The association between vitamin D deficiency and MRI markers of brain health in a community sample

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ABSTRACT

Background and aim

Vitamin D deficiency has been linked to an increased risk of dementia. However, to strengthen current evidence and establish whether vitamin D can indeed play a role in early prevention of neurodegeneration, knowledge on underlying pathways is crucial. Therefore it was the aim of this study to investigate the association of vitamin D status with brain tissue volumes, hippocampus volume, white matter integrity, and markers of cerebral small vessel disease (CSVD) in a dementia-free population.

Methods

In this cross-sectional analysis, 2,716 participants free of dementia from the population-based Rotterdam Study underwent serum 25(OH)D concentration assessment and brain magnetic resonance imaging (MRI) scanning between 2006 and 2009. Outcomes of interest included brain tissue volume (total, white matter, grey matter and hippocampus volume), white matter integrity (fractional anisotropy (FA) and mean diffusivity (MD)), and markers of CSVD (white matter hyper intensity (WMH) volume, presence of lacunes and microbleeds). Associations between vitamin D status, both in categories and continuous, and these brain measurements were assessed using multivariable linear and logistic regression models, adjusting for lifestyle and other disease risk factors.

Results

We observed that vitamin D deficiency (25(OH)D <30 nmol/L) was independently associated with smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume as compared to a sufficient vitamin D status (≥50 nmol/L). Vitamin D per 10 nmol/L increment and an insufficient (30-50 nmol/L) as compared to sufficient vitamin D status were not associated with the brain measures of interest. Moreover, vitamin D status was not associated with grey matter volume, white matter integrity or CSVD markers.

Conclusions

In this dementia-free population, vitamin D deficiency was associated with a smaller brain tissue volume and hippocampus volume. More research, in particular longitudinal, is needed to further elucidate the role of vitamin D in neurodegeneration.

INTRODUCTION

Vitamin D deficiency has been repeatedly linked to higher risk of dementia.[1-8] However, to strengthen current evidence and establish whether vitamin D can indeed play a role in early prevention of neurodegeneration, knowledge on underlying pathways is crucial. It has been hypothesized that the association of vitamin D with dementia might be explained by direct effects of vitamin D levels on brain health.

Indeed, previous studies found that higher vitamin D concentrations were associated with larger grey matter volume,[5] and that lower levels were associated with decreased white matter microstructural integrity (as reflected in lower fractional anisotropy (FA) and higher mean diffusivity (MD)),[9] and with markers of cerebral small vessel disease (CSVD) (white matter hyperintensity (WMH) volume and presence of lacunes and microbleeds).[10, 11] Contrary, another study reported an association of higher vitamin D concentrations with smaller white matter volume.[12] Interestingly, some studies reported that vitamin D deficiency was associated specifically with smaller hippocampus volumes,[13, 14] a part of the brain that plays a major role in learning and memory.[15] However, these previous studies had mostly small to moderate sample sizes, some included participants with dementia, and many studies did not extensively adjust for important confounding factors such as age and other lifestyle factors.[5, 10, 12]

Therefore, we studied the association of vitamin D status with several brain measures, including brain tissue volume (total, white matter, grey matter and hippocampus volume), white matter integrity (FA and MD), and markers of CSVD, in a large middle- and older-aged dementia-free population-based sample.

METHODS

Study design and study population

This cross-sectional study was embedded within the Rotterdam Study, an ongoing population-based prospective cohort study in the Netherlands investigating determinants and consequences of ageing since 1990.[16] At study entry and subsequently every three to four years participants were interviewed and underwent extensive examinations at the dedicated research centre in the district of Ommoord. By 2008, 14,926 individuals aged 45 years and over participated in the Rotterdam Study.

