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ORIGINAL ARTICLE

Vitamin D and white matter hyperintensities: results of the population-based Heinz Nixdorf Recall Study and 1000BRAINS

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Abstract

Background and purpose: Cross-sectional studies showed an inverse association between serum 25-hydroxyvitamin D (25OHD) and white matter hyperintensities (WMHs) whereas the few longitudinal studies did not. The association between baseline 25OHD and WMHs at 10-year follow-up in the Heinz Nixdorf Recall Study plus 1000BRAINS was investigated.

Methods: Data of 505 participants (49% women, 56.2 ± 6.6 years) with 25OHD at baseline (2000–2003) and WMH volume and grade of WMHs using the Fazekas classification at 10-year follow-up were analysed. The association between deseasonalized 25OHD and the base-10 logarithm of WMH volume was evaluated by multiple linear regression, adjusted for age, sex, education, smoking, alcohol consumption, sports, diabetes mellitus, systolic blood pressure and total cholesterol. β-estimators were transformed back (10^{β}). Using multiple logistic regression, odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to evaluate the association between deseasonalized 25OHD and Fazekas grades (0, absence and 1, punctate foci vs. 2, beginning and 3, large confluence).

Results: Mean 25OHD was 17.0 ± 8.2 ng/ml, and mean deseasonalized 25OHD was 16.9 ± 7.5 ng/ml. Mean WMH volume was 16.6 ± 17.4 ml, range 1–132 ml. Most grade 2–3 WMHs were found to be periventricular (39% of the participants), parietal (32%) and frontal (31%) (temporal 6%, occipital 3%). The linear regression showed an inverse association between 25OHD and WMH volume. On average, a 25OHD increase of 1 ng/ml was associated with a reduced WMH volume by a factor of 0.99 (95% CI 0.98; 1.00) (fully adjusted). There was also some indication for an inverse association between 25OHD and extent of periventricular (OR 0.98 [95% CI 0.96; 1.01]), frontal (0.99 [0.97; 1.02]) and parietal (0.98 [0.95; 1.00]) WMHs according to the Fazekas classification.

Conclusions: Lower 25OHD may be a risk factor for the occurrence of WMHs.

KEYWORDS
25OHD, brain, hypovitaminosis D, leukoaraiosis, magnetic resonance imaging, vitamin D, white matter lesions

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INTRODUCTION

Many cross-sectional studies show that lower serum 25-hydroxyvitamin D (25OHD) is associated with a greater extent of white matter hyperintensities (WMHs) [1–6]. This relationship was not observed in the few longitudinal studies available [7–9]. Nonspecific age-related morphological brain changes are characterized by an increase in WMHs, also called leukoaraiosis [10]. WMHs can be visualized in magnetic resonance imaging (MRI) and typically appear rather symmetrically within the white matter of both hemispheres. They cause disruptions of the cortico-subcortical white matter tracts connecting brain regions [11]. WMHs do not appear to cause symptomatic burdens during early stages, but people with more extensive WMHs tend to have reduced cognitive and functional abilities. WMHs are associated with an increased risk of stroke [12], brain atrophy, cognitive and motor decay [13], dementia [8] and even death [14,15]. Deep WMHs are associated with cerebral ischaemic small vessel disease and hypertension, whilst periventricular WMHs are generally imputed to age-related subcortical brain atrophy [16–18]. Since vitamin D influences immunomodulatory and neuroprotective processes in the brain [19], the question arises as to whether lower vitamin D levels cause WMHs. The aim of this study was to investigate the association between baseline 25OHD and the extent of WMHs at 10-year follow-up measured with 3 T MRI based on the data of the large longitudinal population-based Heinz Nixdorf Recall (HNR) Study and 1000BRAINS.

METHODS

Study population

The study population was based on the HNR study and 1000BRAINS. Both studies have been described in detail elsewhere [20,21]. Briefly, the HNR study is an ongoing population-based study that started in 2000–2003 (t_0). Men and women aged 45–75 years at t_0 from the general population living in three large adjacent cities (Bochum, Essen, Muelheim/Ruhr) were recruited from a random sample derived from mandatory citizen registries. The response rate was 56%. In total 4814 participants (50% women) were enrolled [22]. The study was certified and recertified according to DIN EN ISO 9001:2000/2008. 1000BRAINS started in 2011 and is an epidemiological, neuroscientific study which focuses on structural and functional variability in the human brain during the process of ageing [20]. Participants were recruited from the 10-year follow-up (second follow-up, t_2) of the HNR study, amongst others. Additional to the HNR study protocol, 1000BRAINS conducted multimodal brain imaging with 3 T MRI, neuropsychological tests, further laboratory tests, genetic examinations and introduced additional questionnaires. All participants provided written informed consent, gave permission for future measurements, and both studies were approved by the institutional ethics committee.

Figure 1 shows the flowchart of the study population. Out of 4814 participants at t_0, 1727 participants without follow-up were excluded at t_2; of the remaining participants 436 were excluded without 25OHD measurements at t_0 or t_2, 2080 participants without MRI at t_2, 27 because of motion artefacts in MRI, 19 participants with history of stroke and 20 without Fazekas classification due to missing sequence. Our final study population comprised 505 participants.

Measurements

Computer-assisted face-to-face interviews, clinical examinations, comprehensive laboratory tests and MRI were conducted according to standard protocols. The questionnaires referred to behavioural risk factors (smoking, drinking of alcoholic beverages, physical activity), full medical history and sociodemographic characteristics.

25OHD

Serum 25OHD was measured as a prehormone of active vitamin D by the LIAISON® 25 OH Vitamin D TOTAL Assay by DiaSorin which is a direct competitive chemiluminescent immunoassay using coated magnetic microparticles. DiaSorin states dynamic range 4–150 ng/ml, functional sensitivity ≥4 ng/ml, concordance correlation coefficient 0.95, sufficiency 30–100 ng/ml, insufficiency <30–10 ng/ml, deficiency <10 ng/ml [23]. 25OHD was measured on thawed serum samples (stored at −80°C). Participants were asked about vitamin substitution at t_0 and t_2.

Exposure variable

The exposure variable was deseasonalized 25OHD at t_0. For deseasonalization, 25OHD residuals of the total HNR population at t_0 with n = 4131 25OHD readings were estimated, standardized by calendar week of measurement. Residuals were added to the mean 25OHD value of 16.02 ng/ml. The same was done to estimate 25OHD residuals at t_2 (n = 2982, mean 25OHD 15.78 ng/ml). The further analysis comprises only participants with MRI.

