Associations of lower vitamin D concentrations with cognitive decline and long-term risk of dementia and Alzheimer's disease in older adults

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ABSTRACT

INTRODUCTION: Hypovitaminosis D has been associated with several chronic conditions; yet its association with cognitive decline and the risk of dementia and Alzheimer's Disease (AD) has been inconsistent.

METHODS: The study population consisted of 916 participants from the Three-City-Bordeaux cohort aged 65+, non-demented at baseline, with assessment of vitamin D status and who were followed for up to 12 years.

RESULTS: In multivariate analysis, compared to individuals with 25(OH)D sufficiency (n=151), participants with 25(OH)D deficiency (n=218) exhibited a faster cognitive decline. A total of 177 dementia cases (124 AD) occurred: 25(OH)D deficiency was associated with a nearly three-fold increased risk of AD (Hazard ratio =2.85, 95% Confidence Interval 1.37-5.97).

DISCUSSION: This large prospective study of French older adults suggests that maintaining adequate vitamin D status in older age could contribute to slow down cognitive decline and to delay or prevent the onset of dementia, especially of AD aetiology.

1. BACKGROUND

Experimental studies have suggested that hypovitaminosis D could mediate neurodegenerative processes involved in Alzheimer's disease (AD) [1-3]. In humans, cases-control studies indicated that individuals with dementia or AD had lower circulating concentrations of 25(OH)D [4-5]. Furthermore, several longitudinal studies have found an association between lower baseline 25(OH)D concentrations and accelerated cognitive decline, although conflicting results persist [3, 5-13]. Discrepancies would be explained by gender specific associations, different education level and/or specific cognitive domains assessed. Vitamin D status has also been related to the risk of dementia and AD. Results from Littlejohns et al. [14] and Afzal et al. [15], summarized in a meta-analysis [16], reported a risk of all-cause dementia multiplied by up to 1.2 among older persons with 25(OH)D deficient status (i.e. lower than 50 nmol/L), when Littlejohns et al. observed a 1.6 increased risk for AD specifically. Nevertheless, as for cognitive decline over time, conflicting results exist as well, as reported in US populations or among Finnish men, where vitamin D deficiency was not associated with the risk for dementia or dementia leading to hospitalization [10-11, 17]. However, in all longitudinal studies about dementia risk, limitations should be underlined, in particular the lack of consideration of the Apolipoprotein E e4 (*ApoE4*) genotype and of the overall diet quality, two important potential confounding factors in the relationship between vitamin D status and dementia risk [18-19]. Indeed, for example, *ApoE4* carriers may have higher 25(OH)D concentrations, most likely due to an increased intestinal absorption of dietary vitamin D and a lower renal excretion [20], and a significant interaction between *ApoE4* status and 25(OH)D concentrations has been reported in the relationship between vitamin D and memory function [19].

Therefore, the present analysis was designed to investigate the relationship of 25(OH)D status with cognitive decline and the incidence of all-cause dementia and AD over 12 years in a large population-based sample of French older persons, taking into account the *ApoE4* genotype and overall diet quality.

2. METHODS

2.1 Participants

The Three-City (3C) study is a prospective cohort study on vascular risk factors of dementia; the methodology has been described elsewhere [21]. The 3C study protocol was approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research at Kremlin-Bicêtre University Hospital (Paris). A sample of 9,294 community dwellers aged 65 years and over was selected in 1999-2000 from the electoral rolls of three French cities (Bordeaux, Dijon and Montpellier). All participants gave written informed consent. Five follow-up examinations were performed 2y (wave 1), 4y (wave 2), 7y (wave 3), 10y (wave 4) and 12y (wave 5) after baseline

examination. Data collection included socio-demographic information, lifestyle, symptoms and medical complaints, medical history, medication use, blood pressure, tobacco use, anthropometrical data, neuropsychological testing, and blood sampling. The present analysis is based on the baseline and waves 1 to 5 data in Bordeaux, the only 3C center where the standard data collection was completed with measurement of 25(OH)D in plasma at baseline.
Among the 2,104 participants enrolled at baseline in Bordeaux, we excluded 77 individuals with prevalent dementia. We determined plasma 25(OH)D concentrations for 955 participants (52.4% of participants who agreed the blood drawing), and then excluded those with missing data for covariates (n=39), leaving 916 participants, with at least one follow-up re-examination over 12 years. Among them, 874 (95.4%) were examined at wave 1; 860 (93.9%) were examined at wave 2; 894 (97.6%) were examined at wave 3; 787 (85.6%) were examined at wave 4 and 651 (71.1%) were examined at wave 5. Between baseline and wave 5, 144 participants deceased and among them, 88 (61.1%) deceased between wave 4 and wave 5.

2.2 Evaluation of cognitive functions and diagnosis of dementia Trained psychologists administered a battery of neuropsychological tests at each visit [21]. Five tests were administered at the baseline and different follow-up of the 3C study, as earlier described [22]: the Mini Mental State Examination (MMSE), the Isaacs Set Test (IST), the Benton Visual Retention Test (BVRT), the Trail Making Test (TMT), A and B and the Free and Cued Selective Reminding Test (FCSRT) (see supplementary material). These cognitive tests were administered at each follow-up except for the FCSRT, which was not administered at baseline and 4y (wave 2), and TMT-A and TMT-B which were not administered at 2y (wave 1).
The diagnosis of dementia was based on a clinical procedure: i) at each wave, participants who were suspected of dementia on the basis of their present neuropsychological performances and decline relatively to a previous examination were examined by a neurologist; ii) an independent committee of neurologists reviewed all potential cases of dementia to obtain a consensus on the diagnosis and aetiology according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, and the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria, as previously described [22].

