The Journal of Nutrition. First published ahead of print July 1, 2015 as doi: 10.3945/jn.115.214197.

The Journal of Nutrition
Nutrition and Disease

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[1,2]

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Abstract

Background: Previous studies have shown beneficial associations between 25-hydroxyvitamin D [25(OH)D] status and cognitive performance, but results are inconclusive. Studies on 25(OH)D status and brain volumetric measures may provide more insight in the potential role of vitamin D in cognitive performance.

Objectives: The aims of this study were to cross-sectionally investigate the association between vitamin D status and brain tissue volumes in 217 Dutch community-dwelling older adults aged ≥65 y and to examine whether surrogate markers of glucose homeostasis act as modifiers in these associations.

Methods: Serum 25(OH)D, plasma glucose, and plasma insulin were analyzed, serving as exposure measures. Estimates of total brain volume, gray matter volume, and white matter volume were obtained using MRI, serving as outcome measures. Associations of serum 25(OH)D, plasma glucose, and plasma insulin concentrations with brain tissue volumes were evaluated using multiple linear regression analyses. Potential effect modification by glucose homeostasis in the association between 25(OH)D and brain volumetric measures was examined by stratification and testing for interaction.

Results: After full adjustment, higher serum 25(OH)D concentrations and lower plasma glucose concentrations were associated with larger gray matter volume, [β ± SE: 0.20 ± 0.08 mL (P = 0.02) and −3.26 ± 1.59 mL (P = 0.04), respectively]. There was no association between serum 25(OH)D and plasma insulin concentration with total brain volume and white matter volume. Furthermore, there was no evidence for a mediation or modification effect of plasma glucose in the association between serum 25(OH)D and brain tissue volumes.

Conclusion: Higher serum 25(OH)D and lower plasma glucose are associated with larger gray matter volume, but not white matter or total brain volume, in a population of Dutch adults aged ≥65 y. This trial was registered at clinicaltrials.gov as NCT00696514. *J Nutr* doi: 10.3945/jn.115.214197.

Keywords: vitamin D, plasma glucose, plasma insulin, total brain volume, gray matter, white matter

Introduction

Despite great efforts an effective treatment for Alzheimer disease has not yet been developed (1). Therefore, identification of factors that may prevent or slow down the development of Alzheimer disease is important. Vitamin D has been proposed as being one of those potential preventive factors (2). Support for this hypothesis originated from preclinical studies showing that

[1] B vitamins for the Prevention of Osteoporotic Fractures study is supported and funded by Netherlands Organization for Health Research and Development (ZonMw, grant 6130.0031), The Hague; unrestricted grant from NZO (Dutch Dairy Association), Zoetermeer; Orthica, Almere; Netherlands Consortium Healthy Ageing Leiden/Rotterdam; Ministry of Economic Affairs, Agriculture and Innovation (project KB-15-004-003), The Hague; Wageningen University, Wageningen; VU University Medical Center, Amsterdam; Erasmus Medical Center, Rotterdam.

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[2] Author disclosures: NL van der Zwaluw, JP van Wijngaarden, RA Dhonukshe-Rutten, PH in 't Veld, PA Smeets, RP Kessels, and O van de Rest, no conflicts of interest. EM Brouwer-Brolsma, EJ Feskens, and LC de Groot report to have filed a patent related to vitamin D and cognitive executive function. All organizations are based in Netherlands. The sponsors did not have any role in the design or implementation of the study, data collection, data management, data analysis, data interpretation, or in the preparation, review, or approval of the manuscript.

Manuscript received March 24, 2015. Initial review completed April 22, 2015. Revision accepted June 11, 2015.
doi: 10.3945/jn.115.214197.

vitamin D beneficially influences several cerebrovascular disease risk factors, synaptic plasticity, and synthesis of neurotransmitters and neurotrophins and slows down synaptic and neuronal loss (2).