Magnetic resonance imaging

Participants were excluded from MRI according to the standard ethical and safety guidelines for MRI examination for people who exclusively receive the examination for scientific purposes: coronary-artery scans, cardiac pacemakers, surgical implants or prosthesis in trunk or head, claustrophobia, history of neurosurgery, presence of tattoos or permanent make-up on the head. Relative contraindications were dental implants and bridges; the scans were discontinued if the objects led to artefacts [20]. MRI scans were performed on a 3

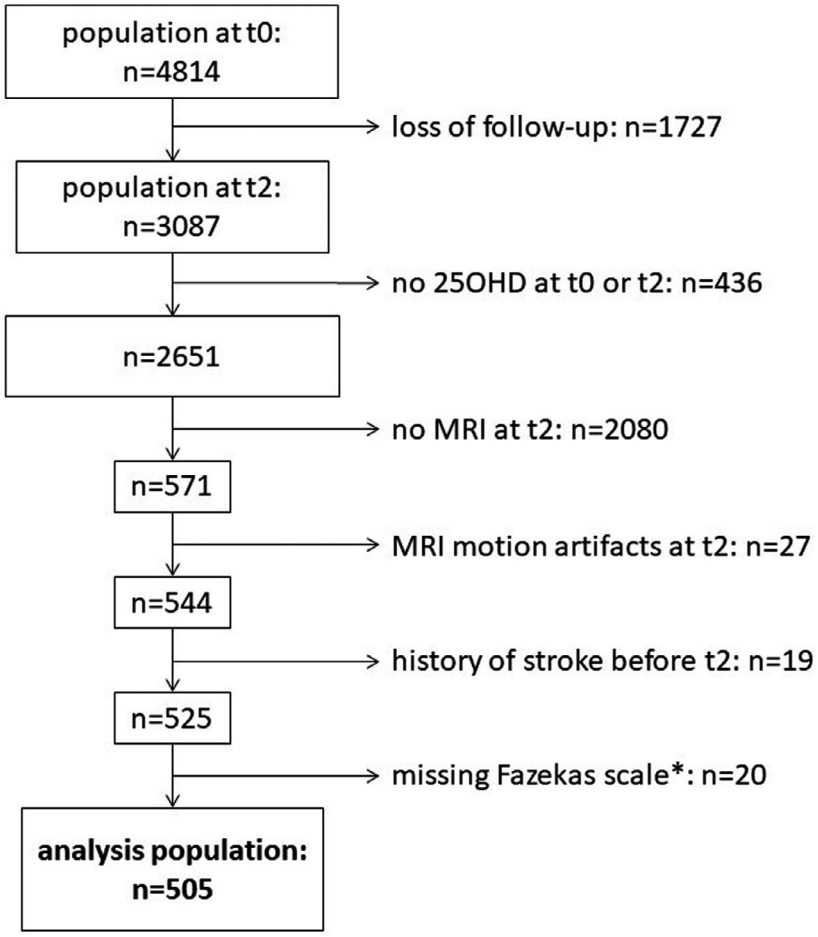

FIGURE 1 Flowchart of the study population. *Others, but no fluid attenuated inversion recovery (FLAIR) sequence to perform the Fazekas classification was available

T MR scanner (Tim-TRIO, Siemens Medical Systems) in the Research Centre Jülich, Germany.

Outcome variables

Outcome variables were (i) the MRI volume of WMHs in millilitres and (ii) the location and extent of WMHs in MRI according to the Fazekas classification [24].

1. WMH volume: For the detection of the WMH volume an anatomical 3D T1-weighted magnetization prepared-rapid gradient echo (MPRAGE) sequence and a T2-weighted fluid attenuated inversion recovery (FLAIR) sequence was used. WMH volume was measured using the Lesion Segmentation Tool for the software tool SPM8, version 1.2.3, 2013, as implemented in MATLAB 2013b (Statistical Parametric Mapping) [25].
2. Fazekas classification: Using FLAIR sequences two independent raters evaluated the location and extent of WMHs independently according to the qualitative rating scale of Fazekas et al. [24]. The rating scale was modified because it was specified by allocation of the WMHs to frontal, parietal, occipital and temporal lobes, and periventricular [26]. Periventricular hyperintensities were graded as 0, absence; 1, 'caps' or pencil-thin lining; 2, smooth 'halo'; and 3, irregular periventricular hyperintensities extending into the deep white matter. Separate deep WMH signals were rated as 0, absence; 1, punctate foci; 2, beginning confluence of foci; 3, large confluent areas [24,26].

Confounder variables at *t*0

Included confounder variables were defined as follows. Body mass index was calculated from height and weight. Education was classified according to the International Standard Classification of Education (ISCED-97) as total years of formal education, combining school and vocational training. 'Low education' was defined as <13 years of education, otherwise 'high education'. 'Current smoking' was defined as a history of cigarette smoking during the past year. 'Past smoking' was defined as quitting smoking more than a year ago, otherwise 'never smoking'. Alcohol drinking behaviour was assessed via a self-report questionnaire asking about the average consumption of different beverages within the last 4 weeks. The proportion of pure alcohol per beverage was then multiplied with the frequency of drinking. All beverages per person were summed up, resulting in the amount of total consumption of pure alcohol in grams per week [27]. 'Sports' was defined as 'yes' when practised in the last 4 weeks before the interview, otherwise 'no'. Fasting blood

glucose was measured. Participants were classified as diabetics when glucose exceeded ≥126 mg/dl or they reported use of insulin or oral hypoglycaemic agents [28]. Blood pressure was measured using an oscillometric method (Omron; Netherlands). The mean value of the second and third of three measurements taken at least 2 min apart was used [29]. Total cholesterol was measured with a standard enzymatic method [30].

Statistical analysis

Further results are based on the analysis of the data of 505 participants with 25OHD readings at t_0 and MRI at t_2. Descriptive statistics were used to present characteristics of the study population. The correlation between deseasonalized 25OHD at t_0 and t_2 was measured by the Pearson correlation coefficient. Mean deseasonalized 25OHD and standard deviation at t_0 was calculated according to the regional Fazekas classification. The association between deseasonalized 25OHD at t_0 and the base-10 logarithm of WMH volume at t_2 was evaluated by univariate and multiple linear regression to assess predictors and their corresponding 95% confidence intervals (95% CI), adjusting for age, sex, education, smoking, alcohol consumption, sports, diabetes mellitus, systolic blood pressure and total cholesterol. β-estimators were transformed back according to the formula 10^{β}. Using univariate and multiple logistic regression, odds ratios (ORs) and 95% CI were calculated to evaluate the association between deseasonalized 25OHD at t_0 and extent of WHMs at t_2 according to Fazekas (grades 0–1 vs. grades 2–3). If a 95% CI includes 1, the p value is >0.05 and estimates are not significant. All analyses were performed using SAS 9.4 (Statistical Analysis System Corp.).

