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Vitamin D status and depressive symptoms in older adults: a role for physical functioning?

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Keywords: vitamin D; depressive symptoms; physical functioning; older persons; cohort study.

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ABSTRACT

Objectives: Depressive symptoms and low vitamin D status are common in older persons and may be associated, but findings are inconsistent. This study investigated whether 25-hydroxyvitamin D (25(OH)D) concentrations are associated with depressive symptoms in older adults, both cross-sectionally and longitudinally. Furthermore, it was examined whether physical functioning could explain this relationship, to gain a better understanding of the underlying mechanisms.

Design, Setting, Participants: Data from two independent prospective cohorts of the Longitudinal Aging Study Amsterdam were used: an older cohort (≥65 years, *N*=1282, assessed from 1995-2002) and a younger-old cohort (55-65 years, *N*=737, assessed from 2002-2009).

Measurements: Depressive symptoms were measured at baseline and after three and six years with the Center of Epidemiological Studies Depression Scale. Cross-sectional and longitudinal linear regression techniques were used to examine the relationship between 25(OH)D and depressive symptoms. The mediating role of physical functioning was examined in the longitudinal models.

Results: Cross-sectionally, associations were not significant after adjustment for confounders. Longitudinally, women in the older cohort with baseline 25(OH)D concentrations up to 75 nmol/L experienced 17-24% more depressive symptoms in the following six years, compared to women with 25(OH)D concentrations >75 nmol/L. Reduced physical performance partially mediated this relationship. In men and in the younger-old cohort, no significant associations were observed.

Conclusions: Older women showed an inverse relationship between 25(OH)D and depressive symptoms over time. Partially, this association may be explained by declining physical functioning.

Keywords: vitamin D; depressive symptoms; physical functioning; older persons; cohort study.

OBJECTIVE

Worldwide, depression is a leading cause of disability, resulting in significant individual and societal burden (1). In addition, low 25-hydroxyvitamin D (25(OH)D) concentrations are common, especially among older persons (2, 3). Meta-analyses have shown an inverse association between 25(OH)D concentrations and depressive symptoms, but findings are inconsistent and longitudinal studies are scarce (4, 5).

A large Italian population-based cohort of older adults found that persons with low baseline 25(OH)D had more depressive symptoms after three and six years, compared to persons with higher baseline 25(OH)D (6). Chan et al. found an inverse cross-sectional, but no prospective association between 25(OH)D and depressive symptoms in older persons (7), whereas Williams et al. observed the opposite pattern (8). Furthermore, older adults with low 25(OH)D had an almost threefold risk of major depressive disorder (MDD) after one year, compared to persons with normal 25(OH)D concentrations in a population with cardiovascular disease (9).

Biologically, a link between vitamin D status and depressive symptoms is plausible, due to protective functions of vitamin D metabolites in the brain (10-12) and presence of the vitamin D receptor in depression-related brain areas such as the hippocampus (13). To better understand the mechanisms underlying the presumed association between 25(OH)D and depression, a mediating role of physical functioning in this relationship can also be considered. Physical functioning is associated with both 25(OH)D and depression: low 25(OH)D concentrations were associated with lower and declining physical performance (14, 15), and bidirectional cross-sectional and prospective relationships between physical functioning and depression have been observed (16-20).

The present cohort study investigated whether 25(OH)D concentrations are associated with the severity (cross-sectionally) and the course and onset (longitudinally) of depressive symptoms in older adults. Data from two independent cohorts of the Longitudinal Aging Study Amsterdam (LASA) were used (21). Part of this data was also used in a cross-sectional study by Hoogendijk et al. (2), on which the present study elaborates. Hoogendijk observed that depressive symptoms (as determinant) were associated with lower 25(OH)D in one LASA cohort. The present study additionally investigated a second LASA cohort, examined longitudinal associations and investigated whether physical functioning explains the association between 25(OH)D and depressive symptoms. By studying two

independent cohorts, we aimed to provide more insight into the relationship between 25(OH)D and depressive symptoms across a wide age range.

METHODS

Design and study sample

LASA is an ongoing population-based prospective cohort study that investigates the predictors, consequences and time course of physical, cognitive, emotional and social functioning in multiple cohorts of older adults (21). Participants were recruited from municipality registries in three regions in the Netherlands, together comprising a representative sample of the Dutch older population. Every three years, LASA participants are invited for interviews and questionnaires. Interviews are conducted at the participants' homes by trained interviewers.

Data from two LASA cohorts were used. In 1992, 3107 participants aged 55-85 years were included in the first LASA cohort ("older cohort"). As 25(OH)D was first assessed in 1995/96, we took this second measurement cycle as baseline for this cohort. Participants of ≥65 years who agreed to have an additional medical interview (N=1509) were asked to donate a blood sample. Serum 25(OH)D was available from 1320 participants.

The second LASA cohort commenced in 2002/03 with 1002 participants aged 55-65 years ("younger-old cohort"). Of the 919 participants who agreed to the medical interview, 739 participants had data on 25(OH)D.

Follow-up time for the present study was six years. Follow-up data were collected at the subsequent two measurement cycles: in 1998/99 and 2001/02 (older cohort), and in 2005/06 and 2008/09 (younger-old cohort).