2.3 Plasma 25(OH)D assessment
Plasma 25(OH)D concentrations were assessed from fasting blood samples collected at baseline and stored at -80°C, with a one-step immunoassay (ARCHITECT 25-OH Vitamin D assay, Abbott Diagnostics, Germany) as described elsewhere [23]. Instead of using raw plasma 25(OH)D concentration and adjusting for season in multivariate models as done in several previous analyses, we used a "de-seasonalized" plasma 25(OH)D concentration variable [15, 24]. We first regressed the measured 25(OH)D concentrations (in nmol/L) on calendar time using the following periodic function:

$$y_t = \beta_0 + \beta_1 \sin\left(\frac{2\pi t}{365}\right) + \beta_2 \cos\left(\frac{2\pi t}{365}\right)$$

where y_t denotes measured plasma 25(OH)D concentration, t denotes the day of the year the sample was collected, and β_j (j = 0, 1, 2) are estimated regression coefficients; we then extracted the residuals from this model (which represent the differences between each individual's actual 25(OH)D concentration and the concentration predicted by calendar time). Because residuals, by definition, have a mean of zero and negative as well as positive values, a constant can be added to every value to convey the sense of an actual concentration value [25]; we thus added the residuals of this regression model to the seasonal average to create a de-seasonalized vitamin D concentration for each individual. This provides a way to adjust for the seasonal variation of 25(OH)D given that blood samples were collected throughout the year. "January 1" de-seasonalized values were arbitrarily chosen for analysis, though in a periodic function any date would be expected to have been equally

informative (and subject to the same limitations). The consideration of de-seasonalized values was an *a priori* decision; this computation being largely used in the field of multiple sclerosis [26-28]. According to the definition of the World Health Organization [29] and the US Institute of Medicine [30], a sufficient 25(OH)D status was defined as a de-seasonalized plasma 25(OH)D concentration over 50 nmol/L, 25(OH)D insufficiency was defined as a de-seasonalized plasma 25(OH)D concentration between 25 nmol/L and 50 nmol/L, and de-seasonalized 25(OH)D deficiency at less than 25 nmol/L (to convert to ng/mL, divide by 2.496). Recent results supported the hypothesis that vitamin D might be protective in the context of dementia in the region of 50 nmol/L [14].

2.4 Other variables

The baseline interview included socio-demographic information such as age, gender, education, and income. Vascular risk factors included Body Mass Index (BMI in kg/m^2 computed as the measured weight/height² ratio; categorized as <21 kg/m², 21 to 27 kg/m² and 27kg/m² and over) [31], hypercholesterolemia (total cholesterol ≥ 6.2 mmol/L or treated), hypertriglyceridemia (total triglycerides ≥ 1.7 mmol/L or treated), smoking status (never, ex-smoker and current smoker), history of cardiovascular or cerebrovascular disease, measured hypertension (blood pressure ≥ 140/90 mmHg or treated) and diabetes (fasting blood glucose ≥ 7.2 mmol/L or treated). At baseline, plasma lipid levels [total cholesterol (TC) and triglycerides (TG)] and glucose were evaluated by routine enzymatic methods.

Apolipoprotein E ε4 (*ApoE4*) genotype was considered dichotomously: presence of at least one ε4 allele vs. no ε4 allele. Depressive symptomatology was assessed with the Center for Epidemiological Studies-Depression scale (CES-D) [32]. The number of drugs consumed per day was considered as a proxy of co-morbidities. Vitamin D supplementation use was recorded at baseline as earlier agreed according to the anatomical therapeutic chemical (ATC) classification [23]. Practice of physical activity was defined as regular when doing sport regularly, or having at least one hour of leisure activity per day [33]. This exposure variable included outdoor leisure activities and was used as a proxy for significant time spent outdoors in our analyses [23]. Overall diet quality was assessed by the Mediterranean diet score [34]. This score was computed as described earlier, based on a comprehensive dietary survey (food frequency questionnaire and 24H recall) administered at wave 1 (N=833 among 916 participants involved in the present analysis) [22].

2.5 Statistical analysis

Baseline characteristics (socio-demographic and health indicators) were compared across categories of de-seasonalized plasma 25(OH)D status using multinomial logistic regression analyses adjusted for age and sex.

We computed a Z-score of global cognition at baseline and each follow-up (except wave 1), using performances on MMSE, IST, BVRT, TMT-A and TMT-B. FCSRT performances were considered separately, since this test was not administered at baseline and the 4y visit. We used mixed models to examine the association between the 25(OH)D status and the evolution of cognitive performances over time. The outcomes of interest were the repeated measures of individual cognitive z-scores and of the FCSRT performances separately [22].

We then estimated the multivariate association between vitamin D status (i.e. 25(OH)D deficiency and insufficiency compared to sufficiency) and risk of all-cause dementia over 12 years using Cox proportional hazards model with delayed entry and age as time scale [35].

Both Cox proportional hazard models and mixed models were adjusted for potential confounders, including sex and education in Model 1 and additionally for income, CES-D score, number of drugs per day, *ApoE4* carrier status, BMI, practice of physical exercise, diabetes, history of cardiovascular diseases and stroke, hypertension, hypercholesterolemia, hypertriglyceridemia, smoking status, and Mediterranean diet score in Model 2. We secondarily investigated the multivariate association between de-seasonalized plasma 25(OH)D status and risk of incident AD over time. Interestingly, the control for vitamin D supplement use was not possible since no user developed the disease (AD) during the follow-up. In fully adjusted models, we also noted the strength of the association between

the presence of at least one *ApoE4* allele and the risk for AD. Hazard ratio (HR) and 95% confidence intervals (CI) were calculated. We tested potential interactions between 25(OH)D status and *ApoE4* genotype on the risk of dementia and AD.
Missing values were treated as follows. Only physical activity and Mediterranean diet score had missing values; data were missing for 12% and 9% of the sample, respectively. Therefore, specific missing categories were created for these two variables.
In supplementary analyses, we assessed the robustness of our analyses to potential reverse causation (that would occur if incipient cognitive impairment at baseline led to lower vitamin D status) by further adjusting the models for baseline cognitive status (i.e. cognitive Z-score). We further assessed the robustness after excluding individuals who developed the disease between baseline and wave 2 (i.e. over 4y). We also tested the relevance of 25(OH)D thresholds by considering 25(OH)D concentrations as continuous variable. Finally, parallel analyses using as-measured 25(OH)D values were also performed.
In all statistical models, all hypotheses were tested and satisfied. The SAS statistical package (Version 9.2 SAS Institute) was used for these analyses.

3. RESULTS

The study sample consisted of 916 participants (571 women, 345 men) with plasma 25(OH)D assessment, aged 73.3 years-old on average at baseline, who were re-examined at least once over 10.8 years of follow-up on average (median of follow-up 11.4 years). Mean de-seasonalized plasma 25(OH)D was 35.8 nmol/L (SD 16.7 nmol/L) and the prevalences of 25(OH)D deficiency (<25 nmol/L) and insufficiency ([25; 50] nmol/L) were 24% and 60%, respectively.