RESULTS

Our study population comprised 505 participants (49% women, 56.2 ± 6.6 years). Participants were younger than the 4309 excluded persons (50% women, 60.0 ± 7.8 years), because older persons more often fulfilled exclusion criteria for MRI. Table 1 shows the characteristics of the study population. For men, there were no changes in mean vitamin D levels and substitution. For women, mean as-measured and deseasonalized 25OHD decreased from 17.4 ± 8.8 to 15.6 ± 7.8 ng/ml and 17.1 ± 8.1 to 15.9 ± 7.3 ng/ml, respectively,

TABLE 1 Characteristics of the study population, n = 505, n (%), mean ± SD

Time		Men	Women	Total
	n (%)	260 (51)	245 (49)	505 (100)
t_0	Age, years	56.8 ± 6.7	55.6 ± 6.5	56.2 ± 6.6
	BMI, kg/m^2	27.3 ± 3.7	26.8 ± 4.6	27.1 ± 4.1
	Education, high	98 (38)	53 (22)	151 (30)
	Smoking			
	Current	48 (19)	51 (21)	99 (20)
	Past	122 (47)	69 (28)	191 (38)
	Never	90 (35)	125 (51)	215 (43)
	Alcohol consumption, g/week	102.7 ± 128.5	32.7 ± 63.4	68.9 ± 108.1
	Missing	1	3	4
	Sports, yes	174 (67)	155 (63)	329 (65)
	Diabetes mellitus, yes	24 (9)	11 (5)	35 (7)
	Systolic RR, mmHg	134.2 ± 18.6	122.8 ± 16.7	128.7 ± 18.6
	Total cholesterol, mg/dl	224.6 ± 35.0	225.1 ± 38.4	224.8 ± 36.6
	25OHD, ng/ml	16.5 ± 7.5	17.4 ± 8.8	17.0 ± 8.2
	Deseasonalized 25OHD, ng/ml	16.7 ± 6.9	17.1 ± 8.1	16.9 ± 7.5
	Oral vitamin D intake, yes	2 (1)	6 (2)	8 (2)
t_2	25OHD, ng/ml	16.7 ± 7.6	15.6 ± 7.8	16.1 ± 7.7
	Deseasonalized 25OHD, ng/ml	16.6 ± 6.6	15.9 ± 7.3	16.3 ± 6.9
	Oral vitamin D intake, yes	2 (1)	17 (7)	19 (4)
	Volume WMHs, ml	18.9 ± 17.9	14.1 ± 16.5	16.6 ± 17.4

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMI, body mass index; RR, blood pressure; t_0, baseline examination; t_2, 10-year follow-up; WMHs, white matter hyperintensities.

whilst the percentage of vitamin D intake in women increased from 2% to 7%. The correlation between deseasonalized 25OHD at t_0 and t_2 is moderate (Pearson correlation coefficient r = 0.42). Men had a higher education than women (38% vs. 22%), were less likely to be non-smokers (35% vs. 51%), consumed more alcohol (103 g/week vs. 33 g/week), more often reported sports (67% vs. 63%), had more often diabetes mellitus (9% vs. 5%) and higher mean systolic blood pressure (134 mmHg vs. 123 mmHg), but had no differences in mean total cholesterol compared to women. At t_2 the mean WMH volume was 16.6 ± 17.4 ml with a range of 1–132 ml. Mean WMH volumes were higher in men than in women (18.9 ± 17.9 ml vs. 14.1 ± 16.5 ml).

Table 2 shows the allocation of WMHs and mean deseasonalized 25OHD at t_0 according to the regional Fazekas classification at t_2. Most grade 2–3 lesions were found periventricular (39% participants with grade 2–3 lesions) and in the frontal (32%) and parietal lobe (31%). In temporal and occipital lobes, grade 2–3 lesions were present only in 6% and 3% of the participants, respectively. Mean WMH volumes stratified by regional Fazekas grading were higher in men compared to women.

Figure 2 shows a weak but significant inverse association between WMH volume at t_2 and deseasonalized 25OHD at t_0 (also see Table S1). On average, a 25OHD increase of 1 ng/ml is associated with a reduced WMH volume by a factor of 0.99 (95% CI 0.98; 1.00). The effect is stronger in women than in men (fully adjusted 0.98 [0.97; 0.99] vs. 0.99 [0.98; 1.01]). Additional adjustment for diastolic blood pressure does not change the estimators.

Figure 3 shows the association between the regional WMH grades at t_2 according to the Fazekas classification and the deseasonalized 25OHD at t_0 (also see Tables S2 and S3). In frontal and parietal lobes as well as periventricularly, there were weak, non-significant, inverse associations. An increase in deseasonalized 25OHD at t_0 by 1 ng/ml results in a 0.98 (0.96; 1.01) times reduced chance of having periventricular grade 2–3 WMHs, 0.99 (0.97; 1.02) in frontal and 0.98 (0.95; 1.00) in parietal lobes. Temporal and occipital WMHs were rare; OR and 95% CI were not meaningful.

DISCUSSION

In this large population-based German cohort study of middle-aged and elderly adults, lower baseline 25OHD was found to be associated with a higher extent of WMHs 10 years later. The association was stronger in women.

The results of previous studies on this topic are heterogeneous. The majority of cross-sectional studies found an association between lower 25OHD and a higher extent of WMHs [1–6,31]. Our own cross-sectional analysis is in line with this; the results will be published soon. But, there are also cross-sectional studies that found no association [31–34]. The first large prospective population-based study by Michos et al. (50% of 1622 participants) showed no association between 25OHD and the extent of WMHs [7]. Those authors used a different evaluation scale for WMHs. Karakis et al. used a similar study design to ours, without brain imaging at baseline, and did not find an association between baseline 25OHD and WMHs, total brain volume or silent cerebral infarction at follow-up, but low 25OHD levels were associated with reduced hippocampal volumes. Participants with low 25OHD had worse results in trail-making and visual organization tests, indicating poorer cognitive and motor functions [8]. The prospective study of Littlejohns et al. used MRI scans with a follow-up period of 5 years to evaluate changes in WMHs, ventricular volume and the presence of infarcts. They concluded that 25OHD serum concentration was not associated with the development or deterioration of WMHs [9]. Our findings differ from the results of those previous studies with a longitudinal design [7–9].