From 2005 onward, magnetic resonance imaging (MRI) scanning of the brain was included in the study protocol.[17] Between 2006 and 2009 blood samples were obtained from 3,425 participants. Of those 3,425 participants, 466 had no MRI data available and 125 had missing vitamin D data. From this group we further excluded participants with implausible MRI scan data (extremely low tissue volumes) (N = 22), participants with

prevalent dementia (N = 3), insufficient data to determine dementia status (N = 26) and those with a score < 24 on the Mini-Mental State Examination (MMSE) (N = 67).[18] None of these participants had cortical brain infarcts on MRI. The final population of analysis consisted of 2,716 participants, with a mean time interval between MRI scan and blood sample collection of 0.1 months (standard deviation (SD): 1.1).

Standard protocol approvals, registrations, and participant consents

The Rotterdam Study has been approved by the medical ethics committee of Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Vitamin D (25(OH)D) assessment

Vitamin D status was determined from 25-hydroxyvitamin D (25(OH)D) concentrations in blood. Fasting blood samples were collected at baseline. Serum 25(OH)D concentration was measured using an electrochemiluminescense-based assay (COBAS Roche Diagnostics GmbH, Germany). This assay has a functional sensitivity of 10 nmol/L (CV 18.5%), measuring a range of 7.5 nmol/L to 175 nmol/L, within-run precision of ≤6.5%, and intermediate precision of ≤ 11.5%.[19, 20] For the current analyses, vitamin D status was categorized into a 25(OH)D concentration of <30 nmol/L considered as deficient, a 25(OH)D concentration of 30-50 nmol/L was considered insufficient, and a 25(OH)D concentration ≥50 nmol/L was considered sufficient based on the vitamin D guidelines of the Institute of Medicine (USA).[8]

Assessment of brain volumetry, white matter microstructure and markers of CSVD

Brain MRI was performed using 1.5-T MRI system with a dedicated 8-channel head-coil (software version 11x; General Electric Healthcare, Milwaukee, WI).[17] The scan protocol included a T1 weighted sequence (voxel sixe 0.49 x 0.49 x 1.6 mm^3), a proton-density weighted sequence (voxel size 0.6 x 0.98 x 1.6 mm^3), and a fluid-attenuated inversion recovery sequence (voxel size 0.78 x 1.12 x 2.5 mm^3). To quantify intracranial volume, brain tissue volume, grey matter volume, white matter volume, and WMH volume, automated brain tissue classification was used.[17] This quantification strategy was based on a *k*-nearest neighbour classification algorithm.[21] Supratentorial intracranial volume was estimated by summing grey matter and white matter (the sum of normal-appearing white matter and WMH volume). To obtain hippocampus volume, T1-weighted MRI's were processed using FreeSurfer (version 5.1).[17, 22]

To obtain microstructural measures of the white matter, diffusion tensor imaging (DTI) (voxel size 3.3 x 2.2 x 3.5 mm^3) was used.[23] A single shot, diffusion weighted spin echo echo-planar imaging sequence was performed with maximum *b* value of 1,000 s/mm^2 in 25 noncollinear directions; 3 b_0 volumes were acquired without diffusion weighting. Using a standardized processing pipeline, diffusion data were pre-processed.[24] From this (in combination with the tissue segmentation), we derived global mean FA and MD in the normal-appearing white matter. FA is the degree of anisotropy in the normal-appearing white matter and is given as a ratio ranging from 0 (isotropic or non-directional) to 1 (unidirectional). MD is expressed in square millimetres per second. Visual ratings were performed for the presence of lacunes or microbleeds by trained raters.[17]