Vitamin D may also be indirectly associated with brain functioning, with glucose intolerance acting as a mediator. Specifically, on a mechanistic level, 1,25-hydroxyvitamin D [1,25(OH)D][8] has been shown to promote pancreatic β cell function as well as insulin action (3). Several observational studies support these potential effects by showing beneficial associations between 25-hydroxyvitamin D [25(OH)D] status and fasting serum/plasma glucose, serum/plasma insulin, and glucose clearance as well as type 2 diabetes mellitus (3, 4). There is also considerable evidence that maintenance of circulating glucose concentrations within a healthy range is beneficial for brain function (5, 6). Furthermore, it has been hypothesized that vitamin D deficiency may be particularly harmful for brain health in the presence of a second neurobiological trigger (7–9). Because glucose intolerance may be such a neurobiological trigger, we therefore hypothesized that markers of glucose homeostasis may also act as modifiers of the association between vitamin D and brain volume.

Several human studies provided evidence for a potential role of vitamin D to benefit cognitive function. Meta-analyses indicate that persons with 25(OH)D concentrations of ≥50 nmol/L have better global cognitive performance (10), episodic memory, information processing speed, mental shifting abilities, and information updating ability than those with a 25(OH)D status of <50 nmol/L (11).

Cognitive decline has been associated with the rate of brain atrophy as measured with MRI (12, 13). Via studying gray and white matter loss, MRI may be a valuable tool to gain more insight in the association between 25(OH)D status and brain function. The use of MRI is a relatively new area in vitamin D research and only a few studies explored the associations between vitamin D and measures of brain volume (14). Because there is a large heterogeneity in study populations and outcome measures and because results are inconclusive (14), more evidence is needed. We therefore investigated associations of serum 25(OH)D with total brain volume, gray matter volume, and white matter volume in Dutch older adults. The second aim of this study was to explore the potential synergism of vitamin D and glucose homeostasis in association with brain volume.

Methods

Participants. This observational study was performed using data of the B Vitamins for the Prevention of Osteoporotic Fractures (B-PROOF) study, a randomized, double-blind, placebo-controlled trial designed to assess the efficacy of daily oral supplementation of vitamin B-12 (500 μg) and folic acid (400 μg) on bone fractures in older adults aged ≥65 y who have mild hyperhomocysteinemia (12–50 μmol/L). Given the known beneficial effect of vitamin D on bone health, 15 μg of cholecalciferol was added to both placebo and treatment tablets. In total, 2919 participants were enrolled in the B-PROOF study, of which 856 were enrolled at the study site of Wageningen University. At the Wageningen University study site, 413 participants were invited to participate in the MRI analysis; 218 participants underwent an MRI scan. For the current analyses, data could be used of participants who underwent an MRI scan and had available serum 25(OH)D data (n = 217; **Figure 1**). When comparing this subsample to the total Wageningen University and Research Center sample, the MRI group (mean ± SD: 72 ± 6) was somewhat younger than the total population (mean ± SD: 73 ± 6; P = 0.007). No significant differences between the 2 groups were observed with respect to sex, education level, serum 25(OH)D status, and Mini-Mental State Examination (MMSE) score. More specific details on the total B-PROOF study population have been reported previously (15). The Medical Ethics Committee of Wageningen University and Research Center approved the study protocol and all participants gave written informed consent. The B-PROOF study has been registered with Netherlands Trial Register at www.trialregister.nl as NTR 1333 since 1 June 2008 and with clinicaltrials.gov as NCT00696514 since 9 June 2008.

