Chapter 6

The association between vitamin D status and parameters for bone density and quality is modified by body mass index

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ABSTRACT

Objective

The association of vitamin D status with bone mineral density (BMD) and Quantitative Ultrasound measurements (QUS) has been inconsistent in previous studies, probably caused by moderating effects. This study explored 1) the association of vitamin D status with QUS and BMD, and 2) whether these associations were modified by body mass index (BMI), age, gender or physical activity.

Methods

Two independent cohorts of the Longitudinal Aging Study Amsterdam (LASA-I, 1995/1996, aged ≥65; LASA-II, 2008/2009, aged 61-71) and baseline measurement of the B-vitamins for the PRevention Of Osteoporotic Fractures (B-PROOF) study (2008-2011, aged 65+) were used. QUS-measurements (Broadband Ultrasound Attenuation (BUA) and Speed of Sound (SOS)) were performed at the calcaneus in all three cohorts (N = 1235, N = 365, N = 1319); BMD was measured by Dual-energy X-ray absorptiometry (DXA) in B-PROOF (N = 1162 and 1192, for specific sites) and LASA-I (N = 492 and 503).

Results

The associations of vitamin D status with BUA and BMD were modified by BMI. Only in persons with low-to-normal BMI (< 25kg/m^2), serum 25(OH)D < 25 nmol/L was associated with lower BUA as compared to the reference group (≥ 50 nmol/L) in LASA-I and B-PROOF. Furthermore, in LASA-I, these individuals had lower BMD at the hip and lumbar spine. In LASA-II, no associations with BUA were observed. Vitamin D status was not associated with SOS and these associations were not modified by the effect modifiers tested.

Conclusion

The association between vitamin D status and BUA and BMD was modified by BMI in the older aged cohorts: there was only an association in individuals with BMI< 25 kg/m^2.

INTRODUCTION

Vitamin D deficiency is common in older individuals, with a prevalence up to 90% depending on the definition of deficiency used, age, gender, lifestyle and the method used for the assessment of vitamin D status [1;2]. The classical function of vitamin D is to increase calcium absorption from the gut in order to permit the mineralization of bone [2]. However, in the last years, vitamin D has also been proposed to play a role in physical performance, the immune system, the cardiovascular system, the nervous system and related diseases [3-8].

Adequate levels of serum 25-hydroxyvitamin D (25(OH)D) are essential for developing and maintaining bone health [2]. In addition, vitamin D deficiency is associated with higher risks of falls and fractures, especially in the older population [9]. Falls and fractures are a predictor of future morbidity and mortality [10;11]. Therefore, monitoring individual's bone health can be valuable, because intervention to improve bone health, with for example, bisphosphonates, is available [12].

Nowadays, the gold standard for the measurement of bone mineral density (BMD) is Dual-energy X-ray absorptiometry (DXA), which can generally only be performed in hospitals. In addition, measurements are relatively time-consuming and expensive [13-15]. An alternative method is quantitative ultrasound measurement (QUS) at the calcaneus or other peripheral skeletal sites. This method has some advantages over DXA: it is simple to use and portable, it does not use ionizing radiation and it provides information on the quality of bone. QUS measures the speed at which sound propagates through bone (speed of sound (SOS) in m/s) and the pattern of attenuation of ultrasonic frequencies in bone (broadband ultrasound attenuation (BUA) in dB/MHz) [13;15]. QUS parameters are associated with bone mineral density and both predict fracture risk [16;17].

Data on the association of vitamin D status with both BMD and QUS are contradicting; some studies found significant positive associations, whereas others did not [13;14;18-21]. These contradictory findings may be caused by differences in patient characteristics, i.e., caused by a moderating effect of patient characteristics. For example, previous studies observed different results in different ethnic groups [19] or at different levels of vitamin D binding protein [21]. It could be hypothesized that other factors, such as body mass index (BMI), gender, age and physical activity, play a role in the association of vitamin D status with BMD and QUS. Higher BMI and higher physical activity are related to better bone health [22;23]. Therefore, we hypothesized that low vitamin D status has less impact in individuals with high BMI or a high physical activity level.

This study aimed to determine whether 25(OH)D levels were associated with BMD and QUS measurements. In addition, the influence of several potential effect modifiers was studied. Furthermore, plots were made to visually estimate the optimal cut-off points of

serum 25(OH)D with respect to QUS. To answer these questions data from three different cohorts were used.

METHODS

Study participants

Data for this study were collected within the framework of the Longitudinal Aging Study Amsterdam (LASA) and the B-vitamins for the PRevention Of Osteoporotic Fractures study (B-PROOF). LASA is an ongoing cohort study on the different aspects of aging, whereas B-PROOF is a multicentre randomized double-blind placebo-controlled trial on the effects of B-vitamins on the prevention of osteoporotic fractures.