Covariate assessment

Trained interviewers conducted home interviews obtaining information on smoking, alcohol consumption and education. Smoking status was categorized as never, past or current smoker. Educational level was categorized as primary, lower, intermediate, and higher education. Physical activity data was collected using the LASA Physical Activity Questionnaire.[25] Metabolic equivalent of tasks (MET) scores were computed by summing the time spent in light, moderate and vigorous activity in MET-hours per week.[26] Depressive symptoms were assessed using the Centre for Epidemiology Depression Scale (CES-D) and were considered present with a score of ≥16.[27] Blood pressure was measured twice in sitting position with a random-zero sphygmomanometer and an average was computed. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and serum glucose were measured in non-fasting participants using an automated enzymatic procedure. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, and/or a diastolic blood pressure of ≥90, and/or antihypertensive medication use.[16] Diabetes was defined as fasting serum glucose of ≥7.0 mmol/L and/or use of antidiabetic medication.[16] Height and weight were measured at the research centre. Body mass index (BMI) was computed as weight divided by meters squared.[16] The MMSE is a validated screening tool and used to screen participants on their mental status.[28]

Statistical analyses

First, we present descriptive statistics for exposures, outcomes and covariates, for the whole study population and stratified by vitamin D categories. Subsequently, we used multivariable linear regression to examine associations of vitamin D status with brain tissue volumes (brain tissue volume, white matter volume, grey matter volume, and hippocampus volume), WMH volume (log-transformed), and global (standardized) measures of white matter integrity (FA and MD). Multivariable logistic regression was used to examine associations of vitamin D status with the presence of lacunes and microbleeds. In all analyses, vitamin D status was analysed both as a continuous variable and categorized

into deficient, insufficient, and sufficient vitamin D status, using sufficient as reference.[2] Assumptions of linearity and normality were checked using PP-plots and scatterplots of residuals, respectively. Assumption of no multicollinearity was checked using VIF values (<10) and homoscedasticity was checked by plotting standardized residuals against predicted residuals. We selected confounders based on theory or literature.[29] Model 1 was adjusted for age, age^2 (to account for non-linear age effects), sex, season of blood draw and intracranial volume (to account for inter-individual differences in head size). Model 2 was additionally adjusted for education, BMI, physical activity, smoking, alcohol consumption, prevalent diabetes mellitus, hypertension and hypercholesterolemia. We additionally adjusted for normal-appearing white matter volume in the analysis for vitamin D status with white matter microstructure. Multiple imputation (m = 10) with the expectation-maximization method was used for missing values of covariates (<3.16%), using data on exposure, outcome and other explanatory variables as predictors. As sensitivity analyses, we repeated our analyses excluding participants with a CES-D score ≥16 (N = 123), indicating presence of depressive symptoms.[30] Moreover, to exclude the possibility of reverse causation by cognitive status, we first repeated our analyses only in participants with a MMSE score of ≥ 29 (N = 1,373). Subsequently, we included participants with lower MMSE scores by two points at a time, down to a minimum score of 25. Level of statistical significance was set at 0.05 and two-tailed analyses were performed using IBM SPSS statistics version 24 (SPSS Inc., Armonk, NY, USA) and using RStudio; integrated development environment for R, version 3.5.1 (RStudio, Boston, MA).

RESULTS

Table 1 shows the baseline characteristics of the study participants. Mean age was 56.9 years (standard deviation (SD) 6.4), 55.4% was female. Mean 25(OH)D was 60.9 nmol/L (SD: 27.8) and blood drawing for vitamin D assessment was mainly performed in autumn (33.7 %). Of our study population, 12.4% was vitamin D deficient (25(OH)D<30 nmol/L), 25.1% had an insufficient vitamin D status (25(OH)D 30-50 nmol/L), and 62.4% of the population had a sufficient vitamin D status (25(OH)D ≥50 nmol/L). Generally, participants with lover levels of vitamin D had a higher BMI, were more often smokers, drank less alcohol, were less physically active and had a higher prevalence of hypertension (Table 1).

