The Effects of Vitamin D Insufficiency and Seasonal Decrease on Cognition

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ABSTRACT: ***Background:*** Vitamin D3 (cholecalciferol) deficiency has been associated with dementia and cognitive decline. Which cognitive domains are most associated with D3 levels and how seasonal fluctuations in levels relate to cognition is unclear. We addressed these questions using a prospective observational study examining associations between D3 levels and cognition among individuals living in northern latitudes (54°N) in summer and winter. ***Methods:*** Healthy adult participants underwent testing in summer and winter of D3 levels and cognition, using the Symbol Digit Modalities Test, phonemic fluency, Digit Span and CANTAB® battery. ***Results:*** Of 32 participants tested in the summer, 46% were D3 insufficient (<75 nmol/L) and performed worse on Digit Span Backward (DS-B) (M=5.8, SD=2) than those who were sufficient (M=7.9, SD=2), p=0.018. In multivariate analyses, sufficiency status was an independent predictor of DS-B, (b=0.41, p=0.02). The majority (63%) of 19 participants tested in winter were D3 insufficient, with levels declining by a median of 15 nmol/L overall. Those with insufficient levels performed worse (i.e., higher scores) on the CANTAB® Spatial Working Memory (SWM) task (M=36.1, SD=6 versus M=29.3, SD=8), p=0.05). Those with larger drops in levels (≥15 nmol/L) showed decline/less improvement on the CANTAB® One Touch Stockings of Cambridge (OTS) task, (M=0.50, SD=1.9 versus M=-2.11, SD=2.6, p=0.01), a test of working memory/executive functioning. ***Conclusions:*** Vitamin D3 insufficiency and seasonal declines ≥15 nmol/L were associated with inferior working memory/executive functioning. While our findings require confirmation, they suggest that sufficient D3 levels should be maintained year-round, likely necessitating supplementation, at least during winter at higher latitudes.

RÉSUMÉ: Effets d'un déficit et d'une diminution saisonnière en vitamine D et sur la cognition. ***Contexte :*** Le déficit en vitamine D3 (cholécalciférol) a été associé à la démence et au déclin cognitif. Toutefois, on ne sait pas à quels domaines de la cognition les niveaux de D3 sont le plus associés et comment les fluctuations saisonnières des niveaux sont reliées à la cognition. Nous avons procédé à une étude d'observation prospective examinant l'association entre les niveaux de vitamine D3 et la cognition chez des individus vivant dans des régions nordiques (n = 54) en été et en hiver afin d'examiner cette question. ***Méthode :*** Des adultes en bonne santé ont subi des dosages de vitamine D3 et une évaluation de la cognition en été et en hiver. Les tests suivants ont été utilisés : le Symbol Digit Modalities Test (SDMT), le test de fluence phonémique, le Digit Span et la batterie CANTAB®. ***Résultats :*** Parmi les 32 participants évalués pendant l'été, 46% avaient un déficit en vitamine D3 (< 75 nml/L) et ont obtenu des résultats inférieurs au Digit Span Backward (DS-B) (μ = 5,8 ; écart type (ét) = 2) que ceux dont les taux étaient supérieurs (μ = 7,9 ; ét = 2), p = 0,018. À l'analyse multivariée le statut normal était un facteur de prédiction indépendant du DS-B, (b = 0,41 ; p = 0,02). La majorité (63%) des 19 participants évalués pendant l'hiver présentait un déficit en vitamine D3 et les niveaux diminuaient selon une valeur médiane de 15 nmol/L globalement. Ceux qui présentaient un déficit avaient des scores moins bons (plus élevés) au CANTAB® Spatial Working Memory (SWM) (μ= 36 ; ét = 6 versus μ = 29,3 ; ét = 8), p = 0,05. Ceux dont les niveaux étaient plus faibles (≤ 15 nmol/L) avaient une baisse/moins d'amélioration au CANTAB® One Touch Stockings of Cambridge (OTS), (μ= 0,50 ; ét= 1,9 versus μ= -2,11 ; ét= 2,6 ; p = 0,01), un test de mémoire à court terme/ des fonctions exécutives. ***Conclusions :*** Un déficit en vitamine D3 et une diminution saisonnière ≥ 15 nmol/L étaient associés à une mémoire à court terme / des fonctions exécutives moindres. Bien que ces observations méritent confirmation, elles suggèrent que des niveaux suffisants de vitamine D3 devraient être maintenus toute l'année, ce qui nécessite vraisemblablement une supplémentation en vitamine D3, du moins pendant l'hiver, aux latitudes plus nordiques.