What role vitamin D plays in the brain is not yet fully understood. Endogenous 25OHD and 1,25-dihydroxyvitamin D (1,25D) biosynthesis, expression of CYP groups, enzymes to activate and deactivate vitamin D, vitamin D receptors (VDRs) and protein-disulfide-isomerase family A members (PDIA3) have been detected in almost the entire brain [19]. A relatively new theory is that the conventional endocrine vitamin D concept is not sufficient to describe the cerebral effects. For some years now, there has been growing evidence that the active form of vitamin D, 1,25D, is produced locally like an autacoid, acts locally and is also inactivated locally. 25OHD levels in the serum are not affected [19]. It is discussed that the brain stores vitamin D in addition to fatty tissue, skin and muscle tissue [35]. VDRs have been detected in neuronal cells such as astrocytes, microglia and neurons [36,37]. The VDR is predominantly found in the nucleus and forms a complex with 1,25D and the retinoid X receptor. The complex binds to vitamin D response elements, which are located on the DNA within the regulatory regions of the target genes. The complex complements a multitude of chromatin-active coregulatory complexes and thus enables gene selective transcription of multiple genes [38]. The PDIA3 expression in the brain is many times greater than in the kidney [19]. Whilst 1,25D activates genetic actions via the VDR, PDIA3 activates non-genetic actions in the brain [19]. In a single human brain, the highest 25OHD concentrations were found in the myelin-rich corpus callosum, followed by the hypothalamus, middle temporal cortex, medulla, pons, middle frontal cortex, prefrontal cortex and cerebellum [39]. A current hypothesis is that the lipophilic 25OHD is stored in the myelin sheaths of microglial cells. In demyelinating or neurodegenerative processes, 25OHD is locally activated to 1,25D in microglial cells and a complex immunomodulatory and neuroprotective process is activated. These processes run independently from the endocrine vitamin D system [35]. Thus, determining serum levels of 25OHD may not be sufficient to measure cerebral vitamin D status. This might explain why the studies that analyse the effect of 25OHD on the brain come to different results.

Vitamin D appears to have a trophic function in the differentiation and maturation of neurons by controlling the rate of mitosis and the production and release of neurotrophins [19]. The association between hypovitaminosis D and WMHs may also be related to the association between vitamin D and vascular risk [40]. Low vitamin D makes the brain more sensitive to vascular stress, which may explain part of the hypovitaminosis D related WMHs [4]. The WMHs observed in older adults with hypovitaminosis D can probably not

TABLE 2 Deseasonalized 25OHD at t_0 and WMH volume at t_2 according to the regional Fazekas classification at t_2

		Men, *n* = 260			Women, *n* = 245[a]			Total, *n* = 505		
	Fazekas grade	*n* (%)	WMH volume mean; range	Deseasonalized 25OHD, t_0 mean ± SD	*n* (%)	WMH volume mean; range	Deseasonalized 25OHD, t_0 mean ± SD	*n* (%)	WMH volume mean; range	Deseasonalized 25OHD, t_0 mean ± SD
Periventricular	0	2 (0.8)	8; 5–12	18.4 ± 10.1	1 (0.4)	3	11.6	3 (0.6)	7; 3–12	16.2 ± 8.2
	1	150 (58)	11; 1–56	16.8 ± 7.1	153 (62)	8; 1–46	18.0 ± 8.2	303 (60)	9; 1–56	17.4 ± 7.7
	2	78 (30)	23; 5–68	16.1 ± 6.9	55 (22)	19; 3–67	15.6 ± 8.2	133 (26)	21; 3–68	15.9 ± 7.4
	3	30 (12)	49; 11–132	17.7 ± 5.8	36 (15)	35; 5–123	15.9 ± 7.2	66 (13)	41; 5–132	16.7 ± 6.6
Frontal	0	44 (17)	8; 1–21	17.5 ± 7.5	38 (16)	7; 2–46	19.4 ± 9.1	82 (16)	7; 1–46	18.4 ± 8.3
	1	136 (52)	14; 2–56	16.4 ± 6.4	127 (52)	9; 1–44	17.0 ± 7.6	263 (52)	12; 1–56	16.7 ± 7.0
	2	53 (20)	25; 5–68	16.2 ± 7.7	47 (19)	19; 5–76	15.5 ± 8.4	100 (20)	22; 5–76	15.9 ± 8.0
	3	27 (10)	47; 4–132	17.9 ± 6.8	33 (13)	33; 4–123	17.2 ± 8.0	60 (12)	40; 4–132	17.5 ± 7.4
Parietal	0	65 (25)	9; 1–40	16.0 ± 7.0	49 (20)	5; 1–19	20.3 ± 8.2	114 (23)	8; 1–40	17.8 ± 7.8
	1	113 (43)	14; 2–56	17.5 ± 6.8	119 (49)	10; 1–46	16.6 ± 7.9	232 (46)	12; 1–56	17.0 ± 7.3
	2	48 (18)	26; 6–58	15.3 ± 7.1	44 (18)	18; 3–106	14.9 ± 6.5	92 (18)	22; 3–106	15.0 ± 6.8
	3	34 (13)	43; 12–132	17.3 ± 6.8	32 (13)	36; 4–123	17.4 ± 9.5	66 (13)	40; 4–132	17.4 ± 8.1
Temporal	0	153 (59)	13; 1–88	16.9 ± 7.0	158 (65)	10; 1–110	17.8 ± 8.5	311 (62)	11; 1–110	17.4 ± 7.8
	1	90 (35)	21; 3–76	15.9 ± 6.7	72 (30)	20; 2–106	15.4 ± 6.3	162 (32)	21; 2–106	15.6 ± 6.5
	2	13 (5)	45; 12–86	17.8 ± 7.2	11 (5)	29; 3–67	16.9 ± 5.9	24 (5)	38; 3–86	17.4 ± 6.5
	3	4 (2)	93; 52–132	21.9 ± 7.5	3 (1)	58; 24–123	23.6 ± 20.0	7 (1)	78; 24–132	22.6 ± 12.7
Occipital	0	223 (86)	17; 1–95	16.7 ± 7.0	215 (88)	12; 1–123	17.1 ± 8.2	438 (87)	15; 1–123	16.9 ± 7.6
	1	28 (11)	25; 2–132	15.9 ± 6.8	24 (10)	25; 1–110	17.5 ± 7.1	52 (10)	25; 1–132	16.6 ± 6.9
	2	6 (2)	40; 4–86	18.5 ± 5.4	5 (1)	55; 16–106	14.4 ± 6.6	11 (2)	47; 4–106	16.6 ± 6.1
	3	3 (1)	51; 25–76	20.7 ± 8.7	0			3 *n*	51; 25–76	20.7 ± 8.7

Abbreviations: 25OHD, 25-hydroxyvitamin D in ng/ml; t_0, baseline; t_2, 10-year follow-up; WMH volume, volume of white matter hyperintensities in ml.