The sampling and data collection procedures of LASA are described elsewhere in detail [24;25]. Briefly, a random age and sex-stratified sample was drawn from population registries of 11 municipalities in three different regions of the Netherlands. At the start in 1992, all 3107 participants were aged 55-85 years (LASA-I). An additional cohort started in 2002 (n=1002, aged 55-65 years, LASA-II). The study was approved by the Medical Ethics Committee of the VU University Medical Center, and all participants gave informed consent. For the current study, the second measurement cycle of the first cohort (LASA-I) was used (1995/1996). Participants aged 65 years and older, as of January 1st, 1996, and participating in the medical interview in addition to the main interview, were invited for QUS measurements. Data on QUS were available for 1327 individuals. After exclusion due to missing values for potential confounders (N = 92), the total study sample consisted of 1235 participants. Participants living in Amsterdam and surroundings were invited to undergo a DXA measurement. BMD data in LASA-I were available for 535 participants. After exclusion because of hip protheses in situ at the measured site (N = 13), and missing values for potential confounders (N = 30), the study sample consisted of 492 (hip measurements) and 503 (lumbar spine measurements) participants. In addition, the third measurement cycle of LASA-II (2008/2009, aged 61-71 years) was used. QUS measurements were performed in a random subsample, N = 464. After exclusion due to missing values regarding potential confounders (N = 99), the total study sample consisted of 365 individuals.

The sampling and data collection procedures of the multicentre B-PROOF study are described elsewhere [26]. In short, the participants were mainly recruited via population registries and general practitioners. All participants were screened for plasma homocysteine and were only included if they had homocysteine concentrations ≥12 µmol/L (n = 2919, aged 65 years and older). All participants gave informed consent before the start

of the study. The study was approved by the Medical Ethics Committee (MEC) of the Wageningen University and the MEC of the VU Medical Center (Amsterdam) and Erasmus MC (Rotterdam) confirmed local feasibility. For the present study, only baseline data of participants with either BMD or QUS measurements were used (n = 1823). Valid QUS measurements were available for 1364 participants, BMD measurements were performed in 1223 participants. After exclusion due to missing values for potential confounders, the study sample consisted of 1319 (QUS), 1162 (femoral neck BMD) or 1193 (lumbar spine BMD) participants.

To answer the first research question on vitamin D in relation to QUS measurements, LASA-II and B-PROOF were used. To answer the research question on vitamin D in relation to BMD, only B-PROOF was used. Results of these analyses in LASA-I were published previously [20]. To answer the second question, i.e., whether the associations were modified by several effect modifiers, all three cohorts were used. In the previous analyses within LASA-I, no analyses on interactions were reported [20]. Therefore, we re-analysed this data including interaction terms.

QUS measurements

In LASA-I, QUS measurements were performed using the Cuba Clinical Instrument (McCue, Winchester, UK). In LASA-II and in B-PROOF, measurements were performed using the Hologic Sahara bone densitometer (Hologic Inc., USA).

In both studies BUA and SOS were measured twice on both left and right calcaneus. In LASA-II and in B-PROOF a third measurement was performed if the first two measurements differed for more than 10 %. After each measurement the foot was repositioned. Mean values per foot were calculated using the first two measurements; if these values differed for more than 10 %, the mean of the first and third (or second and third) measurement was calculated. Finally, mean values for BUA and SOS were calculated by calculating the mean of both measurements of the right and the left foot.

Bone mineral density measurements

In a subsample of the first cohort of LASA, Hologic QDR 2000 scanner (Hologic Inc., Waltham, MA, USA) was used to measure BMD. In the B-PROOF study different devices were used in two participating centres. In the VU University Medical Center the Hologic QDR 4500 Delphi device (Hologic Inc., USA) was used and in the Erasmus MC the GE Lunar Prodigy device (GE Healthcare, USA) was used. The two devices were cross-calibrated;

measurements performed with the Hologic device were transformed into values comparable to the values measured with the GE Lunar Prodigy device.

Serum 25(OH)D measurements

Morning blood samples were drawn in 1995/1996 of the participants of LASA-I. Participants were allowed to take tea and toast, but no dairy products. In 2008/2009, fasting blood samples were drawn of the participants of LASA-II. Participants were not allowed to take any food or drinks from midnight. The samples were centrifuged and stored at -20 ˚C until determination in 1997/1998 and 2010/2011 for LASA-I and LASA-II samples, respectively. Serum 25(OH)D was measured using a competitive binding protein assay (1997/1998: Nichols Diagnostics) and a radioimmunoassay in 2010/2011 (DiaSorin). The interassay coefficients of variation were 10% with both assays.