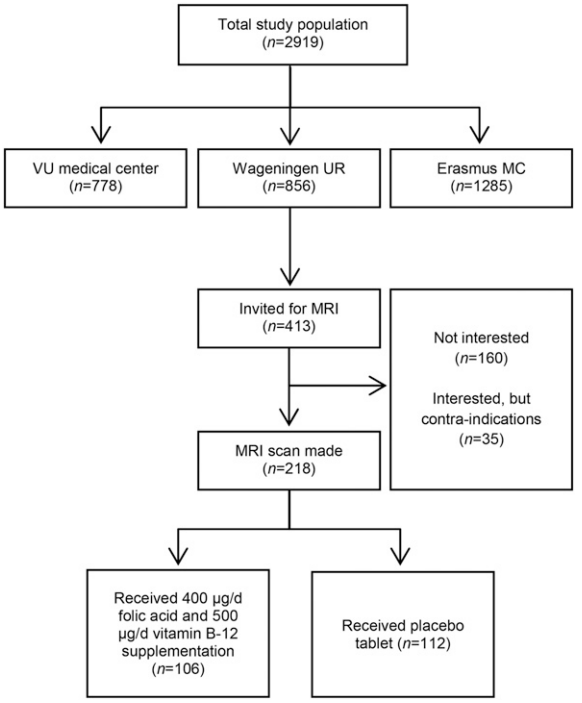

FIGURE 1 Flowchart of the 217 Dutch older adults aged ≥65 y who were enrolled in the MRI measurements of the B-PROOF study. In total, 2919 participants were enrolled in the B-PROOF study, of which 856 were enrolled at the study site of Wageningen University. At the Wageningen University study site, 413 participants were invited to participate in the MRI analysis; 218 participants underwent an MRI scan; for the present study, one participant was excluded from the analyses because of unknown serum 25(OH)D status. B-PROOF, B Vitamins for the Prevention of Osteoporotic Fractures; Erasmus MC, Erasmus Medical Center Rotterdam. VU medical center, Free University Medical Center Amsterdam; Wageningen UR, Wageningen University and Research Center; 25(OH)D, 25-hydroxyvitamin D.

Biochemical analyses. Blood samples were drawn in the morning when participants were fasting or had consumed a restricted breakfast, including 1–2 slices of bread with or without minimal sandwich filling (i.e., some margarine, jam, or cheese) or a small bowl or glass of skimmed or semiskimmed milk or yogurt, up to 1 h before blood collection. Samples were stored at −80°C until determination. Serum 25(OH)D in baseline samples was measured by isotope dilution–online solid-phase extraction liquid chromatography–tandem MS (16). Plasma glucose concentrations were analyzed using a hexokinase method (Gluco-quant; Roche Diagnostics). Plasma insulin concentrations were determined using an immunometric assay (ADVIA Centaur Immunoassay System; Siemens Medical Solutions Diagnostics).

MRI. Cranial volumetric MRI scans were performed 2 y after the baseline measurements, at Hospital Gelderse Vallei (Ede, Netherlands) on a 3-Tesla Siemens Magnetom Verio (Siemens), with a 32-channel head coil. We analyzed the T1-weighted scan [magnetization-prepared rapid acquisition gradient echo, repetition time = 2300 ms, echo time = 3.0 ms, inversion time = 900 ms, 9° flip angle, field of view = 256 × 256 mm, 192 sagittal slices, voxel size = 1 × 1 × 1 mm of 1 × 1 × 1 mm, acceleration

[8] Abbreviations used: B-PROOF, B Vitamins for the Prevention Of Osteoporotic Fractures; MMSE, Mini-Mental State Examination; VBM, voxel-based morphometry; 1,25(OH)D, 1,25 hydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

factor (generalized autocalibrating partially parallel acquisition) = 2]. The voxel-based morphometry (VBM) toolbox within statistical parametric mapping 8 (17) and Functional MRI of the brain Software Library (FSL)-VBM version 6.0 (18) were used for segmentation (19). T1-weighted images were reoriented to match the standard template images in FSL-VBM. VBM spatially normalizes participants' brain images to a standard space and then automated segments of gray matter, white matter, and cerebrospinal fluid, using a unified tissue segmentation approach (20). Gray and white matter volumes were summed to calculate total brain volume. Gray matter, white matter, and cerebrospinal fluid measures were summed to calculate intracranial volume.