[a] *n* = 1 women with missing parietal, temporal and occipital Fazekas classification, due to motion artefacts.

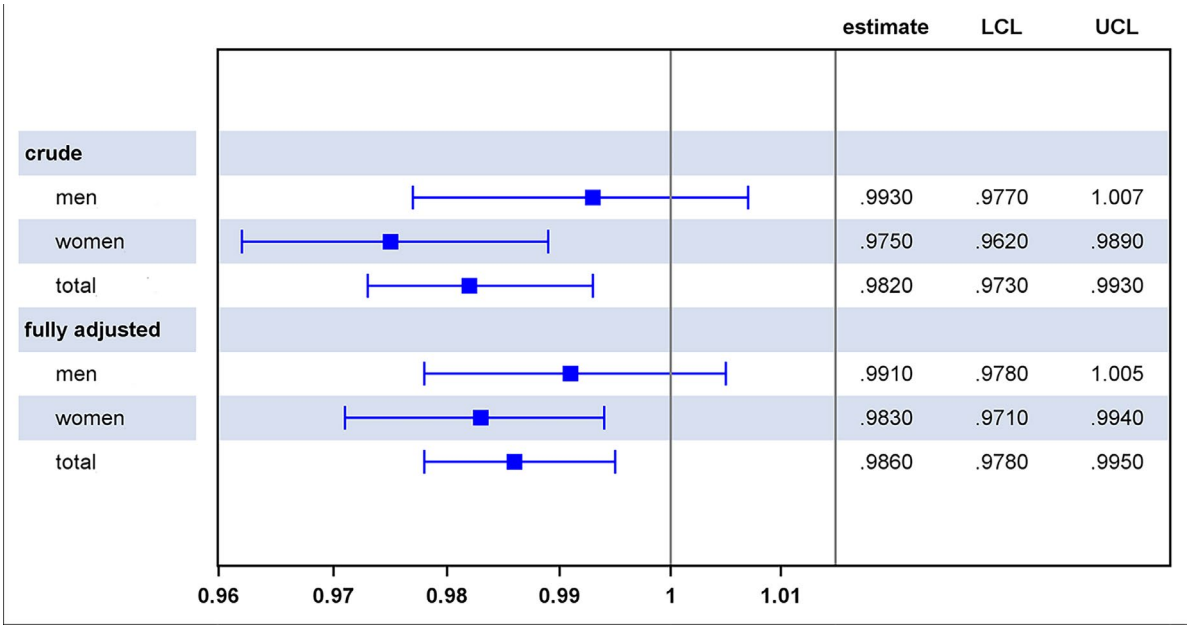

FIGURE 2 Association between deseasonalized 25OHD at t_0 and WMH volume at t_2. LCL, lower confidence limit; UCL, upper confidence limit. Results of the univariate and multiple linear regression [Colour figure can be viewed at wileyonlinelibrary.com]