The participants of B-PROOF were fasting or had a light breakfast. Samples were stored at -80˚C until determination in 2011/2012. Serum 25(OH)D was measured by isotope dilution-online solid phase liquid chromatography – tandem mass spectrometry (ID-XLC-MS/MS)[1;6]. The interasssay coefficient of variation was 9% at the level of 25 nmol/L and 6% at the level of 63 nmol/L. All analyses were performed at the Endocrine Laboratory of the VU University Medical Center.

Potential effect modifiers

Age, gender, physical activity and BMI were examined as potential effect modifiers. As was explained in the introduction, BMD and QUS values have been reported to be higher in participants with higher BMI or a higher level of physical activity [22;23]. Therefore, it can be hypothesized that vitamin D deficiency is less harmful for bone when BMI or physical activity level is high.

It is known that vitamin D metabolism differs between sexes [27] and also changes with advancing age [28]. Therefore, it can be hypothesized that vitamin D also has different influences on bone within different sexes or age categories.

Physical activity was assessed in all cohorts using the LASA Physical Activity questionnaire, which is a validated interviewer-administered questionnaire on the duration and frequencies of the activities of the last two weeks [29]. BMI was calculated by dividing measured weight in kilograms by measured height in square meters.

Potential confounders

Potential confounders included age, gender, level of education, smoking, alcohol consumption, creatinine, season of blood collection, physical activity, BMI, chronic diseases, the use of vitamin supplements, and the level of urbanization.

The highest attained level of education was converted into years of education and subsequently divided into three groups: low (≤ 9 years), intermediate (10-12 years), and high level (> 12 years). Smoking behaviour (never, former, and current smoker) and alcohol consumption (none, light, moderate and (very) excessive drinker) were both based on self-report. Alcohol consumption was divided in the mentioned categories according to the number of days alcohol was consumed and the number of drinks per time [30]. Serum creatinine was measured with the Hitachi 747 analyser (LASA) or the enzymatic colorimetric Roche CREA plus assay (B-PROOF). Season of blood collection was dichotomized in summer (April-September) and winter (October-March). The number of chronic diseases in both LASA cohorts was assessed by asking questions on seven major chronic diseases: chronic obstructive pulmonary disease, cardiac disease, stroke, peripheral arterial disease, diabetes mellitus, cancer, and rheumatoid arthritis/osteoarthritis. In B-PROOF, chronic diseases were less extensively assessed; information on kidney disease, cardiac disease, diabetes mellitus, and transient ischemic attack/stroke was available for a subsample only. The degree of urbanization was assessed using the classification of Statistics Netherlands, which recodes the postal codes of The Netherlands into five categories, based on the number of addresses per square kilometre [31]. Vitamin supplement use was based on self-report, by asking a question on over-the-counter vitamin tablets use.

Statistical analysis

Serum 25(OH)D was divided into three categories, due to non-linearity with the outcomes measures: <25 nmol/L, 25-50 nmol/L and ≥ 50 nmol/L, the last serving as reference category in all analyses. Because in LASA-II only a few participants had serum 25(OH)D < 25 nmol/L, we created only two categories: < 50 nmol/L and ≥ 50 nmol/L. In the Netherlands, the required level of serum 25(OH)D is 50 nmol/L or higher for persons of 50 years and older, similar to the guidelines of the Institute of Medicine [32;33].

Multiple linear regression analyses were used to determine the associations between vitamin D status and QUS measurements and BMD. Assumptions of linear regression analyses were tested by normal probability plots and histograms.

All independent continuous variables were tested on linearity. Only BMI had a non-linear relationship with the outcomes and therefore, BMI was divided into low (< 20

kg/m^2), normal (20-25 kg/m^2), and high (≥ 25 kg/m^2) BMI. To test for effect modification, interaction terms between serum 25(OH)D and the potential effect modifiers (age, gender, physical activity and BMI) were included in the regression models. A *P*-value < 0.1 for the interaction term(s) was considered significant. If a significant interaction term was found, all deciles of the continuous variables were tested separately with different interaction terms to determine the optimal cut-off point for defining subgroups. If a common cut-off point exist in the literature, this cut-off point was chosen if the optimal decile was close to that point. Subsequently, stratified analyses were run to estimate the association of serum 25(OH)D with BMD and QUS for each subgroup.

To test for confounding, all potential confounders were added separately to the univariable model. Parameters that changed the regression coefficient at least 10% were added to the models. For all models, a *P*-value <0.05 was considered significant. Sensitivity analyses were performed by adding multi vitamin supplement use in all cohorts and chronic diseases in B-PROOF to all models. All analyses were performed using SPSS version 20.