Covariates. Height was measured at baseline with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.5 kg with a calibrated analog scale. BMI was calculated as weight/height squared. Data on education level (primary, secondary, or higher education), smoking status (nonsmoker or current smoker), physical activity (expressed as kcal/d) (21), and alcohol consumption (light, moderate, or excessive) (22) were collected by means of questionnaires. The 15-item Geriatric Depression Scale was used as a screening tool for the number of depressive feelings (23). The MMSE was used to assess global cognitive status in this population (24). Based on previous analyses, yet unpublished, season of blood collection was divided into summer–fall (June–November) and winter–spring (December–May) and added to the regression model as a dichotomous variable.

Statistical analyses. Participants' characteristics were reported as means with SDs or percentages. Medians with IQRs were used to report skewed variables. Characteristics are shown for the total MRI group, stratified according to the cutoff values for vitamin D deficiency that are currently used by the Institute of Medicine, specifically, <50 nmol/L vs. ≥50 nmol/L (25), and stratified for low (<5.6 mmol/L) and high (≥5.6 mmol/L) plasma glucose levels. Differences between participants with serum vitamin D concentrations of <50 nmol/L and those with serum vitamin D levels of ≥50 nmol/L—and between participants with plasma glucose concentrations of <5.6 mmol/L and those with plasma glucose concentrations of ≥5.6 mmol/L—were tested using the χ^2-test in case of categorical variables or the Student's *t* test in case continuous variables.

Multiple regression analyses were performed to explore the associations between serum 25(OH)D and brain volumetric measures. All analyses were adjusted for intracranial volume (crude model); crude model + age and sex (model 1); model 1 + BMI, education, smoking, alcohol consumption, habitual physical activity, and season (model 2); and model 2 + the number of depressive symptoms (model 3).

TABLE 1 Characteristics of 217 Dutch older adults aged ≥65 y with complete serum 25(OH)D data and MRI data[1]

	Total population	Serum 25(OH)D, <50 nmol/L (*n* = 71)	Serum 25(OH)D, ≥50 nmol/L (*n* = 146)	Plasma glucose, <5.6 mmol/L (*n* = 110)	Plasma glucose, ≥5.6 mmol/L (*n* = 107)
Serum 25(OH)D, nmol/L	61 ± 23	36 ± 10	73 ± 18*	63 ± 26	59 ± 20
Age, y	72 ± 6	72 ± 6	71 ± 6	71 ± 6	72 ± 5
Sex, *n* (%)					
Men	124 (57)	39 (55)	85 (58)	55 (50)	69 (64)*
Women	93 (43)	32 (45)	61 (42)	55 (50)	38 (36)
BMI, kg/m^2	27.5 ± 4.1	27.3 ± 4.3	27.7 ± 4.0	26.6 ± 3.7	28.5 ± 4.4*
Educational level, *n* (%)					
Primary	89 (41)	27 (38)	62 (43)	44 (40)	45 (42)*
Secondary	52 (24)	21 (30)	31 (21)	20 (18)	32 (30)
Higher	76 (35)	23 (32)	53 (36)	46 (42)	30 (28)
Alcohol intake, *n* (%)					
Light	146 (67)	53 (75)	93 (64)	80 (73)	66 (62)
Moderate	61 (28)	16 (22)	45 (31)	25 (23)	36 (33)
Excessive	10 (5)	2 (3)	8 (5)	5 (4)	5 (5)
Smoking history, *n* (%)					
Nonsmoker	63 (29)	18 (25)	45 (31)	40 (36)	23 (22)*
Smoker	15 (7)	6 (9)	9 (6)	3 (3)	12 (11)
Former smoker	139 (64)	47 (66)	92 (63)	67 (61)	72 (67)
Physical activity level, kcal/d	665 ± 418	595 ± 403	698 ± 422	662 ± 420	667 ± 417
Plasma glucose, mmol/L[2]	5.9 ± 1.3	5.8 ± 1.2	5.9 ± 1.3	5.1 ± 0.5	6.7 ± 1.3*
Plasma insulin, pmol/L[3]	67 (102)	64 (110)	69 (102)	49 (51)	101 (125)*
Depressive symptoms, *n*	1 (1)	1 (1)	1 (1)	0 (1)	1 (2)
Season, *n* (%)					
December–May	51 (24)	23 (32)	28 (19)*	16 (15)	35 (33)*
June–November	166 (76)	48 (68)	118 (81)	94 (85)	72 (67)
MMSE score, points	29 (2)	29 (2)	29 (1)	29 (2)	29 (2)
Gray matter volume, mL (% of ICV)	574 ± 56 (42)	568 ± 57 (42)	578 ± 55 (42)	573 ± 54 (42)	576 ± 58 (42)
White matter volume, mL (% of ICV)	493 ± 61 (36)	493 ± 62 (36)	493 ± 61 (36)	490 ± 59 (36)	497 ± 64 (36)
Total brain volume, mL (% of ICV)	1067 ± 108 (78)	1061 ± 109 (78)	1071 ± 108 (78)	1063 ± 104 (78)	1073 ± 112 (77)
Vitamin B-12 and folic acid supplementation before MRI, *n* (%)	106 (49)	38 (54)	68 (47)	54 (49)	52 (49)