All participants gave written informed consent prior to the start of the study. The LASA study was approved by the Medical Ethics Committee of the VU University Medical Center (VUmc). Detailed information about LASA and its participants can be found elsewhere (21).

Measurements

Depressive symptoms

The Center for Epidemiological Studies Depression Scale (CES-D) (22) was assessed at baseline, three and six years in both cohorts. The CES-D is a widely-used screening instrument that contains 20 items about depressive symptoms as experienced in the previous week. Scores range from 0-60, with higher scores indicating more depressive symptoms. A score of ≥16 is indicative for clinically relevant depressive symptoms. The CES-D displays high reliability (23) and good criterion validity (24) in several older populations.

Blood sampling and measurement of 25(OH)D

Morning blood samples for the assessment of serum 25(OH)D (in nmol/L) were obtained in 1995/96 (older cohort) and 2002/03 (younger-old cohort). Participants were allowed to have a light breakfast without dairy products. Samples were centrifuged and stored at -20°C until 25(OH)D determination in 1997/98 (older cohort) and 2010/11 (younger-old cohort). The analyses were carried out by the VUmc Endocrine Laboratory. A competitive protein-binding assay (Nichols Diagnostics Capistrano, CA, USA; interassay coefficient of variation (CV): 10%) was used for the sample determinations in the older cohort, whereas a radioimmunoassay (Diasorin, Stillwater, Minnesota, USA; interassay CV: 10%) was used for the younger-old cohort. For the statistical analyses of the present study, 25(OH)D was divided into four categories using commonly used cutoff values: <30 nmol/L (deficiency), 30-50 nmol/L (insufficiency), 50-75 nmol/L and >75 nmol/L (25, 26).

Potential effect modifier and confounders

Gender was examined as a potential effect modifier, as previous studies observed different associations between 25(OH)D and depressive symptoms in men and women (6, 9). Education level (years), smoking habits (never, former, current smoker), alcohol consumption (grams/week, 10g per consumption), presence of the most prevalent somatic chronic diseases among Dutch older adults (asthma/chronic obstructive pulmonary disease, cardiac disease (myocardial infarction, coronary artery disease, heart failure, disease of the cardiac valves, arrhythmia), peripheral arterial disease, diabetes mellitus, cerebrovascular accident/stroke, osteoarthritis/rheumatoid arthritis, cancer, hypertension, and other diseases that are present for at least three months) and general cognitive

functioning (Mini-Mental State Examination) (27) were obtained during the interviews and questionnaires. To control for seasonal variations in 25(OH)D concentrations (28), the blood collection dates were dichotomized into winter (October-March) and summer (April-September). Body mass index (BMI) was calculated by dividing measured body weight (in kilograms) by measured body height squared (in meters). Information on physical activity in the previous two weeks (walking, cycling, sports, heavy household activities, in minutes/day, plus the question whether these activities were representative compared to the previous year) was taken from the LASA Physical Activity Questionnaire (LAPAQ) (29).

Potential mediating variables

To cover a broad domain of physical functioning, both objective performance tests and self-reported functional limitations were examined for their possible mediating role in the association between 25(OH)D and depressive symptoms. A modified version of the Short Physical Performance Battery (SPPB (15, 30)) was used to assess physical performance. The SPPB includes three tests: walking speed (walking three meters, turning around and walking back three meters as fast as possible), chair stands (standing up from a chair without using hands five times consecutively, as fast as possible) and standing balance (standing with one foot directly in front of the other for up to 10 seconds). In the older cohort, the total score of the SPPB ranged from 0-12. As the balance test was not administered to the younger-old cohort at baseline, the SPPB score for this cohort was composed of the walking speed and chair stands scores only, resulting in a score range of 0-8. Higher scores indicate better physical performance.

Functional limitations were measured with six questions assessing common daily activities: climbing stairs, cutting toenails, walking five minutes without resting, rising from a chair, (un)dressing and using own/public transportation (31). The participants indicated whether they had difficulty performing these activities (score range: 0-6). Higher scores indicate more functional limitations.

Statistical analyses

As the two cohorts differed in age range, assessment period and 25(OH)D assay, analyses were conducted separately for both cohorts. All analyses were conducted with SPSS version 22 (SPSS Inc. Chicago, IL, USA), except for the mediation analyses, which were conducted with Mplus (version 7.2) and R statistical software (version 3.2.5). A double-sided *p*-value of <.05 was considered statistically significant. Baseline characteristics were compared between 25(OH)D categories using Pearson chi square tests for categorical variables or non-parametric Kruskal-Wallis tests for skewed continuous variables. Cross-sectional and longitudinal non-response analyses comparing included and excluded participants were conducted with Pearson chi square tests for categorical variables or with non-parametric Mann-Whitney tests for skewed continuous variables.

As the distribution of the CES-D scores was skewed to the right, these scores were log-transformed using the formula Ln(1+CES-D score). The four 25(OH)D categories were entered as three dummy variables in the regression analyses, with the >75 nmol/L group as reference category. The Bs, standard errors (SEs) and confidence intervals (CIs) of the regression analyses were transformed back to obtain interpretable ratios (e^B = ratio). These ratios reflect the percentage of change in the outcome per one unit change in the determinant. As these ratios and resulting percentages were calculated from a log-transformed scale, note that they do not correspond to the same change in CES-D across all CES-D scores: higher scores change more than lower scores. For instance: with a ratio of 1.23 and corresponding percentage change of 23%, a CES-D score of 20 changes 4.6 points (23% of 20), whereas a CES-D score of 10 changes 2.3 points (23% of 10).