Finally, we used restricted cubic spline plots to visually estimate an optimal cutoff point for serum 25(OH)D in the relationship with QUS measurements. Cubic splines are piecewise polynomial functions that are constrained to join smoothly at points called knots. Restricted cubic spline functions use all data points to estimate the risk at each level of exposure. Cubic spline functions were tested in regression models at three knots using spline plots and likelihood ratio tests. Spline plots were only made if a significant association was found in the multivariable regression analyses. All spline regression analyses were performed using R version 2.15.0 [34].

RESULTS

The participant characteristics are shown in Table 1. Mean serum 25(OH)D (SD) concentrations were 53.8(24.1), 70.1 (22.2) and 56.3 (24.3) nmol/L, for LASA-I, LASA-II, and B-PROOF, respectively.

Table 2 presents the multivariable results for the cross-sectional analyses of the association between vitamin D status and BUA measurements. In all three cohorts, no significant associations were found when analyzing the total population. In B-PROOF and LASA-I, significant effect modification by BMI was observed (p < 0.1), and therefore the associations shown are stratified for those with BMI < 25 kg/m^2 and ≥ 25 kg/m^2. Because the optimal cut-off was close to 25 kg/m^2 in both cohorts, this value was used as cut-off point.

Table 1. Characteristics of the study populations

	LASA-I N = 1235	LASA-II N = 365	B-PROOF N = 1823[1]
Gender, % women	51.5	49.3	49.5
Age, years	75.4 (6.5)	65.6 (2.9)	73.5 (6.3)
Serum 25(OH)D, nmol/L	53.8 (24.1)	70.1 (22.2)	56.3 (24.3)
< 25 nmol/L, %	10.4	-	7.2
25-50 nmol/L, %	37.2	19.5[2]	36.0
≥ 50 nmol/L, %	52.4	80.5	56.7
BUA, dB/MHz	70.9 (20.2)	72.0 (19.6)	73.3 (19.2)
SOS, m/sec	1622.6 (49.5)	1546.0 (33.1)	1542.0 (36.6)
BMD Femoral neck , g/cm^2	0.66 (0.14)	-	0.89 (0.23)
BMD Total hip, g/cm^2	0.70 (0.13)	-	-
BMD L1-L4, g/cm^2	0.98 (0.19)	-	1.18 (0.23)
BMD trochanter, g/cm^2	0.85 (0.16)	-	-
No. of chronic diseases	1 (0-2)[3]	1 (0-2)[3]	0 (0-1)[4]
Physical activity, min/day	135 (79-205)	150 (10-210)	133 (87-195)
Level of education, %			
Low (≤ 9 years)	61.1	35.3	53.6
Intermediate (10-12 years)	27.2	39.5	21.0
High (> 12 years)	11.7	25.2	25.5
Degree of urbanization, no. addresses/km^2, %			
Rural (<500)	22.4	21.1	3.9
Low (500-1000)	20.8	22.7	13.7
Moderate (1000-1500)	13.9	16.7	20.4
High (1500-2500)	17.8	22.7	42.1
Very high (>2500)	25.1	16.7	19.9
Season of blood collection, % winter	54.3	12.1	46.8
Body Mass Index , %			
< 20 kg/m^2	3.8	1.9	1.8
20-25 kg/m^2	30.7	29.0	28.0
≥ 25 kg/m^2	65.5	71.0	70.2
Smoking behaviour, %			
Non-smoker	35.7	25.5	33.2
Former- smoker	46.4	55.5	57.0
Current smoker	17.9	19.9	9.7
Alcohol use, %			
Nondrinker	24.0	8.2	13.8
Light drinker	50.3	52.3	52.5
Moderate drinker	19.8	33.4	29.4
(very) excessive drinker	5.9	6.1	4.3
Creatinine, µmol/L	94.1 (26.6)	80.5 (15.6)	83.6 (17.6)

Values are means (SD), median (interquartile range), or percentages.

[1] Number of participants is based on the participants with either QUS and/or BMD measurements

[2] Percentage for serum 25(OH)D < 50 nmol/L

[3] Chronic diseases from seven majors: chronic obstructive pulmonary disease, cardiac disease, peripheral arterial disease, stroke, diabetes mellitus, rheumatoid arthritis/osteoarthritis, and cancer.

[4] Chronic diseases from four diseases: kidney disease, diabetes mellitus, cardiac disease, and transient ischemic attack/stroke, N = 1361

In the low-to-normal BMI-group (< 25 kg/m^2), a low vitamin D status (< 25 nmol/L) was significantly associated with lower BUA scores, both in LASA-I and B-PROOF. In persons with low-to-normal BMI, individuals with serum 25(OH)D <25 nmol/L had 7.1 (95 % confidence interval (CI) 0.9-13.3, LASA-I) and 6.6 (95 % CI 0.4-12.8, B-PROOF) dB/MHz lower mean BUA values as compared to individuals with serum 25(OH)D ≥ 50 nmol/L. In persons within the high BMI group in LASA-I and B-PROOF and in all individuals in LASA-II, no associations between vitamin D status and BUA values were found. A significant interaction effect for gender was observed in LASA-I, with the strongest association of vitamin D and BUA in men. However, for men and women separately, the results were not statistically significant (data not shown). No interactions were observed within the LASA-II cohort.