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Vitamin D_3 (cholecalciferol) insufficiency has been associated with cognitive impairment and dementia. Despite considerable study variability, recent meta-analyses[1,2] reported an overall significant relationship between vitamin D deficiency and lower global cognitive functioning as assessed by Mini-Mental Status Exam (MMSE) scores[1,2]. Similarly, analyses of case control studies revealed that vitamin D levels are 6-15 nmol/L lower in Alzheimer's patients than age-matched controls. Meta-analytic pooling of longitudinal studies[3,4] revealed that vitamin D deficient individuals had significantly increased odds of 2.5, 95% confidence interval (CIs) [1.74-3.56], p<0.0001, of incident cognitive impairment at follow-up[2].

Despite the expanding literature on the role of vitamin D in cognition, several questions remain unanswered. Which cognitive domain(s) most affected by vitamin D insufficiency is unclear as only a few trials have attempted to address this

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question[5-8]. Because of heterogeneity in methodology, particularly specific tests used, these studies were not included in the recent meta-analyses. Further, it is not known how seasonal changes in D_3 status relate to changes in cognitive functioning, as this has not been previously examined. Vitamin D insufficiency (<75 nmol/L) is extremely common in northern latitudes, affecting 65% or more of the Canadian population[9] and 77% of United States inhabitants[10]. A recent nationwide study in the United Kingdom reported rates of insufficiency as high as 61% in the summer/fall and 87% in the winter/spring among 45-year-old individuals[11]. Ultraviolet-B (UV-B) ray exposure from sunlight, the primary determinant of vitamin D status, is insufficient during several months of the year in the north[12], and even profuse summer sunlight exposure cannot compensate for lack of adequate UV-B rays in winter[13].

We attempted to address these questions using a prospective observational study examining the association between vitamin D levels and cognition in summer/early fall (July-October 2010) and winter (November 2010-March 2011). Specifically, we sought to determine: 1) which cognitive domains are most associated with vitamin D status (insufficiency defined as <75 nmol/L) at the two time points—summer and winter—and, 2) what effect the degree of seasonal decline in vitamin D levels has on cognition. Although vitamin D insufficiency has been variously defined in the literature, levels <75 nmol/L are thought to be inadequate to maintain skeletal health and 75nmol/L is the level at which parathyroid hormone (PTH) levels becomes maximally suppressed[14]. Therefore, we chose this level as our cut-off value. Importantly, however, the optimal level for cognition is not yet known.

Methods

Participants

We enrolled participants from northern British Columbia, Canada (54° north latitude) who responded to advertisements posted at the local university and college, three local seniors' centers, the local hospital, and several local family physicians' offices. Participants were included if they were over 20 years (yrs) old, literate in English, and did not have a physical or visual impediment that would limit their ability to participate in the paper-based and computerized cognitive testing. Those who had been diagnosed with dementia, or had a brain tumor, history of brain injury or symptomatic stroke were excluded. All procedures were approved by research ethics committees from the University of British Columbia, the University of Northern British Columbia, and the Northern Health region. Written informed consent was obtained from all participants.