Cross-sectional analyses

Multiple linear regression analyses with the 25(OH)D categories as determinants and the CES-D score as outcome were conducted. To assess effect modification, gender and interaction terms of the 25(OH)D categories with gender (25(OH)D dummies * gender) were added to the unadjusted model. If an interaction term had a *p*-value of <.10, stratified analyses for men and women were conducted. If no effect modification was present, gender was added as a confounder to the analyses. Adjustments for demographic variables (age, (gender,) education level, season of blood collection) were made in

Model 1; additional adjustments for lifestyle/health confounders (smoking, alcohol consumption, BMI, number of chronic diseases, physical activity and cognitive functioning) were made in Model 2.

Longitudinal analyses

To study the course of depressive symptoms over time, linear mixed-models analyses were conducted with the 25(OH)D categories at baseline as determinants, the CES-D scores after three years and six years as outcome and the baseline CES-D score as covariate. These analyses included participants who had baseline 25(OH)D and CES-D data and at least one follow-up CES-D measurement. A random intercept was added to the longitudinal CES-D variable to control for dependency of individual measures over time. A time-interaction term was added to the models to examine possible differences in the association between three and six years of follow-up.

To study whether 25(OH)D predicts the onset of clinically relevant depressive symptoms over time, logistic regression analyses were conducted in a subgroup of participants without depressive symptoms at baseline (CES-D <16) and at least one follow-up measurement. The outcome was defined as presence of clinically relevant depressive symptoms (CES-D ≥16) in the six-year follow-up period. Models and effect modification procedures were similar to the cross-sectional analyses.

Finally, the possible mediating effect of physical performance and functional limitations in the relationship between 25(OH)D and depressive symptoms was examined. To account for the temporal, stepwise character of this hypothesized relationship, two longitudinal mediation models were fitted in which physical performance and functional limitations after three years of follow-up were considered as the mediator variables. Using structural equation modeling, the effect of 25(OH)D on physical functioning and the effect of physical functioning on depressive symptoms, adjusted for 25(OH)D, were simultaneously modelled. Mediation was examined only in analyses that showed a statistically significant relationship between 25(OH)D and depressive symptoms. With these mediation analyses, the total effect of 25(OH)D on depressive symptoms was separated into direct and indirect effects. The direct effect represents the effect of 25(OH)D on depressive symptoms, adjusted for physical functioning, whereas the indirect effect represents the multiplied effects of 25(OH)D on physical functioning and physical functioning on depressive symptoms, adjusted for 25(OH)D. This indirect

effect can be seen as the mediating effect of physical functioning in the association of 25(OH)D with depressive symptoms (32). Because of the usually skewed distribution of indirect effects, we used 95% Monte Carlo simulated confidence intervals (20,000 replications) (33). The mediation analyses were performed separately for physical performance and functional limitations and were conducted in the adjusted models (Model 2).

Pooled analyses

In additional analyses, both cohorts were pooled to increase the N and to investigate the consistency of the findings. Since the cohorts used different 25(OH)D assays, the 25(OH)D values of the older cohort (Nichols assay) were calibrated towards the values of the younger-old cohort (Diasorin assay), using the formula Diasorin=3.7778+0.8889*Nichols (28). The cross-sectional and longitudinal regression analyses were repeated in the pooled dataset, with cohort as additional confounder.

RESULTS

Of 1320 participants with a 25(OH)D measurement in the older cohort, 38 participants were excluded due to missing CES-D scores, leaving 1282 participants available for analysis. In this cohort, 217 participants (16.9%) had vitamin D deficiency (25(OH)D <30 nmol/L) and 400 participants (31.2%) had insufficient 25(OH)D concentrations (30-50 nmol/L). Clinically relevant depressive symptoms (CES-D ≥16) were present in 193 participants (15.1%) at baseline, in 202 of 1071 participants (18.9%) at three years and in 169 of 853 participants (19.8%) at six years of follow-up. Non-response analyses comparing analyzed participants (N=1282) with initially eligible participants who were excluded from the cross-sectional analyses (N=227) showed that non-respondents were older (U=104475.5, p<.001), less educated (U=130516.0, p=.02), smoked more ($\chi^2(2)$=8.9, p=.01), drank less alcohol (U=121669.5, p<.001), had more depressive symptoms (U=96413.0, p=.01), were less physically active (U=74118.0, p<.001), had worse cognitive functioning (U=95058.0, p<.001) and physical performance (U=72670.5, p<.001) and more functional limitations (U=87667.5, p=<.001), compared to included participants. Non-response analyses comparing participants who were excluded from the longitudinal analyses (N=207) with included participants (N=1075) showed similar results as above. Furthermore,

excluded participants were more often male ($\chi^2(1)=34.6$, $p<.001$) and had more chronic diseases ($U=538854.0$, $p=.01$).