3.1 Description of studied sample

Participants with 25(OH)D deficiency were significantly older and more often women than those with 25(OH)D insufficiency and sufficiency (**Table 1**). After adjustment for age and sex, participants with 25(OH)D deficiency used significantly more drugs, suffered more often from hypercholesterolemia and hypertriglyceridemia. The use of vitamin D supplementation was significantly associated with the 25(OH)D status of participants with a dose-response relationship.
During follow-up, the independent committee of neurologists validated 177 incident dementia cases (including 124 AD), among whom 62 participants exhibited 25(OH)D deficiency (including 43 AD) and 98 participants 25(OH)D insufficiency (including 72 AD). Kaplan-Meier curves for unadjusted rates of incident all-cause dementia and AD show clear differences in risk by 25(OH)D concentrations (**Figure 1**).

3.2 Vitamin D status and cognitive decline over time

At baseline, 25(OH) insufficiency and 25(OH)D deficiency were significantly associated with lower Z-scores of cognition but not with FCSRT performances (**Table 2**). In longitudinal analyses, 25(OH)D deficiency was significantly associated with faster cognitive decline, as expressed as cognitive Z-score, and with faster verbal episodic memory decline, as expressed as FCSRT performances, over time.

3.3 Vitamin D status and risk for dementia and AD

In multivariate models, compared with participants with vitamin D sufficiency, those with vitamin D deficiency and insufficiency had a significantly doubled risk of all-cause dementia (HR=2.12, 95% CI 1.21-3.71 for deficiency and HR=1.98, 95% CI 1.17-3.36, for insufficiency, P for trend across categories=0.02; **Table 3**). Associations appeared even stronger for the risk of AD, with risks almost tripled in both deficient categories compared to sufficient concentrations (HR=2.78 (95% CI 1.37-5.68) for insufficiency and HR=2.85 (95% CI 1.36-5.97) for deficiency; P trend=0.02). In this last analysis, carrying at least one *ApoE4* allele was also independently associated with an increased risk for AD (HR=1.86, 95%CI 1.21-2.87).

Interactions between *ApoE4* and plasma 25(OH)D concentrations for the risk of all-cause dementia or AD were not statistically significant (P=0.25 and P=0.54, respectively).

3.4 Supplementary analyses

In supplementary analyses, associations between 25(OH)D status and risk for all-cause dementia were no more significant when adjusted for baseline cognitive Z-score (Supplementary Table 1). Conversely, risk estimates remained generally high and statistically significant for AD risk. In another set of supplementary analyses, we excluded 11 participants who developed dementia in the first 4 years of follow-up, including 8 AD. Among the remaining 905 participants, compared to a 25(OH)D sufficient status, 25(OH)D deficiency and 25(OH)D insufficiency were both significantly associated with an increased risk for all-cause dementia (HR=2.22, 95%CI 1.24-3.99 and HR=2.05, 95%CI 1.17-3.58, p=0.016, respectively) and AD (HR=2.96, 95%CI 1.39-6.44 and HR=2.86, 95%CI 1.35-6.03, p=0.017,respectively).

To explore whether there are any threshold effects, we considered 25(OH)D concentrations as continuous variable: for each additional unit of 25(OH)D concentration, we observed a significantly decreased risk of all-cause dementia (HR=0.98, 95%CI 0.97-0.99) and AD (HR=0.98, 95%CI 0.97-0.99) in fully adjusted models. Finally, using as-measured 25(OH)D values did not alter the associations with cognitive decline and risk for dementia/AD.

4. DISCUSSION

In this large prospective cohort study with long-term follow-up for dementia, vitamin D deficiency (i.e. plasma 25(OH)D lower than 25 nmol/L) was associated with a faster cognitive decline, and with a nearly three-fold increased risk of AD over 12 years of follow-up. These associations were independent of major confounders including overall diet quality and *ApoE4* genotype. The association of vitamin D to AD appeared particularly strong and was even higher than that observed with *ApoE4.* Overall, our findings suggest that maintaining plasma 25(OH)D concentrations at or above 50 nmol/L in older persons may contribute to preserve brain health and lower risk of AD.

In the literature, reports about 25(OH)D status and cognitive decline are mixed so far, and appeared to be dependent on several factors such as gender, age and cognitive domains assessed [3, 36-37]. Indeed, which cognitive domain would be particularly affected in case of 25(OH)D deficiency is still not fully elucidated [3]. Results from the present study provided additional insights into this last issue.At baseline, compared with 25(OH)D sufficiency, we observed lower global cognitive performances, except for verbal episodic memory, assessed by the FCSRT, for participants with 25(OH)D deficiency. Over time, a faster cognitive decline was observed among all cognitive domains assessed, including episodic memory: these differences are plausible given the different nature of the tests: MMSE and FCSRT, assessing respectively global cognitive abilities and episodic memory, are known as the hallmark of pathological cognitive aging [38-39].

To our knowledge, several longitudinal studies have assessed the risk of dementia or AD based on plasma 25(OH)D concentrations [10-11, 14-17, 40]. As early as 2011, a small prospective study of about 40 French community-dwelling high-functioning women showed that vitamin D deficiency was significantly associated with the onset of non-Alzheimer dementia, but not AD, over 7y of follow-up [40]. Our findings are consistent with at least two large prospective studies which recently evidenced associations of lower vitamin D to higher risk of dementia/AD among older persons. A US study of 1,658 older community-dwellers found an increased risk of AD and all-cause-dementia among participants with 25(OH)D deficiency/insufficiency, with strengths of associations very close to ours [14]. Accordingly, reduced levels of de-seasonalized plasma 25(OH)D were also associated with an increased risk of AD, alone or combined with vascular dementia (VAD), in the National Danish Patient Registry [15]. However, there have been also conflicting results in the literature, which contrast with the present findings. Two longitudinal studies from Finland and the US failed to find an association between plasma 25(OH)D concentrations and the risk of dementia leading to hospitalization; these

data are difficult to compare with ours because of the diagnostic heterogeneity of the patients (i.e. hospitalization recorded using registry in both studies), their characteristics (40-45years-old at baseline in both studies), and the specific thresholds used to categorize the 25(OH)D status (i.e. sample-specific quantiles) [11, 17]. Additionally, in the Framingham Heart Study, no association was found between baseline vitamin D status and risk for dementia or AD over 9 years, in spite of lower cognitive performances in some domains and smaller hippocampal volumes among participants with 25(OH)D <25 nmol/L at baseline [10]. However, the relatively young age of participants (60+ years-old at baseline) and the low prevalence of vitamin D deficiency in this US sample (6%) may partly explain the Framingham findings.

Results from experimental studies and the evidence of an association between lower 25(OH)D concentrations in humans and a faster cognitive decline have led to clinical trials examining the therapeutic effect of vitamin D supplementation on cognition. However, research has been limited and findings have been inconsistent so far. A small study reported a benefit of vitamin D supplementation, alone or in combination with an anti-AD drug, on cognition among patients with AD [41], while several other interventional studies have failed to demonstrate any effect on cognition among patients with AD or in primary prevention among women enrolled in the Women's Health Initiative [42-44]. Several large randomized controlled trials assessing the impact of vitamin D on cognition, either as a primary or secondary outcome, are currently in progress (for details, see clinicaltrials.gov) [45-47].