Measurements

Participants completed a questionnaire, which included demographic information, general health, supplement use, physical activity, dietary and sun exposure practices, and the Beck Depression Inventory-II (BDI-II). Body mass index (BMI) and skin shade of proximal medial and dorsal forearm using von Luschan Skin Shades[15,16] were also assessed. This scale consists of 36 possible skin shades, numbered from lightest to darkest, with scores of 10 or less corresponding to skin that is light or very light. Standardized cognitive testing consisted of the Symbol Digit Modalities Test (SDMT) to assess information processing speed[17], phonemic fluency (PRW) to assess executive functioning[18], digit span forward (DS-F) and backward (DS-B) to assess attention/working memory[18], and the CANTAB® battery[19,20], which includes measures of: 1) Learning/Memory: Verbal Recognition Memory (VRM), Pattern Recognition Memory (PRM), Paired Associate Learning (PAL); 2) Working Memory: Spatial Working Memory (SWM), and; 3) Executive Functioning: One-Touch Stockings of Cambridge (OTS). Blood collection was performed immediately after cognitive testing. Vitamin D_3 levels were analyzed via high performance liquid chromatography tandem mass spectrometry. Intact PTH (iPTH), ionized calcium and phosphorous were also obtained. Data collection consisted of obtaining all measures listed above for each participant in the summer/early fall (July-October 2010) and then repeating them again in the winter (November 2010-March 2011), about four months after the initial assessment. The data collections protocols were identical for summer and winter, with the same order of cognitive testing maintained, except different test versions were utilized in a counter-balanced fashion to minimize practice effects. For each participant's testing sessions (i.e., summer and winter), laboratory blood-work was obtained after completion of cognitive testing with an approximate delay of two weeks before vitamin D level results were received, thus blinding the researcher to vitamin D status. Additionally, during the winter data collection period, the researcher was blinded to cognitive test results and vitamin D levels from the summer data collection period, as this data was maintained in a separate file that was not required for input of the winter period data.

Analysis

Cross-Sectional Analyses. Participants were divided into groups depending on vitamin D status: Insufficient (<75 nmol/L) and Sufficient (≥75 nmol/L). For both summer and winter, we compared mean values for demographic data as well as cognitive and mood scores, using t-tests or Welch's test for unequal variances, as appropriate. Fisher's exact tests were used to compare differences in proportion. Linear regression (stepwise, controlling for age, education, sex) was utilized to determine whether vitamin D sufficiency status was an independent predictor of the cognitive tests that differentiated between groups on univariate analyses.

Longitudinal Analyses. To determine seasonal changes, winter values for vitamin D levels, cognitive and mood scores were subtracted from summer values for each participant, yielding difference scores. Participants with larger declines in D3 levels (≥15 nmol/L, determined by a median split) were compared to those with smaller declines in D_3 (<15 nmol/L) on cognitive and mood difference scores using t-tests or Welch's test for unequal variances, as appropriate.

Analyses were performed using Statistical Package for the Social Sciences (SPSS) 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Variables not normally distributed, were transformed via square root conversions (VRM, SWM--winter and seasonal difference scores; and OTS--summer scores only), or log transformations (vitamin D—winter levels) into normal distributions prior to parametric testing. The PRM scores could not be adequately transformed into a normal distribution and

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therefore, were analyzed using the nonparametric Mann-Whitney test. Corrections were made for multiple comparisons using the Bonferroni correction, whereby $\alpha=0.05/n$, with n being the number of comparisons (i.e., tests). Accordingly, only p-values <0.005 were considered statistically significant. However, because this was an exploratory study, we also considered variables to be of importance for further study if they reached a two-tailed level of significance with p values <0.05 and had a large effect size (>0.5). Post-hoc correlational analyses, including Pearson's and partial correlation (corrected for age and education) were performed on the variables that were deemed significant/worthy of further study in the main analyses.

Results

Cross-Sectional Analyses

Summer Period. The 32 participants ranged in age from 21 to 75 yrs (M=52, SD=16) with 8-23 yrs of education (M=14, SD=3) and were predominantly (72%; n=23) female. None of the participants had significant medical comorbidities, such as renal or cardiac failure, or active cancer or systemic infection. Approximately 69% (n=22) were of European descent (Caucasian). Vitamin D levels ranged from 46 to133 nmol/L but nearly half the participants (44%, n=14) were vitamin D insufficient. Most participants (63%; n=20) were taking vitamin D supplementation (100-4000 IU/day). There were no significant differences between Sufficient and Insufficient groups regarding age, years of education, amount of vitamin D or calcium supplementation, intake of vitamin D-rich foods, amount of exercise, skin shade/ethnicity or BMI. There was, however, a non-significant trend toward higher reported sun exposure in the Sufficient group (p<0.1), with approximately 2/3 of participants receiving 20 minutes of sun exposure (without sunscreen) twice per week or more, while only about 1/3 of the Insufficient group reported such levels. Other lab values, including iPTH, ionized calcium and phosphorous, did not differ between groups (see Appendix Table A).