Of the 739 participants with a 25(OH)D measurement in the younger-old cohort, two participants were excluded from analysis due to either a very high 25(OH)D concentration (182 nmol/L) or a missing CES-D score. Of the resulting 737 participants, 56 participants had vitamin D deficiency (7.6%) and 243 participants had insufficient vitamin D status (33.0%). Clinically relevant depressive symptoms were present in 100 participants (13.6%) at baseline, in 95 of 703 participants (13.5%) after three years and in 69 of 648 participants (10.6%) after six years. Cross-sectional non-response analyses in this cohort revealed that non-respondents (N=182) smoked more ($\chi^2(2)=14,5$, $p=.001$) compared to included participants (N=737). Longitudinal non-response analyses showed that excluded participants (N=33) had more depressive symptoms ($U=39562.0$, $p=.04$), were more often smokers ($\chi^2(2)=11,3$, $p=.004$) and had more functional limitations ($U=33340.0$, $p<.001$) compared to included participants (N=704). **Table 1** displays baseline characteristics of both cohorts.

Cross-sectional analyses

Table 2 presents the results of the baseline regression analyses. Gender was not an effect modifier ($p>.10$ in both cohorts). In the older cohort, participants with 25(OH)D <50 nmol/L had significantly more depressive symptoms compared to participants with 25(OH)D >75 nmol/L (<30 nmol/L: *ratio*=1.25, 95% CI: 1.0-1.5; 30-50 nmol/L: *ratio*=1.17, 95% CI: 1.0-1.4). However, this association was attenuated after adjustment for lifestyle/health variables. Similarly, in the younger-old cohort, no statistically significant cross-sectional relationship between 25(OH)D and depressive symptoms was seen in the adjusted model.

Longitudinal analyses

In the older cohort, gender was a significant effect modifier for the third 25(OH)D dummy ($p=.05$), so the mixed-models analyses were stratified for men and women (**Table 3**). No significant associations were observed in men, whereas women in baseline 25(OH)D categories below 75 nmol/L experienced more depressive symptoms after three and six years, compared to women with 25(OH)D of >75

nmol/L (adjusted model: <30nmol/L: *ratio*=1.23, 95% CI:1.02-1.49; 30-50 nmol/L: *ratio*=1.17, 95% CI:1.00-1.37; 50-75 nmol/L: *ratio*=1.24,95% CI:1.06-1.45). Corresponding to these ratios, participants with 25(OH)D concentrations below 30 nmol/L had a 23% higher CES-D score over six years than persons with 25(OH)D >75 nmol/L. Similarly, participants with 25(OH)D concentrations of 30-50 nmol/L had a 17% higher CES-D score over six years, and persons with 25(OH)D concentrations of 50-75 nmol/L had a 24% higher CES-D score over six years compared to persons with 25(OH)D >75 nmol/L.

In the younger-old cohort, gender was not an effect modifier (p>.10). After adjustment for lifestyle/health confounders, a significant relationship between baseline 25(OH)D and depressive symptoms over time was no longer observed (Table 3).

Time-interaction terms were not significant in both cohorts (p>.10), indicating that the effect of 25(OH)D on the course of depressive symptoms did not differ between three and six years of follow-up.

In the logistic regression analysis of the older cohort, gender was an effect modifier for the first and third 25(OH)D dummy (p=.05 and .01, respectively). Hence, the analyses were stratified for men and women. Similar to the mixed-models analyses, significant associations were observed in women: women with 25(OH)D concentrations <30 nmol/L had higher odds of developing clinically relevant depressive symptoms over six years, compared to women with 25(OH)D >75 nmol/L (odds ratio (OR) in adjusted model: 2.9, p=.04, 95% CI: 1.1-8.0). In men, a significantly lower odds of developing depressive symptoms over six years was observed for the group with 25(OH)D concentrations of 50-75 nmol/L, compared to the >75 nmol/L group (adjusted OR: 0.4, p=.02, 95% CI: 0.2-0.9). However, due to the small number of cases in the stratified analyses, the reliability of these results is uncertain. In the younger-old cohort, no associations between 25(OH)D and the onset of depressive symptoms were observed (adjusted ORs: 0.7-1.1, p>.6).

Mediation of physical functioning

The mediation analyses for physical performance and functional limitations were performed for the longitudinal mixed-models analysis of the older women, as this analysis was statistically significant

(**Table 4**). The indirect effect (mediation effect) of physical performance was statistically significant for the 30-50 nmol/L 25(OH)D category, compared to the reference category of >75 nmol/L (*ratio* of indirect effect: 1.03, bootstrapped 95% CI: 1.0-1.1). The corresponding percentage mediation of 20.6% suggests that physical performance after three years partially mediates the longitudinal association between 25(OH)D and depressive symptoms. The indirect effects of the other 25(H)D categories of physical performance and the indirect effects of functional limitations were not statistically significant, but the substantial mediation percentages of the <30 and 30-50 nmol/L categories suggest that physical functioning may have a modest mediating role in the relationship between 25(OH)D and depressive symptoms.