From a mechanistic point of view, a solid biological foundation for the brain health benefits of vitamin D has been provided by animal and human studies [2-3, 48]. Vitamin D has a major role in brain development and maturation, and vitamin D Receptors (VDR) are present in several brain areas, including those related to learning and memory functions. The vitamin D-related homeostasis of calcium has been largely reported; moreover, vitamin D is involved in several pathways critical to brain health, including neurotransmission, neuro-protection, modulation of immune response, inhibition of pro-inflammatory agents, and regulation of oxidative stress [2-3, 49]. Furthermore, our findings appeared particularly strong for AD and accordingly, vitamin D has been associated with a higher clearance of amyloid-β, resulting in a decreased number of amyloid plaques in experimental studies [2]. Moreover, the neurodegeneration induced by amyloid-β appears to be mediated by the inhibition of the VDR expression, and increased catabolism of vitamin D [2]. The vitamin D binding protein has even been found to interact with amyloid-β by inhibition of its fibrilization, aggregation and oligomerization [50].

Some methodological limitations of our study should be stressed. First, hypovitaminosis D is a condition highly prevalent in older adults [51-52], leading to a low prevalence of 25(OH)D sufficiency, and a small sample size in the reference group. However, we still observed an association between 25(OH)D status and the risk for dementia/AD when considering vitamin D concentrations as a continuous variable. A single plasma 25(OH)D measurement was available at baseline. However, previous analyses have reported that a single measurement is a reasonable predictor of long-term exposure to plasma 25(OH)D [53]. We did not account for all factors known to influence circulating 25(OH)D concentration, such as the genetic variants of the VDR gene [2]. The lack of consideration of vitamin D supplement use as possible confounders could also be considered as a limit, while a very small proportion of participants (less than 5%) were vitamin D supplement users and the French policy of vitamin D supplementation of selected foods was scarce. In addition, participants not included in our study were significantly older, less educated, and had a worse general health status at baseline than included participants (**Supplementary Table 2**), and selection of participants in better general health may have biased our findings towards the null. Moreover, reverse causality cannot be completely excluded, notably regarding the risk for all-cause dementia, although our result regarding AD was maintained after controlling for cognitive performances at the time of blood draw or after exclusion of individuals in the preclinical phase of dementia.

Despite these limitations, the strengths of the present study are its large size, the longitudinal population-based design with long-term follow-up, the identification of incident dementia and AD by an independent committee of neurologists, and the definition of vitamin D sufficiency in accordance to thresholds clinically relevant to brain health in the literature [14]. Finally, we accounted for numerous potential confounders, including overall diet quality, and the *ApoE4* genotype, but we cannot exclude that residual confounding may persist.

In this large prospective study of French older persons, we found a strong association between lower baseline plasma concentrations of 25(OH)D and i) a faster cognitive decline and ii) an increased risk of dementia and AD over the decade following blood draw. Although the worldwide hypovitaminosis D cannot alone explain rates of dementia,[52, 54] our results add to the growing body of evidence linking vitamin D status to optimal brain functioning, and suggest that maintaining adequate vitamin D status could contribute to slow down cognitive decline and to delay or prevent the onset of dementia and AD among older individuals. The mechanisms underlying this protective association, especially those linking specifically vitamin D to AD, deserve further research.

References

[1] Annweiler C, Bartha R, Goncalves S, Karras SN, Millet P, Feron F, et al. Vitamin D-related changes in intracranial volume in older adults: A quantitative neuroimaging study. Maturitas. 2015;80:312-7.
[2] Gezen-Ak D, Yilmazer S, Dursun E. Why vitamin D in Alzheimer's disease? The hypothesis. J Alzheimers Dis. 2014;40:257-69.
[3] Landel V, Annweiler C, Millet P, Morello M, Feron F. Vitamin D, Cognition and Alzheimer's Disease: The Therapeutic Benefit is in the D-Tails. J Alzheimers Dis. 2016;53:419-44.
[4] Annweiler C, Llewellyn DJ, Beauchet O. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis. 2013;33:659-74.
[5] Balion C, Griffith LE, Strifler L, Henderson M, Patterson C, Heckman G, et al. Vitamin D, cognition, and dementia: a systematic review and meta-analysis. Neurology. 2012;79:1397-405.
[6] Miller JW, Harvey DJ, Beckett LA, Green R, Farias ST, Reed BR, et al. Vitamin D Status and Rates of Cognitive Decline in a Multiethnic Cohort of Older Adults. JAMA Neurol. 2015;72:1295-303.
[7] Moon JH, Lim S, Han JW, Kim KM, Choi SH, Kim KW, et al. Serum 25-hydroxyvitamin D level and the risk of mild cognitive impairment and dementia: the Korean Longitudinal Study on Health and Aging (KLoSHA). Clin Endocrinol (Oxf). 2015;83:36-42.
[8] Annweiler C, Montero-Odasso M, Llewellyn DJ, Richard-Devantoy S, Duque G, Beauchet O. Meta-analysis of memory and executive dysfunctions in relation to vitamin D. J Alzheimers Dis. 2013;37:147-71.
[9] Overman MJ, Pendleton N, O'Neill TW, Bartfai G, Casanueva FF, Finn JD, et al. Evaluation of cognitive subdomains, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D in the European Male Ageing Study. European journal of nutrition. 2016.
[10] Karakis I, Pase MP, Beiser A, Booth SL, Jacques PF, Rogers G, 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;51:451-61.
[11] Schneider AL, Lutsey PL, Alonso A, Gottesman RF, Sharrett AR, Carson KA, et al. Vitamin D and cognitive function and dementia risk in a biracial cohort: the ARIC Brain MRI Study. Eur J Neurol. 2014;21:1211-8, e69-70.