Participants in the Insufficient group scored lower (M=5.8, SD=2) than those in the Sufficient group (M=7.9, SD=2) on DS-B, a test of working memory, p=0.018, *Cohen's d*=1.05). Further, vitamin D sufficiency status was found to be an independent predictor of DS-B score (stepwise linear regression controlling for age, education, sex), R^2=0.17, p=0.01 (*adjusted* R^2= 0.14; b=0.41, p=0.02). Scores on other cognitive measures did not differ significantly between groups (Table 1), nor was there a difference in depression scores, as measured by the BDI-II, in which the Insufficient group (M=11, SD=7) scored similarly to the Sufficient group (M=13, SD=13), p=0.6. Post-hoc correlation analyses were performed on vitamin D levels and DS-B scores revealing a Pearson's r=.36, p=0.041 and partial correlation corrected for age and years of education, r=.27, p=0.15, which correspond to medium effect sizes.

Winter Period. Of 22 participants who returned for follow-up testing, which occurred approximately 16 weeks (M=16, SD=2.6) after the initial assessment, three did not have vitamin D lab values (due to lab error) and thus were excluded from analyses. Ten participants (of the original 32) did not return for follow-up, with the most common reason being "bad winter driving conditions." Of the 19 participants with complete data, vitamin D levels ranged from 27 to 124 nmol/L, with the

Table 1: Cognitive scores for vitamin D insufficient and sufficient groups during the summer period

Cognitive Test	Insufficient (n=14)		Sufficient (n=18)			
	Mean	SD	Mean	SD	Statistical Value (df)	p-value
SDMT	38.5	13.0	39.9	8.0	-.38(30)	0.24
VF	12.5	5.0	13.1	4.0	-.35(30)	0.73
DS-F	9.6	2.0	10.2	2.0	-.60(30)	0.55
DS-B	5.8	2.0	7.9	2.0	-2.5(30)	0.02
VRM-Free Recall	7.2	3.0	7.1	3.0	0.054(30)	0.96
VRM-Delayed	32.5	4.0	32.4	4.0	-0.51(30)	0.62
PRM-Delayed	82	17.0	85	15.0	-.57(30)	0.47
SWM-Strategy	33.0	7.0	32.7	7.0	-0.51(30)	0.62
OTS-Problems Solved	15.4	3.0	16.3	3.0	0.50(30)	0.62
PAL-Memory	14.3	4.0	12.9	3.0	1.06(30)	0.29

SDMT-Symbol Digits Modalities Test (number of symbols converted into numbers in 90 seconds); VF-Verbal (phonemic) Fluency (number of words provided in one minute); DS-F-Digit Span Forwards (number of digits repeated correctly); DS-B-Digit Span Backward (number of digits correctly repeated in reverse order); VRM-Free Recall-Verbal Memory with Free Recall (number of words correctly recalled immediately after presentation, up to a maximum of 18); VRM-Delayed-Verbal Recognition Memory (number of words correctly identified from a list with a maximum score of 36). PRM-Pattern Recognition Memory (percentage of patterns correctly identified after a 20 minute delay, with a maximum score of 100); SWM-Strategy--Spatial Working Memory-Strategy (efficiency of heuristic strategy used to uncover appropriate boxes, with a minimum score of 8, which is best, and a maximum score of 56); OTS—One Touch Stockings of Cambridge (number of problems solved on first attempt, with a maximum score of 20); PAL-Paired Associates Learning (memory for items correctly located on the first trial, summed across the stages completed with a maximum score of 21).