Pooled analyses

The cross-sectional and longitudinal regression analyses were repeated in the pooled dataset. Cross-sectionally, participants in 25(OH)D categories up to 50 nmol/L experienced significantly more depressive symptoms than participants with 25(OH)D >75 nmol/L in the adjusted model (<30nmol/L: *ratio*=1.20, 95% CI: 1.03-1.40; 30-50 nmol/L: *ratio*=1.16, 95% CI: 1.03-1.30; **Supplemental Table 1**). Corresponding to these ratios, participants with 25(OH)D <30 nmol/L had a 20% higher CES-D score than persons with 25(OH)D >75 nmol/L. Similarly, participants with 25(OH)D concentrations of 30-50 nmol/L had a 16% higher CES-D score compared to persons with 25(OH)D >75 nmol/L. The longitudinal pooled analyses revealed no significant associations between 25(OH)D and depressive symptoms in the adjusted models (**Supplemental Table 2**). Due to multiple cohort differences, these results should be interpreted with caution.

Additional analysis

As an exploratory sensitivity analysis, we examined whether the results would be different if we excluded participants who indicated that their physical activity (confounder, LAPAQ) of the previous weeks was not representative for the rest of the year. We repeated the tests for interaction of gender and the cross-sectional and longitudinal regression analyses without these participants (-28.2% and -31.4% in the older and younger-old cohort, respectively) and the results were very similar to the

original analyses and the conclusions did not change (results not shown but available from the author on request).

CONCLUSIONS

This study investigated the baseline and prospective six-year association between 25(OH)D concentrations and depressive symptoms in two large population-based cohorts of older adults. Cross-sectionally, this association was not significant after adjustment for confounders. However, the longitudinal analyses revealed a marked difference between men and women in the older cohort (≥65 years at baseline): women with 25(OH)D concentrations <75 nmol/L at baseline experienced 17-24% more depressive symptoms in the following six years than women with 25(OH)D >75 nmol/L. Low physical performance partially mediated this relationship. No such associations were observed in men or in the younger-old cohort (55-65 years at baseline). According to Geerlings et al., a change of five points in the CES-D score can be regarded as a meaningful change in depressive symptoms (34). Hence, it depends on the initial CES-D score whether change in 25(OH)D status is associated with a meaningful change in depressive symptoms over six years. Higher initial CES-D scores are associated with more relevant change.

The observed differences between the two cohorts could be explained by the better general health status of the younger-old cohort. These participants had higher 25(OH)D concentrations, better physical functioning, fewer chronic diseases and were more physically active compared to the older cohort. This may have enabled the younger persons to better withstand negative effects of low 25(OH)D on mood. On the other hand, the smaller sample size of the younger-old cohort may have limited the power of our analyses.

To increase power and to investigate the consistency of the results, both cohorts were pooled in additional analyses. Cross-sectionally, these analyses demonstrated significantly more depressive symptoms in persons with lower 25(OH)D concentrations (up to 20%), confirming the consistency of the associations in both cohorts. In the longitudinal pooled analyses however, the adjusted associations were not significant. By comparing the longitudinal analyses presented in Table 3 and Supplemental Table 2, it can be seen that the associations of the separate cohorts seem to cancel each other out in the

pooled analyses, especially in the 30-50 and 50-75 nmol/L categories. This may be explained by considerable cohort differences regarding health status and assessment periods. Due to cohort differences, the results of the pooled analyses should be interpreted with caution.

In the older cohort, a longitudinal association between baseline 25(OH)D and the course of depressive symptoms was observed in women only. This gender difference could be attributable to the generally lower 25(OH)D concentrations in women compared to men (25(OH)D <50 nmol/L in 56.9% of women and 38.9% of men). Milaneschi et al. found a similar gender difference in the InCHIANTI study (6). In contrast, no interaction with gender was observed in the Health ABC study (8).

It was hypothesized that low vitamin D status would reduce physical functioning, which in turn would increase depressive symptoms (14, 15, 18-20). Indeed, we observed partial mediation of the association of 25(OH)D with depressive symptoms by physical performance. This provides evidence for a potential mediating role of physical functioning in the relationship between low 25(OH)D and increasing depressive symptoms. To the best of our knowledge, this explanatory role has not previously been investigated prospectively. At most, these variables were treated as confounders in former studies (6-9, 35). The influence of physical functioning may in fact be an important reason why we did not observe significant associations in men and in the younger-old participants. Men in the older cohort had better physical performance than women (p<.001). Similarly, participants in the younger-old cohort generally had higher physical function scores compared to the older cohort (see Table 1). It can be speculated that having adequate physical functioning may act as a ‘buffer’ to safeguard older persons from the negative impact of low 25(OH)D on their mood. It should be noted that this mediating role of physical functioning is still exploratory and should be confirmed by other studies.

Hoogendijk et al. partly used the same data as the present study and did find a significant cross-sectional association between depression status and 25(OH)D concentrations (2). This disparity can be explained by their use of different confounders and different operationalization of depression. Hoogendijk categorized depression status into ‘no depression’ (CESD <16), ‘minor depression’ (CESD ≥16) and ‘MDD’ (diagnosis after psychiatric interview) and analyzed it as determinant instead of an outcome. We chose not to use the MDD variable as outcome, because the number of participants

with MDD is very limited within the LASA study, which would substantially reduce the power of our analyses.

The present study combines several strengths. The use of data from two large, population-based, independent cohorts with a long follow-up increases the value and generalizability of our results. In addition, we studied the influence of 25(OH)D on the severity, course and onset of depressive symptoms using both cross-sectional and longitudinal analysis techniques, adjusted for relevant confounders. By studying 25(OH)D at baseline, physical functioning after three years and depressive symptoms over six years, we explored the potential mediating role of physical functioning longitudinally. This method takes into account the temporal character of the underlying mechanism. To the best of our knowledge, this is the first study that explored physical functioning as a possible mediating factor in the relationship between vitamin D status and depressive symptoms.