Table 2 shows the association of vitamin D with brain tissue volumes. Modelled per 10 nmol/L increment, we did not find associations between vitamin D concentration and brain tissue volumes, white matter integrity nor the presence of markers of CSVD. However, for categories of vitamin D status, we observed that participants with a deficient vitamin D status had smaller total brain tissue volume than those with a sufficient

vitamin D status (difference in mL brain volume as compared to sufficient vitamin D status: -5.02 [95% CI: -8.70, -1.35]). This association with total brain volume slightly attenuated after adjustment for confounders in model 2 (difference: -4.36 [95% CI: -8.07, -0.65]) (Table 2). Vitamin D deficiency as compared to a sufficient vitamin D status was associated with smaller white matter volumes (difference: -6.21 [95% CI: -10.21, -2.20] and difference: -5.67 [95% CI: -9.76, -1.59]; model 1 and 2 respectively), but not with grey matter volume (Table 2). We also observed that a vitamin D deficiency compared to a sufficient vitamin D status was associated with smaller hippocampus volume in both

Table 1. Population characteristics

Characteristics	Total sample (N = 2,716)	Vitamin D deficient (N = 338; 12.4%)	Vitamin D insufficient (N = 682; 25.1%)	Vitamin D sufficient (N = 1,696; 62.4%)
25(OH)D, nmol/L	60.9 ± 27.8	21.9 ± 4.9	39.3 ± 5.8	77.3 ± 21.3
Age, years	56.6 ± 6.4	56.3 ± 7.4	56.7 ± 6.7	56.6 ± 6.0
Age, range	45.5 – 87.8	45.6 – 87.8	45.5 – 87.1	45.8 – 84.1
Female, %	55.4	57.4	55.2	54.8
Education, %				
Primary	9.4	11.1	9.9	9.1
Lower	33.9	30.6	32.5	35.3
Intermediate	28.9	27.4	28.7	28.9
Higher	27.7	30.6	28.9	26.4
Body mass index, kg/m^2	27.5 ± 4.3	28.9 ± 5.2	28.2 ± 4.7	27.0 ± 3.9
Smoking, %				
Never	29.9	34.7	30.5	28.7
Ever	43.9	35.3	40.9	46.4
Current	26.3	30.0	28.6	24.9
Alcohol, grams/wk[a]	11.6 (2.6 – 23.4)	9.1 (1.6 – 21.8)	10.4 (1.8 – 22.6)	12.2 (3.4 – 24.2)
Physical activity, MET-h/wk[a]	50.6 (22.4 – 87.7)	43.0 (15.0 – 79.2)	46.0 (18.0 – 82.0)	54.3 (24.5 – 93.1)
Diabetes, %	9.0	14.6	12.3	6.6
Hypertension, %	47.2	51.3	50.5	44.9
Hypercholesterolemia, %	4.3	2.3	4.2	4.8
CES-D score[a]	3.0 (1.0 – 7.0)	4.0 (1.0 – 10.0)	3.0 (1.0 – 8.0)	3.0 (1.0 – 7.0)

Values are mean ± SD or median (interquartile range) when indicated ([a]) for continuous variables, and percentages for dichotomous variables. MET: metabolic equivalent of task. CES-D: Center for epidemiologic studies depression scale. CHD: coronary heart disease. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/litre. IQR: interquartile range. Dates of season of blood draw: winter 21 December – 20 March; spring 21 March – 20 June; summer 21 June – 20 September; autumn 21 September – 20 December. Values are based on imputed data. Numbers missing per variable were 2 for BMI, 5 for smoking, 732 for alcohol in grams per week, 736 for MET/h per week, 8 for education, 26 for CHD, 22 for hypertension, 5 for total cholesterol, 7 for HDL cholesterol, 10 for systolic and for diastolic blood pressure and 261 for season of blood draw.