[12] Bartali B, Devore E, Grodstein F, Kang JH. Plasma vitamin D levels and cognitive function in aging women: the nurses' health study. J Nutr Health Aging. 2014;18:400-6.
[13] Assmann KE, Touvier M, Andreeva VA, Deschasaux M, Constans T, Hercberg S, et al. Midlife plasma vitamin D concentrations and performance in different cognitive domains assessed 13 years later. Br J Nutr. 2015;113:1628-37.
[14] Littlejohns TJ, Henley WE, Lang IA, Annweiler C, Beauchet O, Chaves PH, et al. Vitamin D and the risk of dementia and Alzheimer disease. Neurology. 2014;83:920-8.
[15] Afzal S, Bojesen SE, Nordestgaard BG. Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. Alzheimers Dement. 2014;10:296-302.
[16] Shen L, Ji HF. Vitamin D deficiency is associated with increased risk of Alzheimer's disease and dementia: evidence from meta-analysis. Nutr J. 2015;14:76.
[17] Knekt P, Saaksjarvi K, Jarvinen R, Marniemi J, Mannisto S, Kanerva N, et al. Serum 25-hydroxyvitamin d concentration and risk of dementia. Epidemiology. 2014;25:799-804.
[18] Feart C, Samieri C, Barberger-Gateau P. Mediterranean diet and cognitive health: an update of available knowledge. Current opinion in clinical nutrition and metabolic care. 2015;18:51-62.
[19] Maddock J, Cavadino A, Power C, Hypponen E. 25-hydroxyvitamin D, APOE varepsilon4 genotype and cognitive function: findings from the 1958 British birth cohort. European journal of clinical nutrition. 2015;69:505-8.
[20] Egert S, Rimbach G, Huebbe P. ApoE genotype: from geographic distribution to function and responsiveness to dietary factors. The Proceedings of the Nutrition Society. 2012;71:410-24.
[21] The 3C Study Group. Vascular factors and risk of dementia: design of the Three-City Study and baseline characteristics of the study population. Neuroepidemiology. 2003;22:316-25.
[22] Féart C, Samieri C, Rondeau V, Amieva H, Portet F, Dartigues JF, et al. Adherence to a mediterranean diet, cognitive decline, and risk of dementia. JAMA - Journal of the American Medical Association. 2009;302:638-48.
[23] Cougnard-Gregoire A, Merle BM, Korobelnik JF, Rougier MB, Delyfer MN, Feart C, et al. Vitamin D Deficiency in Community-Dwelling Elderly Is Not Associated with Age-Related Macular Degeneration. The Journal of nutrition. 2015;145:1865-72.
[24] 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;21:1436-e95.
[25] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American journal of clinical nutrition. 1997;65:1220S-8S; discussion 9S-31S.
[26] van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol. 2007;254:581-90.
[27] Simpson S, Jr., Taylor B, Blizzard L, Ponsonby AL, Pittas F, Tremlett H, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. Annals of neurology. 2010;68:193-203.
[28] Gelfand JM, Cree BA, McElroy J, Oksenberg J, Green R, Mowry EM, et al. Vitamin D in African Americans with multiple sclerosis. Neurology. 2011;76:1824-30.
[29] Prevention and management of osteoporosis. World Health Organ Tech Rep Ser. 2003;921:1-164, back cover.
[30] Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53-8.

[31] Feart C, Jutand MA, Larrieu S, Letenneur L, Delcourt C, Combe N, et al. Energy, macronutrient and fatty acid intake of French elderly community dwellers and association with socio-demographic characteristics: data from the Bordeaux sample of the Three-City Study. Br J Nutr. 2007;98:1046-57.
[32] Radloff LS. The CES-D scale : a self-report depression scale for research in the general population. Appl Psychol Meas. 1977;1:385-401.
[33] Feart C, Lorrain S, Ginder Coupez V, Samieri C, Letenneur L, Paineau D, et al. Adherence to a Mediterranean diet and risk of fractures in French older persons. Osteoporos Int. 2013;24:3031-41.
[34] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003;348:2599-608.
[35] Lamarca R, Alonso J, Gomez G, Munoz A. Left-truncated data with age as time scale: an alternative for survival analysis in the elderly population. The journals of gerontology. 1998;53:M337-43.
[36] Slinin Y, Paudel M, Taylor BC, Ishani A, Rossom R, Yaffe K, et al. Association between serum 25(OH) vitamin D and the risk of cognitive decline in older women. The journals of gerontology. 2012;67:1092-8.
[37] Slinin Y, Paudel ML, Taylor BC, Fink HA, Ishani A, Canales MT, et al. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology. 2010;74:33-41.
[38] Amieva H, Le Goff M, Millet X, Orgogozo JM, Peres K, Barberger-Gateau P, et al. Prodromal Alzheimer's disease: successive emergence of the clinical symptoms. Annals of neurology. 2008;64:492-8.
[39] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet neurology. 2007;6:734-46.
[40] Annweiler C, Rolland Y, Schott AM, Blain H, Vellas B, Beauchet O. Serum vitamin D deficiency as a predictor of incident non-Alzheimer dementias: a 7-year longitudinal study. Dementia and geriatric cognitive disorders. 2011;32:273-8.
[41] Annweiler C, Herrmann FR, Fantino B, Brugg B, Beauchet O. Effectiveness of the combination of memantine plus vitamin D on cognition in patients with Alzheimer disease: a pre-post pilot study. Cogn Behav Neurol. 2012;25:121-7.
[42] Stein MS, Scherer SC, Ladd KS, Harrison LC. A randomized controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease. J Alzheimers Dis. 2011;26:477-84.
[43] Przybelski R, Agrawal S, Krueger D, Engelke JA, Walbrun F, Binkley N. Rapid correction of low vitamin D status in nursing home residents. Osteoporos Int. 2008;19:1621-8.
[44] Rossom RC, Espeland MA, Manson JE, Dysken MW, Johnson KC, Lane DS, et al. Calcium and vitamin D supplementation and cognitive impairment in the women's health initiative. J Am Geriatr Soc. 2012;60:2197-205.
[45] Annweiler C. Vitamin D in dementia prevention. Ann N Y Acad Sci. 2016;1367:57-63.
[46] Grant WB. Using findings from observational studies to guide vitamin D randomized controlled trials. Journal of internal medicine. 2015;277:83-6.
[47] Anastasiou CA, Yannakoulia M, Scarmeas N. Vitamin D and cognition: an update of the current evidence. J Alzheimers Dis. 2014;42 Suppl 3:S71-80.
[48] Annweiler C, Dursun E, Feron F, Gezen-Ak D, Kalueff AV, Littlejohns T, et al. 'Vitamin D and cognition in older adults': updated international recommendations. Journal of internal medicine. 2015;277:45-57.

