population are particularly health-conscious and use more vitamin D supplements during the winter/spring season, creating a false positive association. Cod liver oil supplement has traditionally represented a major source of vitamin D in Norway,[47] with about 35% of both the male and female populations using such supplement in the 1990s.[48] Whole-year usage

among females is associated with poor perceived health but is otherwise associated with a healthy diet,[47] suggesting that use of supplements is not in general matched to the vitamin D needs.[47] In any case, the significant relationship observed among women may be spurious due to the multiple statistical tests carried out. Thus this association cannot yet be regarded as causal.

A basic biological relationship between vitamin D status and risk of LBP would be expected to be similar in different populations. Unless the lack of overall association seen in the present study represents a chance finding, the result should also apply to other populations. However, the population distribution of vitamin D status may vary substantially between countries because of differences in sunlight exposure and dietary conditions. Thus it is not obvious that our main result can be generalized to other widely different populations, and further studies are still called for.

CONCLUSIONS

In this population-based nested case-control study, no overall association between vitamin D status and risk of LBP was found. The positive association observed in women with blood samples drawn in the winter/spring season needs confirmation from other studies.

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Competing interests None declared.

Ethics approval The work was approved by the Regional Committee for Medical and Health Research Ethics in Central Norway, and HUNT was also approved by the Norwegian Data Inspectorate. Each participant in the HUNT2 and HUNT3 surveys signed a written informed consent regarding the collection and use of data for research purposes.

Data sharing statement The data set analysed belongs to a third party, the HUNT study (the Nord-Trøndelag Health Study). The authors of the current manuscript are not affiliated with the project as such, but have been given permission to analyse the data after obtaining the necessary Norwegian permits. Because of the confidentiality requirements according to Norwegian law, a data set of this kind with information from a complete county at the individual level cannot be made public. However, research groups wishing to analyse data from the HUNT study may apply to the HUNT organisation (http://www.ntnu.edu/hunt) to get access to the data, after having obtained the permits needed according to Norwegian law.

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Figure legend

Figure 1 Flow chart showing participants for statistical analysis in the nested case-control study.

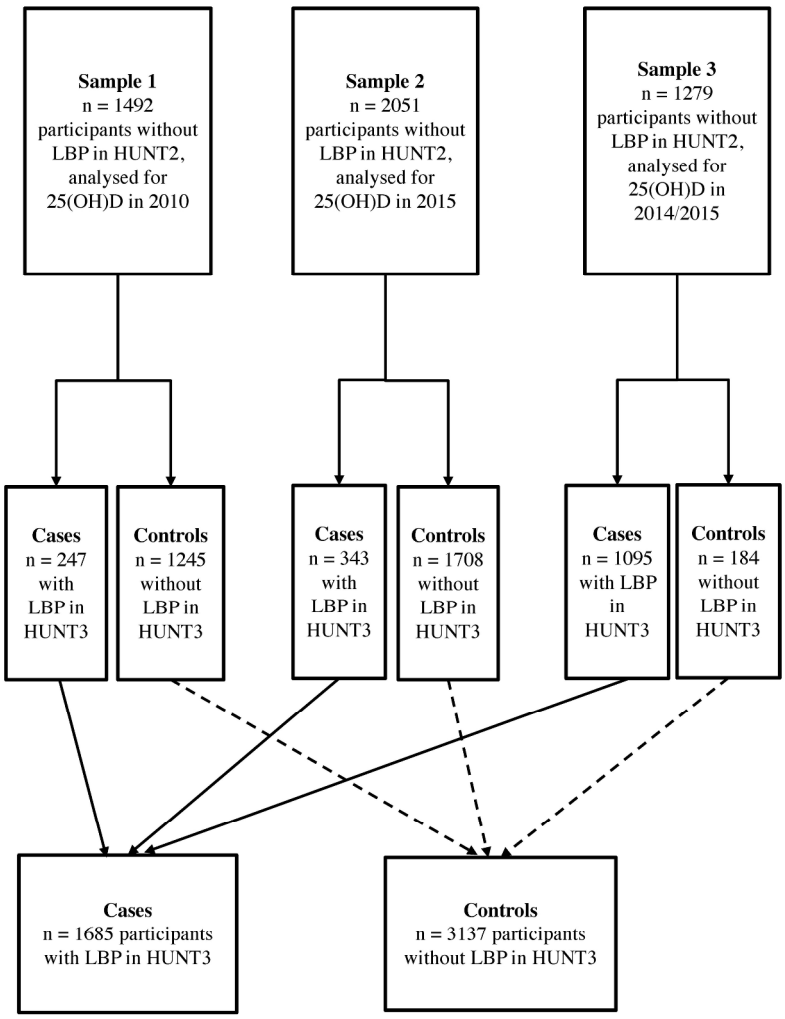

Figure 1 Flow chart showing participants for statistical analysis in the nested case-control study.

254x338mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any pre-specified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	9	Describe any efforts to address potential sources of bias	12-13
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-9
		(b) Indicate number of participants with missing data for each variable of interest	6
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	8
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10
		(*b*) Report category boundaries when continuous variables were categorized	6-7
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.