[1] Values are means ± SDs or medians (IQRs). *Different from the corresponding group, $P < 0.05$. Differences between participants with serum vitamin D concentrations of ≤50 nmol/L and those with serum vitamin D levels of ≥50 nmol/L—and between participants with plasma glucose concentrations of <5.6 mmol/L and those with plasma glucose concentrations ≥5.6 mmol/L—are tested using the χ^2-test in case of categorical variables or the Student's *t* test in case of continuous variables. ICV, intracranial volume; MMSE, Mini-Mental State Examination; 25(OH)D, 25-hydroxyvitamin D.

[2] For fasting glucose the American Diabetic Association considers a plasma concentration between 5.6 and 6.9 mmol/L indicative of an impaired glucose metabolism (prediabetes); a concentration of ≥7.0 may indicate the presence of diabetes (26).

[3] For fasting insulin the normal reference range in Netherlands is considered to be <118 pmol/L for normal-weight persons and <180 pmol/L for obese persons (27).

TABLE 2 Linear regression analyses for serum 25(OH)D with absolute brain volumetric measures using data of 217 Dutch older adults aged ≥65 y[1]

	Total brain volume, mL		Gray matter volume, mL		White matter volume, mL	
	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Crude model	0.26 ± 0.11	0.02	0.25 ± 0.09	0.005	0.01 ± 0.08	0.93
Model 1[2]	0.12 ± 0.08	0.13	0.18 ± 0.08	0.02	−0.06 ± 0.08	0.43
Model 2[3]	0.13 ± 0.08	0.11	0.19 ± 0.08	0.02	−0.06 ± 0.08	0.47
Model 3[4]	0.14 ± 0.08	0.09	0.20 ± 0.08	0.02	−0.06 ± 0.08	0.46

[1] 25(OH)D in nmol/L. 25(OH)D, 25-hydroxyvitamin D.
[2] Adjusted for intracranial volume, age, and sex.
[3] Adjusted for intracranial volume, age, sex, BMI, education, alcohol, smoking, physical activity, and season.
[4] Adjusted for intracranial volume, age, sex, BMI, education, alcohol, smoking, physical activity, season, and the number of depressive symptoms.

Multiple regression analysis was also performed to assess the strength of the associations between plasma glucose, plasma insulin, and brain volumetric measures. Again, all analyses were adjusted for intracranial volume (crude model); crude model + age and sex (model 1); model 1 + BMI, education, smoking, alcohol consumption, and habitual physical activity (model 2); model 2 + the number of depressive symptoms (model 3); and model 3 + serum 25(OH)D (model 4; results of model 4 were only shown in the text of the Results section).

Effect modification by plasma glucose was examined by stratifying the data for low and high plasma glucose levels (median split) and by testing for interaction. Mediation was examined by adding plasma glucose, independently, to fully adjusted regression models for serum 25(OH)D and brain volumetric measures. The α was set at 0.05 and 2-tailed analyses were performed. Analyses were performed using the statistical package SAS, version 9.1 (SAS Institute, Inc.).