[49] Brouwer-Brolsma EM, de Groot LC. Vitamin D and cognition in older adults: an update of recent findings. Current opinion in clinical nutrition and metabolic care. 2015;18:11-6.
[50] Moon M, Song H, Hong HJ, Nam DW, Cha MY, Oh MS, et al. Vitamin D-binding protein interacts with Abeta and suppresses Abeta-mediated pathology. Cell Death Differ. 2013;20:630-8.
[51] Annweiler C, Fantino B, Le Gall D, Schott AM, Berrut G, Beauchet O. Severe vitamin D deficiency is associated with advanced-stage dementia in geriatric inpatients. J Am Geriatr Soc. 2011;59:169-71.
[52] Cashman KD, Dowling KG, Skrabakova Z, Gonzalez-Gross M, Valtuena J, De Henauw S, et al. Vitamin D deficiency in Europe: pandemic? The American journal of clinical nutrition. 2016;103:1033-44.
[53] Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. American journal of epidemiology. 2010;171:903-8.
[54] Howland RH. Association Between Vitamin D Status and Cognitive Decline. JAMA Neurol. 2016;73:762.

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Key words: vitamin D, Alzheimer Disease, dementia, cognitive decline, prospective studies, risk factors in epidemiology

Figure Legend

Figure 1. Kaplan-Meier curves for unadjusted rates of all-cause dementia (left panel) and Alzheimer's disease (right panel) by vitamin D status (de-seasonalized plasma 25(OH)D concentrations). De-seasonalized 25(OH)D status was defined as follows: deficiency (< 25 nmol/L - blue line), insufficiency (25 to 50 nmol/L - red line) and sufficiency (> 50 nmol/L - green line)

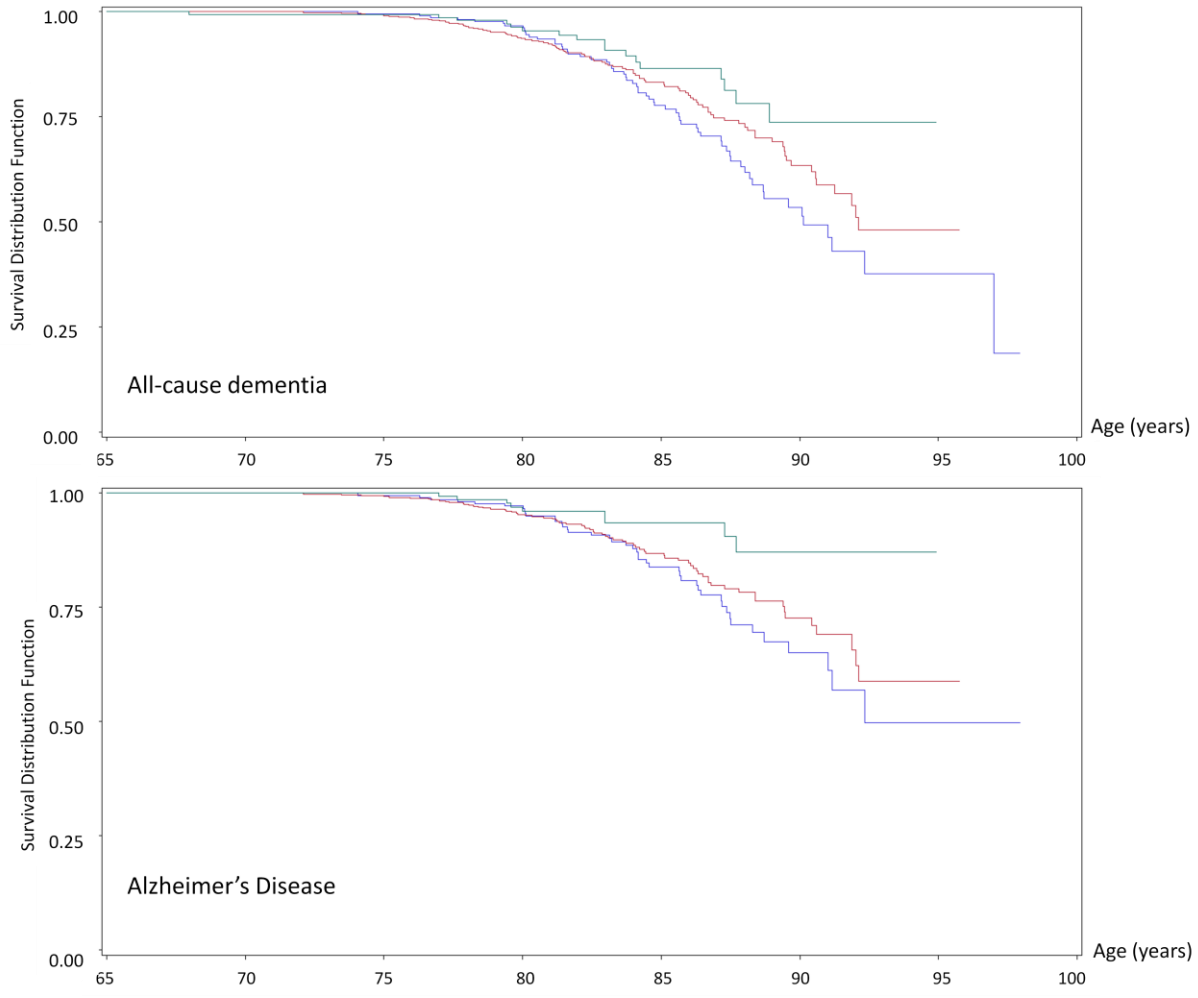

Table 1. Baseline socio-demographic characteristics and health indicators based on vitamin D status of participants from the Bordeaux sample of the Three-City study, 1999-2000 (N = 916)