Table 2: Cognitive scores for vitamin D insufficient and sufficient groups during the winter period

Cognitive Test	Insufficient (n=12)		Sufficient (n=7)			
	Mean	SD	Mean	SD	Statistical Value (df)	p-value
SDMT	43.2	9.0	44.4	10.0	-0.27(17)	0.79
VF	12.4	4.0	13.1	3.0	-0.33(17)	0.74
DS-F	9.7	2.0	9.4	2.0	0.24(9.1)*	0.82
DS-B^	6.2	2.0	7.0	1.0	-1.71(17)	0.11
VRM-Free Recall	8.6	4.0	5.4	3.0	1.69(17)	0.11
VRM-Delayed	33.8	4.0	29.4	2.0	1.74(17)	0.10
PRM-Delayed	89.6	9.0	67.9	19.0	1.76(7.7)*	0.10
SWM-Strategy	36.1	6.0	29	8.0	-2.12(17)	0.05
OTS-Problems Solved	17.3	2.0	16.7	2.0	0.69(17)	0.54
PAL-Memory	14.1	4.0	12.7	4.0	1.68(17)	0.11

*Welch's Test for unequal variances. SDMT-Symbol Digits Modalities Test (number of symbols converted into numbers in 90 seconds); VF-Verbal (phonemic) Fluency (number of words provided in one minute); DS-F-Digit Span Forwards (number of digits repeated correctly); DS-B-Digit Span Backward (number of digits correctly repeated in reverse order); VRM-Free Recall-Verbal Memory with Free Recall (number of words correctly recalled immediately after presentation, up to a maximum of 18); VRM-Delayed-Verbal Recognition Memory (number of words correctly identified from a list with a maximum score of 36). PRM-Pattern Recognition Memory (percentage of patterns correctly identified after a 20 minute delay, with a maximum score of 100); SWM-Strategy--Spatial Working Memory-Strategy (efficiency of heuristic strategy used to uncover appropriate boxes, with a minimum score of 8, which is best, and a maximum score of 56); OTS—One Touch Stockings of Cambridge (number of problems solved on first attempt, with a maximum score of 20); PAL-Paired Associates Learning (memory for items correctly located on the first trial, summed across the stages completed with a maximum score of 21).

majority (63%, *n*=12) being vitamin D insufficient. Most participants (58%; *n*=11) were taking vitamin D supplements (125-4000 IU/day). There were no significant differences between Sufficient and Insufficient groups with regards to age, years of education, amount of vitamin D or calcium supplementation, intake of vitamin D-rich foods, amount of sun exposure, amount of exercise, or skin shade/ethnicity. Body mass index was found to be lower in the Sufficient group (*M*=22.5, *SD*=3) compared to the Insufficient group (*M*=27.9, *SD*=4; *t*(17)=3.07, *p*=.007, uncorrected for multiple comparisons). Other lab values, including iPTH, ionized calcium and phosphorous, did not differ significantly between groups (see Appendix Table B). Importantly, the 13 participants who were not included in the Winter Period analyses did not differ significantly from those that were included with regards to demographic, cognitive, or lab variables.

Participants with insufficient vitamin D levels performed worse (i.e., higher scores) on CANTAB® Spatial Working Memory test-Strategy score (SWM) (*M*=36.1, *SD*=6) as compared to those with sufficient levels (M=29.3, SD=8), $t_{(15.7)}$=2.1, *p*=0.05, *Cohen's d*=1.01 and there was a small trend toward worse performance on DS-B (*M*=6.2, *SD*=2 versus *M*=7.0, *SD*=1), $t_{(17)}$=-1.7, *p*=0.1. There were no significant differences between groups on other cognitive measures (Table 2). Levels of depression, as measured by the BDI-II scale, also did not differ significantly between groups. Post-hoc correlation analyses were performed on vitamin D levels and SWM scores revealing a Pearson's *r*=.41, *p*=0.058 and partial correlation corrected for age and years of education, *r*=.36, *p*=0.16. Although not statistically significant, these associations correspond to medium effect sizes.