Table 2. The association between vitamin D status and brain volumetry

Vitamin D status		Brain tissue volume	Grey matter volume	White matter volume	Hippocampus volume
		Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	0.33 (-0.10, 0.75)	0.01 (-0.40, 0.42)	0.32 (-0.14, 0.79)	0.01 (-0.00, 0.02)
	Model 2	0.31 (-0.13, 0.75)	0.01 (-0.42, 0.43)	0.39 (-0.09, 0.88)	0.01 (-0.00, 0.02)
Vitamin D sufficient		*Reference*	*Reference*	*Reference*	*Reference*
Vitamin D insufficient	Model 1	-0.72 (-3.50, 2.06)	0.42 (-2.26, 3.09)	-1.14 (-4.17, 1.89)	-0.04 (-0.11, 0.03)
	Model 2	-0.26 (-3.06, 2.53)	0.53 (-2.19, 3.24)	-0.79 (-3.86, 2.29)	-0.02 (-0.09, 0.05)
Vitamin D deficient	Model 1	**-5.02 (-8.70, -1.35)**	1.18 (-2.35, 4.72)	**-6.21(-10.21, -2.20)**	**-0.12 (-0.21, -0.03)**
	Model 2	**-4.36 (-8.07, -0.65)**	1.31 (-2.29, 4.91)	**-5.67 (-9.76, -1.59)**	**-0.10 (-0.19, -0.01)**

Difference in mL represents the difference in brain volume per 10 nmol/L increase in vitamin D concentration or the difference in brain volume for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. mL: milliliter. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Vitamin D sufficient: 25(OH)D ≥ 50nmol/L. Vitamin D insufficient: 25(OH)D 30-50 nmol/L. Vitamin D deficient: (25(OH)D < 30 nmol/L. Model 1 is adjusted for age, age^2, sex, season of blood draw and intracranial volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension, and hypercholesterolemia. Statistically significant values ($p<0.05$) are indicated in **bold.**

models (difference model 1: -0.12 [95% CI: -0.21, -0.03]; difference model 2: -0.10 [95% CI: -0.19, -0.01]) (Table 2). No differences were observed for insufficient as compared to sufficient vitamin D status. Also, 25(OH)D concentration or status was not associated with focal MRI-markers such as WMH volume, lacunes and microbleeds (Table 3) or with global measures of white matter integrity (FA and MD) (Table 4).

No significant interaction effects were found for gender, depressive symptoms or season ($p >0.05$). In the sensitivity analysis excluding all participants who scored ≥16 on the CES-D (depressive symptoms present) we observed similar results as reported in the whole study population (Supplementary table 1). There was no indication of reverse causality by cognitive status as results between vitamin D status and brain tissue volumes on MMSE scores were comparable to results in the entire population (Supplementary tables 2-4).

Table 3. The association between vitamin D status and markers of CSVD

Vitamin D status		White matter lesion volume*	Lacunes	Microbleeds
		Difference (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	-0.01 (-0.02, 0.01)	0.96 (0.89, 1.04)	0.98 (0.94, 1.03)
	Model 2	0.00 (-0.01, 0.01)	0.98 (0.90, 1.06)	0.98 (0.94, 1.03)
Vitamin D sufficient *(25(OH)D ≥ 50nmol/L)*		*Reference*	*Reference*	*Reference*
Vitamin D insufficient *(25(OH)D 30-50 nmol/L)*	Model 1	0.06 (-0.01, 0.12)	0.99 (0.59, 1.66)	1.04 (0.77, 1.41)
	Model 2	0.03 (-0.03, 0.10)	0.93 (0.55, 1.57)	1.04 (0.76, 1.41)
Vitamin D deficient *(25(OH)D < 30 nmol/L)*	Model 1	0.05 (-0.04, 0.13)	1.29 (0.69, 2.41)	1.13 (0.77, 1.68)
	Model 2	0.02 (-0.07, 0.10)	1.15 (0.60, 2.20)	1.13 (0.76, 1.68)