Table 2. Associations between vitamin D status and Quantitative Ultrasound Measurements: broadband ultrasound attenuation (BUA) (dB/MHz)

		< 25 nmol/L	25-50 nmol/L	≥50 nmol/L (reference group)	Explained variance (R^2)
LASA-I N = 1235	Whole sample	-1.8 (-5.4;1.7) N = 129	-0.2 (-2.4;2.0) N = 460	0 N = 646	0.271
	BMI < 25 kg/m^2	-7.1 (-13.3;-0.9)* N = 43	-1.7 (-5.7; 2.2) N = 142	0 N = 241	0.316
	BMI ≥ 25 kg/m^2	0.0 (-4.1;4.1) N = 86	-0.2 (-2.8;2.3) N = 318	0 N = 405	0.275
LASA-II N = 365	Whole sample		-0.4 (-5.3;4.4)[1] N = 71	0 N = 294	0.226
B-PROOF N = 1319	Whole sample	-2.7 (-6.3;0.8) N = 94	0.9 (-1.1;2.8) N = 451	0 N = 774	0.266
	BMI < 25 kg/m^2	-6.6 (-12.8;-0.4)* N = 30	-1.2 (-4.7;2.3) N = 132	0 N = 228	0.370
	BMI ≥ 25 kg/m^2	-1.2 (-5.5;3.1) N = 64	1.5 (-0.8;3.8) N = 319	0 N = 546	0.225

Values are unstandardized B (95% confidence interval), indicating the absolute differences in mean BUA (dB/MHz) compared to the reference group (serum 25 (OH)D ≥ 50 nmol/L)
Analyses were separated in two categories of Body Mass Index (BMI) if the interaction term was significant (P < 0.1). Analyses were adjusted for relevant confounders, i.e., age, gender, physical activity and smoking for LASA-I, and age, gender, smoking, alcohol use, level of education, BMI, chronic diseases and degree of urbanisation for LASA-II, and age, gender, and creatinine for B-PROOF.
* P <0.05
[1] results for < 50 nmol/L versus ≥ 50 nmol/L

Table 3 presents the multivariable associations between vitamin D status and SOS measurements. Low vitamin D status was not significantly associated with lower SOS values in any of the cohorts, when analyzing the total populations. Only in B-PROOF, a significant interaction with BMI was observed. Participants with serum 25(OH)D 25-50 nmol/L within the high BMI category had higher SOS values (6.3 (95% CI 1.6;11.0) m/s) than the reference category (serum 25(OH)D ≥ 50 nmol/L). No interactions were found within LASA-I and LASA-II.

Table 3. Associations between vitamin D status and Quantitative Ultrasound Measurements: speed of sound (SOS) (m/s).

		< 25 nmol/L	25-50 nmol/L	≥50 nmol/L (reference group)	Explained variance (R^2)
LASA-I N = 1235	Whole sample	-6.0 (-15.2; 3.2) N = 129	-5.8 (-11.6; -0.01)* N = 460	0 N = 646	0.185
LASA-II N = 365	Whole sample		-2.0 (-10.4;6,5)[1] N = 71	0 N = 294	0.178
B-PROOF N = 1319	Whole sample	2.2 (-5.3;9.8) N = 94	5.0 (0.9;9.0)* N = 451	0 N = 774	0.149
	BMI < 25 kg/m^2	-4.3 (-18.7;10.2) N = 30	1.7 (-6.3;9.7) N = 132	0 N = 228	0.208
	BMI ≥ 25 kg/m^2	3.8 (-5.1;12.7) N = 64	6.3 (1.6;11.0)** N = 319	0 N = 546	0.127

Values are unstandardized B (95% confidence interval), indicating the absolute differences in mean SOS (m/s) compared to the reference group (serum 25 (OH)D ≥ 50 nmol/L)
Analyses were separated in two categories of Body Mass Index (BMI) if the interaction term was significant ($P < 0.1$). Analyses were adjusted for relevant confounders, i.e age, gender, smoking, alcohol consumption and degree of urbanisation for LASA-I, and age, gender, smoking, serum creatinine, season of blood collection, level of urbanization, alcohol consumption, and physical activity for LASA-II, and age, gender, creatinine, smoking, physical activity, season of blood collection, and degree of urbanisation for B-PROOF.
[1] results for < 50 nmol/L versus ≥ 50 nmol/L
* $P < 0.05$, ** $P < 0.001$