Nevertheless, this study also has some limitations. Serum 25(OH)D was only measured at baseline, although it was shown that 25(OH)D concentrations are relatively stable over time (28, 36). Furthermore, as depressive symptoms were measured only once every three years, we did not have information about intermediate time points. Due to the fluctuating nature of depression, we may have missed episodes of depressive symptoms. Unfortunately, some potential confounders were not measured in the LASA measurement cycles that we used. Due to this, we were unable to adjust for variables such as diet and vitamin D supplement use, possibly resulting in residual confounding. Furthermore, over 99% of LASA participants are Caucasian (28) which may have limited the generalizability of our finding to other ethnicities, as evidence suggests that polymorphisms of the vitamin D binding protein and hence bioavailability of vitamin D differs between ethnicities (ref Powe et al 2013). The non-response analyses showed that included participants were healthier than excluded persons, which may limit generalizability. In addition, the physical activity measure (LAPAQ) only gives information about activities in the previous two weeks, and no information about the longer-term habitual activity pattern of the participants. Therefore, we conducted a sensitivity analysis without participants who indicated that their activities were not representative for the previous year. Results of these analyses were similar to the regular analyses. Although this suggests that lack of information on past-year physical activity does not influence the conclusions, it cannot be ruled out that longer-term

habitual physical activity may still be a potential confounding factor. Finally, cohort differences, such as different time periods and 25(OH)D assays, may have compromised the comparability of the two cohorts.

In conclusion, this study showed that older women with 25(OH)D concentrations below 75 nmol/L at baseline experienced 17-24% more depressive symptoms over six years, compared to women with 25(OH)D concentrations >75 nmol/L. This relationship may be partially explained by reduced physical functioning. To the best of our knowledge, this longitudinal mediating role of physical functioning has not been studied before. Our results suggest that having 25(OH)D concentrations >75 nmol/L and adequate physical functioning is especially important for the mental health of older women. Randomized controlled trials investigating both vitamin D supplementation and behavioral interventions to improve physical functioning should examine the causality of these associations further, which may aid treatment or prevention strategies for depression.

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Table 1. Baseline characteristics of the two LASA cohorts.

	Older cohort (*N*=1282)					**Younger-old cohort (*N*=737)**				
Serum 25(OH)D	**<30 nmol/L (*N*=217)**	**30-50 nmol/L (*N*=400)**	**50-75 nmol/L (*N*=434)**	**>75 nmol/L (*N*=231)**	***p***	**<30 nmol/L (*N*=56)**	**30-50 nmol/L (*N*=243)**	**50-75 nmol/L (*N*=310)**	**>75 nmol/L (*N*=128)**	***p***
Depressive symptoms:										
CES-D score [a]	9 [4-15]	7 [3-13]	5 [2-10]	4 [2-9]	<.001	10 [3-16]	6 [3-12]	5 [2-10]	6 [2-9]	.01
CES-D ≥16 [b]	46 (21.2)	70 (17.5)	52 (12.0)	25 (10.8)	.002	15 (26.8)	38 (15.6)	32 (10.3)	15 (11.7)	.01
Women [b]	135 (62.2)	240 (60.0)	201 (46.3)	83 (35.9)	<.001	28 (50.0)	135 (55.6)	158 (51.0)	78 (60.9)	.39
Age [a]	80.9 [75.5-84.5]	76.3 [70.9-82.1]	72.8 [68.8-78.5]	70.7 [67.6-75.8]	<.001	61.7 [58.0-63.2]	59.7 [56.9-62.6]	60.4 [57.5-62.6]	59.5 [56.6-62.0]	.06
Education (years) [a]	9 [6-11]	9 [6-11]	9 [6-11]	9 [6-11]	.04	11 [7-15]	10 [9-12]	10 [9-11]	10 [9-11]	.44
Season of blood collection: [b]					<.001					.07
Winter	147 (67.7)	226 (56.8)	215 (49.7)	108 (46.8)		42 (75.0)	182 (74.9)	213 (68.7)	86 (67.2)	
Summer	70 (32.3)	172 (43.2)	218 (50.3)	123 (53.2)		14 (25.0)	61 (25.1)	97 (31.3)	42 (32.8)	
Smoking [b]					.03					<.001
Current	45 (20.7)	75 (18.8)	68 (15.7)	42 (18.2)		22 (39.3)	85 (35.0)	72 (23.2)	23 (18.0)	
Former	75 (34.6)	167 (41.8)	231 (53.2)	121 (52.4)		21 (37.5)	108 (44.4)	161 (51.9)	67 (52.3)	
Never	97 (44.7)	158 (39.5)	135 (31.1)	68 (29.4)		13 (23.2)	50 (20.6)	77 (24.8)	38 (29.7)	
Alcohol consumption (gr/wk) [a]	10 [0-70]	20 [0-70]	30 [5-120]	60 [10-210]	<.001	60 [5-210]	70 [20-200]	70 [30-210]	70 [45-210]	.23
Body mass index (kg/m^2) [a]	26.8 [23.5-29.6]	27.3 [24.7-30.0]	26.5 [24.3-29.2]	25.6 [23.4-28.2]	<.001	26.5 [24.1-29.1]	27.6 [24.5-30.3]	26.6 [24.2-29.1]	26.4 [24.6-29.0]	.26
Number of chronic diseases [a]	2 [1-3]	2 [1-3]	2 [1-2]	1 [1-2]	<.001	2 [1-3]	1 [0-2]	1 [0-2]	1 [0-2]	.09