* log-transformed. Difference represents the difference in the log of white matter lesion volume per 10 nmol/L increase in vitamin D concentration or the difference in the log of white matter lesion volume for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. Odds ratio represents the odds of having lacunes or microbleeds per 10 nmol/L increase in vitamin D concentration or the odds of having lacunes or microbleeds for vitamin D insufficient and deficient status as compared to sufficient vitamin D status. CSVD: cerebral small vessel disease. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Model 1 is adjusted for age, age^2, sex, season of blood draw and intracranial volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension and hypercholesterolemia. Statistically significant values ($p<0.05$) are indicated in **bold.**

Table 4. The association between vitamin D status and white matter microstructure

Vitamin D status		Fractional anisotropy	Mean diffusivity
		Difference in SD (95% CI)	Difference in SD (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	0.00 (-0.01, 0.02)	-0.00 (-0.02, 0.01)
	Model 2	0.00 (-0.01, 0.02)	-0.00 (-0.02, 0.01)
Vitamin D sufficient *(25(OH)D ≥ 50nmol/L)*		*Reference*	*Reference*
Vitamin D insufficient *(25(OH)D 30-50 nmol/L)*	Model 1	-0.07 (-0.16, 0.02)	0.03 (-0.05, 0.11)
	Model 2	-0.07 (-0.15, 0.02)	0.02 (-0.06, 0.10)
Vitamin D deficient *(25(OH)D < 30 nmol/L)*	Model 1	-0.03 (-0.14, 0.09)	0.01 (-0.09, 0.11)
	Model 2	-0.02 (-0.14, 0.10)	-0.01 (-0.12, 0.09)

Difference in SD represents the difference in standard deviation fractional anisotropy or mean diffusivity per 10 nmol/L increase or the difference in standard deviation fractional anisotorpy or mean difusitivy for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. SD: standard deviation. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Model 1 is adjusted for age, age^2, sex, season of blood draw, intracranial volume and normal-appearing white matter volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension, and hypercholesterolemia. Statistically significant values ($p<0.05$) are indicated in **bold.**

DISCUSSION

In this large population-based study of Dutch middle-aged and older adults free from dementia we observed smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume in persons with vitamin D deficiency as compared to persons with a sufficient vitamin D status. Vitamin D status was not associated with grey matter volume, white matter integrity, or with markers of CSVD.

Strengths of our study include the population-based setting, the large sample size and the standardized quantitative assessment of brain tissue volume, white matter integrity and markers of CSVD. However, some limitations should also be acknowledged. First, due to the cross-sectional design of this study, no causation or temporal direction of the association can be established. Second, although we adjusted for a wide range of covariates, residual confounding might be present from unmeasured or incompletely measured confounders, such as an overall healthier lifestyle resulting in a higher vitamin D status. Third, the Rotterdam Study population incorporated an almost exclusively Caucasian population, limiting generalizability towards other populations.

In our study we found an association of vitamin D status with total brain tissue and white matter volume but not with grey matter volume. These results are not only statistically significant, but based on the observed effect sizes may also be clinically relevant. In another population-based sample it was found that one year of ageing was equivalent to a 5.40 mL smaller brain tissue volume and a 2.30 mL smaller white matter volume.[31] Thus, the averagely 4.36 mL smaller brain tissue volume and 5.67 mL smaller white matter volume that we observed in those with vitamin D deficiency as compared to those with sufficient levels may indicate significant accelerated neurodegeneration on top of normal age-related changes. Previous studies on brain health in relation to vitamin D were inconsistent and have shown associations of vitamin D status with larger or even smaller total brain tissue, grey matter, and/or white matter volume.[5, 12, 32, 33] However, most of these studies did not exclude participants with cognitive impairment or dementia, thereby introducing the possibility to measure a reverse effect of cognitive decline on neurodegeneration rather than the effects of vitamin D status. We performed additional sensitivity analyses in our sample to rule out the reverse causality caused by preclinical cognitive impairment as an explanation for our results. In line with our results, another large prospective community-based study, the Framingham Heart Study, consisting of 1,139 participants (mean age 59.3 years), also found an association of vitamin D deficiency with lower total brain tissue volume.[33] However, further replication is needed, preferable from studies that have repeated measurements of both vitamin D status and brain measurements to infer on temporality.