Longitudinal Analyses

Vitamin D levels decreased an average of 17 nmol/L (*median*=15) from summer to winter. Participants with larger declines in vitamin D levels (≥15 nmol/L; *n*=10) had more of a decline on follow-up testing on the One Touch Stockings of Cambridge (OTS) task, (decrease of 0.5 points) as compared to those with smaller declines in vitamin D (<15 nmol/L;*n*=9), who actually improved by 2.1 points, *t*(17)=-2.54, *p*<0.01, *Cohen's d*=1.00). This task assesses working memory/executive functioning. Seasonal changes in performance were not significantly different between these groups on other cognitive measures or the BDI-II (see Table 3). Post-hoc correlation analyses were performed between vitamin D difference scores and OTS difference scores revealing a Pearson's *r*=.43, *p*=0.067 and partial correlation corrected for age and years of education, *r*=.57, *p*=0.018, which correspond to medium and large effect sizes, respectively.

Discussion

Vitamin D sufficiency status was related to cognition and, specifically, working memory, as assessed by the digit span-backwards (DS-B) and CANTAB® Spatial Working Memory tasks. In addition, greater declines in vitamin D levels from summer to winter were accompanied by greater declines in executive functioning, as measured by CANTAB® One Touch Stockings of Cambridge task.

Although our study was exploratory and not adequately powered to make any firm conclusions, our effect sizes were quite large, suggesting that working memory and executive

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Table 3: Difference scores (summer-winter) on cognitive tests for participants with small (<15 nmol/L) versus large (≥15 nmol/L) seasonal decreases in vitamin D levels

Cognitive Test	Small Drop <15 nmol/L (n=9)		Large Drop ≥15 nmol/L (n=10)			
	Mean	SD	Mean	SD	Statistical Value (df)	p-value
SDMT	-2.77	5.6	-1.2	8.1	-0.454(17)	0.65
VF	-1.67	3.4	0.5	2.2	-1.69(17)	0.10
DS-F	1.11	2.0	1.20	1.3	-0.49(17)	0.63
DS-B	-0.67	2.0	-0.20	2.0	-0.51(17)	0.62
VRM-Free Recall	-0.67	2.0	-0.70	2.5	28.50*	0.18
VRM-Delayed	-1.67	4.6	0.70	2.4	0.272(17)	0.79
PRM-Delayed	4.63	21	2.50	21	0.731(17)	0.98
SWM-Strategy	2.20	5.3	-1.22	8.3	1.09(17)	0.29
OTS-Problems Solved	-2.11	2.6	0.50	1.9	-2.54(17)	0.02
PAL-Memory	-0.89	2.9	0.70	2.8	-1.18(17)	0.25

*Mann-Whitney U. SDMT-Symbol Digits Modalities Test (number of symbols converted into numbers in 90 seconds); VF-Verbal (phonemic) Fluency (number of words provided in one minute); DS-F-Digit Span Forwards (number of digits repeated correctly); DS-B-Digit Span Backward (number of digits correctly repeated in reverse order); VRM-Free Recall-Verbal Memory with Free Recall (number of words correctly recalled immediately after presentation, up to a maximum of 18); VRM-Delayed-Verbal Recognition Memory (number of words correctly identified from a list with a maximum score of 36). PRM-Pattern Recognition Memory (percentage of patterns correctly identified after a 20 minute delay, with a maximum score of 100); SWM-Strategy--Spatial Working Memory-Strategy (efficiency of heuristic strategy used to uncover appropriate boxes, with a minimum score of 8, which is best, and a maximum score of 56); OTS—One Touch Stockings of Cambridge (number of problems solved on first attempt, with a maximum score of 20); PAL-Paired Associates Learning (memory for items correctly located on the first trial, summed across the stages completed with a maximum score of 21).