Table 4 presents the results of the multivariable associations of vitamin D status and BMD measured at several body sites. When analyzing the whole cohorts, only in LASA-I, low vitamin D status (<25 nmol/L) was associated with lower BMD of the trochanter (-0.05 (-0.08;-0.01) as compared to the reference group). In LASA-I, all associations were modified by BMI; these associations were more pronounced within the low-to-normal BMI group (< 25kg/m^2) than in the high BMI group. Vitamin D status was not clearly associated with BMD measured at any site in B-PROOF. Only individuals with serum 25(OH)D 25-50 nmol/L had significantly lower BMD of the lumbar spine as compared to the reference group (serum 25(OH)D ≥ 50 nmol/L). Gender modified the relationship of vitamin D status with the BMD of the total hip and lumbar spine. However, for men and women separately

the results were not statistically significant (data not shown). Table 5 presents some sample characteristics according to the different BMI groups in LASA-I and B-PROOF. These characteristics were not given for LASA-II as no significant interactions with BMI were found within this cohort.

Table 4. Associations between vitamin D status and bone mineral density (BMD) (g/cm^2) of different sites, in BPROOF and LASA-I

			< 25 nmol/L	25-50 nmol/L	≥50 nmol/l	Explained variance (R^2)
LASA-I	Femoral neck BMD N = 492	Whole sample	-0.01 (-0.05;0.03) N = 56	0.01 (-0.02;0.03) N = 193	0 N = 243	0.152
		BMI < 25 kg/m^2	-0.05 (-0.11;0.01) N = 19	-0.00 (-0.04;0.04) N = 58	0 N = 91	0.196
		BMI ≥ 25 kg/m^2	0.00 (-0.04;0.05) N = 37	0.01 (-0.02;0.04) N = 135	0 N = 152	0.157
	Lumbar spine BMD N = 503	Whole sample	-0.03 (-0.08;0.03) N = 59	0.03 (-0.01;0.06) N = 197	0 N = 247	0.139
		BMI < 25 kg/m^2	-0.11 (-0.19;-0.02)* N = 19	0.01(-0.04;0.07) N = 59	0 N = 93	0.204
		BMI ≥ 25 kg/m^2	-0.01 (-0.08;0.06) N = 40	0.02 (-0.03;0.06) N = 138	0 N = 154	0.148
	Trochanter BMD N = 492	Whole sample	-0.05 (-0.08;-0.01)* N = 56	0.01 (-0.02;0.03) N = 193	0 N = 243	0.269
		BMI < 25 kg/m^2	-0.09 (-0.15;-0.03)** N = 19	-0.01 (-0.05;0.03) N = 58	0 N = 91	0.330
		BMI ≥ 25 kg/m^2	-0.04 (-0.08;0.01) N = 37	0.00 (-0.03;0.03) N = 135	0 N = 152	0.282
	Total hip BMD N = 492	Whole sample	-0.03 (-0.08;0.01) N = 56	0.01 (-0.02;0.03) N = 193	0 N = 243	0.254
		BMI < 25 kg/m^2	-0.09 (-0.16;-0.02)* N = 19	-0.02 (-0.07;0.03) N = 58	0 N = 91	0.302
		BMI ≥ 25 kg/m^2	-0.02 (-0.07;0.03) N = 37	0.01 (-0.03;0.04) N = 135	0 N = 152	0.279
B-PROOF	Femoral neck BMD N = 1162	Whole sample	-0.03 (-0.06;-0.00) N = 84	0.00 (-0.02; 0.01) N = 445	0 N = 633	0.279
	Lumbar spine BMD N = 1193	Whole sample	-0.01 (-0.05; 0.04) N = 87	-0.03 (-0.05;0.00)* N = 459	0 N = 647	0.224

Values are unstandardized B (95% confidence interval), indicating the absolute differences in mean BMD (g/cm^2) compared to the reference group (serum 25 (OH)D ≥ 50 nmol/L). Analyses were separated in two categories of Body Mass Index (BMI) if the interaction term was significant ($P < 0.1$).

Analyses were adjusted for relevant confounders, i.e. age, gender, BMI, creatinine, smoking, degree of urbanisation, and season of blood collection for B-PROOF, and age, gender, smoking and level of urbanisation in LASA-I.