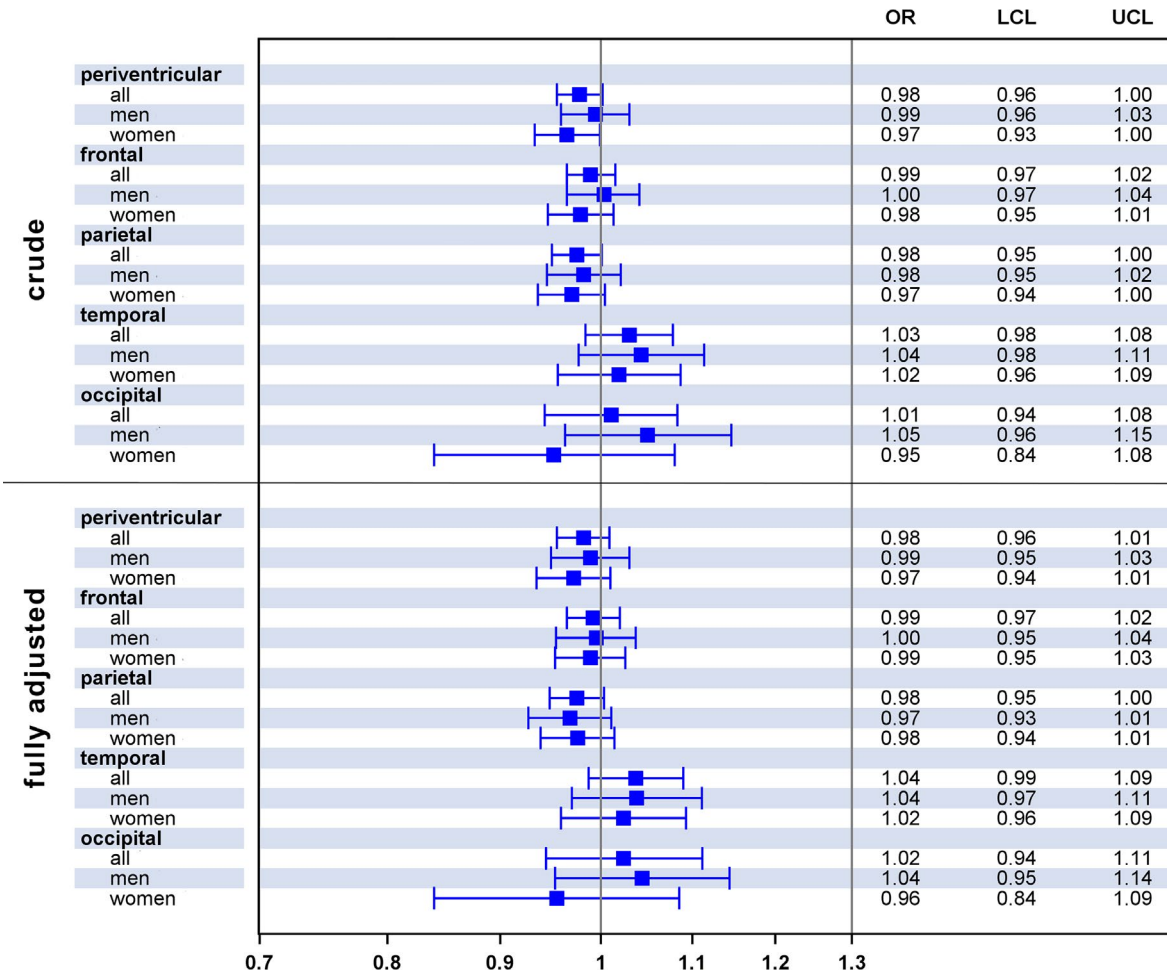

FIGURE 3 Association between classification of WMH at t_2 according to Fazekas (reference grade 0–1) and deseasonalized 25OHD at t_0. LCL, lower confidence limit; OR, odds ratio; UCL, upper confidence limit. Results of the univariate and multiple logistic regression (sex-stratified temporal and occipital estimates were only age adjusted due to small numbers) [Colour figure can be viewed at wileyonlinelibrary.com]

be fully explained by cerebral ischaemic disease of the small vessels, but also by a loss of subcortical white matter [4]. Low 25OHD was associated with periventricular rather than deep WMHs [4]. There is an association between vitamin D deficiency and inflammation, which can lead to brain damage [41,42]. In vitro experiments showed that vitamin D stimulates the growth of nerve cells and induces the production of nerve growth factors [43]. All in all the exact pathophysiological mechanisms that explain why a low vitamin D status could cause WMHs are not fully understood.

As already mentioned above, neuroimaging studies also found other associations between low 25OHD and brain abnormalities and were controversial, too. 25OHD depletion was associated with lower total-brain volume [4,44], larger lateral ventricles [4], smaller hippocampal volume [8] and thinner cingulate gyri [45]. Higher 25OHD levels were associated with larger grey matter volume [34]. However, there are also repeated studies in which these associations or parts of these associations could not be confirmed [34]. Older people with 25OHD deficiency were more prone to cognitive and motor disorders than people with sufficient levels [2,4,46,47], but the association could not always be established [19,48]. Even persons with particularly high 25OHD values or persons with vitamin D substitution had poorer cognitive performance [19,49]. The same applies to dementia [8]. Here again, determining serum 25OHD may not always be sufficient to analyse the effect of vitamin D on the brain.

Care must be taken when comparing studies on WMHs because the detection and definition of WMHs differ. Different terms are used synonymously such as WMHs, leukoaraiosis, vascular dementia, vascular cognitive impairment or subcortical ischaemic vascular dementia [50]. All terms include hyperintense MRI signals within the white matter but some of them differ in their clinical prognosis and eventually in their pathological pathways. An important factor is the MRI sequence itself because the changes only appear in T2- and FLAIR-weighted sequences as hyperintensities. In T1-weighted MRIs and computed tomography scans, they appear hypointense and are more difficult to distinguish from healthy white matter [50]. In our study, the term WMH was used without referring to a clinical equivalent but to an unspecific, symmetric change of white matter signals within MRI scans of an ageing brain [10].

Depending on different current guidelines, a vitamin D deficiency exists if the serum 25OHD level falls below 30 or 20 ng/dl [51–53]. Using these cut-offs, well over 50% of our study population had a vitamin D deficiency [54]. Also 50% or more of the adult European and US population has a vitamin D deficiency [54–56]. Randomized controlled trials, which should actually show that vitamin D substitution reduces the disease risk of various diseases, were unfortunately not promising at all. A number of possible reasons are being discussed: reverse causality, covariation, undetected long-term effects, heterogeneities in populations, different individual responses to vitamin D supplementation, and the topic of nutrient-synergistic co-nutrients [35]. Vitamin D deficiency in preclinical studies has been experimentally induced in the brain prior to the changes [57,58], but a scenario of reverse causality seems likely, as lower brain volume can lead to loss of autonomy and inadequate dietary intake of vitamin D as well as insufficient exposure to sunlight, resulting in vitamin D deficiency [56]. In our study, however, only mobile participants received an MRI because they had to make a long journey to the examination centre.

The results of our study lead to the following questions with clinical relevance. (i) Does sufficient vitamin D status decrease development of WMHs? (ii) Will vitamin D substitution prevent WMHs? Our study shows that there is an association between lower serum 25OHD and WMHs, but the effect is very small. Vitamin D is only one of many factors which lead to decreased neuronal health. Mainly vitamin D is produced by adequate sun exposure. Whether adequate sun exposure is sufficient to avoid WMHs or whether vitamin D substitution is necessary has to be found out in clinical trials.

The strengths of our study are as follows. Our longitudinally designed study is representative of white Caucasian middle-aged and elderly adults. Our participants were relatively healthy and showed no signs of more serious cerebral impairment until the initial examination, which makes it easier to interpret the effect of vitamin D on the normally biologically ageing brain. The WMH data were assessed visually by two independent scientific assistants who were blind to the participants' data and also by a quantitative software tool. Our follow-up period of 10 years was much longer than in most other studies. Different approaches to deseasonalization are used in the literature without explaining them in more detail. Often, a distinction is only made according to the four seasons, which is a very rough approach. Since vitamin D was measured in a large number of people, it was even possible to take the calendar week into account. This is particularly relevant in spring (start of dermal vitamin D production in our latitudes) and autumn (end of vitamin D production) as the start and end are not fixed by calendar but depend on the weather [54].

The limitations of our study are that no information about WMHs was available at t_0, so there is no information about progress or worsening of WMHs. Only relatively healthy participants received an MRI, so no conclusions could be drawn about people with limited mobility or positive stroke anamnesis. Despite several adjustments, possible residual confounding cannot be excluded.

CONCLUSIONS

Our population-based study shows that lower baseline 25OHD may be a risk factor for more pronounced WMHs 10 years later, but a weak one. Our results support the outcome of many cross-sectional studies but is in contrast to previous longitudinal studies. It is unclear whether serum 25OHD is a suitable marker for central nervous vitamin D homeostasis. Since only 25OHD is used in epidemiological studies, heterogeneous results are obtained. Further research to understand the role of vitamin D storage in the human brain is needed. For this purpose, vitamin D metabolites in the brain must be quantified and further knowledge is needed about the exact physiological effects of vitamin D in the brain. With regard to the clinical relevance and substitution of vitamin D, further population-based longitudinal studies with more than just serum 25OHD as a marker for vitamin D status are required to prove a clear benefit of sufficient vitamin D for brain health.