functioning are the cognitive domains most associated with vitamin D status. Thus, our results support and extend those of previous findings in studies using lower vitamin D cut-off values and different population groups, including older adults[7,8], Europeans[6,8], and men[6]. Specifically, among elders (65-99 yrs) enrolled in the NAME Study, vitamin D levels >50 nmol/L were associated with better performance on several tasks of executive functioning/attention, even after controlling for several potential confounders. Similarly, among middle-aged and older (40-79 yrs) European men as part of the European Male Aging Study[6], those with low vitamin D levels (<35 nmol/L) performed worse on digit symbol substitution, after correcting for multiple variables. In healthy European adults (55-87 yrs) enrolled in the ZENITH study[8], vitamin D levels were significantly correlated with performance on the CANTAB® Spatial Working Memory task, as we found in our cross-sectional analysis in winter. While reverse causation (i.e., that cognitive impairment leads to low vitamin D levels due to lack of outdoor activity) has been questioned regarding cross-sectional dementia case control results, it seems less likely in this relatively healthy sample with no overt dementia or physical impairment. Indeed, when seemingly related factors like impaired mobility and physical inactivity have been controlled for in large population-based studies, the relationship between low vitamin D status and cognition persists[21].

Greater seasonal decline in vitamin D levels was accompanied by greater decline in executive functioning, as measured by CANTAB® One Touch Stockings of Cambridge task. In contrast, those with smaller declines (<15 nmol/L) actually improved, presumably due to practice effects. While it could be argued that these results may be at least partly due to regression toward the mean, the finding is consistent with those from both our cross-sectional analyses and other cross-sectional studies[6-8] mentioned above, that vitamin D levels are most associated with executive functioning/working memory.

While mechanisms underlying the effects of vitamin D on cognition are not clear, there are several biologically plausible reasons why vitamin D might be important. The presence of vitamin D receptors in the brain, along with 1,α-hydroxylase, the enzyme that converts the active form of vitamin D (calcitriol) from its precursor, suggests an important role for vitamin D in brain processes[22]. Vitamin D has also been shown to increase acetylcholine levels[23], hippocampal neuron densities[22], enhance neuroprotection[24], decrease pro-inflammatory cytokines[25] and enhance amyloid-beta clearance[26], which are processes importantly implicated in age-related cognitive decline and dementia. That there were no differences between groups regarding vitamin D intake from food, supplementation or sun exposure suggests that, in addition to age, BMI, ethnicity, and skin shade, there are other mediators of vitamin D levels that may vary by individual, such as vitamin D receptor genotype, which may also be importantly involved in cognition. Indeed, carriers of BsmI, TaqI polymorphisms and haplotype 2(Bat) were shown to have worse cognitive test performance than other

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genotypes in a large population-based study of elderly individuals[27] and in a case-control study, those with Alzheimer's were 2.3 times more likely to have the Aa genotype than normal controls[28].

Our study had some limitations, particularly the relatively high attrition rate and the small sample size. Due to time constraints of this study (less than one year), we were only able to enroll 32 participants during the summer. Following a combination of lab error (n=3) and subject attrition (n=10), only 19 participants were available for follow-up testing. Importantly, the participants who could not be included in the Winter Period and Longitudinal Analyses, did not differ significantly from those included in the analyses on any of the demographic, cognitive, or lab values during the Summer Period. However, because we were unable to obtain winter vitamin D levels on these individuals, we cannot rule out a possible withdrawal bias on sufficiency status during the winter months (i.e., those who withdrew may have been more, or conversely less, likely to develop vitamin D insufficiency in the Winter Period). With regards to sample size, a post-hoc power analysis reveals that a minimum of 60 participants (30 per group) would have been required in order to reach the conservative level of significance of 0.005 (adjusted for the number of comparisons), assuming a beta level of 0.2, or a power level of 80%. However, of the comparisons that reached a significance level of <0.05 (uncorrected for multiple comparisons), all had very large effect sizes of 1.00 or greater and all were tests of working memory/executive functioning. The consistency of these findings and the large effect sizes argue against possible Type I errors. However, this study should still be viewed as exploratory and in need of further confirmation from larger trials. Finally, we were able to measure and analyze several potential confounding variables but we were unable to control for all of them in multivariable analyses due to the small sample size. Of note, none of these variables, aside from BMI in the Winter Period, even approached an uncorrected level of significance on univariate analyses.