	25(OH)D status[a]			
	25(OH)D Deficiency (< 25 nmol/L) (n = 218)	25(OH)D Insufficiency (25-50 nmol/L) (n = 547)	25(OH)D Sufficiency (> 50 nmol/L) (n = 151)	P[b]
Socio-demographic characteristics				
Age (y), mean (SD)	74.6 (4.6)	72.9 (4.6)*	72.9 (4.1)†	$<10^{-3}$
Women, n (%)	165 (75.7)	330 (60.3)*	76 (50.3)†	$<10^{-3}$
Education, n (%)				0.89
No or primary	70 (32.1)	172 (31.4)	40 (26.5)	
Secondary	60 (27.5)	168 (25.2)	47 (31.1)	
High school	45 (20.6)	130 (23.8)	26 (23.8)	
University	43 (19.8)	107 (19.6)	28 (18.6)	
Monthly income (euros),n (%)				0.31
<750	21 (9.8)	33 (6.1)	7 (4.6)	
[750 ; 1500]	78 (36.5)	167 (30.6)	39 (25.8)	
[1500 ; 2250]	56 (26.2)	143 (26.2)	42 (27.8)	
≥ 2250	49 (22.9)	177 (32.5)	56 (37.1)	
Refused to answer	10 (4.6)	25 (4.6)	7 (4.6)	
Health indicators				
Depressive symptomatology, n (%)	19 (8.7)	30 (5.5)	7 (4.6)	0.60
Number of drugs per day, mean (SD)	4.5 (2.6)	4.0 (2.6)*	4.0 (2.6)	0.02
ApoE4 carrier, n (%)	45 (20.6)	91 (16.6)	25 (16.6)	0.29
BMI (kg/m²), n (%)				0.02
< 21	23 (10.6)	30 (5.5)	13 (8.6)	
[21 ; 27]	117 (53.7)	284 (51.9)	87 (57.6)	
≥ 27	78 (35.8)	233 (42.6)	51 (33.8)	
Practice of physical exercise, n (%)				0.41
No	137 (62.8)	301 (55.0)	72 (47.7)	
Yes	51 (23.4)	182 (33.3)	63 (41.7)	
No answer	30 (13.8)	64 (11.7)	16 (10.6)	
Diabetes, n (%)	23 (10.6)	41 (7.5)	11 (7.3)	0.14
History of cardiovascular disease or stroke, n (%)	20 (9.2)	48 (8.8)	11 (7.3)	0.60
Hypertension, n (%)	162 (74.3)	414 (75.7)	115 (76.2)	0.68
Hypercholesterolemia, n (%)	150 (68.8)	313 (57.2)*	79 (52.3)†	0.02
Hypertriglyceridemia, n (%)	106 (48.6)	220 (40.2)*	51 (33.8)†	<0.01
Smoking, n (%)				0.59
Never	154 (70.6)	345 (63.1)	88 (58.3)	
Ex-smoker	51 (23.4)	182 (33.3)	53 (35.1)	
Current smoker	13 (6.0)	20 (3.7)	10 (6.6)	
Vitamin D supplementation, n (%)	2 (0.9)	12 (2.2)*	30 (19.9)†‡	$<10^{-3}$

MeDi adherence, n (%)				0.61
Low (score 0-3)	65 (29.8)	167 (30.5)	43 (28.5)	
Moderate (score 4-5)	92 (42.2)	228 (41.7)	60 (39.7)	
High (score 6-9)	38 (17.4)	107 (19.6)	33 (21.8)	
No answer	23 (10.6)	45 (8.2)	15 (10.0)	

Abbreviations: *ApoE4* Apolipoprotein E ε4, BMI Body Mass Index, MeDi Mediterranean Diet, AD Alzheimer's Disease

[a] De-seasonalized 25(OH)D status

[b] P-value of the comparisons of characteristics with vitamin D status were performed with multinomial regression analysis adjusted for age and sex.

* indicated a significant difference between 25(OH)D insufficiency and deficiency

† indicated a significant difference between 25(OH)D sufficiency and deficiency

‡ indicated a significant difference between 25(OH)D sufficiency and insufficiency

Table 2. Change in cognitive performances based on vitamin D status over 12 years of follow-up among older persons living in Bordeaux, The Three-City study (1999-2012).

	Model 1 *				Model 2 *			
	β	95% CI	P	*Overall*	β	95% CI	P	*Overall*
Cognitive Z-score [+]								
Time	-0.057	-0.067;-0.011	<0.0001		-0.057	-0.067;-0.047	<0.0001	
25(OH)D insufficiency, baseline testing	-0.115	-0.220;-0.011	0.031	*0.002*	-0.110	-0.211;-0.009	0.033	*0.007*
25(OH)D deficiency, baseline testing	-0.200	-0.323;-0.077	0.001		-0.167	-0.287;-0.047	0.006	
25(OH)D insufficiency X time	-0.005	-0.016;0.006	0.331	*0.048*	-0.005	-0.016;0.006	0.336	*0.045*
25(OH)D deficiency X time	-0.013	-0.026;0.001	0.052		-0.013	-0.026;-0.001	0.049	
FCSRT †								
Time	-0.083	-0.215;0.049	0.219		-0.081	-0.213;0.051	0.230	
25(OH)D insufficiency, baseline testing	-0.883	-1.998;0.233	0.121	*0.053*	-0.776	-1.883;0.332	0.170	*0.197*
25(OH)D deficiency, baseline testing	-1.322	-2.629;-0.015	0.047		-0.917	-2.229;0.394	0.170	
25(OH)D insufficiency X time	-0.099	-0.249;0.049	0.190	*0.019*	-0.101	-0.250;0.048	0.183	*0.017*
25(OH)D deficiency X time	-0.208	-0.383;-0.033	0.020		-0.211	-0.386;-0.036	0.018	

Abbreviations: MMSE Mini Mental State Examination, BVRT Benton Visual Retention Test, IST Isaacs Set Test, TMT Trail Making Test, FCSRT Free and Cued Selective Reminding Test

[+] The cognitive z-score was computed using performances on MMSE, BVRT, IST, TMT-A and TMT-B at baseline and wave 2 to 5 (N=828 at baseline).

† FCSRT performances were considered between wave 1 and wave 5, except wave 2 (N=810 at wave 1)

[*] Linear mixed models adjusted for age, gender, education, and learning effect (Model 1) and for income, depressive symptomatology, number of drugs per day, Apolipoprotein E ε4 allele, BMI, practice of physical exercise, diabetes, history of cardiovascular diseases and stroke, hypertension, hypercholesterolemia, hypertriglyceridemia, smoking status, and Mediterranean diet score (Model 2).

Table 3. Multivariate associations between vitamin D status and 12-year incident all-cause dementia and Alzheimer's Disease. Three-City Bordeaux study, 1999-2012 (N = 916)

		Model 1			Model 2		
	N incident cases /Total	HR*	95% CI	P for trend	HR*	95% CI	P for trend
De-seasonalized 25(OH)D concentration (nmol/L)							
Risk of all-cause dementia				0.028			0.018
> 50 (sufficiency)	17/151	1.00			1.00		
≥ 25 to ≤ 50 (insufficiency)	98/547	1.65	0.98-2.77		1.98	1.17-3.36	
< 25 (deficiency)	62/218	2.21	1.28-3.80		2.12	1.21-3.71	
Risk of AD				0.033			0.017
> 50 (sufficiency)	9/151	1.00			1.00		
≥ 25 to ≤ 50 (insufficiency)	72/547	2.29	1.14-4.58		2.78	1.37-5.68	
< 25 (deficiency)	43/218	2.96	1.43-6.11		2.85	1.36-5.97	

Abbreviations: AD Alzheimer's Disease, HR Hazard ratio, CI confidence interval

* Cox proportional hazard models with delayed entry adjusted for gender, education (Model 1) and additionally for income, depressive symptomatology, number of drugs per day, Apolipoprotein E ε4 allele, BMI, practice of physical exercise, diabetes, history of cardiovascular diseases and stroke, hypertension, hypercholesterolemia, hypertriglyceridemia, smoking status, and Mediterranean diet score (Model 2).