Results

Participant characteristics are presented in **Table 1**. Participants were 72 ± 6 y old, had a serum 25(OH)D concentration of 61 ± 23 nmol/L and BMI of 27.5 ± 4.1 kg/m^2, and used 665 ± 418 kcal/d for physical activities. Fifty-seven percent of this population was men, 35% had a degree in higher education, and 76% was included in the study during the summer/autumn. The median score on the MMSE was 29, suggesting that this population was cognitively unimpaired, i.e., without any cognitive signs indicative of neurodegenerative disease. No remarkable differences in population characteristics were observed for participants with serum 25(OH)D concentrations of <50 nmol/L and those with concentrations of ≥50 nmol/L. Participants with plasma glucose concentrations of ≥5.6 mmol/L, however, were more likely to be men, smokers, have a higher BMI, have a lower education, and to be included during the winter months than participants with plasma glucose concentrations of <5.6 mmol/L.

Serum vitamin D and brain volumetric measures. Linear regression analyses showed that serum 25(OH)D was significantly associated with gray matter volume (β ± SE: 0.25 ± 0.09 mL; *P* = 0.005) and total brain volume (β ± SE: 0.26 ± 0.11 mL; *P* = 0.02) after adjustment for intracranial volume but not with white matter volume (**Table 2**).The association between serum 25(OH)D and gray matter remained present after further adjustment for age, sex, BMI, education, alcohol, smoking, physical activity, season, and depressive symptoms (β ± SE: 0.20 ± 0.08 mL; *P* = 0.02), indicating that each unit increase in serum 25(OH)D associates with a 0.20-mL (0.01%) increase in gray matter volume. The association between serum 25(OH)D and total brain volume was not significant after full adjustment.

Glucose homeostasis and brain volumetric measures. Higher plasma glucose levels were associated with less gray matter volume (**Table 3**), but not with total brain volume or white matter volume. In the fully adjusted model, each unit increase in plasma glucose was associated with −3.26 mL of lower gray matter volume (β ± SE: −3.26 ± 1.59 mL; *P* = 0.04). After further adjustment for serum 25(OH)D this association became even stronger (β ± SE: −5.53 ± 1.57 mL; *P* = 0.03). No association between plasma glucose and total brain volume or white matter volume was observed, and we did not observe significant associations between plasma insulin and total brain volume, gray matter volume, or white matter volume.

Interplay between serum vitamin D and glucose homeostasis. Incorporating plasma glucose in the full-adjusted models for serum 25(OH)D and gray matter did not support the hypothesis of mediation (β ± SE: 0.20 ± 0.08 mL; *P* = 0.02), and stratification for low and high plasma glucose levels or interaction analyses did not point toward an interplay between serum 25(OH)D with plasma glucose in the association with gray matter volume (*P*-interaction = 0.86), white matter volume (*P*-interaction = 0.32), or total brain volume (*P*- interaction = 0.45; **Table 4**).

Discussion

This study shows that higher serum 25(OH)D concentrations and lower plasma glucose concentrations are associated with larger gray matter volume in a population of cognitively

TABLE 3 Linear regression analyses for plasma glucose and plasma insulin with absolute brain volumetric measures using data of 217 Dutch older adults aged ≥65 y