* $P < 0.05$

** $P < 0.01$

Table 5. Sample characteristics according to different BMI groups in LASA-I and B-PROOF

	LASA-I		B-PROOF	
	BMI < 25 kg/m^2	BMI ≥ 25 kg/m^2	BMI < 25 kg/m^2	BMI ≥ 25 kg/m^2
N	426	809	543	1280
Gender, % women	48.0	53.3	51.4	48.7
Age, years	75.7 (6.6)	75.3 (6.5)	74.2 (6.4)	73.3 (6.2)
Serum 25(OH)D, nmol/L	56.6 (25.2)	52.2 (23.5)	59.0 (25.8)	55.2 (23.5)
No. of chronic diseases	1.0 (0-2)[1]	1.0 (0-2)[1]	0 (0-1)[2]	0 (0-1)[2]

Values are means (SD), median (interquartile range), or percentages.
[1] Chronic diseases from seven majors: chronic obstructive pulmonary disease, cardiac disease, peripheral arterial disease, stroke, diabetes mellitus, rheumatoid arthritis/osteoarthritis, and cancer.
[2] Chronic diseases from four diseases: kidney disease, diabetes mellitus, cardiac disease, and transient ischemic attack/stroke, N = 1361. Characteristics are not given for LASA-II as no significant interactions were found within this cohort.

Figure 1 shows the relationship of serum 25(OH)D with BUA to determine the optimal cut-off point of serum 25(OH)D with respect to BUA. Figure 1a shows the multivariable relationship in participants with BMI ≤ 25 kg/m^2 in LASA-I. No clear cut-off point was observed. Figure 1b shows the same relationship in B-PROOF. Up to levels of approximately 65 nmol/L, mean BUA values increased with increasing serum 25(OH)D.

Sensitivity analyses, i.e., adding chronic diseases in B-PROOF and (multi) vitamin supplement use in all cohorts, did not materially change any of the results (data not shown). However, the significant association of vitamin D status (25-50 vs. > 50 nmol/L) with SOS in B-PROOF disappeared. The distribution of participants in the different vitamin D categories was similar in the two BMI groups in both LASA- and B-PROOF.

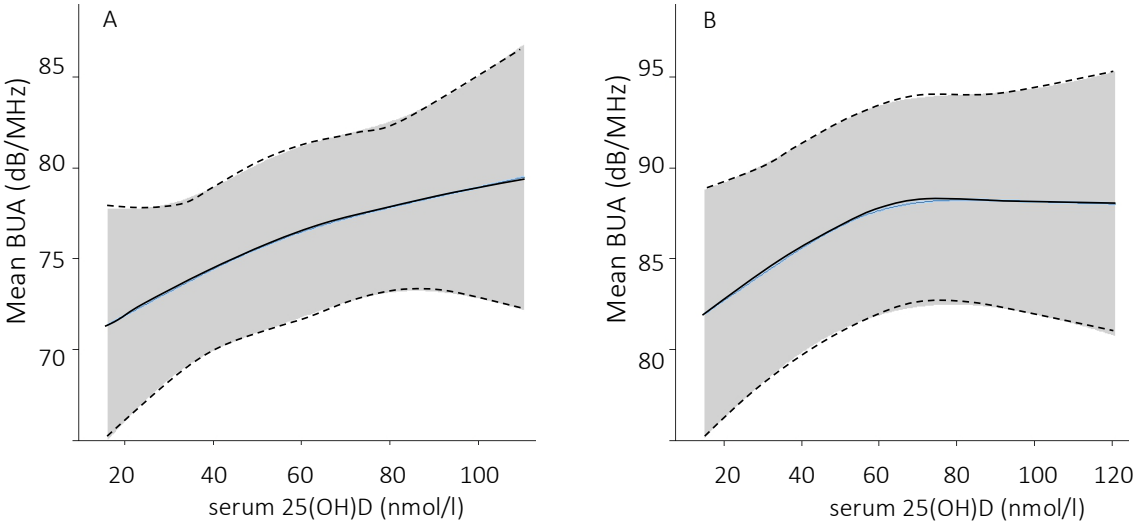

Figure 1. Mean BUA in relation to serum 25(OH)D in persons within the lowest BMI group. Grey area reflects the 95%- confidence interval. a. Analysis within LASA-I (BMI ≤ 25 kg/m^2), adjusted for age, gender, physical activity, and smoking. b. Analysis within B-PROOF (BMI ≤ 25 kg/m^2), adjusted for age, gender, and creatinine.

DISCUSSION

This study showed that the association between vitamin D status and QUS measurements and BMD was modified by BMI. In individuals with low-to-normal BMI, lower vitamin D status was associated with lower BUA and BMD values, whereas no significant associations were found in the high BMI groups. This was only the case in the cohorts with the oldest individuals, while in individuals of the youngest cohort no associations were found at all. In addition, no associations of vitamin D status with SOS values were observed.