Supplementary material

e-Methods

Evaluation of cognitive functions and diagnosis of dementia

Five cognitive tests were administered at baseline and subsequent follow-up:

i. the Mini Mental State Examination (MMSE) is a sum-score, evaluating various dimensions of cognition, used as an index of global cognitive performance. Scores range from 0 to 30.
ii. the Isaacs Set Test (IST) evaluates semantic verbal fluency abilities and speed of verbal production. Individuals have to generate a list of words (with a maximum of 10) belonging to a specific semantic category in 15 seconds. Four semantic categories are used successively (cities, fruits, animals and colors). Score range from 0 to 40.
iii. the Benton Visual Retention Test (BVRT) evaluates immediate visual memory. After the presentation for 10 seconds of a stimulus card displaying a geometric figure, individuals are asked to identify the initial figure among 4 possibilities. Fifteen figures are successively presented and scores range from 0 to 15.
iv. The Trail Making Test (TMT), A and B. The TMT evaluates executive functioning. The TMT-A consists of connecting numbers from 1 to 25 in an ascending manner, whereas in the TMT-B the numbers and letters have to be linked alternately in ascending order. The variable analyzed is the time required to perform each part of the test.
v. the Free and Cued Selective Reminding Test (FCSRT) involves verbal episodic memory. Sixteen words belonging to 16 semantic categories are presented during the encoding phase. Afterwards, three successive recall trials are performed, each trial starting with a free recall inviting participants to retrieve as many words as possible. Then, for words not retrieved, the examiner provides a category cue to enhance recall. We considered the total free recall score as corresponding to the sum of the 3 free recalls ranging from 0 to 48.

e-Results

Supplementary Table 1. Multivariate associations between vitamin D status and 12-year incident all-cause dementia and Alzheimer's Disease. Three-City Bordeaux study, 1999-2012 (N = 828)

	N incident cases/Total	HR*	95% CI	P for trend
De-seasonalized 25(OH)D concentration (nmol/L)				
Risk of all-cause dementia				
> 50 (sufficiency)	15/140	Ref.		0.17
≥ 25 to ≤ 50 (insufficiency)	82/493	1.60	0.92-2.81	
< 25 (deficiency)	49/195	1.54	0.85-2.81	
Risk of AD				
> 50 (sufficiency)	7/140	Ref.		0.04
≥ 25 to ≤ 50 (insufficiency)	59/493	2.60	1.17-5.77	
< 25 (deficiency)	36/195	2.49	1.08-5.72	

Abbreviations: AD Alzheimer's Disease, HR Hazard ratio, CI confidence interval

* Cox proportional hazard models with delayed entry adjusted for gender, education, income, depressive symptomatology, number of drugs per day, Apolipoprotein E ε4 allele, BMI, practice of physical exercise, diabetes, history of cardiovascular diseases and stroke, hypertension, hypercholesterolemia, hypertriglyceridemia, smoking status, Mediterranean diet score and baseline cognitive performances computed as a Z-score including Mini Mental State Examination, Benton Visual Retention Test, Isaacs Set Test, and Trail Making Test performances.

Supplementary Table 2. Baseline socio-demographic characteristics and health indicators of participants included or not in the analysis. Bordeaux sample of the Three-City study (N=2,104)

	Not included (n=1188)	Included (n=916)	P[a]
Socio-demographic characteristics			
Age (y), mean (SD)	75.6 (5.3)	73.3 (4.5)	$<10^{-3}$
Women, n (%)	717 (60.3)	571 (62.3)	0.36
Education, n (%)			$<10^{-3}$
No or primary	481 (40.8)	281 (30.8)	
Secondary	310 (26.3)	244 (26.8)	
High school	220 (18.6)	210 (23.0)	
University	169 (14.3)	177 (19.4)	
Monthly income (euros),n (%)			$<10^{-3}$
<750	147 (12.6)	61 (6.7)	
[750 ; 1500]	430 (37.0)	284 (31.2)	
[1500 ; 2250]	239 (20.5)	241 (26.5)	
≥ 2250	258 (22.2)	282 (31.0)	
Refused to answer	90 (7.7)	42 (4.6)	
Health indicators			
MMSE Score, mean (SD)	26.8 (2.7)	27.7 (1.9)	$<10^{-3}$
Depressive symptomatology, n (%)	106 (9.2)	56 (6.1)	0.01
Number of drugs per day, mean (SD)	5.1 (3.0)	4.1 (2.6)	$<10^{-3}$
ApoE4 carrier, n (%)	204 (21.5)	161 (17.6)	0.03
BMI (kg/m²), n (%)			0.25
< 21	103 (9.1)	66 (7.2)	
[21 ; 27]	576 (50.9)	488 (53.3)	
≥ 27	453 (40.0)	362 (39.5)	
Practice of physical exercise, n (%)			$<10^{-3}$
No	627 (52.8)	510 (55.7)	
Yes	226 (19.0)	296 (32.3)	
No answer	335 (28.2)	110 (12.0)	
Diabetes, n (%)	145 (15.1)	76 (8.3)	$<10^{-3}$
History of cardiovascular disease or stroke, n (%)	148 (12.5)	79 (8.6)	0.01
Hypertension, n (%)	970 (81.9)	691 (75.4)	0.01
Hypercholesterolemia, n (%)	594 (57.1)	542 (59.2)	0.35
Hypertriglyceridemia, n (%)	486 (40.9)	377 (41.2)	0.91
Smoking, n (%)			0.31
Never	766 (64.5)	587 (64.1)	
Ex-smoker	349 (29.4)	286 (31.2)	
Current smoker	72 (6.1)	43 (4.7)	
Vitamin D supplementation, n (%)	63 (5.3)	44 (4.8)	0.60
Incident dementia, n (%)	227 (19.1)	177 (19.3)	0.90
Incident AD, n (%)	145 (12.2)	124 (13.5)	0.36

Abbreviations: MMSE Mini-Mental State Examination, *ApoE4* Apolipoprotein E ε4, BMI Body Mass Index, AD Alzheimer's Disease

[a]P-value for the Chi-Square test between participants included and those not included, except for age, MMSE score, and number of drugs per day whose means were compared by Student's t test.