Cognitive functioning (MMSE) [a]	27 [24-28]	28 [26-29]	28 [26-29]	28 [27-29]	<.001	28 [26-29]	28 [27-29]	28 [27-29]	28 [27-29]	.09
Physical activity (min/day) [a]	21.4	38.6	56.8	61.5	<.001	48.0	66.4	72.9	62.9	.001
	[5.4-54.3]	[15.0-65.4]	[30.0-99.6]	[28.8-102.1]		[16.4-86.6]	[35.4-109.3]	[42.5-129.5]	[37.9-110.0]	
Physical performance [a, c]	6 [2-8]	7 [5-9]	9 [7-10]	9 [8-11]	<.001	6 [4-7]	6 [5-7]	6 [5-7]	6 [5-8]	.01
Functional limitations	2 [0-4]	1 [0-3]	0[0-2]	0 [0-1]	<.001	0 [0-2]	0 [0-1]	0[0-0]	0 [0-1]	<.001

Values are displayed as N (%) for categorical variables or as median [interquartile range] for skewed continuous variables. [a] Differences between 25(OH)D categories tested with Kruskal-Wallis test; [b] Differences between 25(OH)D categories tested with Pearson Chi-square test, *p*-value represents *p* for trend; [c] The physical performance score ranges from 0-12 in the older cohort and from 0-8 in the younger-old cohort. 25(OH)D: 25-hydroxyvitamin D; CES-D: Center for Epidemiological Studies Depression Scale; MMSE: Mini-Mental State Examination.

Table 2. Cross-sectional associations between 25(OH)D and depressive symptoms in the two LASA cohorts.

	Older cohort						**Younger-old cohort**					
	Model 1			**Model 2**			**Model 1**			**Model 2**		
Serum 25(OH)D	***Ratio[a] (SE)***	***t (df)***	***p***	***Ratio[a] (SE)***	***t (df)***	***p***	***Ratio[a] (SE)***	***t (df)***	***p***	***Ratio[a] (SE)***	***t (df)***	***p***
<30 nmol/L	1.25 (1.10)	2.41 (1270)	.016	1.14 (1.10)	1.40 (1238)	.16	1.38 (1.16)	2.21 (729)	.028	1.16 (1.15)	1.05 (710)	.29
30-50 nmol/L	1.17 (1.08)	1.99 (1270)	.047	1.11 (1.08)	1.32 (1238)	.19	1.20 (1.10)	1.87 (729)	.062	1.15 (1.10)	1.47 (710)	.14
50-75 nmol/L	0.99 (1.08)	-0.10 (1270)	.92	0.96 (1.08)	-0.51 (1238)	.61	1.01 (1.10)	0.07 (729)	.94	1.01 (1.10)	0.15 (710)	.88
>75 nmol/L	Ref			Ref			Ref			Ref		

[a] As the outcome variable was ln(1+CESD), Bs and SEs were transformed back to normal scale to obtain interpretable ratios.

Analyzed with multiple linear regression analysis. Model 1: adjusted for age, gender, education and season. Model 2: additionally adjusted for smoking, alcohol use, BMI, chronic diseases, cognitive functioning and physical activity. 25(OH)D: 25-hydroxyvitamin D; CES-D: Center for Epidemiological Studies Depression Scale.

Table 3. Longitudinal associations between 25(OH)D and depressive symptoms in the two LASA cohorts; older cohort stratified for gender.

	Older cohort												**Younger-old cohort**					
	Women						***Men***						***Total sample***					
	Model 1			**Model 2**			**Model 1**			**Model 2**			**Model 1**			**Model 2**		
Serum 25(OH)D	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***	***Ratio[a]*** ***(SE)***	***t*** ***(df)***	***p***
<30 nmol/L	1.30	2.69	.007	1.23	2.13	.034	0.96	-0.35	.73	0.95	-0.51	.61	1.31	2.46	.014	1.16	1.35	.18
	(1.10)	(569)		(1.10)	(553)		(1.11)	(499)		(1.11)	(484)		(1.12)	(687)		(1.12)	(670)	
30-50 nmol/L	1.23	2.61	.009	1.17	1.92	.055	0.97	-0.43	.67	0.96	-0.44	.66	0.99	-0.18	.86	0.93	-0.96	.34
	(1.08)	(545)		(1.08)	(532)		(1.08)	(460)		(1.09)	(446)		(1.07)	(688)		(1.08)	(675)	
50-75 nmol/L	1.26	2.92	.004	1.24	2.70	.007	0.97	-0.37	.71	0.97	-0.47	.64	0.94	-0.85	.40	0.93	-1.04	.30
	(1.08)	(544)		(1.08)	(530)		(1.07)	(458)		(1.07)	(444)		(1.07)	(689)		(1.07)	(674)	
>75 nmol/L	Ref			Ref			Ref			Ref			Ref			Ref		

[a] As the outcome variable was ln(1+CESD), Bs and SEs were transformed back to normal scale to obtain interpretable ratios.