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CONFLICT OF INTEREST

The authors declare no ethical, financial, personal or any other conflict of interest.

AUTHOR CONTRIBUTIONS

Sara Helena Schramm: Conceptualization (lead); formal analysis (lead); methodology (lead); visualization (supporting); writing original draft (lead); writing review and editing (lead). Lea Schliephake: Formal analysis (supporting); investigation (supporting); writing original draft (supporting). Heiko Himpfen: Conceptualization (supporting); data curation (supporting); methodology (supporting). Raimund Erbel: Conceptualization (lead); investigation (equal); project administration (supporting); supervision (lead). Karl-Heinz Jöckel: Conceptualization (lead); funding acquisition (lead); project administration (lead); supervision (lead). Susanne Moebus: Conceptualization (equal); funding acquisition (lead); project administration (equal); supervision (supporting).

DATA AVAILABILITY STATEMENT

The corresponding author has full access to all data in the study and final responsibility for the submission of the article for publication. Due to data security reasons (i.e., data contain potentially participant identifying information), the HNR study and 1000BRAINS do not allow sharing data as a public use file. Data requests can be addressed to recall@uk-essen.de.

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REFERENCES

1. Buell JS, Dawson-Hughes B, Scott TM, et al. 25-Hydroxyvitamin D, dementia, and cerebrovascular pathology in elders receiving home services. *Neurology*. 2010;74(1):18-26.
2. Sakurai T, Ogama N, Toba K. Lower vitamin D is associated with white matter hyperintensity in elderly women with Alzheimer's disease and amnestic mild cognitive impairment. *J Am Geriatr Soc*. 2014;62(10):1993-1994.
3. Prager JM, Thomas C, Ankenbrandt WJ, et al. Association of white matter hyperintensities with low serum 25-hydroxyvitamin D levels. *AJNR Am J Neuroradiol*. 2014;35(6):1145-1149.
4. Annweiler C, Annweiler T, Bartha R, Herrmann FR, Camicioli R, Beauchet O. Vitamin D and white matter abnormalities in older adults: a cross-sectional neuroimaging study. *Eur J Neurol*. 2014;21(12):1436-e95.
5. Annweiler C, Bartha R, Karras SN, Gautier J, Roche F, Beauchet O. Vitamin D and white matter abnormalities in older adults: a quantitative volumetric analysis of brain MRI. *Exp Gerontol*. 2015;63:41-47.
6. Chung P-W, Park K-Y, Kim J-M, et al. 25-hydroxyvitamin D status is associated with chronic cerebral small vessel disease. *Stroke*. 2015;46(1):248-251.
7. Michos ED, Carson KA, Schneider ALC, et al. Vitamin D and subclinical cerebrovascular disease: the Atherosclerosis Risk in Communities brain magnetic resonance imaging study. *JAMA Neurol*. 2014;71(7):863-871.
8. Karakis I, Pase MP, Beiser A, et al. Association of serum vitamin D with the risk of incident dementia and subclinical indices of brain aging: the Framingham Heart Study. *J Alzheimers Dis*. 2016;51(2):451-461.
9. Littlejohns TJ, Kos K, Henley WE, et al. Vitamin D and risk of neuroimaging abnormalities. *PLoS One*. 2016;11(5):e0154896.
10. Bischoff C, von Einsiedel HG. The significance of leukoaraiosis. A current evaluation and differential diagnosis. *Nervenarzt*. 1997;68(8):609-619.
11. Annweiler C, Beauchet O, Celle S, et al. Contribution of brain imaging to the understanding of gait disorders in Alzheimer's disease: a systematic review. *Am J Alzheimers Dis Other Demen*. 2012;27(6):371-380.
12. Berghout BP, Fani L, Heshmatollah A, et al. Vitamin D status and risk of stroke: the Rotterdam Study. *Stroke*. 2019;50(9):2293-2298.
13. Frey BM, Petersen M, Mayer C, Schulz M, Cheng B, Thomalla G. Characterization of white matter hyperintensities in large-scale MRI-studies. *Front Neurol*. 2019;10:238.
14. Schmidt R, Ropele S, Ferro José, et al. Diffusion-weighted imaging and cognition in the leukoariosis and disability in the elderly study. *Stroke*. 2010;41(5):e402-e408.
15. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
16. Fazekas F, Schmidt R, Scheltens P. Pathophysiologic mechanisms in the development of age-related white matter changes of the brain. *Dement Geriatr Cogn Disord*. 1998;9(suppl 1):2-5.
17. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. *Stroke*. 1997;28(3):652-659.
18. Smith CD, Snowdon D, Markesbery WR. Periventricular white matter hyperintensities on MRI: correlation with neuropathologic findings. *J Neuroimaging*. 2000;10(1):13-16.
19. Stephenson A, Mamo JCL, Takechi R, Hackett MJ, Lam V. Genetic, environmental and biomarker considerations delineating the regulatory effects of vitamin D on central nervous system function. *Br J Nutr*. 2019:1-38. https://doi.org/10.1017/S000711451900268X
20. Caspers S, Moebus S, Lux S, et al. Studying variability in human brain aging in a population-based German cohort—rationale and design of 1000BRAINS. *Front Aging Neurosci*. 2014;6:149.
21. Schmermund A, Möhlenkamp S, Stang A, et al. Assessment of clinically silent atherosclerotic disease and established and novel risk factors for predicting myocardial infarction and cardiac death in healthy middle-aged subjects: rationale and design of the Heinz Nixdorf RECALL Study. Risk Factors, Evaluation of Coronary Calcium and Lifestyle. *Am Heart J*. 2002;144(2):212-218.
22. Stang A, Moebus S, Dragano N, et al. Baseline recruitment and analyses of nonresponse of the Heinz Nixdorf Recall Study: identifiability of phone numbers as the major determinant of response. *Eur J Epidemiol*. 2005;20(6):489-496.
23. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography–tandem mass spectrometry methods. *Clin Chem*. 2012;58(3):531-542.
24. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149(2):351-356.
25. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in multiple sclerosis. *NeuroImage*. 2012;59(4):3774-3783.
26. Himpfen HCS, Mahabadi A, Lohmeier V, et al. Early signs of coronary atherosclerosis are associated with early signs of