	Total brain volume, mL				Gray matter volume, mL				White matter volume, mL			
	Plasma glucose, mmol/L		Plasma insulin, pmol/L		Plasma glucose, mmol/L		Plasma insulin, pmol/L		Plasma glucose, mmol/L		Plasma insulin, pmol/L	
	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Crude model	−3.80 ± 2.00	0.06	0.01 ± 0.02	0.37	−4.60 ± 1.70	0.006	0.02 ± 0.01	0.13	0.80 ± 1.50	0.59	−0.01 ± 0.01	0.62
Model 1[1]	−2.70 ± 1.54	0.08	−0.00 ± 0.01	0.76	−3.96 ± 1.50	0.009	0.01 ± 0.01	0.21	1.26 ± 1.42	0.38	−0.01 ± 0.01	0.32
Model 2[2]	−2.54 ± 1.62	0.12	−0.00 ± 0.01	0.83	−3.45 ± 1.60	0.03	0.02 ± 0.01	0.20	0.91 ± 1.51	0.55	−0.01 ± 0.01	0.26
Model 3[3]	−2.38 ± 1.63	0.15	−0.00 ± 0.01	0.85	−3.26 ± 1.59	0.04	0.02 ± 0.01	0.19	0.88 ± 1.52	0.56	−0.01 ± 0.01	0.23

[1] Adjusted for intracranial volume, age, and sex.
[2] Adjusted for intracranial volume, age, sex, BMI, education, alcohol, smoking, and physical activity.
[3] Adjusted for intracranial volume, age, sex, BMI, education, alcohol, smoking, physical activity, and the number of depressive symptoms.

TABLE 4 Linear regression analyses for serum 25(OH)D with absolute brain volumetric measures, using data of 217 Dutch older adults aged ≥65 y, stratified for low (<5.6) and high (≥5.6) plasma glucose concentrations[1]

	Total brain volume, mL					Gray matter volume, mL					White matter volume, mL				
	Plasma glucose, <5.6 mmol/L (n = 110)		Plasma glucose, ≥5.6 mmol/L (n = 107)			Plasma glucose, <5.6 mmol/L (n = 110)		Plasma glucose, ≥5.6 mmol/L (n = 107)			Plasma glucose, <5.6 mmol/L (n = 110)		Plasma glucose, ≥5.6 mmol/L (n = 107)		
	β ± SE	P	β ± SE	P	P-interaction[2]	β ± SE	P	β ± SE	P	P-interaction[2]	β ± SE	P	β ± SE	P	P-interaction[2]
Crude model	0.38 ± 0.12	0.002	−0.01 ± 0.19	0.96		0.27 ± 0.10	0.01	0.19 ± 0.16	0.22		0.11 ± 0.10	0.24	−0.21 ± 0.14	0.15	
Adjusted model[3]	0.20 ± 0.10	0.04	0.17 ± 0.16	0.31	0.45	0.23 ± 0.10	0.02	0.29 ± 0.16	0.07	0.86	−0.03 ± 0.10	0.76	−0.12 ± 0.15	0.39	0.32

[1] 25(OH)D, 25-hydroxyvitamin D.
[2] Interaction is tested for the adjusted model.
[3] Adjusted model is adjusted for intracranial volume, age, sex, BMI, education, alcohol, smoking, physical activity, and season.

unimpaired Dutch older adults. Markers of glucose homeostasis did not mediate or modify the association between serum 25(OH)D status and brain volume.

Serum vitamin D and brain volumetric measures. Previous studies have shown that in the normal aging brain, gray matter loss predominates over white matter loss (28). In this study, a 1-nmol/L increase in serum 25(OH)D was associated with 0.20 mL (0.01%) of larger gray matter volume but not with white matter volume or total brain volume. Our results are in line with previous preclinical research that indicates that the active form of vitamin D, calcitriol, may support neuronal survival, neurogenesis, and synaptogenesis (2) and with previous observational studies that show associations between higher serum 25(OH)D concentrations and better cognitive performance (10, 29–37).