The main finding of the current study is that BMI modifies the associations between vitamin D status and both BUA and BMD in older persons. To our best knowledge, this is the first study to show the importance of BMI in this relationship, because previous studies did not report information of analyses on interaction with BMI. Previous studies found that BMI or fat mass influences QUS measurements [35;36]. In these studies, higher BMI was associated with higher BUA and SOS. Because it is plausible that vitamin D has no or little influence on bone structure and density if an individual's BMI is high, the results of these previous studies are in agreement with our results that only in individuals with low-to-normal BMI vitamin D status is positively associated with BUA and BMD. It can be hypothesized that the influence of vitamin D on bone is less when compared to the effect of mechanical loading of high BMI. Another possible explanation for the importance of BMI can be hypothesized to be found in the fat solubility of vitamin D. Because bone marrow contains fat, the availability of vitamin D in the bone of more obese people may be higher than predicted from the serum concentration of 25(OH)D and therefore the classification based on serum levels may not be a good reflection of the levels in the bone [37].

Previous studies on the association between vitamin D and QUS showed contradictory results [13;14;20;38]. These inconsistent results may be explained by the fact that some of these studies assumed a linear relationship of serum 25(OH)D with BUA and SOS [13;14]. A linear relationship is, however, not probable. In a vitamin D sufficient state, it is not likely that a further increase in vitamin D levels cause a further increase in BUA or SOS. We also showed that a linear relationship is not always present within our datasets. This could explain the inconsistent results between different studies. Another explanation for the different results is that BMI might not be examined as an effect modifier in any of these studies as these studies did not report information on this. We found only an association within individuals with low-to-normal BMI in LASA-I and B-PROOF. An Italian study did find a significant association of vitamin D status with QUS parameters, but these measurements were performed at the phalanges [13]. It is probable that the weight-bearing effect reflected by BMI, and thereby the positive effect of mechanical loading on bone, is of minor influence in the hand as compared to the foot.

The results for the relationship of vitamin D status and BMD differed between the two cohorts (LASA-I and B-PROOF). Previous literature is not conclusive either; there are studies which found significant positive associations between vitamin D status and BMD [18-20;38;39], whereas others did not [18;21;39;40]. Although we did not find any clear significant associations in B-PROOF, the direction was similar to LASA-I: low vitamin D status was related to lower BMD. The differences between study results may be explained by for example, differences in vitamin D binding protein levels. One study showed that vitamin D binding protein modifies the association between 25(OH)D and BMD; bioavailable vitamin D is more related to BMD than total serum 25(OH)D [21]. Most studies, including ours, did not take vitamin D binding protein levels into account. In addition, adjustment for confounders was done differently in these previous published studies. It is obvious that several factors play a role in the described association, such as age, gender, physical activity and BMI. Therefore, some of the results of studies that reported a positive association between vitamin D status and BMD may be partly explained by measured or non-measured confounders.

There was some discrepancy between the results of the analyses of the associations of vitamin D status with QUS measurements and of those with BMD: we found a significant association in B-PROOF for QUS measurements, whereas we did not for BMD. This may be explained by differences between both types of measurements. In the literature, it is suggested that QUS measurements rely more on bone quality (i.e., architecture and elasticity), rather than bone density only as compared to BMD [41]. Moreover, previous research found only moderate correlations between BMD of the hip or lumbar spine and QUS values of the heel [42].

The last finding of our study is that no clear cut-off point for serum 25(OH)D with respect to BUA was revealed in LASA-I, whereas the cut-off point in B-PROOF was around 65 nmol/L. Therefore, on the basis of the results of our study, we could not advise an optimal serum 25(OH)D level with respect to BUA. In addition, the confidence interval around the mean value is wide and therefore there is much uncertainty around the estimates. To the best of our knowledge, there is no previously published study specifically addressing this issue and therefore, more research should be performed to draw any conclusions on this topic.

This study has some limitations. The first limitation is its cross-sectional design and therefore, no inference on causality can be made. In addition, participants in all three cohorts were relatively healthy, partly because in LASA and B-PROOF, most participants had to visit the study center for blood collection and BMD measurement, which may have led to selection bias. Furthermore, the results of the different cohorts could not be compared directly because different assays for the assessment of serum 25(OH)D were

used and different devices for QUS and BMD measurements. The main strengths are the large and independent study samples, with different ages, that were analyzed.

In conclusion, the association of vitamin D status with BUA and BMD was modified by BMI in the older cohorts, with the strongest association of vitamin D on bone in persons with low-to-normal BMI. These results may be of clinical relevance in that different decisions for individuals with different BMI may be considered regarding vitamin D supplementation for bone health. However, this has to be studied further in clinical trials, because previous trials did not report about the influence of BMI [43].

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Conflict of interest

An unconditional grant was received from Merck&Co. for part of the serum 25(OH)D measurements. Evelien Sohl, Renate T de Jongh, Karin MA Swart, Anke W Enneman, Janneke P van Wijngaarden, Suzanne C van Dijk, Annelies C Ham, Nikita L van der Zwaluw, Elske M Brouwer-Brolsma, Nathalie van der Velde, Lisette CPGM de Groot, Saskia J te Velde, Paul Lips and Natasja M van Schoor declare that they have no conflict of interest.

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