cerebrovascular disease identified as white matter lesions—a 3 Tesla magnetic resonance imaging study. *J Heart Cardiovasc Med*. 2020;3:1-11.
27. Bittner N, Jockwitz C, Mühleisen TW, et al. Combining lifestyle risks to disentangle brain structure and functional connectivity differences in older adults. *Nat Commun*. 2019;10(1):1-13.
28. Moebus S, Stang A, Möhlenkamp S, et al. Association of impaired fasting glucose and coronary artery calcification as a marker of subclinical atherosclerosis in a population-based cohort—results of the Heinz Nixdorf Recall Study. *Diabetologia*. 2009;52(1):81-89.
29. Erbel R, Lehmann N, Möhlenkamp S, et al. Subclinical coronary atherosclerosis predicts cardiovascular risk in different stages of hypertension: result of the Heinz Nixdorf Recall Study. *Hypertension*. 2012;59(1):44-53.
30. Erbel R, Lehmann N, Churzidse S, et al. Gender-specific association of coronary artery calcium and lipoprotein parameters: the Heinz Nixdorf Recall Study. *Atherosclerosis*. 2013;229(2):531-540.
31. Annweiler C, Montero-Odasso M, Hachinski V, Seshadri S, Bartha R, Beauchet O. Vitamin D concentration and lateral cerebral ventricle volume in older adults. *Mol Nutr Food Res*. 2013;57(2):267-276.
32. Vicente P, Herr M, Mahieux F, Ankri J. Vitamin D and neuropsychological assessment of cognitive functions: a study of their relationships in a sample of 244 patients attending a memory clinic. *Geriatr Psychol Neuropsychiatr Vieil*. 2015;13(4):452-461.
33. Bowman GL, Silbert LC, Howieson D, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. *Neurology*. 2012;78(4):241-249.
34. Brouwer-Brolsma EM, van der Zwaluw NL, van Wijngaarden JP, et al. Higher serum 25-hydroxyvitamin D and lower plasma glucose are associated with larger gray matter volume but not with white matter or total brain volume in Dutch community-dwelling older adults. *J Nutr*. 2015;145(8):1817-1823.
35. Chabrol T, Wion D. Randomized clinical trials of oral vitamin D supplementation in need of a paradigm change: the vitamin D autacoid paradigm. *Med Hypotheses*. 2019;134:109417.
36. Eyles DW, Liu PY, Josh P, Cui X. Intracellular distribution of the vitamin D receptor in the brain: comparison with classic target tissues and redistribution with development. *Neuroscience*. 2014;268:1-9.
37. Smolders J, Schuurman KG, Strien MEV, et al. Expression of vitamin D receptor and metabolizing enzymes in multiple sclerosis-affected brain tissue. *J Neuropathol Exp Neurol*. 2013;72(2):91-105.
38. Kato S. The function of vitamin D receptor in vitamin D action. *J Biochem*. 2000;127(5):717-722.
39. Fu X, Dolnikowski GG, Patterson WB, et al. Determination of vitamin D and its metabolites in human brain using an ultra-pressure LC-tandem mass spectra method. *Curr Dev Nutr*. 2019;3(7):nzz074.
40. Wang J, Zhou JJ, Robertson GR, Lee VW. Vitamin D in vascular calcification: a double-edged sword? *Nutrients*. 2018;10(5):652.
41. Briones TL, Darwish H. Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. *J Neuroinflammation*. 2012;9:244.
42. Ibi M, Sawada H, Nakanishi M, et al. Protective effects of 1 alpha,25-(OH)(2)D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology*. 2001;40(6):761-771.
43. Brown J, Bianco JI, McGrath JJ, Eyles DW. 1,25-dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. *Neurosci Lett*. 2003;343(2):139-143.
44. Hooshmand B, Lökk J, Solomon A, et al. Vitamin D in relation to cognitive impairment, cerebrospinal fluid biomarkers, and brain volumes. *J Gerontol A Biol Sci Med Sci*. 2014;69(9):1132-1138.
45. Foucault G, Duval GT, Simon R, Beauchet O, Dinomais M, Annweiler C. Serum vitamin D and cingulate cortex thickness in older adults: quantitative MRI of the brain. *Curr Alzheimer Res*. 2019;16(11):1063-1071.
46. Annweiler C, Montero-Odasso M, Schott AM, Berrut G, Fantino B, Beauchet O. Fall prevention and vitamin D in the elderly: an overview of the key role of the non-bone effects. *J Neuroeng Rehabil*. 2010;7:50.
47. Annweiler C, Schott A-M, Montero-Odasso M, et al. Cross-sectional association between serum vitamin D concentration and walking speed measured at usual and fast pace among older women: the EPIDOS study. *J Bone Miner Res*. 2010;25(8):1858-1866.
48. Schneider A, Zhao DI, Lutsey P, et al. Serum vitamin D concentrations and cognitive change over 20 years: the Atherosclerosis Risk in Communities Neurocognitive Study. *Neuroepidemiology*. 2018;51(3–4):131-137.
49. Granic A, Hill TR, Kirkwood TBL, et al. Serum 25-hydroxyvitamin D and cognitive decline in the very old: the Newcastle 85+ Study. *Eur J Neurol*. 2015;22(1):106-e7.
50. Wardlaw JM, Valdés Hernández MC, Muñoz-Maniega S. What are white matter hyperintensities made of? Relevance to vascular cognitive impairment. *J Am Heart Assoc*. 2015;4(6):e001140.
51. National Institutes of Health. Vitamin D Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/. Accessed October 16, 2020.
52. Dachverband Osteologie (DVO). DVO Leitlinie Osteoporose 2017. https://dv-osteologie.org/osteoporose-leitlinien. Accessed October 16, 2020.
53. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-1930.
54. Schramm S, Lahner H, Jöckel K-H, et al. Impact of season and different vitamin D thresholds on prevalence of vitamin D deficiency in epidemiological cohorts—a note of caution. *Endocrine*. 2017;56(3):658-666.
55. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-281.
56. Annweiler C, Souberbielle JC, Schott AM, de Decker L, Berrut G, Beauchet O. Vitamin D in the elderly: 5 points to remember. *Geriatr Psychol Neuropsychiatr Vieil*. 2011;9(3):259-267.
57. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. *Neuroscience*. 2003;118(3):641-653.
58. Féron F, Burne T, Brown J, et al. Developmental Vitamin D3 deficiency alters the adult rat brain. *Brain Res Bull*. 2005;65(2):141-148.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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