The earliest studies on brain volume were conducted in rats and showed that a prenatal vitamin D deficiency resulted in larger total brain volumes, larger lateral ventricles (38, 39), reduced cortical thickness, and increased cell proliferation (38). Studies evaluating the association between serum 25(OH)D and brain volumetric measures in humans are sparse (14) but suggest that lower vitamin D concentrations associate with larger ventricle volumes (40), white matter hyperintensities (41), more vascular pathologies (42), and a higher rate of cortical thinning (43). In addition, higher plasma 25(OH)D concentrations have been associated with larger volumetric measures of white matter, amygdala, thalamus, and anterior cingulate gyrus in 28 patients referred to a memory clinic (44). Conversely, several other studies did not observe associations between 25(OH)D status and total brain volume (42, 45), gray matter volume (45), or volumes of the hippocampus (42), amygdala (42), parahippocampal gyrus (42), and temporal horn (40). Overall, the number of studies examining associations between 25(OH)D status and brain volume are sparse and generally had relatively small sample sizes and differed largely in type of population studied and the particular brain volumes examined. Hence, as yet, no firm conclusions can be drawn based on these data.

Glucose homeostasis and brain volumetric measures. Previous research has shown that an impaired glucose tolerance may lead to advanced protein glycation, oxidative stress, increased secretion of amyloid, decreased breakdown of amyloid disease, microvascular brain complications, brain infarcts, and brain atrophy (6, 46). Our findings add that higher plasma glucose levels are associated with less gray matter, which has also been observed by others (47–49). Although our cross-sectional design makes it not possible to draw conclusions about cognitive decline over time, our findings are consistent with the hypothesis that increased blood glucose levels may play a role in the pathogenesis of cognitive decline and dementia.

Interplay between serum vitamin D and glucose homeostasis. In this population, higher serum 25(OH)D concentrations and lower plasma glucose concentrations were associated with larger gray matter volume, but plasma glucose concentrations did not seem to modify or mediate the association between serum 25(OH)D and gray matter volume. The hypothesis that glucose homeostasis could (partially) explain or modify the association between serum 25(OH)D and brain volume has not yet been extensively studied. To date, to our knowledge, only 4 studies have examined whether the association between serum 25(OH)D and cognitive function was influenced when a marker of glucose homeostasis was added (30, 31, 37, 50). These studies

showed either no change (30, 31, 37) or a partial attenuation of the association between serum 25(OH)D concentrations and cognitive performance (50).

Methodologic aspects. Several methodologic issues of the present study warrant further discussion. First, our results are limited because of the observational study design. Second, participants were selected to have a mildly elevated homocysteine level. Although ~50% of the Dutch elderly population has a mildly elevated homocysteine level (51), our design limits the generalizability of these results to the total elderly population. Third, plasma glucose and plasma insulin concentrations presented in this study cannot be considered completely fasting, because participants were allowed to consume a light breakfast before blood sampling. Therefore, participants may have been misclassified as having "high" plasma glucose levels—because of the consumption of a light breakfast—while not having an impaired glucose metabolism. If misclassification occurred, it is expected to be nondifferential misclassification, thus, leading to an underestimation of the actual associations. Finally, MMSE data indicate that this study included cognitively unimpaired elderly, which may have reduced our ability to detect associations between serum 25(OH)D or plasma insulin and white matter volume and total brain volume. Strengths of this study include the possibility to control for a large number of potential covariates and the possibility to further explore the role of glucose homeostasis in the association between serum 25(OH)D and brain volume.

Conclusions. This study showed that higher serum 25(OH)D concentrations and lower plasma glucose concentrations are associated with larger gray matter volume. No associations between serum 25(OH)D concentrations and total brain volume or white matter volume were observed. Our data do not indicate that the association between serum 25(OH)D and gray matter volume is mediated or modified by plasma glucose concentrations. Future prospective studies and well-designed randomized controlled trials are warranted to examine whether vitamin D treatment could play a significant role in slowing down the progress of brain atrophy.

Acknowledgments

We thank M Hillen-Tijdink and L Ottenheijm who helped this trial succeed. We also thank P Schaapsmeerders for help with analyzing the MRI scans. RAD-R and LCdG were responsible for the study design of the B-PROOF study; EMB-B, NLvdZ, JPvW, and PHi'tV conducted research; PAS and RPK provided knowledge and expertise of the MRI data acquisition and analyses; EJF contributed with her expertise on nutrition and glucose homeostasis; OvdR contributed with her expertise on nutrition and cognition; and EMB-B analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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