Analyzed with linear mixed-models analysis, with the CES-D score after 3 and 6 years as outcome and baseline CES-D as covariate. Model 1: adjusted for age, (gender,) education and season. Model 2: additionally adjusted for smoking, alcohol use, BMI, chronic diseases, cognitive functioning and physical activity. 25(OH)D: 25-hydroxyvitamin D; CES-D: Center for Epidemiological Studies Depression Scale.

Table 4. Mediation effects of physical functioning (after three years) in the longitudinal association between baseline 25(OH)D and depressive symptoms over six years in women of the older LASA cohort.

	Physical performance			**Functional limitations**		
Serum 25(OH)D	***Indirect effect*** [a]	***95% CI*** [b]	***% mediation***[c]	***Indirect effect*** [a]	***95% CI*** [b]	***% mediation***[c]
<30 nmol/L	1.03	0.99 - 1.07	12.4	1.04	0.99 - 1.09	16.7
30-50 nmol/L	1.03*	1.00 - 1.07	20.6	1.02	0.99 - 1.06	13.3
50-75 nmol/L	1.01	0.98 - 1.04	3.3	1.02	0.98 - 1.05	7.4
>75 nmol/L	Ref			Ref		

As the outcome variable was ln(1+CESD), the Bs and confidence intervals of the indirect effects were transformed back to normal scale to obtain interpretable ratios. Mediation analyses were performed in the adjusted Model 2. 25(OH)D: 25-hydroxyvitamin D; CI: confidence interval.

[a] The indirect effect is the mediating effect of physical functioning on the association between 25(OH)D and depressive symptoms. It represents the multiplied effects of 25(OH)D on physical functioning and physical functioning on depressive symptoms, adjusted for 25(OH)D.

[b] Bootstrapped confidence intervals with Monte Carlo simulation.

[c] Percentage mediation calculated by (indirect effect / total effect)*100. Total effects are displayed in Table 3.

*Statistically significant indirect effect.

SUPPLEMENTAL DIGITAL CONTENT 1:

Supplemental Table 1. Cross-sectional associations between 25(OH)D and depressive symptoms in the pooled analysis of the two LASA cohorts.

	Pooled cohort					
	Model 1			Model 2		
Serum 25(OH)D	***Ratio[a] (SE)***	***t (df)***	***p***	***Ratio[a] (SE)***	***t (df)***	***p***
<30 nmol/L	1.33 (1.08)	3.50 (2006)	<.001	1.20 (1.08)	2.30 (1962)	.022
30-50 nmol/L	1.21 (1.06)	3.22 (2006)	.001	1.16 (1.06)	2.55 (1962)	.011
50-75 nmol/L	1.04 (1.06)	0.72 (2006)	.473	1.03 (1.06)	0.58 (1962)	.563
>75 nmol/L	Ref			Ref		

[a] As the outcome variable was ln(1+CESD), Bs and SEs were transformed back to normal scale to obtain interpretable ratios. Analyzed with multiple linear regression analysis. Model 1: adjusted for age, gender, education, season and cohort. Model 2: additionally adjusted for smoking, alcohol use, BMI, chronic diseases, cognitive functioning and physical activity. 25(OH)D: 25-hydroxyvitamin D; CES-D: Center for Epidemiological Studies Depression Scale.

SUPPLEMENTAL DIGITAL CONTENT 2:

Supplemental Table 2. Longitudinal associations between 25(OH)D and depressive symptoms in the pooled analysis of the two LASA cohorts, stratified for gender.

	Women						**Men**					
	Model 1			**Model 2**			**Model 1**			**Model 2**		
Serum 25(OH)D	***Ratio*[a] (SE)**	***t (df)***	***p***	***Ratio*[a] (SE)**	***t (df)***	***p***	***Ratio*[a] (SE)**	***t (df)***	***p***	***Ratio*[a] (SE)**	***t (df)***	***p***
<30 nmol/L	1.16 (1.09)	1.82 (973)	.069	1.06 (1.09)	0.66 (949)	.51	1.24 (1.10)	2.12 (830)	.034	1.19 (1.11)	1.73 (808)	.085
30-50 nmol/L	1.09 (1.06)	1.47 (919)	.14	1.02 (1.06)	0.29 (897)	.77	0.98 (1.07)	-0.26 (784)	.80	0.98 (1.07)	-0.35 (769)	.72
50-75 nmol/L	1.08 (1.06)	1.37 (917)	.17	1.06 (1.06)	1.09 (897)	.28	0.99 (1.06)	-0.14 (786)	.89	0.99 (1.06)	-0.22 (769)	.83
>75 nmol/L	Ref			Ref			Ref			Ref		

[a] As the outcome variable was ln(1+CESD), Bs and SEs were transformed back to normal scale to obtain interpretable ratios.

Analyzed with linear mixed-model analysis, with the CES-D score after 3 and 6 years as outcome and baseline CES-D as covariate. Model 1: adjusted for age, education, season and cohort. Model 2: additionally adjusted for smoking, alcohol use, BMI, chronic diseases, cognitive functioning and physical activity. 25(OH)D: 25-hydroxyvitamin D; CES-D: Center for Epidemiological Studies Depression Scale.