Interestingly, we also observed an association of vitamin D deficiency with smaller volumes of the hippocampus compared to those with sufficient vitamin D levels. This

was also observed in three previous population-based studies.[14, 32, 33] Unfortunately only one of these three previous studies took cognitive status into account.[14, 32, 33] The hippocampus plays a critical role in cognition, especially in declarative memory and is one of the first parts in the brain to be affected in the case of Alzheimer's disease.[34, 35] Although total evidence remains sparse, the results from our study and these previous studies combined suggest a promising potential role of vitamin D for brain health.[5, 32, 33] And in light of above findings one could hypothesize that a sufficient vitamin D status promotes brain health directly through larger brain tissue volume and possibly even through larger tissue volume of the hippocampus. When interpreting these results, it is of importance to take into account that a higher BMI is related to vitamin D deficiency and that a high BMI has been reported to increase the risk of dementia. In our population we saw that participants with vitamin D deficiency have a higher BMI than participants with sufficient or insufficient vitamin D levels. We adjusted for BMI in our models, but because of the cross-sectional nature of our study could not further explore the role that BMI and changes in BMI may have in these associations. Longitudinal studies are warranted to study effects of overweight and obesity in the association between vitamin D and brain health.

A potential direct effect of vitamin D and brain health may go through neuronal health. Indeed, it has been found that higher levels of vitamin D support neuronal growth, maintenance and survival by the up-regulation of neurothrophins such as neurotrophin-3 (NT-3), glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF).[1, 36-38] Lower concentrations of BDNF have been found to be associated with reduced hippocampus volume.[39] Vitamin D can also modulate neurogenesis in the hippocampus and is neuroprotective as it activates the downregulation of L-type-voltage sensitive calcium channel (LVCC), which causes exitotoxic cell death in the hippocampus and upregulating vitamin D receptors.[1, 39, 40] Moreover, vitamin D deficiency may result in a lack of protective effects related to enhancement of amyloid-beta peptide clearance across the blood brain barrier.[40] Unfortunately, we do not have these kind of measures in the Rotterdam Study and are therefore unable to replicate those potential associations.

Contrary to the few other studies on these outcomes, we did not find an association between vitamin D status and white matter integrity or markers of CSVD.[10, 11, 14] One study among participants with mild cognitive impairment (N = 54) found an association between vitamin D deficiency and disruptions of neural white matter integrity, primarily in the frontal regions of the brain.[14] Regrettably, this study did not adjust profoundly for possible confounding factors. As such, significant results may still be (partly) confounded, increasing the risk of an overestimation of the true effect. In our study, we only used global measures of white matter integrity, which might limit sensitivity for possible associations between vitamin D and white matter integrity in specific regions

of the brain. For example, in light of our results it could be hypothesized that vitamin D deficiency is associated with degeneration in white matter fibre bundles connecting parts of the brain which are important for cognition. Moreover, other studies found an inverse association between vitamin D concentration and WMH volume,[14] as well as an inverse association between vitamin D concentration and the number of lacunes and microbleeds.[10, 11] But those latter two studies included participants with acute ischemic stroke or transient ischemic attack, therefore possibly introducing selection bias as those participants may already be further along the neurodegenerative-pathway due to these cerebrovascular events then participants without a history of cerebrovascular events.[10]

In conclusion, our study shows that vitamin D deficiency is associated with smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume. These results suggest that an adequate vitamin D status is important for structural brain health, and probably most importantly, for hippocampus volume. This association with structural brain health may partly explain previously reported associations between vitamin D, cognitive decline and the risk of dementia. Although more research is needed to replicate these findings, our results support the importance of achieving a sufficient vitamin D status, for example by increasing vitamin D intake, supplementation, or through sufficient sun exposure.

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