Our study also had several strengths. We utilized a prospective design, collecting comprehensive cognitive data and vitamin D levels (and other markers of calcium metabolism) at two time points with the a priori purpose of examining the association between vitamin D and cognition. All other studies, to our knowledge, have been post hoc analyses of previously collected data and none have measured vitamin D on more than one occasion and related changes in levels to changes in cognition. We used different test versions, which were counterbalanced to minimize practice effects, and measured several demographic and health-related variables, which may be importantly involved.

In conclusion, vitamin D insufficiency, defined as <75 nmol/L, was associated with relatively inferior working memory performance, while greater declines in vitamin D levels from summer to winter were accompanied by greater declines in executive functioning. Although this was a small, exploratory study, these findings suggest that adequate vitamin D levels should be maintained year-round, likely necessitating supplementation, at least during the winter months, and especially at higher latitudes. Given the potential impact of these findings on public health, further study is warranted. In particular, larger prospective longitudinal studies possibly controlling for vitamin D receptor genetics[27,28], should be conducted to further assess the potential positive effects on working memory/executive functioning. To what extent supplementation will alter D_3 levels and affect different cognitive domains is not known but is currently being assessed by our group.

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Appendix Table A: Additional demographic variables for vitamin D insufficient and sufficient groups during the summer period

Variable	Insufficient (n=14)		Sufficient (n=18)	
	Mean	SD	Mean	SD
Age	47.1	16.4	55.4	15.8
Education (yrs)	13.8	3.5	14.4	3.2
Body Mass Index	26.1	5.4	26.6	7.4
BDI-II Score	11.0	7	13.1	13
Vit D Suppl (IU)	757	1070	811	1104
Calcium Suppl (mg)	239	263	358	432
Calcium (ionized)	1.29	0.05	1.36	0.3
PTH	4.64	2.3	4.30	1.5
Phosphorous	1.1	0.15	1.2	0.19
	%		%	
Sex (%male)	36		22	
Ethnicity-%Caucasian	64		72	
Physical Activity*	83		43	
Vitamin D in Diet^	33		29	
Sun Exposure#	33		29	
Vascular Risk Factors~	42		42	
Skin Shade-Prox&	67		71	
Skin Shade-Distal&	67		85	

*% exercising for at least 20 minutes, 2-3 times per week or more; ^ consumption of vitamin D rich foods (salmon, other fish and seafood) more than once per week); # % sun exposure (skin exposed, without sunscreen) for at least 20 minutes, 2-3 times per week or more; ~% 1 or more vascular risk factors including: hypertension, dyslipidemia, diabetes, atrial fibrillation, ischemic heart disease;& von Luschan skin shade >10.

Appendix Table B: Additional variables for vitamin D insufficient and sufficient groups during the winter period

Variable	Insufficient (n=12)		Sufficient (n=7)	
	Mean	SD	Mean	SD
Age	49.2	15	58.1	16
Education (yrs)	14.6	4	14.1	3
Body Mass Index+	27.9	4	22.5	3
BDI-II Score	8.6	13	11.0	9
Vit D Suppl (IU)	351	402	832	1498
Calcium Suppl (mg)	208	312	304	369
Calcium (ionized)	1.29	0.04	1.29	0.03
PTH	4.99	2.4	4.59	1.3
Phosphorous	1.2	.4	1.1	.1
	%		%	
Sex (%male)	42		29	
Ethnicity-%Caucasian	92		57	
Physical Activity*	83		43	
Vitamin D in Diet^	33		29	
Sun Exposure#	33		29	
Vascular Risk Factors~	42		42	
Skin Shade-Prox&	67		71	
Skin Shade-Distal&	67		85	

+ $p<0.01$;* % exercising for at least 20 minutes, 2-3 times per week or more; ^ consumption of vitamin D rich foods (salmon, other fish and seafood) more than once per week); # % sun exposure (skin exposed, without sunscreen) for at least 20 minutes, 2-3 times per week or more; ~% 1 or more vascular risk factors including: hypertension, dyslipidemia, diabetes, atrial fibrillation, ischemic heart disease;& von Luschan skin shade >10.

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